PLANTS AND LACTATION:

FROM TRADITION TO THE MECHANISM OF ACTION

ZOURATA LOMPO-OUEDRAOGO

Proefschrift

Ter verkrijging van de graad van doctor
op gezag van de rector magnificus
van Wageningen Universiteit,
Prof. dr. ir. L. Speelman,
in het openbaar te verdedigen
op dinsdag 14 oktober 2003
des namiddags te half twee in de Aula

Propositions (Stellingen)

1. The traditional belief that Acacia nilotica can improve milk production is valid. (this thesis)

2. A widespread use of plants relies on long-term observations and experiences. (this thesis)

3. Flavonoids from Digitaria exilis (fonio), a variety of millet commonly eaten by inhabitants of African semiarid regions, are strongly interfering with the thyroid hormone system. (Sartelet, H. et al, Nutrition 1996: 12, 100-106).

4. Treatment with high doses of progestins results in excessive secretion of growth hormone by the mammary gland, resulting in acromegaly and breasttumors (Kooistra, H.S. et al, J.Reprod.Fertil. 2000: 119, 217-222).

5. A child never dies, it just goes back to its ancestors.

6. Failure to lactate, like failure to ovulate means failure to reproduce and the latter equals curse which means exclusion.

7. It is worth promoting the research in traditional medicine in order to provide health facilities and medicines to the population in developing countries.

Propositions belonging to the thesis
Plants and lactation: from tradition to the mechanism of action.
Zourata Lompo-Ouedraogo,
Wageningen
October 14, 2003

*To my husband Martin and my children Stephanie and Andy
with love and honour, I dedicate this thesis to them*

Abstract

In most African communities, failure to lactate, like failure to ovulate, means failure to reproduce. Thus plant extracts are being used by African women to induce or stimulate milk production.

An ethnobotanical survey has been carried out in the northern, central, eastern and southern parts of Burkina Faso. In total 79 informants (healers and village elders) and 217 users from 22 villages were interviewed. This survey shows that the use of plants during lactation is common and that the indigenous people have a remarkable knowledge of species and their use as crude drugs. Ethnobotanical data on 90 plant species belonging to 40 families, including medical use and traditional practices, were collected. Agalactia is the most common pathology after parturition, since 34% of the plants are used for induction of lactation. This survey pointed out seven species including *Pennisetum americanum, Vitellaria paradoxa, Cadaba farinosa, Leptadenia hastata, Capsicum frutescens, Crateva adansonii, Acacia nilotica ssp adansonii* to be widely used by women. The first six species are commonly used in human alimentation, albeit the latter appeared to be frequently used together with other plants for the treatment of a wide variety of diseases. Despite the widespread use of *Acacia nilotica ssp adansonii* in folk medicine, little is known about its actual pharmacological mechanism.

In view of the traditional belief that *Acacia nilotica ssp adansonii* (AN) can stimulate milk production in lactating women, experiments were performed to determine the effect of an aqueous extract of AN on milk production in the rat. Female rats that received the AN extract orally during their first lactation, produced about 59% more milk causing an increase in pup growth compared to that in controls ($P<0.01$). Prolactin (PRL) is known to stimulate milk synthesis and secretion. Experiments were performed in peri-pubertal and adult virgin female rats to investigate the effect of AN on PRL synthesis and release. A strong relation between AN administration and PRL release after "acute" and "chronic" treatment has been observed in cycling female rats treated intravenously. PRL synthesis was also stimulated by AN extract in both adult and peri-pubertal animals albeit that in the latter group, estradiol-priming was needed for an efficient effect. The treatment with AN extract results in increased lobulo-alveolar structure development, filled with basophilic secretions and suppression of the terminal end bud development. AN extract stimulates the mammary gland growth and differentiation from the least differentiated structures to fully developed alveoli, showing active milk production. The mechanisms via which these processes occurred have been investigated. The treatment with bromocriptine (CB154), an agonist of dopamine significantly lowered pituitary- and plasma PRL levels compared to controls. A significant increase of pituitary PRL was observed in the group with 3g AN+CB154 as compared to CB154 alone although no significant change was found in plasma PRL.

These results indicate that the AN-extract acts by stimulating the synthesis and release of PRL and probably other hormones, which in turn stimulate growth and differentiation of the mammary gland. AN-extract may also act directly on the mammary gland by stimulating the synthesis of milk components. Nevertheless further research is needed to elucidate the possible mechanism of action of AN-extract.

Preface

It is my great pleasure to express my thanks to God for all He has done in my life and especially for giving me good health, strength and determination during these years of study. To him, I return all the Glory and honour for this work.

Contents

CHAPTER 1

GENERAL INTRODUCTION

For a long time, human milk was the only food for young babies. Human newborns are very immature at birth and thus totally dependent on their mother's milk for a varying time thereafter. However, during the last decade, major changes have occurred in infant feeding: from breast-feeding to bottle-feeding with either human milk or formulae based on cow's milk. It is well-known that the milk of each species is a unique complex secretion containing a large variety of components with special metabolic and nutritional functions, as mimicked at least in part by the wide variety of "humanized formula milk" on the market. Noticeably, it is widely accepted that breast-feeding is more than just nutrition. Fortunately, this assertion is reinforced since 1992 by the recommendations of the WHO and UNICEF for the practice of breast-feeding, even in developed countries.

Recently, many aspects including prevention of infectious diseases and the increasing amount of information about the importance of mother-infant interactions on the psychology of the neonate have increased the necessity to reappraise the role of breast-feeding in both developed and developing countries (Wright & Schanler 2001, Filteau 2000, Ahiadeke 2000). Certainly, the benefits in terms of preventing the malnutrition-infection cycle are especially great in developing countries (Brown *et al.* 1989). Consequently, the international policy (World Health Organization 1993) recommended that all infants should be exclusively breast-fed, i.e. not given any other foods or liquids including water for the first 4-6 months of life. Indeed, there is no doubt on the scientific basis of the importance of breast-feeding (Filteau 2000, Goldman 1993, Anderson *et al.* 1999, Khin-Maung-U *et al.* 1985, and Sazawal *et al.* 1992). However, some apparently controversial evidence exists regarding the applicability of such recommendation in developing communities like in Africa, where breast-feeding is tightly linked with traditional medicine.

African Traditional Medicine

"African Traditional Medicine is not only use of plants but also all the practices, measures, ingredients and procedures of all kinds of materials or not, which from centuries and centuries had enabled the African people to protect themselves against disease, to cure themselves and to alleviate their sufferings." (WHO 1973).

Indeed, the use of natural products with therapeutic properties is as ancient as human civilisation. For a long time, minerals, animals, and plant products were the main sources of drugs. Although in developed countries, indigenous health care practices have been replaced by modern medicine, in developing countries, these practices are still the way of health care for the majority of the population, albeit that these tended to decline during the last few decades. However, an increased interest actually seems to reverse this trend. This

trend reversal is based on a need for new drugs in developed countries and on the fact that many modern drugs are beyond the reach of the majority of the population in developing countries, such as Burkina Faso, West Africa. Unfortunately, traditional knowledge has gradually changed. This alteration is due to the fact that the transmission of the knowledge is essentially oral and that traditional healers often pass away without having had the opportunity to hand down their knowledge, in addition to the progressive disappearance of useful plants caused by drought and deforestation.

Having thus noted the impact of traditional medicine on health care in Africa, the necessity of officially integrating its activities into National Health Programmes is under discussion in most countries. An integration of the two types of medicine can only be most efficient when traditional medicine is well documented and the biological activities of the plants used are established. This raises a number of questions:
1- What is the indigenous knowledge in medicinal plant use?
2- What are the traditional practices and prescriptions?
3- How many people have used these prescriptions?
4- Are they efficient and safe?
5- What are the mechanisms of action?

One of the difficulties in answering these questions is that African traditional medicine appears to rely on cultural aspects. These cultural aspects are based on the concept that 3 supernatural powers are at the origin of change in human well-being: The force of evil (sorcery), the gods, and the spirits emanating from ancestors which can be good or evil. The balance between good and evil spirits determines the health of the individual. Disease is regarded as a punishment for bad behaviour. Evil spirits can be chased away or appeased by certain procedures including the utilisation of certain plants. This is particularly true as far as reproduction is concerned. Plants from various habitats are traditionally used to stimulate libido and fertility, or to inhibit those during evil moon, to protect pregnant women from the evil eye, to inhibit or stimulate milk production *etc*. In the latter case, however, plant use during lactation has actually tended to decline due to new recommendations of the WHO (WHO 1992). Consequently, the significance of plant use during lactation in the African traditional communities might be worth reappraising.

Breast-feeding in the African traditional community

In most African communities, failure to lactate, like failure to ovulate, means failure to reproduce. Thus, breast-feeding implies more than just nutrition: it encompasses aspects of cultural, environmental, and psychosocial significance, operating between the mother and the baby as well as within the community.
The traditional belief is that the newborn is a reincarnation of one of the ancestors of the family. The day, time, and conditions of birth are the indicators that will determine his given name and his destiny. Furthermore, an infant looks neither like his father nor his mother but resembles his grandfather or grandmother, particularly when the latter are deceased. Therefore, infringement of traditional rules during pregnancy is considered the

main cause of complications observed during parturition and leading to agalactia, hypoglactia, or mastitis and sometimes to the "return" (i.e. the death) of the infant. Since traditional medicine and breast-feeding are tightly interconnected, it is not surprising that breast-feeding is based on the same concept that supernatural powers lie at the origin of human well-being and that the newborn can be protected against evils by the administration of certain plants together with certain procedures. This concept may explain to some extent, that exclusive breast-feeding is rarely practiced in African communities (Shirima *et al.* 2001, Senanayake *et al.* 1999) where herbal tea is usually used during the first few days after birth. Indeed, maternal colostrum and milk have an important function as protective agents against infections in addition to their nutritional role. Early lack of maternal colostrum and milk in a sufficient quantity and quality might consequently affect early infant well-being and development, of which the latter determines its resistance against infections. Although 99% of the mothers are breast-feeding in Burkina Faso, as well as in most of the developing countries, infant morbidity and mortality are still high. The confounding question is whether or not the high rate of infant malnutrition is due to the traditional practices or to insufficiencies in maternal milk quality and quantity.

Indeed, health and nutrition are dramatically interconnected as evidenced by the drastically limited food resources in developing countries. Therefore, maternal malnutrition is thought to be the main, if not only, cause of infant feeding problems and early childhood malnutrition. Some reports show, however, that maternal undernutrition neither affected milk volume and energy levels (Rasmussen 1992, Perez-Escamilla *et al.* 1995, Gonzalez-Cossio *et al.* 1998) nor immune factors (Miranda *et al.* 1983, Chang 1990, Herias *et al.* 1993, Lonnerdal *et al.* 1976, Prentice *et al.* 1983, Filtteau *et al.* 1999b). Reports on the relationship between maternal infection and lactational performance are controversial. Some investigations did not find any adverse effect of maternal infection on milk quality and quantity (Zavaleta *et al.* 1995), while others reported a dramatic decrease of lactational performance (Filteau *et al.* 1999a,b; Willumsen *et al.* 2000) associated with a low weight gain of the child (Filteau *et al.* 1999b), albeit that the bacteria from breast milk have been reported to be non-pathogenic for the infant. On the other hand, an infant from an HIV-infected mother can be infected during lactation.

Many views regarding breast-feeding and traditional practices have been reported. Interestingly, in addition to limited access to modern formula milk, African women traditionally use plant extracts to induce and/or stimulate milk production. Lactation is a complex phenomenon encompassing multi-factorial processes including physiological, biochemical and structural changes of the mammary gland. Milk is consequently a complex product consisting mainly of sugars, lipids (mainly triglycerides), proteins (e.g. casein, lactalbumin) as well as many other factors regulating the immune function of the neonate (Bonati & Campi 2000). Based on new scientific information from biochemistry, immunology, nutrition, endocrinology, and psychophysiology about human milk and breast-feeding, the overall scheme of events that occurs in the mammary gland has been reviewed. This review is necessary to gain insight into the fundamental issue of the physiological adequacy of plant use in terms of induction and/or stimulation of lactation in women.

Mammogenesis and lactogenesis

The ability to lactate depends on the presence of mammary glands, which is a typical feature of mammals. The mammary gland is part of the reproductive apparatus and lactation can be considered as the final phase of reproduction. Of subcutaneous origin, its number, shape and size vary greatly between species. Due to difficulties in the availability of tissue, normal development and differentiation of human breasts are rarely reported. Thus, the descriptive review reported here is based on rodent data. However, it is assumed that the overall patterns of growth and differentiation and the generation of various morphological structures are comparable between rodents and humans (Lydon *et al.* 2000, Shyamala 1999) (see Figure 1).
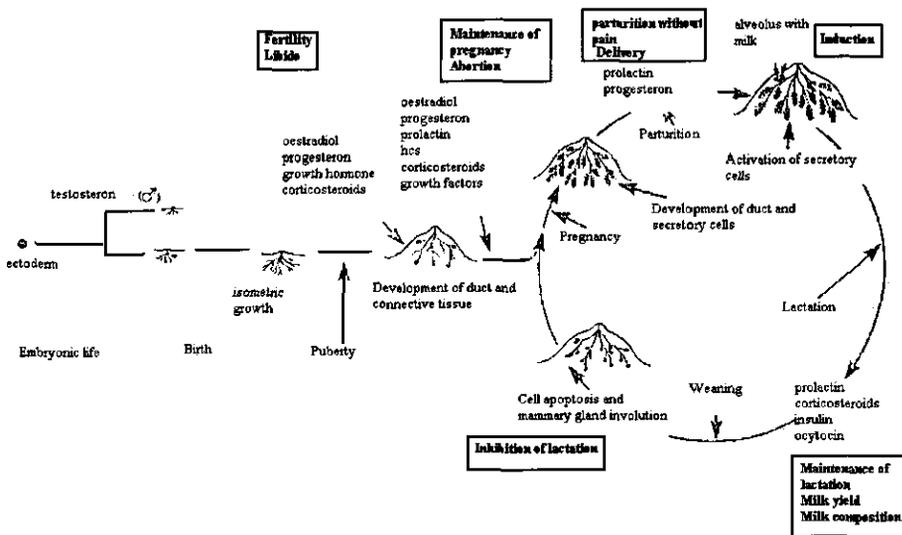
Figure 1 The overall pattern of mammary gland development and physiological targets of plant use during each stage of development.

In rats, the mammary gland forms extensive subcutaneous sheets of tissue that extend from the cervical to the inguinal regions as six ventrolateral pairs, each with its own nipple (Russo & Russo 1978). The mammary gland of newborns is represented by a rudimentary duct system with small club-like ends that regress shortly after birth. In neonates of most species, there is an isometric duct growth of the mammary gland until before the onset of puberty, albeit that this period of growth has not yet been extensively studied in rodents. With the onset of puberty, the mammary gland undergoes allometric growth to establish the ductal elongation with terminal end buds (TEBs) prior to pregnancy (Cowie 1949). The first events of puberty are increases in circulating gonadotropin and estradiol (E_2) levels associated with vaginal opening followed by vaginal cornification several days later

(Nelson *et al*. 1990). At puberty, the mammary gland consists of ducts with TEBs, which are the major site of proliferation (Monaghan *et al*. 1990) leading to the formation of terminal lobular units (Howard & Gusterson 2000) with the advancement of puberty. The epithelial-stromal interactions are the major components of ductal elongation. This ductal elongation and branching continue after puberty until the mammary fat pad is filled. The main characteristics of a mature mammary gland are the absence of terminal end buds and the presence of a highly branched architecture terminated by lobular buds (Horseman 1999) in response to the hormonal change during the estrous cycle. During pregnancy, the lobular buds give rise to alveolar lobules, which increase both in size and number and reach maximal development at the end of pregnancy (Masso-Welch *et al*. 2000).

Thus, lactation is a result of long physiological processes occurring in the mammary gland, mainly during pregnancy until the day of parturition. Indeed, mammary epithelial cells undergo secretory differentiation throughout the estrous cycle (Schedin *et al*. 2000), but pregnancy is required for final differentiation (Robinson *et al*. 1995). At the end of pregnancy, the mammary gland achieves a high rate of proliferation and a profound biochemical transformation within the epithelial cells which later leads to the synthesis of specific components of milk such as proteins (e.g. casein, lactalbumin), lipids (fatty acids) and sugar (e.g. lactose). During that period, the myoepithelial cell layer, which surrounds the alveolar epithelium, has acquired abundant cytoplasmic filaments for contractile function (Joshi *et al*. 1986) during lactation.

Involution of the mammary gland following weaning is characterized by a rapid decrease in weight, volume and number of alveolar structures and in total DNA content, essentially due to the loss of cells by apoptosis (Strange *et al*. 1992, 1995, Walker *et al*. 1989).

Hormonal regulation of mammogenesis and lactogenesis

Extensive studies have clearly established that the developmental biology of mammary glands is very complex involving interactions between ovarian, adrenocortical and pituitary hormones (Nandi 1958, Kleinberg *et al*. 1990, Lyons *et al*. 1995, and Topper & Freeman 1980). Cyclical variations in cell proliferation and steroid receptor expression have been reported in the human breast (Olssen *et al*. 1996, Soderqvist *et al*. 1997) and in rat mammary tissue (Masso-Welch *et al*. 2000). Estrogen (E_2) (Anderson *et al*. 1998) and growth hormone (GH) (Kleinberg *et al*. 2000, Sejrsen *et al*. 1999) are essential for mammary duct development. Progesterone (P) is essential for lobulo-alveolar development (Lydon *et al*. 2000, Shyamala 1999, and Fendrick *et al*. 1998) but not for ductal side branching as previously reported (Russo *et al*. 1991). Corticosteroids must be present for the ovarian steroids to exert their full effect. In contrast to the other hormones, recent reports show that prolactin (PRL) regulates mammary gland organogenesis from the primary duct system to the fully mature non-pregnant gland. It participates with placental lactogen in the differentiation of the lobuloalveolar system from the lobular buds, induces milk protein gene expression and finally stimulates lactation after delivery (Horseman 1999). The ejection of milk requires the action of oxytocin (OX), secreted from the posterior pituitary in response to suckling. The milk stasis that occurs after weaning and

the decrease of lactogenic hormones, in particular PRL, are the main causes of involution of the mammary gland (Nguyen & Pollard 2000). However, the mechanisms involved in the regulation of mammogenesis and lactogenesis are still to be more clearly elucidated.

PRL regulation of lactogenesis

Identified by Riddle *et al.* (1931) and purified in 1933, PRL is a polypeptide hormone produced mainly in pituitary lactotrophic cells as well as in some peripheral tissues, including the mammary gland (Jonathan *et al.* 1996, Iwasaka *et al.* 2000).

Pituitary PRL secretion is under hypothalamic control by both inhibitory and stimulatory factors (Freeman *et al.* 2000) although dominated by inhibitory factors (PIH), mainly dopamine, synthesized by the hypothalamus and released into the portal blood vessels. PRL down regulates its own secretion by increasing the release of PIH's in the portal system.

Although more than 300 functions attributable to the hormone have been reviewed recently (Bole-Feysot *et al.*1998), PRL has the specialised function of stimulating milk synthesis and secretion in mammals. In rats, PRL is secreted during early pregnancy in a diurnal and nocturnal pulse from the anterior pituitary (Andrews *et al.* 2001). The decrease in progesterone and estrogen levels after delivery allows PRL to exert its stimulatory action on the mammary gland epithelial cells and thus induce lactogenesis. It is unclear whether or not the inhibition of lactogenesis during late pregnancy, even though PRL levels are relatively high, is due to the high levels of estrogen or due to progesterone, which may act on the epithelial cell by decreasing PRL receptor numbers. Interestingly, PRL mRNA has been detected in rat mammary glands from mid to late pregnancy and throughout lactation (Iwasaka *et al.* 2000) and no ß-casein was detectable in the mammary gland of PRL receptor (PRLR) knock-out mice (Brisken *et al.* 1999).

During established lactation, the suckling of the neonate stimulates PRL secretion. Continuous production of PRL is necessary for the maintenance of lactation, although no correlation was found between milk yield and plasma PRL levels (Cowie *et al.* 1980), suggesting that PRL may be more of a "survival factor" rather than a regulatory factor for milk secretion.

In humans (Benedek-Jaszmann & Sternthal, 1976) and rodents (Cowie *et al.* 1969, Mena *et al.* 1982, and Flint *et al.* 1998), PRL is the major stimulus to milk synthesis. Bromocriptine (CB154), an analogue of dopamine that inhibits pituitary PRL secretion, completely suppresses milk yield in rats (Flint *et al.* 1998, Farmer *et al.* 1998, and Taylor & Peaker 1975). Yet, when milk production is almost completely inhibited by both CB154 and a specific antiserum to rat GH, replacement therapy with GH partially restored milk production while replacement with PRL was fully effective (Flint *et al.* 1992). Other reports (Mena *et al.* 1982) have suggested the possible transformation of PRL into a presecretory PRL before it is secreted, and that dopaminergic control is exerted on the transformation-phase but not on the release-phase of the hormone. On the other hand,

drugs used for the treatment of psychological disorders such as sulpiride and perphenazine, stimulate PRL secretion and thus induce lactation.

Causes of lactation disruption

Indeed, lactation success depends on many factors. The change in systemic lactogenic hormone levels as reviewed above and the intra-mammary mechanisms of responsiveness to lactogenic hormones and milk removal are, in all species, at the origin of lactation defects. Mammary epithelial cell differentiation and proliferation are thought to be the primary determinants for milk production. Nutritional status at the time of puberty and during pregnancy, associated with a great variability of hormones in the maternal plasma has been reported to be critical to maximise mammary gland cell proliferation (Wilde *et al*. 1986, Park *et al*. 1989, Choi *et al*. 1997, and Kim *et al*. 1998). Moreover, lactation success depends on regular suckling of the neonate (Peaker & Wilde 1996). Without this stimulus, milk secretion stops and mammary involution is induced. Successful parturition under appropriate conditions may also influence lactation since in most cases stress is associated with an increase in PRL levels (Jeffcoate *et al*. 1986) as well as a decrease, particularly under conditions under which levels are normally high (Chatterton *et al*. 2000, Johansson *et al*. 1983).

Possible ways of action of lactogenic plant extracts to induce and /or stimulate milk synthesis

As reviewed above, PRL plays a key role in inducing and stimulating milk synthesis and maintaining lactation. PRL synthesised in the anterior pituitary is released into the portal vessel and transported to the mammary glands to stimulate milk synthesis. E_2 increases the number of PRL-R whereas P suppresses this increase. PRL also upregulates its own receptors as an increase in PRL-R occurs at the time of the increase of plasma PRL levels at parturition. Therefore, the lactogenesis that occurs after parturition can be, to some extend, explained by the removal of the inhibition by P, and the stimulation of production of PRL-R by the increase of PRL itself.

To this end, lactogenic plants may exert their effect by stimulating PRL synthesis in the anterior pituitary, increasing its release into the portal vessels, or increasing the number of PRL receptors on the mammary gland. The stimulatory effect of PRL on its own receptors was found to be discontinuous, as an increase of maternal circulating PRL levels during established lactation does not increase milk production. This indicates that the gland at this stage probably becomes less responsive to PRL, likely a result of the low number of PRL receptors on the mammary gland. This clearly suggests that the number of PRL-R at the beginning of lactation associated with the level of circulating PRL is essential for the maintenance of copious milk production.

Lactogenic plants may also induce milk synthesis by acting directly on the mammary gland to stimulate mammary gland differentiation and cell proliferation, or by stimulating

mammary cell activity as milk production is related to mammary epithelial cell differentiation and proliferation.

Outline of the thesis

The aim of this study is to gain insight into the traditional beliefs and to provide scientific proof for the efficacy of lactogenic plants.

This thesis focusses on (1) the plants used by women for the treatment of lactation deficiencies, (2) the biological effects of the plants used for induction and stimulation of lactation, and (3) the possible underlying mechanisms of action. For this purpose, information on plants with therapeutic properties was collected during field work, regarding which part of the plant is used, mode of preparation and administration, number of cases reported and number of people who have used these prescriptions (chapter 2). The ethnopharmacology, phytochemistry, and toxicology of Acacia nilotica ssp. Adansonii (AN), one of the plants widely used for induction and stimulation of lactation are addressed in chapter 3. In view of the traditional beliefs, experiments were performed to investigate the biological activity of an aqueous extract of AN in a rat model (chapters 4 and 5). Finally, the mechanism via which AN-extract may act to achieve its biologic activities was explored (chapter 6).

References
Ahiadeke C 2000 Breast-feeding, diarrhoea and sanitation as component of infant and child health: a study of large scale survey data from Ghana and Nigeria. *Journal of Biosocial Science* **32** 47-61.
Anderson E, Clarke RB & Howell A 1998 Estrogen responsiveness and control of normal human breast proliferation. *Journal of Mammary Gland Biology and Neoplasia* **3** 37-48.
Anderson TJ 1999 Pathological studies of apoptosis in the normal breast. *Endocrine Related Cancer* **6** 9-12.
Andrews ZB, Kokay IC & Grattan DR 2001 Dissociation of prolactin secretion from tuberoinfundibular dopamine activity in late pregnant rats *Endocrinology* **142** 2719-2724.
Benedek-Jaszmann & Sternthal V 1976 Late suppression of lactation with bromocryptine *Practitioner* **216** 450.
Bole-Feysot C, Goffin V, Edery M & Binart N, Kelly PA 1998 Prolactin (PRL) and its receptor: actions, signal transduction pathways and phenotypes observed in PRL receptors knockout mice. *Endocrine Reviews* **19** 225-268.
Bonati M & Campi R 2000 Breastfeeding and infant illness *American Journal of Public Health* **90** 1478-1479.
Brisken C, Kaur S, Chavarria T, Binart N, Sutherland RL, Weinberg Kelly PA & Ormandy CJ 1999 Prolactin controls mammary gland development via direct and indirect mechanisms. *Developmental Biology* **210** 96-106.
Brown ML & Adelson SF 1969 Infant feeding practices among low and milde income families in Honolulu. *Tropic Geographical Medicine* **21** 53.
Brown KH & Bentley ME 1989 Dietary management of diarrhea. *Mothers Child.* **7** 1-5.
Chang SJ 1990 Antimicrobial proteins of maternal and cord sera and human milk in relation to maternal nutritional status. *American Journal of Clinical Nutrition* **51** 183-7.
Chatterton RT, Hill HP, Aldag JC, Hodges KR, Belknap SM & Zinaman MJ 2000 Relation of plasma oxytocin and prolactin concentration to milk production in mothers of preterm infants: influence of stress. *The Journal of Clinical Endocrinology and Metabolism,* **85** 3661-3668.
Choi YJ, Han IK, Woo JH, Lee HJ, Jang K, Myung KH & Kim YS 1997 Compensatory growth in dairy heifers: the effect of a compensatory growth pattern on growth rate and lactation performance. *Journal of Dairy Science* **80** 519-24.

Cowie AT 1949 The relative growth of the mammary gland in normal, gonadectomized and adrenalectomized rat. *Journal of Endocrinology* **6** 145-147.

Cowie AT, Forsyth IA & Hart IC 1980 Hormonal control of lactation. *Monograph of Endocrinology* **15**:1-XIV, 1-275.

Cowie AT, Hartmann PE & Turvey A 1969 The maintenance of lactation in the rabbit after hypophysectomy. *Journal of Endocrinology* **43** 651-62.

Dietz WH 2001 Breastfeeding may help prevent childhood overweight. *The Journal of the American Medical Association* **285** 2506-2507.

Farmer C, Robert S & Rushen J 1998 Bromocriptine given orally to periparturient of lactating sows inhibits milk production. *Journal of Animal Science* **76** 750-7.

Fendrick JL, Raafat AM & Haslam SZ 1998 Mammary gland growth and development from the postnatal period to the postmenopause: Ovarian steroid receptor ontogeny and regulation in the mouse. *Journal of Mammary Gland Biology and Neoplasia* **3** 7-22.

Filteau SM 2000 Role of breast-feeding in managing malnutrition and infectious disease. *The Proceedings of the Nutrition Society* **59** 565-572.

Filteau SM, Lietz G, Mulokozi G, Bilotta S, Henry CJ & Tomkins AM 1999a Milk cytokines and subclinical breast inflammation in Tanzanian women: effects of dietary red palm oil or sunflower oil supplementation. *Immunology* **97** 595-600.

Filteau SM, Rice AL, Ball JJ, Chakraborty J, Stoltzfus R, de Francisco A & Willumsen JF 1999b Breast milk immune factors in Bangladeshi women supplemented postpartum with retinol or beta-carotene. *American Journal of Clinical Nutrition* **69** 953-8.

Flint DJ & Gardner M 1994 Evidence that growth hormone stimulates milk synthesis by direct action on the mammary gland and that prolactin exerts effects on milk secretion by maintenance of mammary deoxyribonucleic acid content and tight junction status. *Endocrinology* **135** 1119-1124.

Flint DJ & Vernon RG 1998 Effects of food restriction on the responses of the mammary gland and adipose tissue to prolactin and growth hormone in the lactating rat. *Journal of Endocrinology* **156** 299-305.

Flint DJ, Tonner E, Beattie J & Panton D 1992 Investigation of the mechanism of action of growth hormone in stimulating lactation in the rat. *Journal of Endocrinology* **134** 377-383.

Freeman ME, Kanyicska B, Lerant A & Nagy G 2000 Prolactin: Structure, function and regulation. *Physiological Reviews* **80** 1523-1631.

Gillman MW, Rifas-Shiman SL, Camargo CA Jr, Berkey CS, Frazier AL, Rockett HR, Field AE & Colditz GA 2001 Risk of overweight among adolescents who were breastfed as infants. *Journal of the American Medical Association* **285** 2461-2467.

Goldman AS 1993 The immune system of human milk: antimicrobial, antiinflammatory and Immunomodulating properties. *Pediatrics and Infectious Disease Journal* **12** 664-71.

Gonzalez-Cossio T, Habicht JP, Rasmussen KM & Delgado HL 1998 Impact of food supplementation during lactation on infant breast-milk intake and on the proportion of infants exclusively breast-fed. *Journal of Nutrition* **128** 1692-702.

Herias MV, Cruz JR, Gonzalez-Cossio T, Nave F, Carlsson B & Hanson LA 1993 The effect of caloric supplementation on selected milk protective factors in undernourished Guatemalan mothers. *Pediatric Research* **34** 217-21.

Horseman ND 1999 Prolactin and mammary gland development *Journal of Mammary Gland Biology and Neoplasia* **4** 79-88.

Howard BA & Gusterson BA 2000 Human breast development. 5 *Journal of Mammary Gland and Biology of Neoplasia* **5** 119-37.

Iwasaka T, Umemura S, Kakimoto K, Koizumi H & Osamura YR 2000 Expression of prolactin mRNA in rat mammary gland during pregnancy and lactation. *Journal of Histochemistry & Cytochemistry* **48** 355-362.

Jeffcoate WJ, Lincoln NB, Selby C & Herbert M 1986 Correlation between anxiety and serum prolactin in human. *J Psychosom res.* **30** 217-222.

Jonathan NB, Mershon JL, Allen DL & Steinmetz RW 1996 Extrapituitary prolactin: distribution, regulation, functions and clinical aspects. *Endocrine Reviews* **17** 639-669.

Johansson GG, Karonen SL & Laakso ML 1983 Reversal of an elevated plasma level of prolactin during prolonged psychological stress. *Acta Physiol. Scand.* **119** 463-464.

Joshi K, Ellis JTB, Hughes CM, Monaghan P & Neville AM 1986 Cellular proliferation in the rat mammary gland during pregnancy and lactation *Laboratory Investigation* **54** 52-61.

Kim SH, Moon YS, Keller WL & Park CS 1998 Compensatory nutrition-directed mammary cell proliferation and lactation in rats. *British Journal of Nutrition* **79** 177-183.

Khin Maung U & Greenough WB 3rd. 1991 Treatment of the dehydrated child. *Pediatric Annual* **20** 7.

Kleinberg DL Ruan W, Catanese V Newman CB & Feldman M 1990 Non-lactogenic effects of growth hormone on growth and insulin-like growth factor-1 messenger ribonucleic acid of rat mammary gland. *Endocrinology* **126** 3274-3276.

Lonnerdal B, Forsum E, Gebre-Medhin M & Hambraeus L 1976 Breast milk composition in Ethiopian and Swedish mothers. II. Lactose, nitrogen, and protein contents. *American Journal of Clinical Nutrition* **29** 1134-41.

Lydon JP, DeMayo FJ, Funk CR, Mani SK, Hughes AR, Montgomery CA Jr, Shyamala G, Conneely OM & O'Malley BW 1995 Mice lacking progesterone receptor exhibit pleiotropic reproductive abnormalities. *Genes and Development* **15** 2266-78.

Lydon JP, Sivaraman L & Conneely OM 2000 A reappraisal of progesterone action in the mammary gland. *Journal of Mammary Gland Biology and Neoplasia* **5** 325-338.

Lyons WR 1993 Hormonal synergism in mammary growth. *Procedings of the Royal Society (London)* **149** 303-325.

Masso-Welch PA, Darcy KM, Stangle-Castor CN & Ip MM 2000 A developmental atlas of rat mammary gland histology. *Journal of Mammary Gland Biology and Neoplasia* **5** 165-185.

Mena F, Martinez-Escalera G, Aguayo D, Clapp C & Grosvenor CE 1982 Latency and duration of the effects of bromocriptine and prolactin on milk secretion in lactating rabbits. *Journal of Endocrinology* **94** 389-95.

Miranda G, Anglade P, Mahe MF & Erhardt G 1993 Biochemical characterization of the bovine genetic kappa-casein C and E variants. *Animal Genetics* **24** 27-31.

Monaghan P, Perusinghe NP, Cowen P & Gusterson BA 1990 Peripubertal human breast development. *Anatomical Record* **226** 501-8.

Morton JA 1994 The clinical usefulness of breast milk sodium in the assessment of lactogenesis. *Pediatrics* **93** 802-6.

Nandy S 1959 Hormonal control of mammogenesis and lactogenesis in the C3H/He Crgl mouse. *University of California Publications in Zoology* **65** 1-128.

Nelson JF, Karelus K, Felicio LS & Johnson TE 1990 Genetic influences on the timing of puberty in mice. *Biology of Reproduction* **42** 649-55.

Neville MC & Picciano MF 1997 Regulation of milk lipid synthesis and composition *Annual Review of Nutrition* **17** 159-184.

Neville MC 1983 Regulation of mammary gland development and lactation, In Neville MC & Neifert MR (eds), *Lactation: Physiology, nutrition and breast-feeding*. Plenum Press New York, pp 103-140.

Neville MC, McFadden TB & Forsyth I 2002 Hormonal regulation of mammary differentiation and milk Secretion *Journal of Mammary Gland Biology and Neoplasia* **7** 49-66.

Nguyen AV & Pollard JW 2000 Transforming growth factor beta3 induces cell death during the first stage of mammary gland involution. *Development* **127** 3107-18.

Olssen H, Jernstrom H, Alm P, Kreipe H, Ingvar C, Jonsson PE & Ryden S 1996 Proliferation of the human breast epithelium in relation to menstrual cycle phase, hormonal use, and reproductive factors. *Breast Cancer Research and Treatment* **40** 187-196.

Park CS, Baik MG, Keller WL, Berg IE & Erickson GM 1989 Role of compensatory growth in lactation: a stair-step nutrient regimen modulates differentiation and lactation of bovine mammary gland. *Growth Devlopment and Aging* **53** 159-66.

Perez-Escamillia R, Cohen RJ, Brown KH, Landa Rivera L, Canahuati J & Dewy KG 1995 Maternal anthropometric status and lactation performance in low income Honduran population: evidence for the role of the infant. American *Journal of Clinical Nutrition* **61** 528-534.

Prentice MA, Paul AA, Prentice A, and Black AE, Cole TJ & Whitehead RG 1986 Cross-cultural differences in lactational performance. In Hamosh M and Goldman AS (eds.), *Human Lactation 2*, Plenum Publishing Corp., New York, p. 13-44.

Rasmussen KM 1992 The influence of maternal nutrition on lactation. Annual *Reviews in Nutrition* **12** 103-17.

Riddle O & Braucher PF 1931 Studies on the Physiology of reproduction in Birds. Control of the special secretion of the crop-gland in pigeons by an anterior pituitary hormone. *American Journal of Physiology* 97 617-625.

Riddle O, Bates RW & Dykshorn SW 1933 The preparation, identification and assay of prolactin – a hormone of the anterior pituitary. *American Journal of Physiology* 105 191-216.

Robinson GW, McKnight RA, Smith GH & Hennighausen L 1995 Mammary epithelial cells undergo secretory differentiation in cycling virgins but require pregnancy for the establishment of terminal differentiation. *Development* 121 2079-2090.

Russo IH & Russo J 1978 Developmental stage of the rat mammary gland as determinant of its susceptibility to 7,12-dimethylbenz(a)anthracene. *Journal of the National Cancer Institute* 61 1439-1449.

Russo IH & Russo J 1991 Progestagens and mammary gland development: differentiation versus carcinogenesis. *Acta Endocrinolica (Copenhagen)* 125 Suppl 1 7-12.

Russo J & Russo IH 1978 DNA labeling index and structure of the rat mammary gland as determinant of its susceptibility to carcinogenesis. *Journal of the national Cancer Institute* 61 1451-1457.

Sazawal S, Bhan MK, Bhandari N. (1992) Type of milk feeding during acute diarrhoea and the risk of persistent diarrhoea: a case control study. *Acta Paediatrica Supplement* 381 93-7.

Schedin P, Mitrenga T & Kaeck M 2000 Estrous cycle regulation of mammary epithelial cell proliferation, differentiation and death in the Sprague-Dawley rat: A model for investigating the role of estrous cycling in mammary carcinogenesis. *Journal of Mammary Gland Biology and Neoplasia* 5 211-225.

Sejrsen K, Purup S, Vestergaard M, Weber MS & Knight CH 1999 Growth hormone and mammary gland development *Domestic Animal Endocrinology* 17 117-129.

Senanayake MP, Weerawarna H, Karunaratne KW & de Silva TU 1999 Do Babies need water in Srli Lanka? *Ceylon Medical Journal* 44 126-129.

Shah BH, Safdar B, Virani SS, Nawaz Z, Saeed SA & Gilani AH 1997 The antiplatelet aggregatory activity of Acacia nilotica is due to blockade of calcium influx through membrane calcium channels. *Genetica and Pharmacology* 29 251-5.

Shirima R, Greiner T, Kylberg E & Gebre-Medhin M 2001 Exclusive breast-feeding is rarely practised in rural and urban Morogoro, Tanzania. *Public Health Nutrition* 4 147-154.

Shyamala G 1999 Progesterone signaling and mammary gland morphogenesis *Journal of Mammary Gland Biology and Neoplasia* 4 89-104.

Silberstein GB, Van Horn K, Shyamala G & Daniel CW 1994 Essential role of endogenous estrogen in directly stimulating mammary growth demonstrated by implants containing pure antiestrogens. *Endocrinology* 134 84-90.

Soderqvist G, Isaksson E, Schoultz B, Carsltrom K, Tani E & Skoog L 1997 Proliferation of breast epithelial cells in healthy women during menstrual cycle *American Journal of Obstetrics and Gynecology* 176 123-128.

Strange R, Friis RR, Bemis LT & Geske FJ 1995 Programmed cell death during mammary gland involution. *Methods in Cell Biology* 46 355-68.

Strange R, Li F, Saurer S, Burkhardt A & Friis RR 1992 Apoptotic cell death and tissue remodelling during mouse mammary gland involution. *Development* 15 49-58.

Taylor JC & Peaker M 1975 Effects of bromocriptine on milk secretion in the rabbit. *Journal of Endocrinology* 67 313-4.

Topper YJ & Freeman CS 1980 Multiple hormone interactions in the developmental biology of the mammary gland. *Physiological Reviews* 60 1049-1106.

Walker NI, Bennett RE & Kerr JFR 1989 Cell death by apoptosis during involution of the lactating breast in mice and rats. *American Journal of Anatomy*, 185 19-32.

Wilde CJ, Henderson AJ & Knight CH 1986 Metabolic adaptations in goat mammary tissue during pregnancy and lactation. *Journal of Reproduction & Fertility* 76 289-98.

Willumsen JF, Filteau SM, Coutsoudis A, Uebel KE, Newell ML & Tomkins AM 2000 Subclinical mastitis as a risk factor for mother-infant HIV transmission. *Adv Experimental Medical Biology* 478 211-23.

Wright A & Schandler R 2001 The resurgence of breastfeeding at the end of the second millennium. *The Journal of Nutrition* 131 421S-425S.

Zavaleta N, Lanata C, Butron B, Peerson JM, Brown KH & Lonnerdal B 1995 Effect of acute maternal infection on quantity and composition of breast milk. *American Jouranl of Clinical Nutrition* 62 559-63.

CHAPTER 2

PLANTS AND LACTATION: INDIGENOUS KNOWLEDGE AND TRADITIONAL PRACTICES

Lompo-Ouedraogo Z[2], Van Der Heide D[1], Nianogo AJ[2]

[1]Human and Animal Physiology Group, Department of Animal Sciences, Wageningen University, The Netherlands
[2]Unité de Formation et de Recherche en Sciences de la Vie et de la Terre, Université de Ouagadougou, Burkina Faso

Abstract

An ethnobotanical survey was carried out in the northern, central, eastern and southern parts of Burkina Faso inhabited by Yadse, Mosse, Gulmantse, and Bissa ethnic groups. In total 79 informants (healers and village elders) and 217 users from 22 villages were interviewed. Ethnobotanical data on 90 species belonging to 40 families including medical use and traditional practices, were collected. All women interviewed used plant medication to improve their milk production during several lactation periods; about 20% of the users used plants to stop ongoing lactation because of mastitis or death of the infant before weaning. Agalactia is the most common pathology after parturition since 34% of the plants are used for induction of lactation. This might be due to either the effect of socio-cultural factors or retention of placental fragments leading to blockage of the lactogenic hormone surge and thus to disruption of lactogenesis. Although only 1, rarely 2, plant is taken for inhibition, a mixture of at least 2 plants is always used for improvement of lactation, suggesting that some plants might be included just to inhibit the possible toxicity of some of the active plants. This study shows that the use of plants during lactation is common in the study area and that the indigenous peoples have a remarkable knowledge of species and their use as crude drugs.

Introduction

Based on the cases reported (Bognounou *et al.* 1975, Olivier-Bever 1982, Chenu & Ake 1992, Gelfand *et al.* 1985, Nacoulma-Ouedraogo 1996, Van Wyk *et al.* 1997, and Ndubani & Hojer 1999) it can be stated that herbal medicine is still a method of health care in most African communities.

With the advent of modern medicine, indigenous health care practices have tended to decline in importance during the past few decades, particularly in urban areas. However, a number of factors seem to have helped reverse this trend. Firstly, there is an increased interest in herbal medicine across the world (Elvin-Lewis 2001). Secondly, in Africa, most rural areas still lack functional health centers and many drugs are beyond the reach of the majority of the population. Indeed, modern medicine is more efficient for the treatment of specific diseases; however, modern medicine is still a luxury for the majority of Africans.

In Burkina Faso, where 80% of the population lives in rural areas, there is a long tradition of using plant resources for basic needs, such as food, firewood, timber, and medicine. Plants from various habitats, such as forest, scrub, grassland and cultivated fields, are used as crude drugs (Ouedraogo 1984, Guinko 1977, 1984, Guinko & Bognounou 1982, and Bognounou *et al.* 1975, Nacoulma-Ouedraogo 1996). However, this inheritance is slowly disappearing due to the progressive disappearing of useful plants because of droughts, deforestation and overexploitation, as well as interruption of the line of transmission of knowledge. The transmission of knowledge is still essentially oral, from father to son (Van Wyk *et al.* 1997). However, with the new socioeconomic situation, sons often do not live with their parents anymore so that traditional healers pass away without having had the

opportunity to hand down their knowledge. In the present study we have attempted to document the plants used during all steps of reproduction (fertility, abortion, libido, lactation) in the northern, central and eastern parts of Burkina Faso (West Africa). In this paper, however, only plants used during lactation will be discussed.

Materials and Methods

The study area

A field study was carried out in 5 provinces (Yatenga, Kadiogo, Kouritenga, Gourma, and Boulgou) of Burkina Faso (Figure 1). Yadse, Mossé, Mossé, Gulmatse, and Bissa ethnic groups inhabit these provinces, respectively. The landscape consists mainly of plains, between 200 and 400 m. above sea level. The climate of the area is tropical with a rainy season from June to October and a dry season from November to May. The annual rainfall in the study area is between 300 and 600 mm. The vegetation is characterised by thorny steppes, hardwood forests (acacia, anogeissus leiocarpus, crateva adansonii, adansonia digitata), shrubs and grasslands.
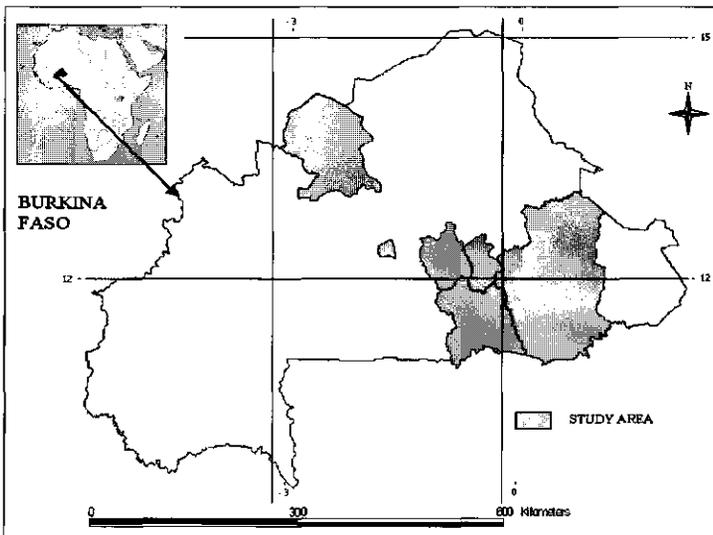

Figure 1: Study area in Burkina Faso

The survey

Ethnobotanical information was gathered during field trips between 1997 and 1999. Interviews and discussions were carried out with local informants, i.e. traditional healers or "tradipraticians," village elders and rural women who are supposed to use these plants

(Table 1). A healer is someone who treats people by means of plants or any item associated with magic. A village elder, who is not necessarily the oldest person in the village, is the one to whom family knowledge is confided. For the interviews, a questionnaire was developed. The questions concerned the plants used, methods of preparation, mode of administration, traditional practices and perception of people with respect to certain related issues. At least three field trips were necessary to collect the information from each informant. The first was used to identify the people in the villages most suited for the interview. The second trip was to collect the information; interviews often lasted all night long, and sometimes, the interview could be recorded. During the third trip, information collected from the informants of a village was used to interview the women of that village. After each interview, voucher herbarium specimens were collected; specimens were later on identified taxonomically with the help of botanical references and by comparisons with identified specimens from the Department of Systematic Botany at the University of Ouagadougou, Burkina Faso.

Results

Informants and users

A total of 296 people from 22 villages within the study area, including 261 females (16 healers, 28 elders and 217 users) and 35 males (18 healers and 17 village elders) were interviewed (Table 1). In total 79 informants and 217 users were interviewed during the study. Of the informants, 89 % received the knowledge from their parents. The remaining 11% (only men) acquired the knowledge from other sources (healers, self-learning or mystic revelation). Although village elders tend to specialize in a disease group (infant and maternal diseases, infant diseases, reproduction, female diseases, psychiatry, etc.), healers are super generalists whose healing relies mainly on the invocation of supernatural forces, in combination with plants or other items.

Table 1: Composition of the informants and the users

	Healers	Elders	Users	Total interviewed
Women	16	28	217	261
Age (Years)	57 - 80	60 - 80	16 - 59	
Men	18	17	0	35
Age (Years)	50 - 71	50 - 71		
Total	34	45	217	296

Ethnobotanical data

Plant use

Species are listed alphabetically by family and genus together with their local name, the plant part used for treatment, the number of times reported by informants, the number of

times reported by women. Only plants mentioned by 5 informants or more are listed. Ethnobotanical data on 90 species with medicinal use, belonging to 40 families, were collected. Plants reported by the informants as being used for medical purposes are illustrated in Figure 2. Of the 90 species, 70 were mentioned by at least 10% of the informants, and 75 were used by at least one user. From the 75 species, 19 were used by at least 10% of the users; however, it appeared that the women did not use 15 species reported by the informants.

⟶ Plants used for induction and stimulation of milk production and its components
As listed in Table 2, sixty-five plant species were reported for the induction and/or stimulation of lactation and improvement of milk quality. It was observed that more plants are used for induction (39 species) than for stimulation alone (6 species) or for the combination of stimulation and induction (7 species) of lactation. The use of plants for improvement of milk quality is more often associated with induction and stimulation (7 species) than with induction alone (3 species) or stimulation alone (4 species). The improvement of milk quality is also often associated with the treatment of mastitis, as seen in Table 3. A change in milk quality due to mastitis was reported by at least 50% of the informants. Although at least 18 % of the users used plants to improve the quality of their milk, only 3 plant species belonging to 3 families were used.

The most popular species for improvement of lactation were Pennisetum americanum (100%), Vitellaria paradoxa (58%), Cadaba farinosa (55%), Acacia nilotica ssp adansonii (50%), Leptadenia hastata (47%), Capsicum frutescens (46%), and Crateva adansonii (40%) (Figure 3). A plant is considered popular when reported by at least 50% of the informants and used by at least 40% of the users.

⟶ Plants used for inhibition of lactation and the treatment of mastitis
Twenty plant species belonging to 16 families were listed as potent agents for the inhibition of lactation and/or the treatment of mastitis (Table 4). Of these 20 species, 10 were used for the treatment of mastitis, 6 for the inhibition of lactation and 4 for both inhibition and mastitis. At the most, 20% and 24% of the users had used at least one plant for the treatment of mastitis and inhibition of lactation, respectively. The most popular plants for inhibition of lactation and/ or treatment of mastitis were *Triumfeta cordifolia* (11%), *Anogeissus leiocarpus* (10%), *Hyptis suaveolens* (8%), *Combretum micranthum*
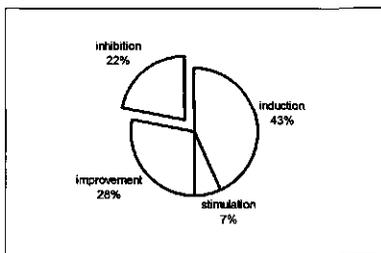

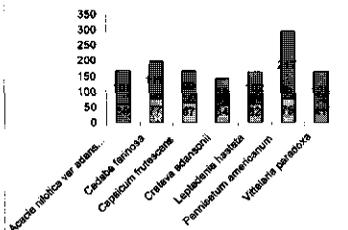**Figure 1** Medical purposes

Figure 2 Plants used for improvement of lactation

(7%), *Arachis hypogea* (6%), and *Bauhinia rufescens* (5.5%) (Figure 4). A plant is considered popular when reported by at least 30% of the informants and used by at least 5% of the users.

Parts of the plant used and medicinal preparation
Analysis of the ethnobotanical data shows that various plant parts are used, as illustrated in Figure 5. The most common parts are leaf (35%), stem with leaf (19%), bark (13%), root, rhizome and tuber (15%), fruit (8%), seed (5%), flower, latex and butter (5%). The collection of different parts of the plant is based on precise criteria. For instance, time (early in the morning, late in the afternoon), position of the moon and the sun (full moon, sunshine), birthday of the infant (on Monday) and the physiological state of the plant (stem with leaves, with or without flowers).

Likewise, medicinal processing seems to depend on the part used, which in turn is related to the availability of the plant over the year and within the region. The most common medicinal preparations were maceration (induction, stimulation and milk quality), decoction (induction, stimulation and inhibition), fumigation (mastitis), paste (inhibition, mastitis and milk quality) and latex (mastitis). It was found that a mixture of at least 2 plants was used for the induction and/or stimulation of lactation and its components, while only 1 or 2 plants were used for inhibition of lactation and or treatment of mastitis.
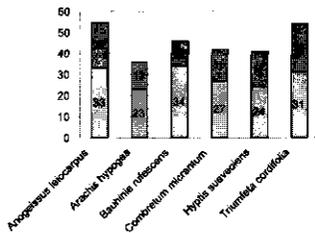
Figure 4 Plants used for inhibition of lactation
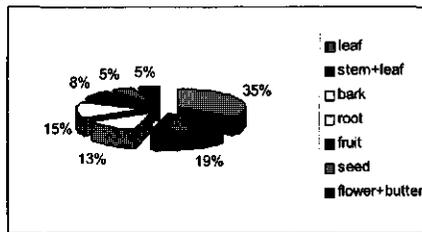
Figure 5 The use of various plant parts

Table 2 List of plants used for induction, stimulation of lactation and improvement of milk quality

Family/species	Local Name	Number of times mentioned by respondents (n=79)	Number of times used by women (n=217)	Part used	Medical use
DRACAENACEAE					
Sanseviria senegambica, Baker	Kaantobga	5	0	leaf	induction
				Rhizom	induction
AMARANTHACEAE					
Alternantera pungens H.B & Kunth	Rakomp noedre	5	1	stem , leaf	induction
Amaranthus spinosus L.	Kurkurgoaga	15	3	stem , leaf	stimulation
Amaranthus viridis L.	Zimini gnanga	7	1	stem , leaf	induction
ANACARDIACEAE					
Ozoroa insignis Del.	Nin-noore	5	0	leaf	induction
ANNONACEAE					
Annona senegalensis Pers.	Barkudga	25	10	bark	induction, stimulation
APOCYNACEAE					
Saba senegalensis (A.DC) Pichon var. *senegalensis*	Wedga	25	19	leaf, fruit	induction
ASCLEPIADACEAE					
Glossonema boveanum (Decne.) Decne.	Kutin-leenga	5	0	stem, leaf	induction
Leptadenia hastata (Pers.) Decne.	Lelongo	62	102	leaf	induction, milk quality
Sarcostemma viminale (L.) R. Br.	Wobgnaado	7	0	stem	induction
ASTERACEAE					
Bidens engleri O.E. Schulz	Kinkiris-sabtulga	17	29	stem, leaf	induction
Vernonia colorata (Willd.) Drake	Koa-safande	24	17	leaf	induction
BASELLACEAE					
Basella alba L.	Epinard	12	2	leaf	stimulation
BOMBACACEAE					
Adansonia digitata L.	Tohega	61	83	leaf, fruit	stimulation, induction
Bombax costatum Pellegr. & Vuillet	Voaka	31	19	bark, leaf	induction
Ceiba pentandra (L.) Gaertn.	Gounga	20	11	bark	induction
CAESALPINIACEAE					
Afzelia africana Smith ex Pers.	Kankalga	6	1	bark	induction
Senna occidentalis (L.) Link	Kinkeliba	21	7	leaf, root	induction
Detarium microcarpum Guill. & Perr.	Kagdega	11	13	leaf	stimulation, milk quality
Parkinsonia aculeata L.	Nasar arzin-tiiga	9	0	leaf, flower	induction
CAPPARACEAE					
Boscia senegalensis Pers. Lam. ex Poir.	Laboiiga	27	11	root	induction
Cadaba farinosa Forssk.	Kiensga	77	119	leaf, root	induction, stimulation, milk quality

31

Maerua angolensis DC	Zilgo	29	17	leaf	induction
Crateva adansonii DC.	Kalguin-tohega	54	88	leaf	induction, stimulation, milk quality

CARICACEAE

Carica papaya L.	Papaye-tiiga	13	2	leaf, fruit, latex	stimulation

COMBRETACEAE

Guiera senegalensis J.F. Gmel.	Wilin-wiiga	45	26	leaf	stimulation

CONVOLVULACEAE

Ipomea batatas (L.) Lam	Wosso	17	5	leaf	induction
Ipomea eriocarpa R. Br.	guitiguidga	37	21	stem, leaf	induction

CUCURBITACEAE

Luffa cylindrica (L) M.J. Roem.	Needego	12	0	fruit	induction, stimulation
Momordica balsamica L.	Mogod-ti-raogo	9	1	leaf	induction

CYPERACEAE

Cyperus esculentus L.	Kiogo	16	7	root	induction

EUPHORBIACEAE

Euphorbia hirta L.	Walbissum	39	57	stem, leaf	induction, stimulation, milk quality
Euphorbia prostata Aiton	Bandakou miugu	21	5	stem, leaf	stimulation, milk quality
Euphorbia pulcherrima Willd. ex Klotzsch	Tacsindo	7	0	flower	induction
Jatropha curcas L.	Wombin bangma	5	0	leaf	induction
Manihot esculenta Crantz.	Bandakou	23	7	tuber	induction
Phyllanthus niruri L.	Tinguin-garga	19	6	root	induction

FABACEAE

Cajanus cajan (L.) Millsp.	Pois d'angole	14	5	leaf	stimulation
Indigofera tinctoria L.	Garga, gare-tiiga	24	9	leaf, root	induction, stimulation milk quality
Stylosanthes erecta P. Beauv.	Sakwisablega	12	4	stem, leaf	induction milk quality

LILIACEAE

Asparagus flagellaris (Kunth) Baker	Baguem-baguem	5	0	root	stimulation
Asphodelus fistulosus L.	Gaog-nangouri	23	14	leaf, seed tuber	stimulation, milk quality

MALVACEAE

Gossypium ssp.	Lamdo	12	7	leaf	induction, milk quality

MIMOSACEAE

Acacia ataxacantha DC.	Goaaga	5	1	leaf	induction, stimulation
Acacia nilotica (L.) .Willd. ex Del. var. *adansonii* (Guill. and Pers) O. Ktze	Peg-nenga	59	109	leaf, seed	induction, stimulation
Entada africana Guill. & Perr.	Sinnogo	23	15	stem, leaf	induction
Parkia biglobosa (Jacq.) R.Br. ex Benth.	Roanga	32	36	bark, leaf	stimulation, milk quality

MORACEAE

Ficus glumosa Delile	Kakan-senega	15	4	stem, leaf	induction
Ficus ingens var. Ingensitalic? (Miq.) Miq.	Kunkwiiga	9	5	bark	induction
Ficus platyphylla Delile	Kamsanwogo	21	11	bark, fruit	induction, stimulation
Ficus sur Forssk	Womsayega	17	11	leaf, bark fruit	induction stimulation
Ficus sycomorus L.	Kakanga	31	14	bark, root	induction

Ficus thonningii Blume	Kuusga	7	0	bark, root	induction
OXALIDACEAE					
Biophytum umbraculum Welw.	Napagb-godeb-nao	17	5	stem, leaf	stimulation, induction, milk quality
POACEAE					
Andropogon gayanus Kunth.	Mopoka	7	1	stem	induction
Pennisetum Glaucum (L.) (K. Schum.) (L.) R.Br.	Kazui	79	217	seed	induction, stimulation, milk quality
RUBIACEAE					
Crossopterix febrifuga (Afzel.ex G.Don) Benth.	Kumbr-waaga	5	0	stem, leaf	induction
Feretia apodanthera Delile	Fininga	11	0	leaf	induction
Spermacoce verticillata L.	Yod peelga	9	2	stem, leaf	induction
SAPINDACEAE					
Paullinia pinnata L.	Noussanou	17	5	stem, leaf	induction
SCROPHULARIACEAE					
Scoparia dulcis L.	Kaafr-mande	11	0	stem, leaf	induction
SOLANACEAE					
Capsicum frutescens L.	Kipare	67	99	fruit	induction
STERCULIACEAE					
Sterculia setigera Delile.	Ponsemporgon	27	9	bark, leaf	induction
VERBENACEAE					
Vitex doniana Sweet	Aadega	17	3	fruit	induction
VITACEAE					
Cissus quadrangularis L.	Wobzanre	15	31	stem, leaf	induction

Table 3 List of plants used for induction, stimulation, and/or mastitis

Family/species	Local Name	Number of times mentioned by respondents (n=79)	Number of times used by women (n=217)	Part used	Medical use
ASCLEPIADACEAE					
Calotropis procera (Aiton) W.T. Aiton)	Poussoumpuuga	6	1	leaf, bark root	induction, stimulation, mastitis
CAESALPINIACEAE					
Bauhinia thonningii Schum.	Baguin-gnaga	51	39	leaf, fruit	induction, stimulation, mastitis, milk quality
LAMIACEAE					
Ocimum basilicum L.	Yulumb-gnuuga	17	32	leaf, seed	induction, mastitis
POACEAE					
Sorghum bicolor (L.) Moench	Kenn-da	49	52	seed	induction, stimulation, milk quality
				stem, ear	mastitis
SAPOTACEAE					
Vitellaria paradoxa C.F. Gaertn.	Taanga	41	125	bark, butter	induction, mastitis
				latex	milk quality

33

Table 4: List of plants used for inhibition and/or mastitis

Family/species	Local Name	Number of times mentioned by respondents (n=79)	Number of times used by women (n=217)	Part used	Medical use
AMARANTHACEAE					
Pupalia lappacea (L.) Juss.	Yong-tabdo	11	2	leaf	mastitis
BALANITACEAE					
Balanites aegyptiaca (L.) Delile	Kieglega	21	5	leaf	inhibition
BIGNONIACEAE					
Stereospermum kunthianum Cham.	Nin-yilenga	15	2	leaf	mastitis
CAESALPINIACEAE					
Bauhinia rufescens L.	Tipohega	34	12	stem, leaf	mastitis
Tamarindus indica L.	Pousga	17	6	leaf	mastitis
COMBRETACEAE					
Anogeissus leiocarpus (DC.) Guill. & Perr.	Siiga	33	22	leaf	inhibition, mastitis
Combretum micranthum G. Don	Randga	27	15	leaf	inhibition, mastitis
CUCURBITACEAE					
Momordica charantia L.	Mogod-tiim	7	0	stem, leaf	mastitis
EUPHORBIACEAE					
Flueggea virosa (Roxb. ex Willd.) Baill.	sugd-daaga	5	1	root	mastitis
Ricinus communis L	Gon-noanga	7	1	leaf	inhibition
FABACEAE					
Arachis hypogaea L.	Sumkam	23	13	flower	inhibition, mastitis
Vigna unguiculata (L.) Walp.	Benga	5	1	seed	mastitis
LAMIACEAE					
Hyptis suaveolens Poit.	Rung-rung gnaaga	24	17	stem, leaf	mastitis
MORINGACEAE					
Moringa oleifera Lam.	Arzin-tiiga	23	7	bark, root, leaf	inhibition
NYMPHAEACEAE					
Nymphaea lotus L.	Gwiina	17	9	leaf	inhibition
PASSIFLORACEAE					
Passiflora foetida L.	Grenadine	5	0	leaf, root	mastitis
POACEAE					
Andropogon pinguipes (Stapf)	Koulmoodo	5	2	stem, leaf	mastitis
RHAMNACEAE					
Ziziphus mauritiana Lam.	Mougounuga	25	7	stem, leaf	inhibition, mastitis
TILIACEAE					
Triumfetta cordifolia A. Rich.	Natoukouli	31	23	root	inhibition
VERBENACEAE					
Vitex diversifolia Baker	Napug-aadega	21	7	bark	inhibition

Table 5 List of the most popular plants used for improvement of lactation

Family/species	Local name	No of times mentioned by the respondents (N = 79)	No of times used by the women (N = 217)	Part used	Medical use
ASCLEPIADACEAE					
Leptadenia hastata (Pers.) Decne.	Lelongo	62	102	stem with leaf	induction, milk quality
CAPPARACEAE					
Cadaba farinosa Forssk.	Kiensga	77	119	leaf	induction, stimulation, milk quality
Crateva adansonii DC.	Kalguin-tohega	54	88	leaf	induction, stimulation, milk quality
MIMOSACEAE					
Acacia nilotica (L.) Willd.ex Del. var. *adansonii* (Guill. & Perr.) O. Ktze	Peg-nenga	59	109	leaf, seed	induction, stimulation, milk quality
POACEAE					
Pennisetum glucaum (L.) R. Br.	Kazui	79	217	seed	induction, stimulation, milk quality
SAPOTACEAE					
Vitellaria paradoxa C.F. Gaertn.	Taanga	41	125	bark, butter	induction, milk quality, mastitis
SOLANACEAE					
Capsicum frutescens L.	Kipare	67	99	fruit	induction

Traditional practices

The concept
Traditional practices are based on the concept that supernatural powers lie at the origin of human well-being: the forces of evil, the gods, and the spirits emanating from the ancestors who can be good or evil. The balance between good and evil spirits determines the health of the individual. Disease is regarded as a punishment for bad behaviour. Evil spirits can however be appeased by the administration of certain plants together with certain procedures.

Induction
According to traditional perceptions, agalactia occurs in women who do not comply with traditional rules (for instance, eating foods normally prohibited by local customs) during their pregnancy. In some areas, physical treatment is applied to the breast before the use of

any plant. The breast is massaged with the butter of *Vitellaria paradoxa*. After this massage therapy, a mixture of plants is administrated for the induction of lactation. Some of the popular prescriptions are listed below.

1 - Fresh leaves of *Acacia nilotica ssp adansonii* are boiled in water. The decoction is mixed with the powder of the seeds of *Pennisetum americanum* and drunk all day long. Lactation occurs within 2 days.

2 - Fresh leaves of *Acacia nilotica ssp adansonii* and *Crateva adansonii* are crushed in a mortar. The paste is mixed with water and filtered (for dry leaves, the decoction is used). The seeds of *Pennisetum americanum* are crushed together with a few fruits of *Capsicum frutescens* and a bit of salt. This powder is mixed with a bit of butter of *Vittelaria paradoxa* to obtain a paste. This paste is then mixed with the filtrate obtained above and drunk throughout the day. The treatment may be repeated the following day, if necessary. This mixture is reported to induce lactation in non-lactating women. Many variants of this recipe are known, based on 1 to 3 of the following plants: *Cadaba farinosa*, *Leptadenia hastata*, *Acacia nilotica ssp adansonii* and *Crateva adansonii* in association with *Pennisetum americanum*, *Capsicum frutescens* and *Vittelaria paradoxa*.

Stimulation

Hypogalactia is considered to be a result of non-observation of traditional rules, only rarely due to "natural" reasons, and occurs about 4 to 6 months after parturition.

As reported by the informant, clinical symptoms observed in the case of hypogalactia are the lack of growth and the continuous cries of the breast-fed infant. In most cases, the latter is the main sign for the mother. For treatment, one mixture of plants is given to the infant to stimulate his appetite and to make him grow faster and stronger, while another mixture is given to the mother.

1 - Fresh leaf-bearing stems of *Piliostigma thonningii* are crushed in a mortar with the powder of *Pennisetum americanum* seeds. Fresh leaf-bearing stems of *Leptadenia hastata* are boiled in water and filtered. The filtrate is added to the paste and drunk throughout the day.

2 - Fresh bark from the East and West sides of the tree *Parkia biglobosa* is macerated overnight. The macerate is mixed with powder of the seeds of *Pennisetum americanum* and drunk on an empty stomach (early in the morning).

3 - Immature fruits and/or fresh stems with leaves of *Saba senegalensis* are crushed in a mortar with seeds of *Pennisetum americanum*. The paste is mixed with a macerate of fruits of *Adansonia digitata* and drunk throughout the day. The treatment is continued for 3 days (for a male infant) or 4 days (for a female infant).

4 - Fresh stems with leaves of *Guiera senegalensis* (collected late in the afternoon) and a stem with leaves of *Euphorbia hirta* are crushed in a mortar and mixed with the powder of *Pennisetum americanum*. The paste is then macerated in water and drunk all day long. The treatment is continued for 14 days. Only a decoction of *Guiera senegalensis* is used together with the powder of *Pennisetum americanum*.

Milk quality and mastitis

Clinical symptoms observed by the informant which indicate low content of milk components during lactation are lack of growth, diarrhoea, continuous crying and

vomiting of the infant, and swelling of the mother's breast. For the diagnosis, the "ant test" is used: an ant is put in a small quantity of the mother's milk; drowning of the ant means the milk is not good. The prescriptions listed below are then used for treatment.

1 – For improvement of milk quality, a paste is cooked from powderised *Sorghum bicolor*. A small quantity of this paste is added to leaves of *Piliostigma thonningii* and boiled in water. The potage is used to wash the breast. The filtrate is drunk all day for 3 (for a male infant) or 4 days (for a female infant).

2 - For mastitis, fruits of *Piliostigma thonningii* are burned in a traditional pot. The ash is mixed with butter of *Vitellaria paradoxa* and applied to the breast. The same paste is also used to increase milk lipid content.

Inhibition and mastitis

Women use plants to inhibit their milk production mainly in the case of abortion or loss of the infant before weaning.

1 - Fresh leaves of *Anogeissus leiocarpus* are boiled in water and drunk during a period of 7 days on an empty stomach.

2 – Fresh leaves of *Combretum micranthum* are boiled in water and drunk during a period of 7 days.

3 – Fresh leaves or seeds of *Vigna unguiculata* are crushed in a small traditional pot. The paste is then applied to the breast each day during a period of 3 to 7 days.

Discussion

The present study clearly demonstrates that inhabitants of various parts of Burkina Faso use plants for their health care, as previously reported (Nacoulma-Ouedraogo 1996, Guinko 1984, 1977, and Bognounou *et al.* 1975). This is particularly true as far as lactation is concerned. Interestingly, not only all women have used at least one plant for either induction or stimulation of lactation, but the plants used appear to be similar within the study area as well. Eleven species were widely used by women for the treatment of agalactia and / or hypogalactia, seven of which were used by at least 40% of the users throughout several lactation periods. Although a mixture of at least 2 plants is used for induction and stimulation in all the prescriptions, such mixtures rarely exist for inhibition of lactation and treatment of mastitis where the number of plants used is 2 or less.

Evidence exists that certain drugs can be transmitted to the infant through breast milk when mothers use herbal remedies during lactation (Elvin-Lewis 2001); some of these drugs may be fatal for the infant. It seems likely that while some of the plants in a given treatment mixture induce or stimulate lactation, other plants may be added to the same mixture to inhibit the possible toxicity of active plants and to prevent intoxication of both mother and infant. Moreover, widespread use of a given plant within a village community is generally based on long-term observations of its efficacy (pharmacological effects) and its safety for mother and infant (toxicological effects). However, variations were observed not only in species and number of plants used, but also in parts used; such variations may

be due to either availability of the plant over the year or to variations in soil nutrient composition.

On the other hand, it appeared that plants were often used for inhibition of lactation and treatment of mastitis, even if only a maximum of 23 cases were reported, suggesting that at least 10% of the women had been obliged to stop an ongoing lactation. However, the exact causes of infant death or mastitis are still not known.

The traditional belief is that the newborn is a reincarnation of one of the ancestors of the family. The day, time, and conditions of birth are the indicators that will determine his given name and his destiny. Furthermore, an infant looks neither like his father nor his mother but resembles his grandfather or grandmother, particularly when the latter are deceased. Therefore, infringement of traditional rules during pregnancy is considered the main cause of complications observed during parturition and leading to agalactia, hypoglactia, or mastitis and sometimes to the "return" (i.e. the death) of the infant.

Lactation is the result of extensive physiological processes occurring in the mammary gland during pregnancy until the day of parturition (Martinet & Houdebine, 1993). Indeed, growth and development of the mammary gland during pregnancy and the hormonal interactions occurring during parturition are indispensable for the onset of milk production. At parturition, the abrupt decrease in both progesterone and estrogen levels associated with the abrupt increase in PRL level and the increase in glucocorticoid and oxytocin levels lead to the onset of lactation. Lactation is in fact a combination of milk secretion (mainly regulated by PRL) and milk removal (regulated by stimulation of the nipple by the infant).

Comparison of the number of plants used for induction (39 species) with the total clearly shows that agalactia is the most common pathology occurring after parturition in the study area. Three key suggestions can be made:

Firstly, the conditions of birth such as maternal stress 2-5 days after labour, as often occurs in primiparae, may affect the onset of lactation. Indeed, the stress response is not only associated with increased PRL levels (Jeffcoate et al. 1986) but also with decreased PRL levels (Chatterton et al. 2000, Johansson et al. 1983), particularly under conditions in which its levels are normally high. Some cultural and social factors, such as food taboos might have been introduced to reduce the stress- induced problems during parturition. Although some explanations of food taboos such as "the child will become a thief when the mother eats eggs during pregnancy" are unsubstantiated, others are based on the general claim that a small baby is easier to deliver than a big one. For that reason, it is in fact a taboo to eat energy-rich food during pregnancy to prevent excessive growth of the baby. Since neither traditional midwives nor modern medical assistants, if present, have any surgical training and/or equipment, traditional ways of controlling food intake during pregnancy must be sustained. Moreover, 70 to 80% of the pregnant women living in towns (pers. comm.), mainly primiparae, undergo surgical incision of the vagina during delivery, not being exposed to the obligation of following traditional rules. The lack of such equipment and knowledge in the villages may to some extent explain the high maternal and neonatal mortality rates, in addition to the abusive use of abortive plants in these

particular cases. On the other hand, pregnant women have restricted access to calories and specific nutrients during some periods when the food supply is inadequate, thus affecting the course of pregnancy and thereby the onset of lactation. Although food taboos may be based on prolonged experience when they relate to pregnant women, chances are most of them are based on superstition when they relate to lactating women.

Secondly, incomplete delivery of the placenta after parturition may occur, leading to blockage of the PRL surge and consequently to alterations in the onset and maintenance of lactation, as previously reported by Anderson (2001). This suggestion is quite plausible since the induction of lactation occurs within one day of treatment with the extract of a plant used for both parturition and delivery of the placenta. It should be noted that some of the plants used for parturition and delivery of the placenta are also used in other African countries, as an abortive (Noumi & Tchkonang 2001).

Thirdly, decreasing progesterone levels can stimulate copious milk secretion only in the presence of lactogenic hormones, in particular PRL (Austin & Short 1984). Moreover, not only a certain level of circulating PRL is needed, but mammary gland PRL receptors must also be sufficiently present and functional, inducing the effect of PRL on the mammary gland cells and thus stimulating milk synthesis. Some plants, such as *Acacia nilotica ssp adansonii,* have been reported to stimulate PRL release in ewe and rat (Sawadogo 1987). These observations suggest that a mixture of *Leptania hastata*(which is used as abortive) and *Acacia nilotica ssp adansonii* could induce milk production; however, their effects on the mammary gland have not yet investigated. Maternal milk yield is regulated by the demands of the infant (Perez-Escamilla *et al.* 1995, Prentice *et al.* 1986), which are in turn a function of the energy content of the milk (Villalpando *et al.* 1999). However, milk secretion requires adequate levels of circulating PRL since the inhibition of PRL secretion and its release after treatment with bromocriptine led to inhibition of milk secretion (Martin *et al.* 1981).

It can be concluded from this survey that all women in the study area have used plant extract during lactation and a mixture of plants is always used to improve lactation for the infant. As far as traditional practice is concerned, we hypothesise that components of the plant used for improvement of milk production could have a direct effect by stimulating mammary secretory cells and/or an indirect effect by stimulating the secretion and release of lactogenic hormones, in particular PRL. Likewise, plants used for inhibition of lactation may have an indirect effect by inhibiting the secretion and release of PRL, which would lead to the interruption of lactation, and a direct effect on the activity of secretory cells since some of the plants are applied directly onto the mammary gland as pastes. However, there is no scientific proof of the traditional belief that these plants can effectively stimulate milk production. Further investigation will be necessary to identify plants with active components, the exact effects of these components, and their mechanisms of action.

References

Anderson AM 2001 Disruption of lactogenesis by retained placental fragments. *Journal of Human Lactation* **17** 142-144.

Austin CR & Short RV 1984 Reproduction in mammals: Book 3, Hormonal control of reproduction. *Cambridge University Press*, Cambridge, London, New York, p. 244.

Bognounou O, Ouédraogo C & Ouédraogo O 1975 Contribution à l'inventaire des plantes médicinales africaines en pays Mossi (region de Ouagadougou). *Bull. Trim. Inform. Sc.* Notes et Documents 4 50-57.

Chatterton RT, Hill HP, Aldag JC, Hodges KR, Belknap SM & Zinaman MJ 2000 Relation of plasma oxytocin and prolactin concentration to milk production in mothers of preterm infants: influence of stress. *Journal of Clinical Endocrinology and Metabolism* **85** 3661-3668.

Chenu J & Ake Assi L 1992 Plantes médicinales tropicales et Camerounaises. Tome 1. *Edition Ren-Ronche Berrebi Véronique*, Barcelone, p. 222.

Cotton MC 1996 Ethnobotany, Principle and Applications *Willey and Sons*, New York, p 1-44.

Elvin-Lewis M 2001 Should we be concerned about herbal remedies. *Journal of Ethnopharmacology* **75** 141-164.

Gelfand M, Mavai S, Drummond RB & Ndemera B 1985 The traditional medical practitioner in Zimbabwe; His principles of practice and pharmacopoeia. *Mambo press*, Harare, 1-411.

Guinko S 1984 Végétation de la Haute-Volta. These de Doctorat es-Sciences, Tome1, *Université de Bordeaux III*, p. 316.

Guinko S 1977 Plantes utilisées dans la médecine traditionnelle en Afrique de l'Ouest. *Ed. Roche Basel*, Switzerland p. 151.

Guinko S, & Bognounou O 1982 Réflexions autour de quelques techniques et plantes galactogènes en Haute-Volta. *Bull. Trim. Inform. Sc.* Note et Documents **13** 5-14.

Jeffcoate WJ, Lincoln NB, Selby C & Herbert M 1986 Correlation between anxiety and serum prolactin in human. *Journal of Psychosomatic Research* **30** 217-222.

Johansson GG, Karonen SL & Laakso ML 1983 Reversal of an elevated plasma level of prolactin during prolonged psychological stress. *Acta Physiologica Scandinavica* **119** 463-464.

Kerharo J & Adam JC 1974 La Pharmacopée Sénégalaise Traditionnelle, Plantes Médicinales et Toxiques. *Edition Vigot-Freres*, Paris

Martin RH, Glass MR, Chapman C, Wilson G & Woods KL 1981 Suppression by bromocryptine of the lactalbumin peak associated with human lactogenesis. *Clinical Endocrinology* **14** 363-366.

Martinet J & Houdebine LM 1993 Biologie de la lactation, Editions INSERM/INRA Editions, p 587.

Michaelsen KF, Larsen PS, Thomsen BL & Samuelson G 1994 The Copenhague Cohort study on infant nutrition and growth: Breast-milk intake, human milk macronutrient content and influencing factors. *American Journal of Clinical Nutrition* **59** 600-611.

Nacoulma-Ouédraogo OG 1996 Plantes médicinales et pratiques médicales traditionnelles au Burkina Faso: Cas du plateau Central. Thèse de Doctorat Es-Sciences Naturelles. *Université de Ouagadougou*, tome II, p. 285.

Ndubani P & Hojer B 1999 Traditional healers and the treatment of sexually transmitted illness in rural Zambia *Journal of Ethnopharmacology* **67** 15-25.

Noumi E & Tchkonang NYC 2001 Plants used as abortifacients in the Sangmelima region of Southern Cameroon. *Journal of Ethnopharmacology* **76** 263-268.

Olivier-Bever B 1982 Medicinal plants in tropical West Africa. 1. Plants acting on cardiovascular system. *Journal of Ethnopharmacology* **5** 1-71.

Ouedraogo SP 1984 Plantes médicinales et médecine traditionnelles au Burkina Faso. Thèse de Diplome d'Etat de Docteur en Pharmacie *Ed. Ellipses-ACCT* p. 196.

Perez-Escamillia R, Cohen RJ, Brown KH, Landa Rivera L, Canahuati J & Dewy KG 1995 Maternal anthropometric status and lactation performance in low income Honduran population: evidence for the role of the infant. *American Journal of Clinical Nutrition* **61** 528-534.

Prentice MA, Paul AA, Prentice A, and Black AE, Cole TJ & Whitehead RG 1986 Cross-cultural differences in lactational performance. In Hamosh M and Goldman AS (eds.), *Human Lactation 2*, Plenum Publishing Corp., New York, p. 13-44.

Sawadogo L 1987 Contribution á l'étude des plantes médicinales et de la pharmacopée traditionelle africaine, Thèse de Doctorat d'Etat, *Universite de Tours*, p. 149.

Sawadogo JM, Some BL & Chauvel FPB 1983 La phytothérapie dans le Gourma Synthèse des travaux de la première semaine départementale de la santé publique de Fada, 24-31 Janvier 1982, *Ed. Laafia*, Burkina Faso.

Travers MT, Barber MC, Tonner E, Quarrie L, Wilde CJ & Flint DJ 1996 The role of prolactin and growth hormone in the regulation of casein gene expression and mammary cell survival: relationships to milk synthesis and secretion. *Endocrinology* 137 1530-1539.

Van Wyk BE, Van Oudtshoorn B & Gericke N 1997 Medicinal plants of South Africa Briza, Pretoria, p. 1-304.

Villalpando S & Del Prado M 1999 Interrelation among dietary energy and fat intakes, maternal body fatness and total lipid in humans. *Journal of Mammary Gland Biology and Neoplasia* 4 285-295.

CHAPTER 3

ACACIA NILOTICA VAR *ADANSONII* (Mimosaceae)

Lompo-Ouedraogo Z[2], Van Der Heide D[1], Nianogo A J[2]

[1]Human and Animal Physiology Group, Department of Animal Sciences, Wageningen University, The Netherlands
[2]Unité de Formation et de Recherche en Sciences de la Vie et de la Terre, Université de Ouagadougou, Burkina Faso

Introduction

The genus *Acacia* (Mimosaceae) contains several species distributed in dry regions of Africa (Barnes *et al.* 1996, Guinko 1992), from Egypt and Mauritania southwards (Woldemeskel *et al.* 1998) to South Africa (Dube *et al.* 2001). Seventeen species are present in Burkina Faso (Guinko 1992) three of which, *Acacia nilotica* var. *adansonii, Acacia nilotica* var. *nilotica* and *Acacia nilotica* var. *tomentosa* are remarkably distributed from the northern to the eastern part of the country. According to Kerharo & Adam (1974), *Acacia nilotica* var. *adansonii* (Guill. et Perr.) O. Ktze is a thorny tree of 10-12 m of length or less, with dark gray bark color, green gray-leaves and golden flowers.

Apart from differences between the forms of the pods and in some areas the color of the trunk, these three species that resemble eachother qua foliage and inflorescence have nearly the same systematic characteristics. The systematics of *Anilotica* species is confusing and most of the time it has been described as *Acacia arabica* Willd. or as *Acacia nilotica* Del. (Kheraro & Adam 1974). Moreover, the possible hybridisation (pollination by bees) and the constant change (with age) of the shape of inflorescence and colors of their flowers makes the determination of Acacia nilotica subspecies difficult. People in the villages however, are able to distinghuish between these 3 species. The local names of Acacia nilotica ssp adansonii are "Pegnenga" for Yadse and Moose people, "Konbonkarga" for Gourmantse and "pelinga gô" for Bissa people. *Acacia nilotica* var. *adansonii* (AN) and thus all the acacia species, is a multipurpose tree that plays a key role in rural development. AN is a source of various products including firewood, charcoal, timber, fodder, seeds for human nutrition, gum and pods for dye and tannin. In addition to its role in rural economy, AN provides good restoration of soil fertility, and plays an important role in soil conservation (Guinko 1992, Barnes *et al.* 1996).

Apart from its importance in rural population survival and its role in the regulation of the ecosystem, Acacia nilotica var. adansonii is widely used in folk medicine for the treatment of various diseases. In the traditional context, a widespread use of a plant relies on a long-term observation of its efficiency and safety. Its multiple therapeutic properties however, are still poorly explored. Indeed, research and development of therapeutic properties from a plant is a multidisciplinary process (Verpoorte 1989) involving botany, pharmacology, phytochemistry, toxicology and biology and may explain, to some extent, the lack of scientific information on African traditional medicine. In this chapter, the ethnopharmacology, phytochemistry, and toxicology of *Acacia nilotica* var. *adansonii* are presented. This information may provide a basis for understanding its multiple therapeutic uses and its biological activity in the particular case of lactation.

Ethnopharmacology and biological activities

Several plants with therapeutic properties regarding lactation related defects have been reported (see chapter 2) with *Acacia nilotica* var. *adansonii* being one of the six plants widely used for induction and stimulation of lactation. Apart from this, *Acacia nilotica* var. *adansonii* appears to be used and / or frequently associated with other plants in parts of Burkina Faso for the treatment of a wide variety of diseases (Personal observation). The

bark and/or pods are frequently associated in preparations for the stimulation of libido and fertility. Decoctions from leaves and bark are widely used to treat infant diarrhoea and to prevent infants from infections that occur during weaning. *Acacia nilotica* ssp *adansonii* is said to be able to regenerate necrotic tissue and to "firm up" a withered breast. Nacoulma-Ouedraogo (1996) reported astringent, antiseptic, anti-inflammatory, hemostatic and tonic properties of a tea prepared from its leaves, flowers, seed and bark. Decoctions or powder of leaves and bark were applied externally in case of leprosy and mouth lacerations. In Niger, the seeds are preferentially used for induction and stimulation of lactation (Guinko 1992). Likewise, *Acacia nilotica* species have been reported to have anti-hyperglycemic (Akhtar & Khan 1985), anti-microbial (Sotohy *et al.* 1997, Abd-El-Nabi 1992), anti-plasmodial (El-Tahir *et al.* 1999), anti-inflammatory, analgesic and anti-pyretic (Dafallah & al-Mustapha 1996) properties. The methanol extract of the pods of *Acacia nilotica* ssp showed anti-hypertension activities (Gilani *et al.* 1999) while aqueous extract of the seeds exhibited spasmogenic and vasoconstrictive actions (Amos *et al.* 1999). *Acacia nilotica* subspecies are traditionally used in Pakistan for the treatment of diarrhoea and Shah *et al.* (1997) recently uncovered their anti-platelet aggregation activity. Methanol (bark and pods) and aqueous (pods) extract of *Acacia nilotica* showed considerable inhibitory effects to HIV-1 protease (Hussein *et al.* 1999) and hepatitis C virus protease (Hussein *et al.* 2000).

Phytochemistry

The phytochemistry of Acacia nilotica has received great interest, mainly because of the use of its tannins for industrial pruposes. However, a number of phytochemical reports do not specify the variety investigated, which is mainly due to the fact that these three varieties had been described as Acacia nilotica Del. pers.

Phytochemistry (method of Ciulei 1982) of the leaves of Acacia nilotica var. adansonii performed in the northern, central and eastern parts of Burkina Faso revealed the presence of polyphenols (tannins), anthracenosids, coumarinic derivatives, cardiotonic glycosides, flavonoids, steroids, and terpenoids (Table 1). The aqueous extract is rich in glucids including rhamnose, arabinose, galactose and also rich in uronic acids such as galacturonic acid. None of those –oses, however, have been found to stimulate PRL release in the ewe apart from pectin and oligogalacturonic acid (Sawadogo 1987, Sepehri et al. 1990).
The extract from the leaves, roots and bark of *Acacia nilotica* ssp contains 18-20% of tannins (Kerharo & Adam 1974). The bark of the roots of *Acacia nilotica* ssp *adansonii* contains up till 36% of tannins. The presence of saponosids has also been reported, but a haemolytic test was found to be negative. *Acacia nilotica* leaves also contain proanthocyanidins and other phenolics (Dube *et al.* 2001).

Toxicology

From the ethnobotanical survey it follows, that women have used *Acacia nilotica* var. *adansonii* for generations without any reports of pathologic effects. In addition, we did not observe any clinical toxicity in rats treated orally with increasing doses (3, 3.5, 4, 4.5, 5,

5.5, 6g/kg) of aqueous extract of leaves of this plant (this thesis), although tannins have been reported to be potentially toxic to ruminants (Reed 1995). The intra-muscular injection, however, caused an inflammatory effect in the area surrounding the injection point in rats. We have also observed a claustration of the animal after intravenous injection of either extract (2-5 min) or saline (2-3 min). Likewise, the extract did not inhibit intestine peristalsis *in vitro* and no apparent toxicity was observed in rats and ewes treated orally with the extract, although a meteorisation after i.v. treatment has been reported in ewes (Sawadogo 1987). Al-Mustafa & Dafallah (2000) reported a significant decrease in body weight and in levels of hemoglobin, serum total protein and total cholesterol in rats fed a diet containing 8% of *Acacia nilotica* for up to 4 weeks. No death or histopathological changes in the liver were observed. On the other hand, a methanol extract of roots of *Acacia nilotica* has shown cytotoxic activity *in vitro* (Kamuhabwa *et al.* 2000).

Conclusion

Regarding its multiple use in traditional medicine, Acacia nilotica might be compared to aspirin in modern medicine. Despite its widespread use in folk medicine in the treatment of various diseases, little is known about its biological effects. Given that 90% of the women in Africa are breast-feeding, and that a lot of them may have lactation-related defects, Acacia nilotica appears to be an attractive source of new therapeutic compounds in the improvement of lactation in a low income population. It is currently not known whether or not Acacia nilotica may effectively induce or stimulate lactation in women. Further studies will elucidate potential stimulatory effects, and possible mechanisms of action in rats.

Table 1: Phytochemistry of leaves of *Acacia nilotica* var *adansonii*

	Anthocians	Coumarins	Flavonoids	Glucids	Quinones	Saponosids	Steroids Triterpene	Tannins
Aqueous Extract	-	-	-	+++		+/-	-	++
Ethanolic extract	++	++	++	++	++	-	-	++
Methylene chloride extract	-	++	++	-		-	++	-

References
Abd-El-Nabi OM, Reisinger EC, Reinthaler FF, Still F, Eibel U & Krejs GJ 1992 Antimicrobial activity of Acacia nilotica (l.) Willd Ex del. var. nilotica. *Journal of Etnopharmacology* **37** 77-79.
Al-Mustapha ZH & Dafallah AA 2000 A study on the toxicology of *Acacia nilotica*. *American Journal of Chinese Medicine* **28** 123-129.
Akhtar MS & Khan QM 1985 Studies on the effect of *Acacia arabica* fruits (kikar) and *Caralluma edulis* roots (Chung) on blood glucose levels in normal and alloxan-diabetic rabbits. *Pakistan Journal of Agriculture and Science* **22** 252-259.

Amos S, Akah PA, Odukwe CJ, Gamaniel KS & Wambede C 1999 The pharmacological effects of an aqueous extract from *Acacia nilotica* seed. *Phytotherapy Research* 13 683-685.

Barnes RD, Filer DL & Milton SJ 1996 *Acacia Karroo* Tropical Forestry Papers, 32 *Oxford Forestry Institute*, Oxford University

Ciulei I 1982 Methodology for analysis of vegetable drug, Practical Manual on Industrial utilization of medicine and aromatic plants *Edited by the Ministry of Chemical Industry. Bucharest*, p 16-27.

Dafallah AA & al-Mustapha Z 1996 Investigation of the anti-inflammatory activity of Acacia nilotica and Hibiscus sabdariffa *American Journal of Chinese Medicine* 24 263-269.

Dube JS, Reed JD & Ndlovu LR 2001 Proanthocyanidins and other phenolics in Acacia leave of South Africa. *Animal Feed Science and Technology* 91 59-67.

El-Tahir A, Satti GM & Khalid SA 1999 Antiplasmodial activity of selected sudanese medicinal plants with emphasis on Acacia nilotica. *Phytotherapy Research* 13 474-478.

Gelani AH, Shaeen F, Zaman M, Janbaz KH, Shah BH & Akhtar MS 1999 Studies on antihypertensive and antispasmodic activities of methanol extract of *Acacia nilotica* pods. *Phytotherapy Research* 13 665-669.

Guinko S 1992 Role des acacia dans le développement rural au Burkina Faso et au Niger, Afrique de l'Ouest. *Etudes flor. Veg. Burkina Faso* 1 3-16, Fankfurt/Ouagadougou

Hussein G, Miyashiro H, Nakamura N, Hattori M, Kakiuchi N & Shimotohno K 2000 Inhibitory effect of Sudanese plant extracts on HIV-1 replication and HIV-1 protease. *Phytotherapy Research* 14 510-516.

Hussein G, Miyashiro H, Nakamura N, Hattori M, Kawahata T, Otake T, Kakiuchi N & Shimotohno K 1999 Inhibitory effect of Sudanese plant extracts on HIV-1 replication and HIV-1 protease. *Phytotherapy Research* 13 31-36.

Kamuhabwa A, Nshimo C & De White P 2000 Cytotoxicity of some medical plant extracts used in Tanzanian traditional medicine. *Journal of Ethnopharmacology* 70, 142-149.

Kerharo J & Adam JC 1974 La Pharmacopée Sénégalaise Traditionnelle, Plantes Médicinales et Toxiques. *Edition Vigot-Freres*, Paris

Nacoulma-Ouédraogo OG 1996 Plantes médicinales et pratiques médicales traditionnelles au Burkina Faso: Cas du plateau Central. Thèse de Doctorat Es-Sciences Naturelles. *Université de Ouagadougou*, tome II, p. 285.

Reed JD 1995 Nutritional toxicology of tannins and related polyphenols in forage legumes, *Journal of Animal Science* 73 1516-1528.

Shah BH, Safdar B, Virani SS, Nawaz Z & Gilani AH 1997 The antiplatelet aggregatory activity of Acacia nilotica is due to blockage of calcium influx through membrane calcium channels. *General Pharmacology* 29 251-255.

Sawadogo L 1987 Contribution á l'étude des plantes médicinales et de la pharmacopée traditionelle africaine, These de Doctorat d'Etat, *Universite de Tours*, p. 149.

Sepehri H, Renard C & Houdebine LM 1990 *B*-glucan and pectic derivatives stimulate PRL secretion from hypophysis in vitro. *Proceedings of the Society for Experimental Biology and Medicine* 194 193-201.

Sotohy SA, Sayed AN & Ahmed MM 1997 Effect of tannin-rich plant, (Acacia nilotica) on some nutritional and bacteriological parameters in goats. *Deutsche Tierarztliche Wochenschrift* 104 432-435.

Verpoorte R 1989 Some phytochemical aspects of medicinal plant research. *Journal of Ethnopharmacology* 25 43-59.

Woldemeskel E & Sinclair FL 1998 Variation in seedling growth, nodulation, and nitrogen fixation of *Acacia nilotica* inoculated with eight rhizobial strains. *Forest Ecology and Management* 104 239-247.

CHAPTER 4

EFFECTS OF AQUEOUS EXTRACTS OF *ACACIA NILOTICA* SSP *ADANSONII* ON MILK PRODUCTION AND PROLACTIN RELEASE IN RATS

Lompo-Ouedraogo Z[1,2], Van Der Heide D[1], Van Der Beek EM[1], Swarts JJM[1] Mattheij JAM[1], Sawadogo L[2].

[1]Human and Animal Physiology Group, Department of Animal Sciences, Wageningen University, The Netherlands
[2]Unité de Formation et de Recherche en Sciences de la Vie et de la Terre, Université de Ouagadougou, Burkina Faso

Abstract

In view of the traditional belief that *Acacia nilotica* ssp *adansonii* (AN) can stimulate milk production in lactating women, experiments were performed to determine the effect of an aqueous extract of AN on milk production in rats. Female rats that received oral doses (280 or 560 mg) of aqueous extract of this plant during their first lactation produced about 59% more milk compared to controls (P<0.01). Pup weight gain was also significantly higher compared to that in the control group. A lower dose, i.e. comparable to that used by women to improve their milk yield, led to about 33% more milk volume with the same growth rate for pups compared to the high dose group. The extract of AN was found to stimulate synthesis and release of PRL significantly (P<0.05). In addition, mammary glands of estrogen-primed rats treated with the extract showed clear lobulo-alveolar development with milk secretion. This study demonstrates that aqueous extract of AN can stimulate milk production and PRL release in the female rat and could consequently have the properties claimed for by lactating women.

Introduction

African women with milk production deficiencies traditionally use plant extracts to induce milk production or to increase milk yield. With limited access to modern milk replacers, breast-feeding is essential for the survival of the newborn. Most plant extracts are generally used in the form of decoction or maceration. The floristic and ethnobotanic aspects of lactogenic plants have been studied extensively (Adjanohoun *et al.* 1979a, 1979b; Bognounou *et al.* 1974, Nacoulma-Ouédraogo 1996); however, little is known about their biological activities. On the other hand, the positive effect of *Asparagus racemosus* extract on milk production in buffaloes has been reported, (Patel & Kanitkar 1969). Some plants have been identified as lactogenic because of a capacity to stimulate the synthesis of lactogenic hormones (PRL, growth hormone, cortisol) and/or ß-endorphin and ß-casein accumulation in the mammary gland *in vivo* and *in vitro* (Sawadogo *et al.* 1987, 1988a, 1988b, 1988c). Indeed, PRL is known to play a key role in mammogenesis and lactogenesis (Zwierzchowski 1993, Hennighausen *et al.* 1997, Horseman 1999, Brisken *et al.* 1999, and Llovera *et al.* 2000) in all species, including the rat. However, for PRL to exert its mitotic effects, the tissue must be exposed to estrogen (Martal *et al.* 1982). In rodents, initiation of lactation has the obligate requirement of PRL (Knight *et al.* 1986, Madon *et al.* 1986). After parturition, PRL induces lactation by direct stimulation of synthesis of milk proteins in epithelial cells and by indirect stimulation of proliferation of secretory cells. However, growth hormone (GH) is needed to support milk production when PRL is reduced (Flint *et al.* 1992). A few plants have been described that stimulate PRL release (Sawadogo 1987) or milk production (Patel & Kanitkar 1969) or mammary gland development (Sabnis *et al.* 1969). However, an exact relationship between these effects for a given plant has not yet been established. In view of the claim that AN is frequently used traditionally to improve lactation, experiments were performed to determine whether AN can stimulate milk production, PRL release and mammary gland development in rats.

Materials and Methods

Preparation of Acacia nilotica ssp adansonii (AN) extract

According to tradition, a nursing woman (± 65 kg) drinks an extract of about 100g dry AN leaves per liter per day. For our experiments, AN fresh leaves were air-dried in shade and pulverized, then boiled in water for 15 min (1:4 w/w). After centrifugation the supernatant was freeze-dried and the dry material was weighed. The yield in crude extract was about 18%. All doses used in the experiments were based on this estimation. For each experiment, a sample of the freeze-dried extract was dissolved in 0.9% NaCl and then centrifuged at 3000 x g for 10 min and stored at +4°C.

Animals

For all experiments, mature Wistar rats were purchased from IFFA-CREDO, France, and maintained on a 12 h light–dark cycle (lights on from 6.00 – 18.00 h). They were housed individually in standard plastic cages with wood chips on the floor and were given food and water *ad libitum*.

Experimental protocols

I. Effect of oral treatment with AN extract on milk production

Experiment A
Eighteen lactating dams, weighing 225-250 g at the beginning of lactation and suckling 8 to 9 pups, were used in this experiment. Females were divided into three experimental groups and received either 2 ml of 0.9% NaCl (n=6) or 280 mg (n=6) or 560 mg AN-extract / kg BW / 2 ml 0.9% NaCl (n=6), respectively.

Experiment B
Fifteen lactating dams, weighing 225-250 g at the beginning of lactation and suckling 8 to 9 pups, were divided into two experimental groups and received either 2 ml 0.9% NaCl (n=7) or 280 mg AN-extract / kg BW/ 2 ml 0.9% NaCl (n=8), respectively.
All animals were treated daily, starting on the evening of day two of lactation. The extract was administered orally with a gavage syringe each day at 18:00 h. Milk production was estimated 18 h (for experiment A) and 23 h (for experiment B) after gavage.
In both experiments, milk production was measured from day 3 to day 15 of lactation. Milk yield and body weight of dams, and weight gain of pups were measured each day.
Every day during the study period (experiment A) the pups were weighed at 7:00 h (w1) and subsequently isolated from their mother for 4 hours (Sampson & Jansen 1984). At 11:00 h the pups were weighed (w2), returned to their mother and allowed to feed for 1 hour. At 12:00 h, they were weighed (w3). In experiment B, the same procedure was followed, but the pups were isolated between 12:00 h and 16:00 h. After weighing at 16:00 h (w4), they were reunited with their mother for 1 hour of feeding and, finally, they were

weighed (w5). They were then left with their mother during the night. For weight measurements, an electronic balance (Sartorius Basic plus) accurate up to 0.01 g was used. Milk yield was estimated as w3-w2 (milk yield 18 h after the gavage) and w5-w4 (milk yield 23 h after the gavage). Daily milk yield was corrected for weight loss due to metabolic processes in the pup (respiration, urination, and defecation) during suckling. The value used was (w2-w1) / 4 or [(w2-w1) + (w4-w3)] / 8 for the first and the second group of animals, respectively. This value was then multiplied by the number of suckling hours per day and added to the daily suckling gain (Sampson & Jansen, 1984). Daily weight gain of pups was calculated from the pup weight at w2.

II. Effect of intravenous (i.v.) injection of AN extract on plasma and pituitary PRL concentration

Eighteen cycling virgin rats aged 90 days and weighing 200-250 g were cannulated in the jugular vein by the method described by Van Dongen (1990). A week after cannulation, the animals were divided into 3 groups and received either 0.2 ml 0.9% NaCl or 45 mg or 110 mg AN- extract / kg BW / 0.2 ml 0.9% NaCl. All doses were given as a single injection each day via the intravenous cannula during a period of six days.
Animals were adjusted to blood sampling procedures two days before the start of the experiment. On day 1 (at diestrous) and day 3 (at estrous) of the treatment, two plasma samples were taken before and five samples after the injection, at 20 min intervals. The blood samples were centrifuged and plasma was stored at $-20°C$ until radioimmunoassay (RIA) for PRL content. On the 7th day of the experiment, the animals were sacrificed by decapitation under ether anesthesia. Pituitaries were collected for PRL extraction. Extraction occurred in a Potter tube at 0°C with PBS pH 7.4, 0.03 M; 10 μl of the homogenate was diluted with phosphate buffer plus 1% BSA and stored at -20°C.
Plasma and pituitary PRL levels were measured using rat reagents distributed by the NIDDK (NIH, Bethesda, MD). PRL values were expressed in terms of the reference standard RP-3. The intra-assay variation was 7.92% and 9.95% for plasma PRL and pituitary PRL, respectively.

III. Effect of intramuscular injection of AN extract on mammary gland tissue

The animals used in this experiment were first-generation descendants of the rats purchased from IFFA-CREDO. Thirty-six virgin female rats aged 60-70 days were divided into 2 groups. The first group first received a subcutaneous injection of estradiol in a dose of 10 μg / 0.1ml sesame oil twice daily for 2 days. Subsequently they were divided into 3 subgroups and received an intramuscular injection (i.m.) of either 0.9% NaCl or 200 mg or 400 mg AN-extract/kg BW twice daily for 5 days. The second group first received an injection of 0.9% NaCl and was then divided into 3 subgroups receiving the same injections as the first group. On the 6th day, all animals were anaesthetized with ether and sacrificed by decapitation. Pituitaries were collected for PRL extraction, as previously described. The two inguinal mammary glands were removed, immediately fixed in alcoholic Bouin and embedded in paraffin after dehydration in a graded series of ethanol and xylene. Paraffin sections (5 μm) of the mammary glands were sliced and stained with

Harris' hematoxilin and eosin. Mammary gland structures were identified according to the criteria of Russo and Russo (1978, 1996) and Masso-Welch et al. (2000) using a Zeiss microscope coupled to an image analysis system.

IV. Effect of the treatment with AN extract after bromocriptine injection

Twenty-four female rats of 90 days were divided into 4 groups. Two groups were treated orally with 2 ml NaCl 0.9% (n=6) or 550 mg AN-extract/kg BW/2 ml NaCl 0.9% (n=6) twice daily during 5 days, respectively. The remaining two groups were treated subcutaneously with bromocriptine (CB154) at the dose of 4.5 mg/kg BW/0.25ml ethanol 70% twice daily during 2 days. Then they were treated orally by gavage with 2 ml NaCl 0.9% (n=6) or 550 mg AN-extract /kg BW/2 ml NaCl 0.9% (n=6) twice daily during 3 days. The 6th day, all animals were anaesthetized with ether and sacrificed by decapitation. Pituitaries were collected for PRL extraction and determined as described above.

Statistical Analysis

Data were analyzed by Student's t-test or one-way ANOVA, followed by either the Bonferroni test or Scheffe test or LSD test, using the statistical package SPSS (version 7.5 for Windows).
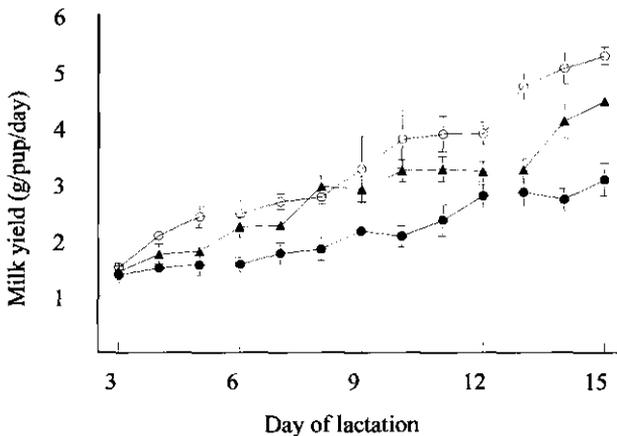
Figure 1 Effect of aqueous extract of AN on milk production
C (●) control group treated with 0.9% NaCl (n = 6); T280 mg (▲) group receiving 280 mg AN-extract / kg BW (n = 6); T560 mg (O) group receiving 560 mg AN-extract / kgBW (n = 6). Values are the means ± SEM. Statistically significant difference is observed during the whole period for the group receiving 560 mg (P<0.01). A significant difference is observed between days 8-11 and days 14-15 for the group receiving 280 mg (P<0.05). Statistically significant differences are given compared to the controls (ANOVA followed by Bonferroni).

Results

Milk production

Milk production of both groups receiving 280 mg and 560 mg AN-extract was higher compared to that of the control group, as illustrated in Figure 1 (experiment I-A). Milk yield increased from 1.38 ± 0.16, 1.44 ± 0.15, and 1.52 ± 0.05 g / pup / day to about 3.07 ± 0.29, 4.46 ± 0.06, and 5.27 ± 0.15 g / pup / day for the controls, and those receiving 280 and 560 mg, respectively. The differences observed were significant from day 2 until the end of treatment, in particular for the 560 mg dose group (ANOVA followed by Bonferroni, $P<0.01$). The mean milk yield was 2.12 ± 0.17, 2.83 ± 0.23 and 3.36 ± 0.32 g / pup / day over the experimental period, respectively (Student's t-test with Bonferroni
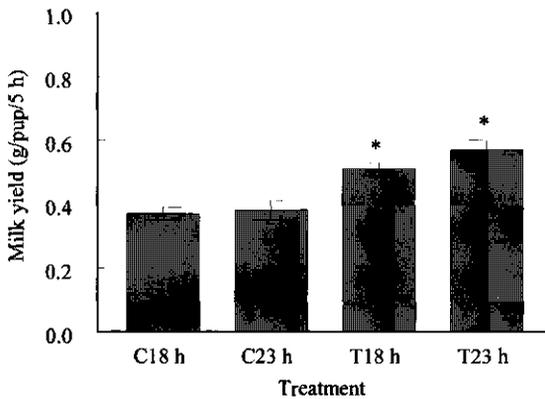
Figure 2 Mean milk production per day. Values are the means ± SEM. Statistically significant differences observed: **P<0.01. *P<0.05. (Student's t- Test with Bonferroni correction). Same treatment as in Figure 1.
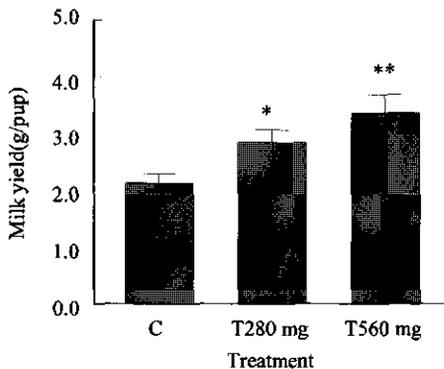
Figure 3. Mean milk production 18 h and 23 h after gavage. Values are the means ± SEM and have been given per 5 h (4 h separation + 1 h suckling).
Milk yield 18 h or 23 h after gavage with either saline (C18 h, C23 h, n = 7) or 280 mg AN-extract (T18 h, T23 h, n = 8). The Scheffe test is used to assess differences between groups, P<0.05.

correction at least P<0.05) (Figure 2).

Milk production data 18 h and 23 h (experiments I-A and B, respectively) after gavage indicated that milk production was significantly increased in all groups receiving the extract at both time points (ANOVA followed by Scheffe P<0.05) (Figure 3). The mean milk yield of the control group was 0.37 ± 0.02 and 0.38 ± 0.03 g / pup / 5 h 18 h and 23 h after gavage with saline, respectively. For the group receiving extract, mean milk yield was 0.51 ± 0.02 and 0.57 ± 0.03 g / pup / 5 h 18 h and 23 h after treatment, respectively.

Body weight

All pups gained weight during the study period (Figure 4) and the rate of weight gain for the treated groups was significantly higher than that for the controls. Body weight increased from 7.83 ± 0.39 to 17.65 ± 1.54 g / pup for the controls, from 8.17 ± 0.66 to 22.94 ± 0.57 g / pup for those receiving 280 mg and from 8.72 ± 0.39 to 25.20 ± 1.51 g / pup for those receiving 560 mg AN-extract. The daily weight gain was 0.86 ± 0.08, 1.42 ± 0.12 and 1.43 ± 0.11 g / pup, respectively (Figure 5). A significant difference was observed between all treated groups and the controls (Student's t-test with Bonferroni correction P<0.01). No significant effect on body weights of dams was seen.

PRL content

To evaluate the effects of extract on PRL secretion and release, PRL concentrations were measured in both plasma and pituitary. In experiment II, the effect of i.v. administration of the extract on the plasma PRL concentration was studied; see Figure 6. A wide variation in individual responses to treatment was observed. However, plasma PRL concentrations were significantly higher in treated animals compared to the controls; in the latter PRL
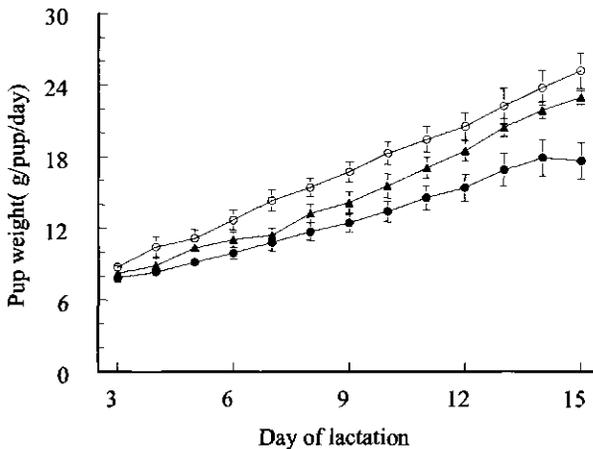
Figure 4 Effect of aqueous extract of AN on pup weight. Values are the means ± SEM. A significant difference was observed between the treated group and the controls (at least P<0.05). C (●) T280 mg (▲) T560 mg (O). Same treatment as in Figure 1.

55

levels remained constant (ANOVA followed by Bonferroni, at least P<0.05). On day 1 ("acute treatment") of treatment, the highest levels were observed 60 min after injection. They remained relatively high until day 3 of treatment where the highest levels were observed earlier (40 min). PRL levels 40 min after injection were 1.23 ± 0.35, 2.95 ± 1.46 and 56.03 ± 22.84 ng / ml for controls, and the groups receiving 45 and 110 mg on day 1 of the treatment, respectively. On day 3 ("chronic treatment"), the plasma levels were, at the same times, 4.02 ± 0.76, 10.53 ± 2.44 and 43.93 ± 16.24 ng / ml for controls, and the group receiving 45 and 110 mg, respectively. Furthermore the pituitary PRL content for the same animals (Figure 7) was significantly increased in the group receiving 110 mg compared to the controls (ANOVA followed by Bonferroni, P< 0.01) although no significant increase was found for the group receiving the lower dose.

Regarding pituitary PRL in animals receiving the extract i.m.(Experiment III), significantly higher PRL concentrations were observed in the E_2-primed animals treated with the extract, as compared to non-primed ones (ANOVA, follow by Bonferroni, at least P<0.05) (Figure 8). Moreover, a significant increase was found for E_2-primed animals receiving extract compared to the E_2-primed control group. In both CB154 treated groups (Figure 9), pituitary PRL content was significantly lower compared to control value (ANOVA, follow by LSD P<0.05). AN treatment significantly increased PRL content compared to both that in animals treated with CB154 alone or with CB154 + AN-extract (P< 0.05), albeit that treatment with extract after CB154 injection had no effect on PRL content.
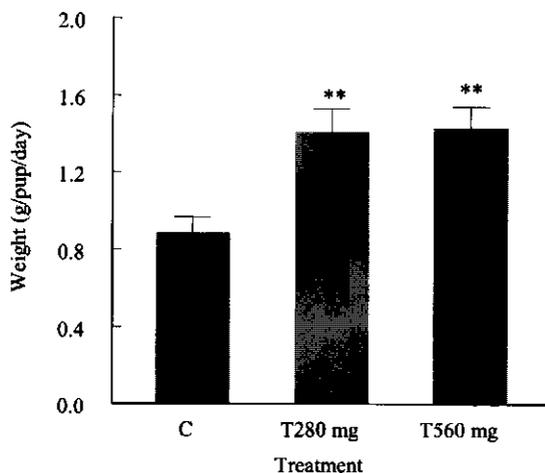
Figure 5 Mean weight gain of the pups. Values are the means ± SEM. ** Statistically significant (P<0.01) (Student's t-test followed by Bonferroni correction). C, T280 mg, and T560 mg. Same treatment as in Figure 1.

Histology of mammary tissue

To understand the relationship between PRL and mammary growth, the histology of rat mammary gland tissues was studied in the third experiment (Figure 9). The mammary glands of non-E_2-primed rats treated with saline show a bare duct system and terminal end buds (TEBs) within an important fat pad (A). Although the mammary gland of the E_2-
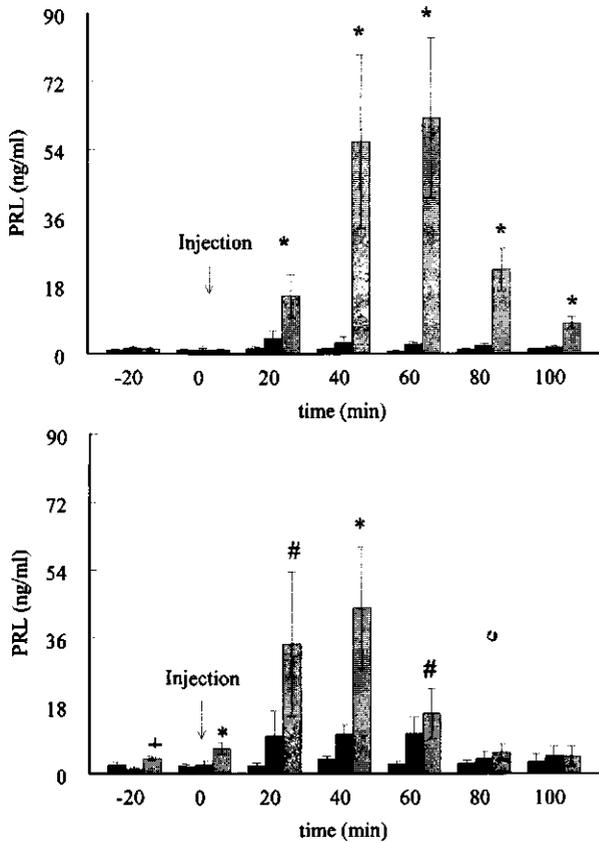
Fig. 6 Plasma PRL profiles (i.v. injection) on day 1 (acute effect) and day 3 (chronic effect). Values are the mean ± SEM. *statistically significant compared to both controls and the group receiving 45 mg (at least P<0.05), # Statistically significant compared to the controls (at least P<0.05) (ANOVA followed by Bonferroni). C (■) control treated with 0.9% NaCl (IV), T45 mg (▓) group receiving 45 mg of plant extract / kg BW (IV), and T110 mg (▒) group receiving 110 mg of plant extract / kg BW (IV). "0" time represents the time of injection of AN extract with samples taken at 20-minute intervals post-injection.

primed animals consisted of ducts branching into ductules (B), no alveolar development was observed. In all E_2-primed and non-primed animals receiving the extract (C-F), ductule branching with alveolar development was observed, as well as lipid droplets in the alveoli. However, there was no evidence of secretion into ducts of the non-primed group receiving 400 mg plant extract / kg BW (E). The largest alveolar structures with basophilic secretions in the lumen of alveoli and ducts were observed in the estrogen-primed group receiving 400 mg of extract.
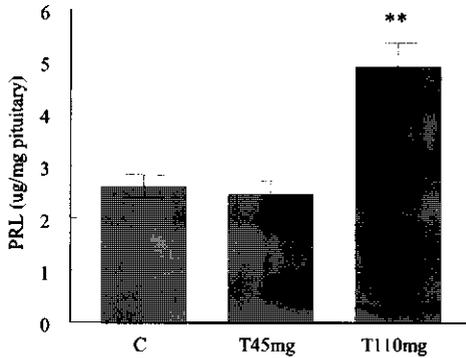
Figure 7 Pituitary PRL content after i.v. injection treatment during six days. Values are the means ± SE. ** Statistically significant (P<0.01) (ANOVA followed by Bonferroni). C: control group receiving 0.9% NaCl (IV, 6 days); T 45 mg: group receiving 45 mg of plant extract / kg BW (IV, 6 days); T110 mg: group receiving 110 mg of plant extract / kg BW (IV, 6 days).
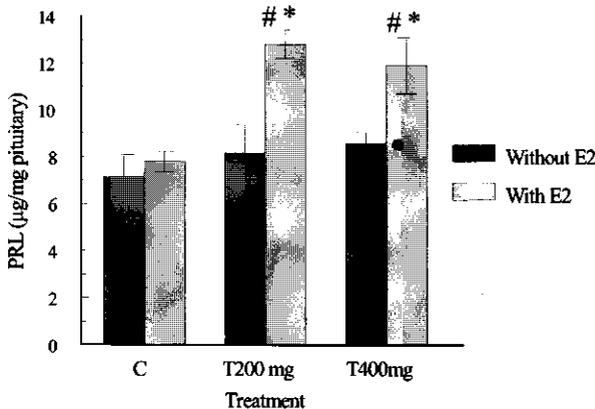
Figure 8 Pituitary PRL content after intramuscular injection. Values are the mean ±SEM. * Statistically significant compared to the control, # Statistically significant compared to the E_2-primed control group (ANOVA followed by Bonferroni, at least P<0.05). Without E_2: not previously treated with estradiol. With E_2: previously treated with estradiol. C: control group receiving 0.9% NaCl (IM, 5 days), T200 mg: group receiving 200 mg of plant extract / kg BW (IM, 5 days), T400 mg: group receiving 400 mg of plant extract / kg BW (IM, 5 days).
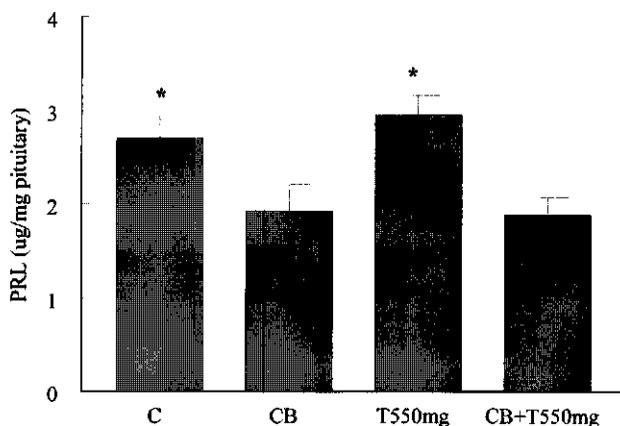
Figure 9 Pituitary PRL content after oral administration of extract and after subcutaneous injection of CB154. Values are the mean ± SEM. * Statistically significant compared to the bromocriptine treated group receiving the extract. (ANOVA followed by LSD, P<0.05). C: control group receiving 0.9% NaCl (oral, 5 days). CB: group receiving CB154 injection (subcutaneous, 5 days). CB+T550 mg: group receiving CB154 injection (subcutaneous, 2 days) + T550 mg (oral, 3 days). T550 mg: group receiving the extract (oral, 5 days).

Discussion

The measurement of milk production in the rat model is difficult. Milk yield estimations for rats by means of pup weight and weight gains have been used in several studies (Morag *et al.* 1975, Sampson & *Jansen* 1984, Kamani *et al.* 1987, Kim *et al.* 1998). It has to be noted that the purpose of this study was essentially to determine whether AN is lactogenic. As expected, milk production was significantly higher in treated animals than in controls. In addition, milk yield appears to be significantly stimulated about 24 h after administration of the extract and pup growth rate was significantly improved. Moreover, pup growth rate in the group receiving the low dose (i.e. in the same range as that used by native women) was similar to that in the group receiving the high dose, whereas the dams produced 20% less milk. This suggests a possible effect of the extract on milk components. However, this suggestion can only be confirmed by studying the composition of the milk. Likewise, extract of *Asparagus racemosus* has been observed to stimulate milk production in buffaloes (Patel and Kanitkar 1969). On the other hand, treatment with some of these plants did not improve rat pup growth (Kamani *et al.* 1987) and milk production in ewes (Sawadogo *et al.* 1989), but it did stimulate PRL secretion in ewes (Sawadogo *et al.* 1989). Interestingly, the results obtained in this study show that not only milk production was increased, but plasma and pituitary PRL levels as well. The mechanism of action is still unknown as well as the active components of the extract. However, we suggest an effect through the hypothalamo-pituitary axis leading to the synthesis and release of PRL, as observed in previous studies (Sawadogo *et al.* 1987, 1989, Sepehri *et al.* 1990, 1992). Moreover, the pituitary PRL content in peri-pubertal
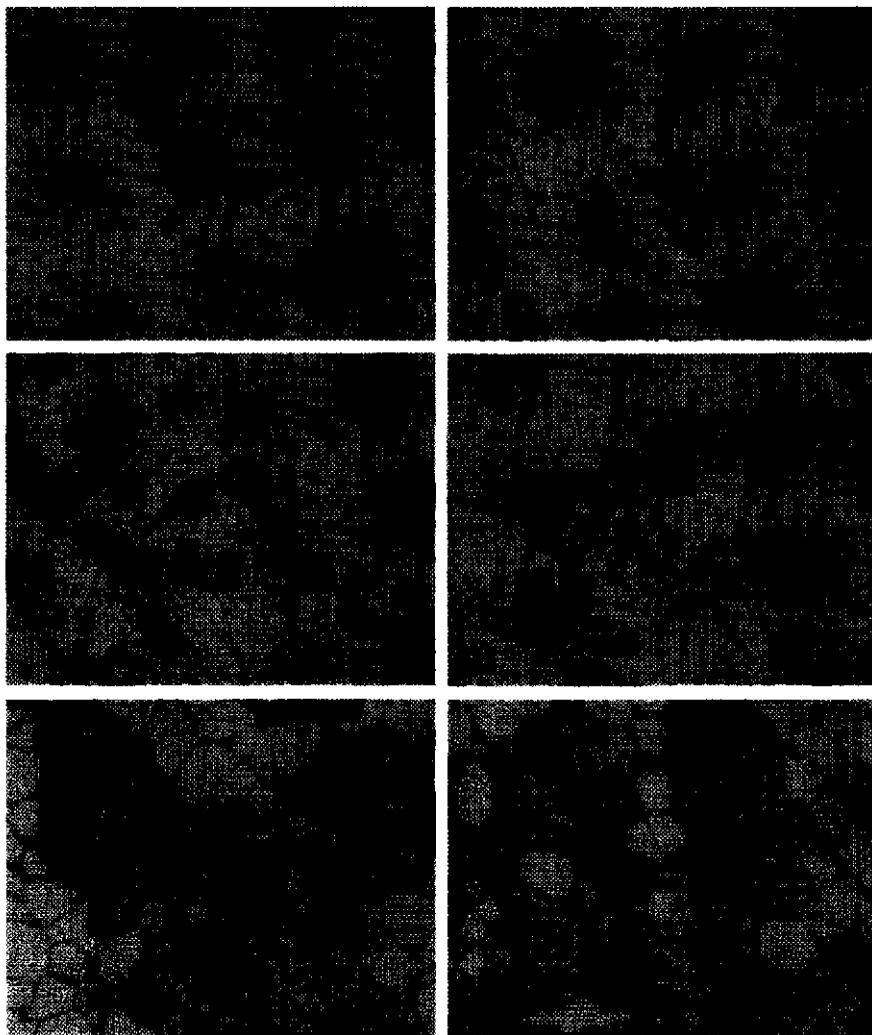
Figure 10 Sections of mammary glands from virgin estrogen (E_2)-primed and non-primed rats, 60 to 70 days old treated with saline or plant extract.

A: Section of mammary gland from a non-primed rat receiving 0.9% NaCl showing ductules and TEBs, with no alveolar development. B: Mammary gland from an E_2-primed rat receiving 0.9% NaCl showing ducts branching into ductule, with fewer alveolar buds (Alv). C: Mammary gland from a non-primed rat receiving 200 mg of plant extract / kg BW, showing duct branching to ductule with modest alveolar structure development. D: Mammary gland from an E_2-primed rat receiving 200 mg of plant extract / kg BW, showing ductule branching with alveolar development, adjacent blood vessels (BV) and lymphatic tissue (ly). E: Mammary gland from a non-primed rat receiving 400 mg of plant extract / kg BW showing a welldefined alveolar structure with lipid droplets within the alveoli. F: Mammary gland from an E_2-primed rat receiving 400 mg of plant extract / kg BW showing a well-defined alveolar structure with lumens filled with basophilic secretions (S). Paraffin sections of 5 μm were stained with Harris hematoxilin and eosin.

animals treated with the extract did not significantly differ from the control as compared to that in cycling females treated intravenously with extract. This indicates that the extract exerts a specific PRL-secretion activity under certain physiological conditions. A strong relation between treatment with extract and PRL release after "acute" as well as "chronic" treatment has been observed. From this, it can be expected that the underlying mechanism of PRL elevation will be related to neuro-endocrine regulation. Our results suggest involvement of dopaminergic activity, since treatment with extract after CB154 injections did not increase pituitary PRL content.

Obviously, PRL is known to stimulate milk synthesis and secretion, but in the last decades, research has also shown a relationship with breast carcinogenesis; the increase in PRL synthesis and release may increase susceptibility to carcinoma (Venugopal *et al.* 1999). In this study, extract of AN was shown to stimulate mammary gland development and the differentiation of the lobulo-alveolar system from the lobular buds with milk secretion within the lumen. Immunohistochemical data on mammary cell proliferation must be obtained before any statement on the carcinogenic effect of the plant can be made. However, women have used this plant for generations without any pathologic effects being reported. Further investigations are in progress and will provide increased understanding of the effect of the extract on mammary gland development.

In conclusion, it can be stated that aqueous extract of AN effectively stimulates milk production as well as PRL synthesis and release in the rat. Therefore, the traditional belief that AN extract can improve milk production in lactating women may be valid.

References

Adjanohoun E, Ake AL, Florret JJ, Guinko S, Koumare M, Amyi AMR & Raynal J 1979a Médecine traditionnelle et Pharmacopée. Contribution aux études ethnobotaniques et floristiques au Mali. *Ed. Agence de Coopération Culturelle et Technique, Paris.*

Adjanohoun E, Amyi AMR, Ake AL, Dan DH, Delmas M, De Souza S, Garba M, Guinko S, Kayonga A, Raynal JL & Saadou M 1979b Médecine traditionnelle et pharmacopée. Contribution aux études ethnobotaniques et floristiques au Niger. *Ed. Agence de Coopération Culturelle et Technique, Paris.*

Bognounou O, Ouedraogo OC & Ouedraogo OG 1974 Contribution à l'inventaire des plantes médicinales africaines en pays Mossi (Région de Ouagadougou- Burkina Faso). *Colloque du CAMES,* Lomé, Togo.

Brisken C, Kaur S, Chavarria TE, Binart N, Sutherland RL, Weinberg RA, Kelly PA & Ormandy CJ 1999 PRL controls mammary gland development via direct and indirect mechanisms. *Developmental Biology* **210** 96-106.

Flint DJ, Tonner E, Beattie J & Panton D 1992 Investigation of the mechanism of action of growth hormone in stimulating lactation in the rat. *Journal of Endocrinology* **134** 377-383.

Hennighaussen L, Robinson GW, Wagner KU & Liu W 1997 PRL signaling in mammary gland development *Journal of Biological Chemistry* **272** 7567-7569.

Horseman ND 1999 Prolactin and mammary gland development *Journal of Mammary Gland Biology and Neoplasia* **4** 79-88.

Kamani HT, Karunanayake EH & Mahindartna MPD 1987 Evaluation of galactoguic activity of *Asparagus falcatus. Ceylon Journal of Medical Sciences* **30** 63-67.

Kim SH, Moon YS, Keller WL & Park CS 1998 Compensatory nutrition-directed mammary cell proliferation and lactation in rats. *British Journal of Nutrition* **79** 177-183.

Knight CH, Calvert DT & Flint DJ 1986 Inhibitory effect of bromocriptine on mammary development and function in lactating mice. *Journal of Endocrinology* **110** 263-270.

Llovera M, Touraine P, Kelly AP, & Goffin V 2000 Involvement of PRL in breast cancer: redefining the molecular targets. *Experimental Gerontology* **35** 41-51.

Madon RJ, Ensor DM, Knight CH & Flint DJ 1986 Effects of an antiserum to rat Growth Hormone on lactation in rat. *Journal of Endocrinology* **111** 117-123.

Martal P & Houdebine LM 1982 Effect of various drugs affecting cytoskeleton and plasma membranes on the induction of DNA synthesis by insulin, epidermal growth factor in mammary explants. *Biologie Cellulaire* **44** 111-116.

Masso-Welch PA, Darcy KM, Stangle-Castor NC & Ip MM 2000 A developmental atlas of rat mammary gland histology. *Journal of Mammary Gland Biology and Neoplasia* **5** 165-185.

Morag M, Popliker F, & Yagil R 1975 Effect of litter size on milk yield in the rat. *Laboratory Animals* **9** 43 - 47.

Nacoulma-Ouedraogo OG 1996 Plantes médicinales et pratiques traditionnelles au Burkina: Cas du plateau central. Thèse de Doctorat Es Sciences Naturelles, *Université de Ouagadougou*, tome **II**, p. 285.

Patel AB & Kanitkar UK 1969 *Asparagus racemosus Willd form. bordi* as a galactogogue in buffaloes. *Indian Veterinary Journal* **46** 718-721.

Russo IH & Russo J 1978 Developmental stage of rat mammary gland as determinant of its susceptibility to 7,12-dimethyl-benz [a] anthracene. *Journal of the National Cancer Institute* **60** 1451-1459.

Russo IH & Russo J 1996 Mammary neoplasia in long-term rodent studies. *Environmental Health Perspectives* **104** 938-967.

Sabnis PB, Gatonde BB & Jetmalani M 1969 Effects of *Asparagus racemosus* on mammary glands of rats. *Indian Journal of Experimental Biology* **6** 55-56.

Sampson DA & Jansen GR 1984 Measurement of milk yield in lactating rat from pup weight and weight gain. *Journal of Pediatry, Gastroenterology and Nutrition* **3** 613-617.

Sawadogo L 1987 Contribution à l'étude des plantes médicinales et de la pharmacopée traditionnelle africaine. *Thèse de Doctorat d'état, Université de Tours*, p 149.

Sawadogo L & Houdebine LM 1988b Induction de la synthèse de caséine-*B* dans la glande mammaire de rates traitées par des extraits de plantes. *Comptes Rendus de l'Academie Sciences Paris* **306** 167-172.

Sawadogo L, Houdebine LM, Thibault JF & Rouau X 1988a Mise en évidence d'extraits de plantes possedant une activité galactogène. *Bulletin Médecine Traditionelle* **2** 133-146.

Sawadogo L, Houdebine LM, Thibault JF, Rouau X & Olivier-Bousquet M, 1988c Effect of pectic substances on PRL and growth hormone secretion in the ewe and on the induction of casein synthesis in the rat. *Reproduction, Nutrition, Development* **28** 293-301.

Sawadogo L, Sepehri H & Houdebine LM 1989 Mise en évidence d'un facteur stimulant la sécrétion de PRL et d'hormone de croissance dans la drèche de brasserie. *Reproduction, Nutrition, Development* **29** 139-146.

Sepehri H, Renard C & Houdebine LM 1990 *B*-glucan and pectic derivatives stimulate PRL secretion from hypophysis in vitro. *Proceedings of the Society for Experimental Biology and Medicine* **194** 193-201.

Sepehri H, Kann G & Houdebine LM 1992 Pouvoir lactogènes potentiel de quelques extraits de plantes iraniennes *Cahiers Agricultures* **1** 35-39.

Venugopal M, Callaway A & Snyderwine EG 1999 2-Amino-1-methyl-6-phenylimidazo [4,5-*b*] pyridine (PhIP) retards mammary gland involution in lactating Sprague-Dawley rats. *Carcinogenesis* **20** 1309-1314.

Zwierzchowski L 1993 Control hormonal de la division de la cellule mammaire et de la synthèse d'ADN, In *Biologie de la lactation*, 59-73. Ed J Martinet & LM Houdebine Editions INSERM/INERA Editions

CHAPTER 5

EFFECT OF CRUDE AQUEOUS EXTRACT OF *ACACIA NILOTICA* VAR *ADANSONII* ON CELL PROLIFERATION AND MAMMARY GLAND DIFFERENTIATION

Lompo-Ouedraogo Z[1,2], Van Der Beek EM[1,3], Van Der Heide D[1], Sawadogo L[2]

[1]Human and Animal Physiology Group, Department of Animal Sciences,
Wageningen University, The Netherlands
[2]Unité de Formation et de Recherche en Sciences de la Vie et de la Terre,
Université de Ouagadougou, Burkina Faso
[3]Neuroendocrinology Section, Department of Biomedical Research,
Numico Research, Wageningen

Abstract

Past research shows that *Acacia nilotica* ssp. *adansonii* (AN) extract can stimulate milk production and prolactin (PRL) synthesis and release in rats. As milk yield and persistency of lactation are related to mammary gland growth and differentiation, the major objective of this study was to investigate the *in vivo* response of mammary epithelium to AN-extract. To this end, peripubertal virgin female rats were treated with AN extract, either oral or i.m., in the presence or absence of exogenous estradiol (E_2). Rats treated with perphenazine or sulpiride, two selective dopamine receptor antagonists, were included as positive controls. One hour before sacrifice, BrdU was administered subcutaneously and mammary gland tissue was collected and processed for BrdU immunocytochemical staining.

The treatment with extract resulted in significantly more lobulo-alveolar structures filled with basophilic secretions and lipid globules compared to controls ($P<0.05$). Terminal end buds (TEBs) were completely absent in the E_2-primed, high dose AN-treated groups when treatment was given intramuscularly. In contrast, AN-extract did not affect TEBs formation in the absence of E_2. The overall mammary response to AN-extract was potentiated by the concomitant presence of E_2, albeit that limited alveolar differentiation was observed in the AN-treated animals compared to the perphenazine or sulpiride treated groups. The BrdU labelling index was significantly increased following oral administration of AN extract compared to control. In both perphenazine and sulpiride-treated animals, the BrdU labelling index was significantly lower than that of AN treated animals.

This study shows that mammary cell proliferation was significantly higher in AN-treated groups compared to control, and that the more the lobular unit is differentiated, the less cell proliferation is present, as measured by the decrease in BrdU labelling index. We conclude that AN-extract is able to stimulate mammary gland growth and differentiation from the least differentiated structures (TEBs) to the fully developed mammary gland showing active milk production.

Introduction

The mammary gland of rats is composed of multiple cell types and can be divided roughly into two compartments (Masso-Welch *et al.* 2000). The epithelial compartment is composed of different epithelial structures with distinct morphology, and functional and proliferative activity (i.e. epithelium of ducts, ductules, terminal end buds (TEBs), alveolar buds, alveoli, and myoepithelial cells). The stromal compartment, which is distinct in thickness, composition, and density, surrounds ducts, ductules, and alveolar lobules and contains many fibroblasts and adipocytes.

It is well known that development and differentiation of mammary gland structures are hormone dependent processes and that blood levels of those hormones vary during the reproductive cycle. The regulation of mammary gland activity is dominated by the inhibitory hypothalamic dopamine (DA) mechanism that directs pituitary prolactin release (PRL). Administration of perphenazine or sulpiride, a selective D2 and D1 receptor

antagonist, respectively, to female rats resulted in an increase in PRL secretion and release, thus stimulating lactation. The perphenazine-induced PRL elevation is associated with a growth response of the mammary gland and a large proliferative response that occurs in the working unit of the gland, the lobule (Stringer *et al.* 1990).

During early development, rising levels of endogenous estradiol (E_2) promote mammary duct branching that ends in highly proliferative structures termed TEBs. TEBs are the major sites of proliferation (Monaghan *et al.* 1990) leading to the formation of lobular units (Howard and Gusterson 2000) with the advancement of puberty. The main characteristic of a mature mammary gland is the absence of TEBs and the presence of a highly branched architecture terminated by lobular buds (Horseman 1999). During pregnancy, the lobular buds give rise to alveolar lobules, which increase both in size and number and reach maximal development at the end of pregnancy (Masso-Welch *et al.* 2000). At the end of pregnancy, the mammary gland has reached a high rate of proliferation and the epithelium has achieved a profound biochemical transformation that leads to the synthesis of specific components of milk such as proteins (casein), lipids (fatty acids), and sugar (lactose). During this period, the myoepithelial cell layer, which surrounds the alveolar epithelium, has acquired abundant cytoplasmic filaments for contractile functions during lactation (Joshi *et al.* 1986). Mammary cell proliferation continues throughout lactation with a peak on day 3 in rats (Joshi *et al.* 1986). The lobulo-alveolar epithelium is subsequently transformed into a secretory epithelium, which synthesises milk components and lactogenic enzymes. Although the great variability of hormones in maternal plasma is required for lactogenesis, a fully differentiated mammary gland is the primary determinant for sufficient milk yield and persistency of lactation (Kim *et al.* 1998). During lactation, prolactin regulates the synthesis of milk in the mammary gland. Previous reports show that extract of *Acacia nilotica* (AN) can increase milk production and PRL secretion and release (Lompo *et al.* submitted) and can induce β-casein accumulation in mammary glands of rats (Sawadogo 1988a). In this study, we investigated the effect of AN extract on mammary cell proliferation using bromodeoxyuridin (BrdU). BrdU is an analogue of thymidin that is incorporated into the DNA of proliferating cells. To this end, different doses of AN-extract were compared with control treatment in peripubertal virgin female rats pretreated with either E_2 or NaCl. Histological evaluation of mammary gland development as well as quantification of selective BrdU incorporation into specific mammary gland structures was performed.

Materials and Methods

Animals

Wistar virgin female rats were obtained from IFFA-CREDO, France. They were kept under controlled light-dark cycles (lights on from 6:00 h to 18:00 h) at 22-28° C and had free access to pelleted food and tap water. Females were weighed every day. The animals used in the present study were offspring from the first generation purchased from IFFA-CREDO.

Experimental design

Experiment 1

Forty-two virgin female rats, aged 60-70 days, were divided into 2 groups. The first group (n=18) first received a subcutaneous injection of E_2 in a dose of 10 µg/0.1 ml sesame oil twice daily for 2 consecutive days. Subsequently, they were divided into 3 subgroups of 6 animals each receiving an i.m. injection of saline, 200 mg or 400 mg-AN extract/kg BW once daily for 5 consecutive days.

The 2nd group of animals (n=24) served as controls for the estrogen pretreatment and received an injection of 0.1 ml of 0.9% NaCl twice daily for 2 consecutive days. They were subsequently divided into 4 groups of 6 animals receiving either saline, 400 mg-AN extract/kg BW, 20 mg perphenazine/kg BW or 20 mg sulpiride/kg BW once daily for 5 consecutive days. AN extract and saline were administered intramuscularly; perphenazine and sulpiride were given subcutaneously (s.c.).

Experiment 2

Thirty virgin females, aged 60-70 days, were divided into 5 groups of 6 animals each. Four groups first received a subcutaneous injection of E_2 in a dose of 10 µg/0.1 ml sesame oil twice daily for 2 consecutive days and were subsequently treated orally with either saline or 1 g or 2 g or 3 g AN extract/kg BW once daily for 5 consecutive days. The remaining group first received 0.1 ml saline twice daily for 2 consecutive days and was then treated orally with 3 g AN extract/kg BW once daily for 5 consecutive days.

On day six for both experiment 1 and 2, females were injected i.p. with 5 mg BrdU/kg BW and 2 hours later sacrificed by decapitation under ether anaesthesia. The inguinal mammary glands were removed and subsequently frozen for immunocytochemical staining. Pituitaries were collected for PRL extraction as described previously (Lompo *et al.* submitted). Pituitary PRL was measured using rat reagent kindly provided by the NIDDK (NIH, Bethesda, MD). PRL values were expressed in terms of the reference standards RP-3. The intra-assay variation was determined using pooled rat serum and amounted to 7.92% and 9.95% for plasma and pituitary PRL levels, respectively.

Immunohistochemistry

Frozen sections

Unfixed rat mammary glands collected as described above were used for immunocytochemical staining. Eighteen cryostat sections (10 µm) taken at regular intervals (every 50 µm) were randomly cut from each frozen mammary gland. The sections were mounted on Superfrost Plus slides coated with 0.1% poly-l-lysine and collected in boxes containing desiccant, wrapped with parafilm, and stored at -20° C until use.

Unfixed frozen sections of the rat intestine treated or not with BrdU, and rat mammary glands not treated with BrdU were used as positive and negative controls of the BrdU staining.

Staining procedure

The immunocytochemical (ICC) staining was performed according to McGinley *et al.* 2000 with some modifications.
Briefly, the sections were allowed to return to room temperature before unwrapping, after which they were fixed in Carnoy's solution for 10 min, followed by methanol for 5 min and subsequently hydrated in distilled water for 30 min.
The sections were then immersed in 2N HCl for 90 min to hydrolyse the DNA. Subsequently, sections were rinsed several times (4 times 10 min) in distilled water and acid hydrolysis was stopped by immersion in 0.1M sodium borate for 5 min followed by 3 rinses with distilled water (3 times 10 min). Endogenous peroxidase activity was blocked by immersing sections in 3% H_2O_2 for 5 min. Sections were rinsed in distilled water (3 times 10 min), followed by TBS pH 7.4 (3 times 5 min). All subsequent steps were performed in a humidity chamber. Primary antibody (mouse anti-BrdU final dilution 1:750 diluted in TBS, pH 7.4) was applied to the sections except for slides used for method control that were incubated with the same amount of TBS. The incubation in primary antiserum was performed at room temperature overnight (17 hrs). The secondary antibody, biotinylated horse anti-mouse IgG, rat preabsorbed (Vector Laboratories, Burlingame, final dilution 1:250 in 10% normal horse serum diluted in TBS) was applied to all sections and incubated for 60 min. Thereafter, sections were incubated with horseradish peroxidase-conjugated streptavidin (Vector Lab., final dilution 1:750) for 90 min and thoroughly washed in TBS (3 times 5 min). Subsequently, 3,3'-diaminobenzidine (DAB, with 0.03% H_2O_2 added to it) was used for 15 min to visualize the staining. Finally, after rinsing in TBS (3 times 10 min), sections were counterstained in diluted Harris haematoxylin (1:10) for 5 min, rinsed in distilled water (3 times 10 min) followed by tap water (3 times 5 min). Sections were dehydrated in a series of graded ethanol, cleared in xylene and cover slipped using DEPEX®.

Counting of BrdU positive cells

To assess BrdU incorporation, a computer-assisted counting procedure was used. BrdU positive cells were analysed using a Zeiss microscope with a CCD-camera, coupled to a SCION image analysis system. All images were captured at a magnification of 100x (or 200x) and collected for analysis, using Scion image. Before capturing an image, the camera was calibrated for area measurements (μm). For counting a random sampling of microscopic fields was used, which reduced the likelihood of field sampling bias in specimen analysis. Each slide contained 6 sections of mammary gland. For each section, the slide was moved to a position at which the higher left corner of the section was in the field of view and 3 images were randomly captured from the left corner to the right corner. Then, the slide was moved to a position at which the lower left corner of the section was in the field of view. Again, 3 images were randomly captured from the left to the right. Thus,

for each section, six images were taken and saved in which the X and Y coordinate values were automatically displayed thanks to the initial calibration. The number of BrdU positive nuclei counted in each image was expressed as the percentage of the total number of cells in the average of all images in the same treatment group.

Counting of mammary gland structures

For the determination of the degree of mammary gland differentiation, the number of structures was counted as previously described using the morphological criteria of Russo and Russo (1978, 1996). Histological analysis was routinely performed on the inguinal mammary tissue to minimise differences in mammary gland morphology. The number of structures was expressed per unit of area (mm^2). Mammary gland composed of only ducts and TEBs was scored as 0. Mammary gland with small lobules composed of 5 or fewer acini were scored as 1, those with 6-10 acini as 2, those with 11-20 acini as 3 and those with more than 20 were scored as 4. Mammary glands with lobules composed of very large acini were scored as 5.

Statistical analysis

Data were expressed as mean percentage BrdU+/cell total/mm^2. Statistical evaluation of PRL data was performed using ANOVA followed by a post-hoc Bonferroni and Scheffe test to identify differences between groups. $P<0.05$ was considered significant.

Results

Mammary gland differentiation

A total of fifty-four to seventy-two randomly collected mammary gland fields in each animal were used to analyse the effects of AN extract on inguinal mammary gland development. This method of quantification has the advantage of including ducts, alveoli or alveolar buds and TEBs in approximately the same proportion as they occur in the whole gland. Mammary glands of control animals pretreated with saline only showed ducts and TEB structures and very few alveoli (Figure 1A), while mammary gland of E_2-primed animals clearly exhibit more TEBs and more alveolar structures (Figure 1B). Perphenazine and sulpiride treatment resulted in large alveolar structures with milk secretion in the lumen (Figure 2). The amount of stromal tissue was clearly decreased compared to control or E_2-primed animals (see Figures 1 and 2). The number of differentiated alveolar structures (lobules type II) was higher in the perphenazine compared to the sulpiride treated animals. In all animals treated with AN-extract, ductules branching to lobule type I and II were present (Figure 3). Figures 3A and 3B show mammary gland from a rat treated intramuscularly with 200 mg AN extract /kg BW, and a rat treated with 400 mg AN-extract/kg BW after priming with E_2, respectively. The size of the mammary gland structures was clearly increased following a higher dose of AN extract. Figures 3C and 3D show typical mammary glands from rats receiving NaCl + 3 g
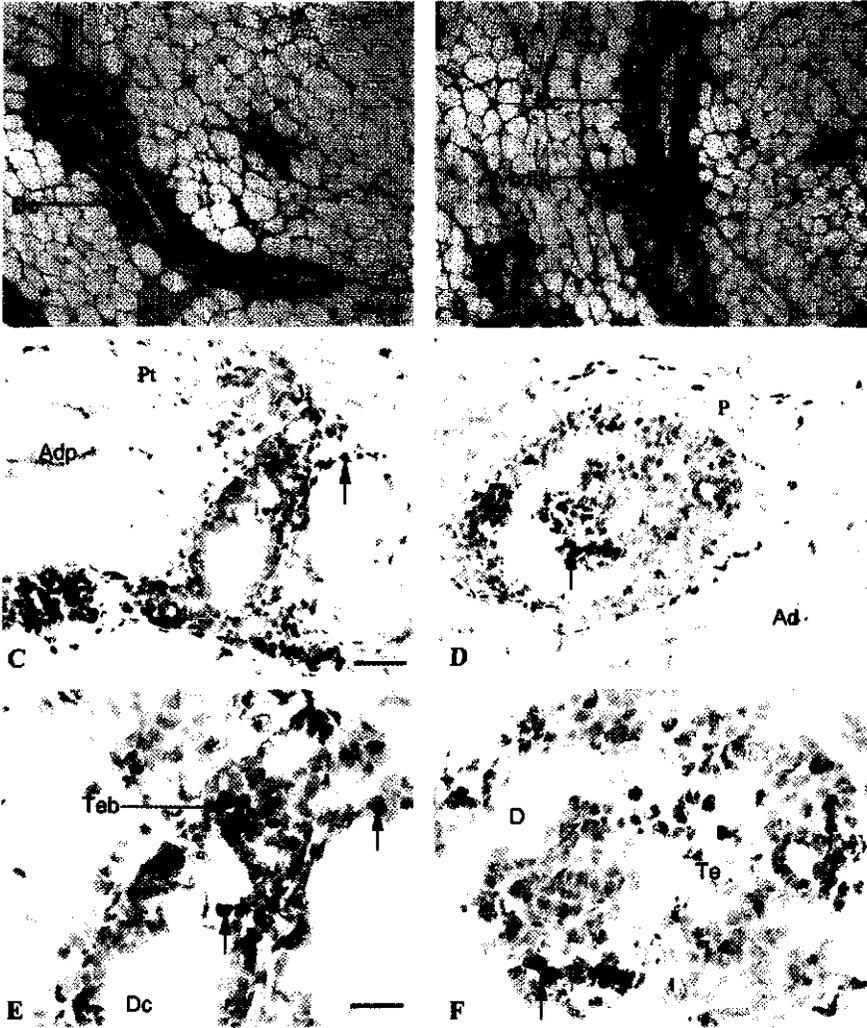
Figure 1: Photographs showing mammary gland development and differentiation in control (NaCl) (panel A, C and E) and E_2-primed animals (panels B, D and F). Panels A and B show mammary gland following haematoxylin-eosin staining at low magnification. Extension of the ducts (Dc) into alveolar buds (Alvbds) can be clearly seen following E_2-treatment. Note the abundance of adipose tissue surrounding the structures. Panels C-F show mammary gland following BrdU immunocytochemical staining at low (panels C, D) and high (panel E, F) magnification. Note the increase in size of the terminal end bud following E_2-treatment (panels D, F). Arrows point to BrdU positive cells within the mammary structure. Adp: adipose tissue; Teb: terminal end bud; Pt: stromal tissue. Magnification panels A and B (100x), panels C and D (200x) and panels E and F (400x).

69

or E_2 + 3 g AN extract/kg BW, respectively. Oral treatment also resulted in a clear differentiation of mammary gland structures in E_2-primed as well as control (NaCl) animals. More stromal tissue was seen surrounding the lobules in AN-treated glands than in the glands from perphenazine or sulpiride treated animals.
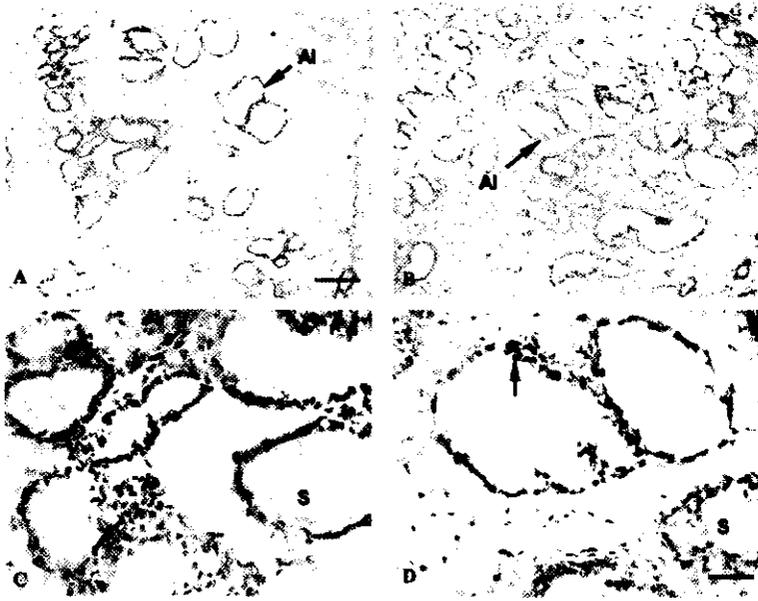
Figure 2 Photographs showing mammary gland development and differentiation following sulpiride (panels A, C) or perphenazine (panels B, D) treatment. Panels A and B show mammary gland following BrdU staining at low magnification (100x). The amount of alveolar structures lobule type II was clearly increased after perphenazine treatment. Note the decrease in the amount of stromal tissue compared to that in control animals in Figure 1. Panels C and D show mammary gland following BrdU immunocytochemical staining at higher magnification (400x). Arrows point to BrdU positive cells. Alv: alveoli; S: milk secretion in lumen.

Quantification of mammary gland structures revealed that animals that received saline treatment, alone or in combination with E_2, had significantly more TEBs than those glands from AN-treated animals where more lobules were observed (Table 1). Intramuscular AN-treatment appeared to stimulate lobulo-alveolar development from TEBs as evidenced by the lower number of TEBs compared to control treatment in the low dose and the complete absence of TEBs in the estrogen-primed high dose AN-treated group. Mammary gland tissue from perphenazine and sulpiride treated animals showed consistently less ducts compared to all other treatment groups and no TEBs were observed. Also, perphenazine treated animals showed a higher degree of structural differentiation compared to sulpiride treated rats, as evidenced by the increase in lobule type II and the absence of lobules type I in perphenazine treated animals. End buds were still clearly present in the mammary gland

of animals treated orally with a low dose of AN-extract, albeit that the number decreased following the higher dose of AN extract. Oral AN-treatment resulted in a higher degree of differentiation of the mammary gland compared to E_2 alone, lobules type II were only present in AN-treated animals. Lobules type I and II were also present in control, NaCl animals that were treated orally with the high dose of AN extract. No clear differences were seen between the number of ductal structures of all treated and untreated animals, except following the pharmacological intervention, where a decrease was demonstrated (see Tables 1 and 2).
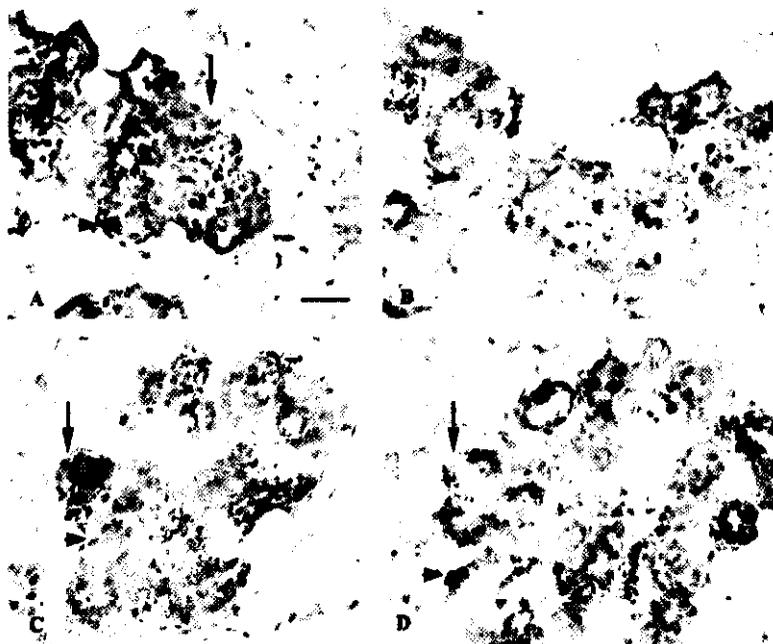
Figure 3 Photographs showing BrdU immunocytochemical staining of mammary gland development and differentiation after 200 mg i.m. (panel A), 400 mg (panel B) or oral treatment (panel C) with AN extract in sodium chloride (control) primed animals. Panel D shows an example of mammary gland from a E_2-primed animal following 3 g AN extract orally. Arrows point to a distinct lobule type II within the alveolar structure, arrowheads point to BrdU positive cells. Magnification 400x.

Mammary cell proliferation

Using BrdU incorporation and immunocytochemical detection, mammary cell proliferation was analysed in ducts and lobules. A low number of BrdU positive cells were clearly visible in the TEBs of control, NaCl as well as E_2-primed animals (Figures 1C-F). BrdU positive cells were occasionally present in the differentiated lobule type I and II structures frequently found in the perphenazine and sulpiride treated animals (Figures 2C-D). Following AN-treatment, either i.m. or orally, the number of BrdU positive cells

71

increased compared to that seen in control animals with or without E_2-priming (see Figures 1 and 3). As seen in Tables 3 and 4, the labelling index was significantly higher in both lobules and ducts in all AN-treated animals compared to that observed in controls. The labelling index in the ductal structures for i.m. treated animals increased from 8% in both NaCl and E_2 + NaCl-treated animals to 15% and 13% in E_2 + 200 mg and E_2 + 400 mg-treated animals, respectively.

Table 1 Number of mammary gland structures in control and E_2-primed animals after i.m. treatment with AN extract or after sulpiride or perphenazine treatment, expressed as number of structures / mm^2 area (mean ± SEM), A: absent.

	D	TEB	L1	L2
NaCl	2.30 ± 0.15	2.09 ± 0.17	A	A
E_2 + NaCl	2.74 ± 0.22	2.70 ± 0.02	2.30 ± 0.07	A
E_2 + 200 mg	2.31 ± 0.06	1.28 ± 0.05	5.87 ± 0.29	4.27 ± 0.23
E_2 + 400 mg	2.39 ± 0.11	A	4.76 ± 0.06	4.43 ± 0.49
Sulpiride	0.74 ± 0.05	A	1.31 ± 0.07	1.11 ± 0.03
Perphenazine	1.40 ± 0.05	A	A	2.52 ± 0.12

In the lobules, the labelling index increased from 9% in E_2 + NaCl-treated animals to 20% and 25% in E_2 + 200 mg and E_2 + 400 mg, respectively. Moreover, the labelling index in lobules type I and II appeared to be dose-dependent, i.e. a dose of E_2 + 400 mg consistently showed a higher labelling index compared to E_2 + 200 mg AN extract. In the ductal structures, the labelling index was much higher in the oral AN-treated animals (Table 4) compared to that in the i.m.-AN groups (see Table 3).

Table 2: Number of mammary gland structures in control and E_2-primed animals after oral treatment with AN extract expressed as number of structures / mm^2 area (mean ± SEM), A: absent.

	D	TEB	L1	L2
NaCl	2.22 ± 0.08	2.09 ± 0.15	A	A
E_2 + NaCl	2.74 ± 0.22	2.70 ± 0.02	2.3 ± 0.07	A
E_2 + 1 g	2.77 ± 0.15	2.92 ± 0.09	3.63 ± 0.33	2.60 ± 0.12
E_2 + 2 g	2.62 ± 0.12	1.78 ± 0.08	3.27 ± 0.25	4.12 ± 0.26
E_2 + 3 g	3.26 ± 0.28	0.54 ± 0.10	4.63 ± 0.22	3.70 ± 0.13
NaCl + 3 g	2.30 ± 0.13	1.41 ± 0.07	2.97 ± 0.42	3.39 ± 0.18

The labelling index in the orally treated animals, however, decreased from 23% to 20% in lobules type I, and from 28% to 17% in lobules type II, for E_2 + 1 g AN extract/kg BW and E_2 + 3 g AN extract/kg BW, respectively. All animals treated with either sulpiride or perphenazine showed a significantly lower labelling index compared to that in the other groups. The differentiation of the mammary structures was clearly influenced by the AN treatment as demonstrated by the changes in both number of alveoli as well as number of cells counted in each alveoli (Table 5). Also, perphenazine treatment resulted in a higher number of alveoli, and cells per alveoli, compared to sulpiride treatment.

Table 3: Labelling index in control and E_2-primed animals after i.m. treatment with AN extract or following sulpiride or perphenazine treatment expressed as percentage BrdU positive cells / total cells within a structure (mean ± SEM), A: absent.

	D	L1	L2
NaCl	7.69 ± 0.01	A	A
E_2 + NaCl	8.23 ± 0.11	9.27 ± 0.55	A
E_2 + 200 mg	15.52 ± 0.59	20.46 ± 0.43	21.3 ± 0.06
E_2 + 400 mg	12.94 ± 0.25	25.27 ± 0.83	27.54 ± 1.8
Sulpiride	A	3.96 ± 0.2	0.62 ± 0.03
Perphenazide	A	A	2.21 ± 0.27

Table 4 Labelling index in control and E_2-primed animals after oral treatment with AN extract expressed as the percentage BrdU positive cell/ total cell within a structure (mean ± SEM), A: absent.

	D	L1	L2
NaCl	7.72 ± 0.09	A	A
E_2 + NaCl	8.52 ± 0.12	10.07 ± 0.5	A
E_2 + 1 g	23.22 ± 0.15	23.65 ± 0.45	27.98 ± 0.79
E_2 + 2 g	19.78 ± 0.25	26.90 ± 0.52	24.84 ± 1.68
E_2 + 3 g	21.17 ± 0.45	20.75 ± 1.18	17.34 ± 0.93
NaCl + 2 g	18.95 ± 0.78	12.40 ± 0.84	17.73 ± 1.6

Pituitary PRL content

Since pituitary PRL content varies during the estrus cycle, as well as during lactation and weaning, prepubertal virgin rats were used to study the effects of AN extract on pituitary PRL levels and mammary gland development. Perphenazine and sulpiride, which act as a selective D2 and D1 receptor antagonist respectively and subsequently affect pituitary PRL levels, were used as positive controls. We found a significant increase in pituitary PRL content in all treated animals, i.e. E_2 + 200 mg, E_2 + 400 mg, sulpiride or perphenazine compared to that in controls i.e. E_2 + NaCl or NaCl alone (Figure 4a). Sulpiride and perphenazine treatment led to a twofold increase in PRL content compared to AN-treated animals. Oral administration of AN extract led to a significant increase in PRL content when given in the presence of E_2-priming (Figure 4b). One as well as 2 or 3g

AN extract given orally, significantly increased pituitary PRL content compared to saline or E_2-primed animals, whereas pituitary PRL levels were comparable between treatment with 3g AN extract without E_2 and saline or E_2-priming alone.

Table 5 Alveoli differentiation in different treatment groups, i.e. the number of alveoli type 1 and 2, and the number of cells per alveoli (mean ± SEM), A: absent.

	Number alv1	Number alv2	Cell/alv1	Cell/alv2
NaCl	A	A	A	A
E_2 + NaCl	3-5	A	8-10	A
E_2 + 200 mg	6-8	8-10	16-24	A
E_2 + 400 mg	10-20	>25	8-10	8-10
Sulpiride	10-20	>25	32-60	20-30
Perphenazide	A	10-20	A	33-78
E_2 + 1 g	2-10	10-20	8-12	8-12
E_2 + 2 g	7-20	>25	8-10	8-10
E_2 + 3 g	10-20	>25	12-24	12-24
NaCl + 3 g	6-10	10-20	16-20	12-14

Discussion

In this paper, we report the effects of AN extract on cell proliferation and mammary gland development in female virgin rats. The rat mammary gland appears to be an excellent model for such studies, although the multiple cell types within the gland make the interpretation of the histology very complex. During mammary gland development, TEBs, which are only present in nonparous rats, are actively growing into ductal structures that differentiate to alveolar buds and lobules. TEBs also regress to form terminal ducts that are able to differentiate into lobules during pregnancy and lactation. In this study, AN extract stimulated mammary gland development resulting in fewer TEBs, and in more lobules as compared to untreated animals, albeit that AN-induced mammary gland development was limited in the absence of E_2. When AN extract was given intramuscularly, TEBs were completely suppressed in the E_2-primed, high dose AN-treated groups indicating that E_2 effectively sensitises the gland to pituitary hormones (Nagasawa & Yanai 1971). Mammary epithelial response to either perphenazine or sulpiride, however, was more robust than that to AN extract. The thickness of the extralobular stroma, as well as the stroma between adjacent alveoli was smaller. Moreover, the lumen of the alveoli was full of basophilic secretions in association with lipid globules very similar to those previously reported in lactating mammary glands (Pitelka 1980, Jeffers 1935). However, there was clear heterogeneity between lobules and individual alveoli within a lobule indicating that alveolar differentiation was still incomplete. Similar heterogeneity was also observed in mammary glands of lactating rats (Masso-Welch *et al.* 2000) despite high levels of circulating PRL during that period. This incomplete alveolar differentiation may, to some extent, explain the continuing cell proliferation during lactation.
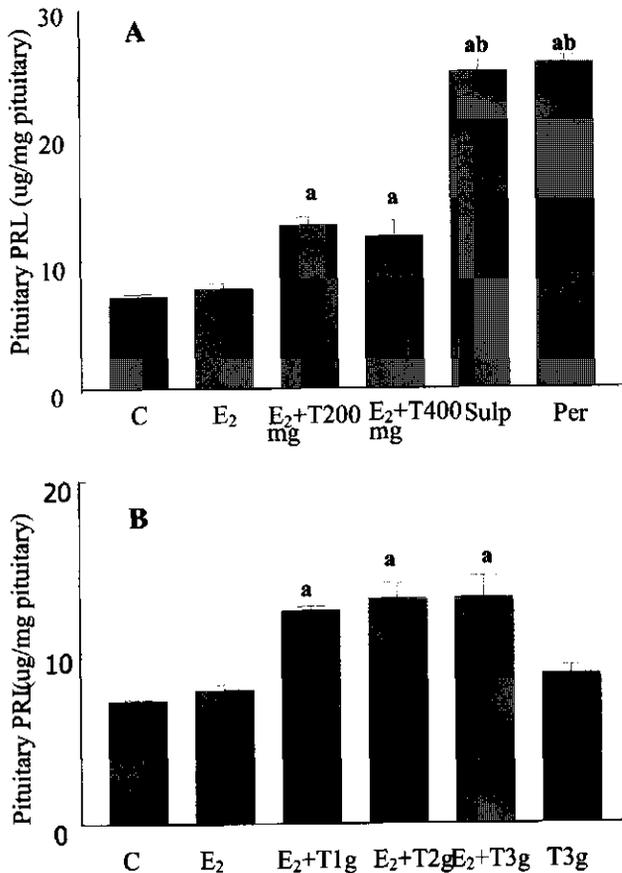
Figure 4 Pituitary PRL content in NaCl control (c) or E_2-primed animals treated intramuscularly with AN extract in a low (200 mg) or high dose (400 mg) or after treatment with perphenazine (Per) or sulpiride (Sulp) (Panel A). Panel B shows pituitary PRL content following oral administration of AN extract in different doses with or without E_2-priming (Panel B): a: significantly different from control and E_2; b: significantly different from AN-treatment (mean ± SEM).

Mammary cell proliferation as demonstrated by the BrdU labelling index was found to be significantly higher in all AN-treated animals compared to control and perphenazine-treated animals. In the lobular structures, which are known to be the working units of the mammary gland, the labelling index was found to increase concomitantly with the increased doses in i.m. AN-treated animals. Yet the labelling decreased following oral AN-treatment in increasing dose. This can be explained by the fact that the more the alveoli were developed in terms of number of cells, the lower the labelling index will be (see Table 5). This finding suggests that more cell proliferation can be found in the less

75

differentiated lobules, supporting the idea that AN extract is stimulating mammary gland differentiation from the less differentiated structures (TEBs) to the most differentiated ones (alveolar structures). Although mammary gland development is the primary determinant of milk yield and persistency of lactation (Kim *et al.* 1998), highly proliferative mammary gland has also been suggested to promote development of mammary carcinoma (Harwell *et al.* 2002, Snyderwine 1999, Thompson *et al.* 1998). In contrast, reduction of the highly proliferative terminal end bud (TEB) structures in a developing mammary gland by differentiation into alveolar buds and lobules, as reported following intramuscular as well as oral treatment with AN extract in this study, has been suggested to be protective against mammary carcinoma. Moreover, despite the high proliferative index observed, we clearly observed myoepithelial cells surrounding the alveoli, whereas their absence would be indicative of invasive carcinoma (Fernig *et al.* 1991). We hypothesise that the risk of carcinogenesis is minimal. This assumption is supported by the fact that AN extract is traditionally used for induction and stimulation of lactation in African women who will certainly undergo more than one pregnancy (Chapter 2). Women, who have carried at least one full-term pregnancy, have lower circulating levels of PRL and a blunted PRL response to the secretagogue (Thordarson *et al.* 1995, Musey *et al.*, 1987). This supports the idea that the risk of mammary carcinoma in African women who used AN extract is very low.

Indeed AN-extract as well as either perphenazine or sulpiride injection led to a significant increase in pituitary PRL content. In agreement with earlier studies (Stringer *et* al. 1990), we have shown that this perphenazine-induced PRL increase is associated with mammary gland growth response and cell proliferation, particularly in the lobular structures. Since PRL is known to induce mammary gland differentiation, the AN-induced increase in pituitary PRL levels may, at least in part, indeed explain the mammary gland growth and cell proliferation observed in this study. However, it is generally admitted that the hormonal environment is altered in parous rats as compared with that in age matched virgin animals. Both GH and PRL are significantly reduced after parturition, and thus may consequently have inhibitory effects on cell proliferation and lactation.

Although the variability of the lobuloalveolar development in the non-pregnant rat has been found to be strain dependent (Imagawa *et* al. 1990), the overall pattern of growth and differentiation and generation of various morphological structures has been assumed to be similar to that in humans. Current literature shows that alveolar differentiation is still incomplete during lactation in rats (rev Masso-Welch *et al.* 2000) and that epithelial cells undergo proliferation during that period (Joshi *et al.* 1986). Finally, AN extract has been found to stimulate milk production and PRL release in the rat (Lompo *et al.* submitted). Based on these results and those from the present study, we hypothesise that AN-extract can indeed induce milk production in women.

Acknowledgements

assistance. Also, we would like to thank Karin Slot (Department of Biochemistry and Cellbiology, Veterinary Faculty, Utrecht University) for her valuable help with the photographs.

References

Fernig DG, Smith JA, and Rudland PS 1991 Relationship of growth factors and differentiation in normal and neoplastic development of the mammary gland. *Cancer Treatment Research* **53** 47-78.

Harwell DME, Strecker TE, Xie B, Pennington KL, McComb RD, and Shull JD 2002 Dietary energy restriction inhibits estrogen-induced mammary, but not pituitary, tumorigenesis in the ACI rat. *Carcinogenesis* **23** 161-169.

Horseman ND 1999 Prolactin and mammary gland development *Journal of Mammary Gland Biology and Neoplasia* **4** 79-88.

Imagawa W, Bandyopadhyay GK, and Nandi S 1990 Regulation of mammary epithelial cell growth in mice and rats. *Endocrine Reviews* **90** 494-523.

Jeffers KR, 1935 Cytology of the mammary gland of the albino rat I. Pregnancy, Lactation, and Involution *American Journal of Anatomy* **56** 257-278.

Joshi K, Ellis JTB, Hugues CM, Monaghan P and Neville AM 1986 Cellular proliferation in the rat mammary gland during pregnancy and lactation *Laboratory Investigation* **54** 52-61.

Kim SH, Moon YS, Keller WL and Park CS 1998 Compensatory nutrition-directed mammary cell proliferation and lactation in rats *British Journal of Nutrition* **79** 177-183.

Lompo-Ouedraogo Z, Van Der Heide D, Van Der Beek EM, Swarts JJM Mattheij JAM, and Sawadogo L Effect of aqueous extract of *Acacia nilotica ssp adansonii* on milk production and prolactin release in the rat, submitted to *Journal of Endocrinology*

Masso-Welch AP, Darcy MK, Stangle-Castor NC and Ip MM 2000 A developmental atlas of rat mammary histology *Journal of Mammary Gland Biology and Neoplasia* **5** 165-185.

McGinley JN, Knott KK and Thompson HJ 2000 Effect of fixation and epitope retrieval on BrdU indices in mammary carcinomas *Journal of Histochemistry & Cytochemistry* **48** 355-362.

Musey VC, Collins DC, Musey PI, Martino-Saltzman D and Preedy JRK 1987 Long term effects of a first pregnancy on the secretion of prolactin *New England Journal of Medicine* **316** 229-234.

Nagasawa H and Yanai R 1971 Increased mammary gland response to pituitary mammotropic hormones by estrogen in rats *Endocrinologica Japonica* **18** 53-56.

Pitelka DR 1980 General morphology and histology of adult gland, In: The mammary gland, *Plenum Press, New York*, 944-965.

Russo IH and Russo J 1996 Mammary gland neoplasia in long-term rodent studies *Environmental Health Perspectives* **104** 938-967.

Russo IH and Russo J 1978 Developmental stage of the rat mammary gland as determinant of its susceptibility to 7,12-dimethylbenz[a]anthracene *Journal of the National Cancer Institute* **61** 1439-1449.

Sawadogo L and Houdebine LM 1988 Induction de la synthèse de caséine-*B* dans la glande mammaire de rates traitées par des extraits de plantes, *Comptes Rendus de l'Academie des Sciences Paris* **306** 167-172.

Snyderwine EG 1999 Mammary gland carcinogenesis by 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine in rats: possible mechanisms *Cancer Letters* **143** 211-215.

Shyamala G 1999 Progesterone signaling in mammary gland morphogenesis *Journal of Mammary Gland Biology and Neoplasia* **4** 89-104.

Abstract

In view of our previous reports that AN extract can stimulate pituitary PRL synthesis and release, as well as mammary gland development and milk production, experiments were performed to determine the mechanism of action. To this end virgin (nulliparous) adult females as well as parous females at 7, 14 and 21 days following weaning of their pups were used. CB154, a potent DA agonist, was used to suppress pituitary PRL synthesis and release in adult virgin female rats prior to oral administration of AN extract. Plasma and pituitary PRL as well as mammary gland development and differentiation were examined following BrdU immunocytochemical staining. Also, detailed histological examination of the mammary gland during involution was performed.

Plasma and pituitary PRL levels were significantly increased in both AN extract and sulpiride treated females with a concomitant lobulo-alveolar development with basophilic secretion in the lumen of alveoli. Prior treatment with CB154 significantly lowered plasma and pituitary PRL levels compared to control, but did not suppress PRL synthesis and release completely. A significant increase of pituitary PRL was still observed in the group treated with 3 g AN + CB154, although plasma PRL levels were not significantly altered. Moreover, treatment with a low dose of 550 mg AN orally was not sufficient to alter either plasma or pituitary PRL concentrations. This suggests that the dosage of CB154 used was sufficient to block PRL synthesis and release after 2 days of treatment. The increase in PRL levels observed after the high dose of AN extract may likely be due to a direct effect of the extract on the level of the pituitary. These findings suggest that AN extract acts through the hypothalamus-pituitary system to stimulate PRL synthesis and release. Yet, other local factors may also contribute to the observed mammary gland development following combined AN + CB-treatment, as suggested by the higher incidence of BrdU positive cells in the surrounding stroma of the mammary epithelial cells.

Introduction

The endocrine system synchronises developmental and functional differentiation of reproductive organs in mammals. In rats, several substances have been reported to influence mammary gland development from puberty until the end of lactation: estrogen (E_2) (Sivaraman et al. 2001, Couse & Korach 1999, Anderson et al.1998, Fendrick et al. 1998, Shyamala 1997, Silberstein et al. 1994, Haslam 1988, Daniel et al. 1987), progesterone (P) (Sivaraman et al. 2001, Shyamala 1999, Robinson et al. 1995), prolactin (PRL) (Gallego et al. 2001, Flint et al.1997, Travers et al. 1996), growth hormone (GH) (Gallego et al. 2001, Sejrsen et al. 1999, Flint et al.1997, Travers et al. 1996, Nandi et al. 1959, oxytocin (OX), thyroid hormones (TH) (Vonderhaar & Greco 1979), glucocorticoids (GLC) (Feng et al. 1995) and insulin (INS) (Hadsell & Bonnette 2000, Deeks et al. 1988). Whereas E_2 and P are primarily responsible for mammary gland development and branching, PRL is essential for alveologenesis and functional differentiation (Brisken et al. 1999). However, the exact contribution of each of these hormones during different stages of development remains to be clarified. Some reports show that E_2, P, PRL, GH and TH are essential for ductal elongation, branching and

alveolar budding (Vonderhaar & Greco 1979, Horseman 1999), and that PRL, GH, TH, OX and INS are required for complete lobulo-alveolar development, milk synthesis, milk secretion and maintenance of lactation. Although some of these hormones (E_2, P, PRL, GH, and OX) may have more inductive effects, others (TH, GLC, and INS) have been reported to have permissive effects, although direct effects on mammary gland can also occur. PRL plays a crucial role in stimulating milk synthesis and secretion. This hormone is responsible for direct stimulation of synthesis within the epithelial cell, and also indirect stimulation of the proliferation of the secretory cells. Most studies have pointed out its essential role in the proliferative phase of alveologenesis in rodents. PRL is a polypeptide hormone produced primarily in the pituitary, but numerous extra-pituitary synthesis sites, including the mammary gland, have recently been reported. Pituitary PRL release is predominantly regulated by PRL inhibiting factors (PIF), the most important being dopamine (DA), released from the hypothalamus. The regulatory system of the production of PRL from the extra-pituitary sites is still unclear. 2-bromo-α-ergocriptine (CB154), a potent selective D2 agonist, inhibits the synthesis and release of pituitary PRL but not of extra-pituitary PRL. On the other hand, numerous factors stimulate pituitary PRL synthesis and release, such as E_2 and sulpiride, a potent D1 receptor antagonist. Our previous report shows that extracts of *Acacia nilotica* ssp *adansonii* can effectively stimulate pituitary PRL synthesis and release, mammary gland development and differentiation, and milk production in rats. A question remaining unanswered thus far is how AN stimulates mammary gland differentiation: as a consequence of an effect on pituitary PRL synthesis and release, and/or through a direct effect on the mammary gland itself. To address this issue, we have used CB154, a potent DA agonist, to suppress pituitary PRL synthesis and release in adult virgin female rats before treatment with AN extract. Plasma and pituitary PRL levels as well as mammary gland development and differentiation were examined. Animals treated with sulpiride were used as positive controls. In addition, the effect of prolonged AN administration on mammary gland development and involution was studied in lactating rats immediately after weaning of their pups.

Materials and methods

Animals

Wistar virgin rats were obtained from IFFA-CREDO, France. They were kept under controlled illumination (lights on from 6:00 h to 18:00 h) at 22-28° C and had access to pelleted food and tap water *ad libitum*. Females were weighed daily.

Experiment I

Twenty-four virgin female rats of 90 days old were divided into 4 groups. Two groups were treated orally with either 2 ml NaCl 0.9% (control = Ctr) (n=6) or 550 mg AN extract/kg BW/2 ml NaCl 0.9% (AN) (n=6) twice daily for 5 consecutive days, respectively. The remaining two groups were treated subcutaneously with bromocriptine

(CB154) at a dose of 4.5 mg/kg BW/0.25 ml ethanol 70% twice daily for 2 consecutive days. Subsequently, they were treated orally with either 2 ml NaCl 0.9% (CBCtr) (n=6) or 550 mg AN extract /kg BW/2 ml NaCl 0.9% (CBAN) (n=6) twice daily for 3 consecutive days. The 6th day, all animals were anaesthetised with ether and sacrificed by decapitation. Pituitaries were collected for PRL extraction as described previously (Chapter 4). The two inguinal mammary glands were removed and immediately fixed in alcoholic Bouin. After dehydration in a graded series of ethanol and xylene, mammary glands were embedded in paraffin. Paraffin sections (5 µm) of the mammary glands were collected and stained with Harris' haematoxylin and eosin, and examined under a light microscope.

Experiment II

Thirty virgin adult females were divided into 5 groups. Two groups were treated orally with either saline (Ctr) or 3 g AN (AN) extract/kg BW for 5 consecutive days. The third group was treated subcutaneously with 20 mg sulpiride (Sul) /kg BW for 5 consecutive days. The last two groups were first treated subcutaneously with 4.5 mg CB154/kg BW for 2 consecutive days and subsequently treated orally with either 3 g AN extract/kg BW (CBAN) or saline for 3 consecutive days (CBCtr).
The sixth day, all animals received 5 mg BrdU/kg BW i.p., 2 hours before sacrifice. Animals were anaesthetised with ether and sacrificed by decapitation. Blood samples were taken and the inguinal mammary glands were removed, and subsequently frozen for further histological and immunocytochemical staining. The pituitary was removed and store at -20° C for PRL extraction. Plasma and pituitary PRL were determined as described previously.

Experiment III

Twenty-five females were used immediately after weaning of their pups for this experiment. A first group of pregnant females (n = 7) was orally administered a daily dose of 560 mg AN extract, starting one week before parturition (ANW1). The other two groups (n = 6 each) were orally given either 280 mg (AN) or 560 mg AN (ANW2) extract, starting the second day of lactation. The last group (n = 6) received saline (Ctr) over the entire period of the experiment.
On days 7, 14, and 21 after weaning of their pups, two females of each group were injected with 5 mg BrdU/kg BW 2 hours before sacrifice. Animals were anaesthetized with ether and sacrificed by decapitation; blood samples were collected for determination of PRL concentrations. The inguinal mammary glands were removed and subsequently frozen for immunocytochemical (ICC) staining. The pituitary was removed for prolactin extraction. Plasma and pituitary prolactin were determined as described previously.

Histology of the mammary gland

Immunocytochemistry for BrdU was performed on unfixed frozen mammary glands from rats, collected from animals in experiments II and III as described previously and stained according to McGinley *et al.* (2000) with some modifications (Chapter 5). Also, the

manual counting procedure was similar to that described previously (Lompo *et al.* chapter 5). Mammary gland differentiation was assessed according to the criteria of Russo and Russo (1978, 1996).

On day 6, mammary glands from the animals in experiment I were removed and immediately fixed in alcoholic Bouin. After dehydration in a graded series of ethanol and xylene, mammary glands were embedded in paraffin. Paraffin sections (5 μm) of the mammary glands were sliced, dried and stained with Harris' haematoxylin and eosin. Mammary gland differentiation was described following light microscopic evaluation.

Statistical Analysis

Statistical evaluation of data involved ANOVA followed by Bonferroni and Scheffe test to identify differences between groups. $P<0.05$ was considered to be significant.

Results

Plasma and pituitary PRL content

Treatment with CB154 alone significantly decreased plasma PRL levels compared to the control group ($P< 0.05$) (Figure 1). A significant increase in plasma PRL levels was observed in the sulpiride and 3 g AN-treated animals compared to the control and CB154 treated animals ($P<0.05$). No significant difference was found between CB154 (1.45 $\pm$ 0.09 ng/ml) and CB154 + 3 g AN (2.49 $\pm$ 0.12 ng/ml) -treated animals, albeit that a slight, non-significant increase in PRL level was observed in the latter group as compared to CB154 alone. Plasma and pituitary changes in PRL were highly comparable in magnitude. Again, pituitary PRL content in animals treated with either sulpiride or 3 g AN extract significantly differed from that in the control animals (Figure 2). We found no significant
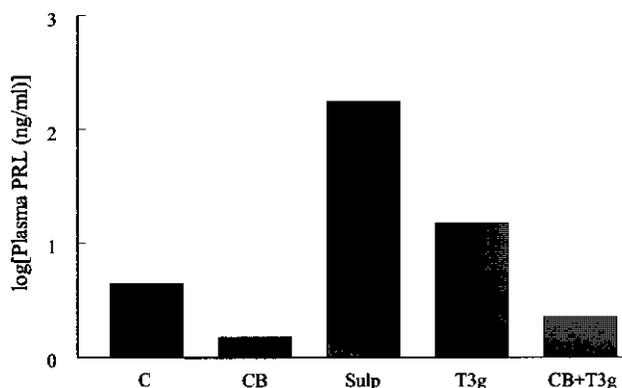
Figure 1 Plasma PRL concentration (ng/ml) in adult virgin female rats following different treatments: C: saline control, CB: bromocriptine, Sulp: sulpiride, T3g: AN extract, CB+T3g: bromocriptine followed by AN extract.

difference between the 560 mg AN-treated animals and control animals (data not shown). Treatment with 3 g AN extract after CB154 treatment significantly increased PRL content compared to that observed in the CB154 treated group, although no significant change was found compared to the control group. After weaning of their pups, plasma PRL levels gradually declined in the Ctr group to reach a basal level around day 21 (Figure 3). PRL levels in the 560 mg AN-treated animals were still relatively high at day 14 as compared to that observed in Ctr animals. Pituitary PRL was also found to markedly decline after weaning in all animals, although the decrease in time after weaning, especially in the AN-treated group, was less dramatic than that of plasma (data not shown).
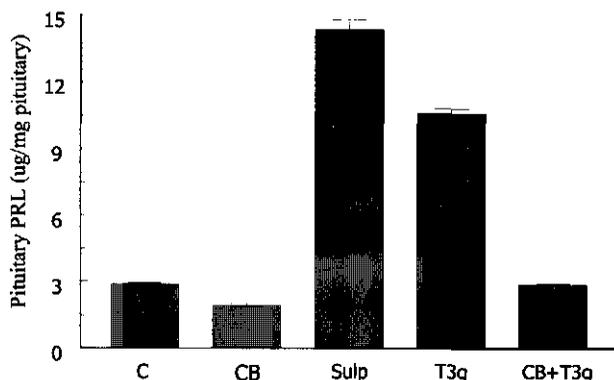
Figure 2 Pituitary PRL content (ng/mg tissue) in adult virgin female rats following different treatments: C: saline control, CB: bromocriptine, Sulp: sulpiride, T3g: AN extract, CB+T3g: bromocriptine followed by AN extract.
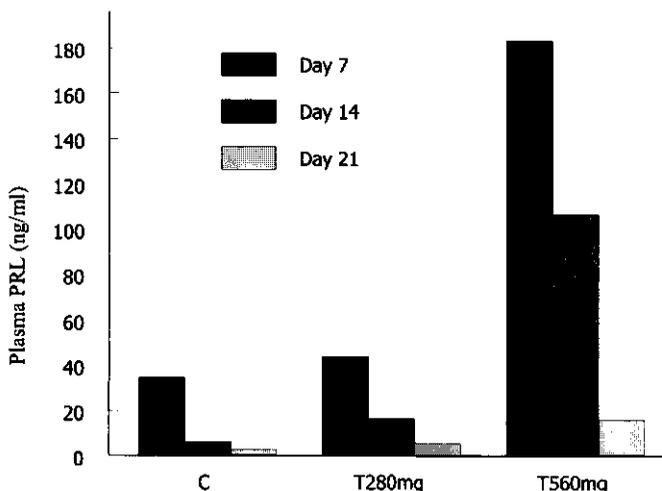
Figure 3 Plasma PRL concentrations (ng/ml) in adult virgin female rats on 7, 14 and 21 days after weaning of their pups for control (c), low dose (T280 mg) and high dose (560 mg) of AN extract administered orally.

Mammary gland development and differentiation

Mammary glands of the control group showed bare duct systems with few alveolar buds, within extensive connective tissue (Figure 4A). More lobulo-alveolar structures filled with basophilic secretions were found in AN-treated animals (Figure 4B) than in the control and in the CB154-treated groups (Figures 4C-D). Large vacuoles were present in some of the cells. Mammary glands of animals treated with CB154 showed an ill-defined alveolar structure with very dark nuclei in the epithelial cells (Figure 4C). Treatment with ANextract after CB154 (Figure 4D) did not reverse the situation, albeit that some mitotic cells were seen (arrowhead).

Detailed histological examination of mammary glands during involution was also performed. Mammary glands from both control (Figures 5A and 5C) and AN-treated animals (Figures 5B and 5D) showed clear lobulo-alveolar structures within the stromal tissue. Compared to control animals, less stromal tissue was seen surrounding lobulo-alveolar structures from AN-treated animals (see Figures 5A-B). Yet, the mammary gland of both AN-treated and control animals at day 21 appeared to be very similar (Figures 5C-D).

To further understand the effect of AN extract on mammary gland differentiation, BrdU staining was performed to identify proliferating cells. BrdU positive cells were clearly visible as middle to dark brown stained cells. Quantification of BrdU positive cells (expressed as the number of BrdU cells / total cells within the structure) showed a higher proliferating index in AN-treated animals as compared to that in control animals. However, no BrdU positive cells were observed in mammary epithelial of rats receiving CB154 alone or with AN extract, albeit that in the latter case, a larger number of BrdU positive cells was found in the stromal tissue surrounding the mammary epithelium (Figure 4E).

Discussion

In this report, the effect of 2-bromo-α-ergocriptine (CB154), a selective dopamine D2 receptor agonist, on PRL synthesis and release and mammary gland development was studied. CB154-treated animals were compared to AN-treated animals, and to control animals treated either with saline or sulpiride alone. Extensive lobulo-alveolar growth with functional development of the mammary gland occurred after both sulpiride and AN-treatment in adult virgin cycling female rats. Mammary gland development is known to differ throughout the cycle with the less differentiated mammary gland consisting of ducts and TEBs, and fewer alveoli being observed during diestrus as compared to other days of the cycle. Histological examination of the mammary glands showed a comparable image in our control animals to that reported previously in mammary glands from diestrus animals (Chapter 4), although the mammary gland of CB154-treated animals also showed apoptotic cells with dark nuclei.

Plasma and pituitary PRL levels were significantly increased in both AN extract and sulpiride-treated animals as compared to that in control and CB154-treated groups as
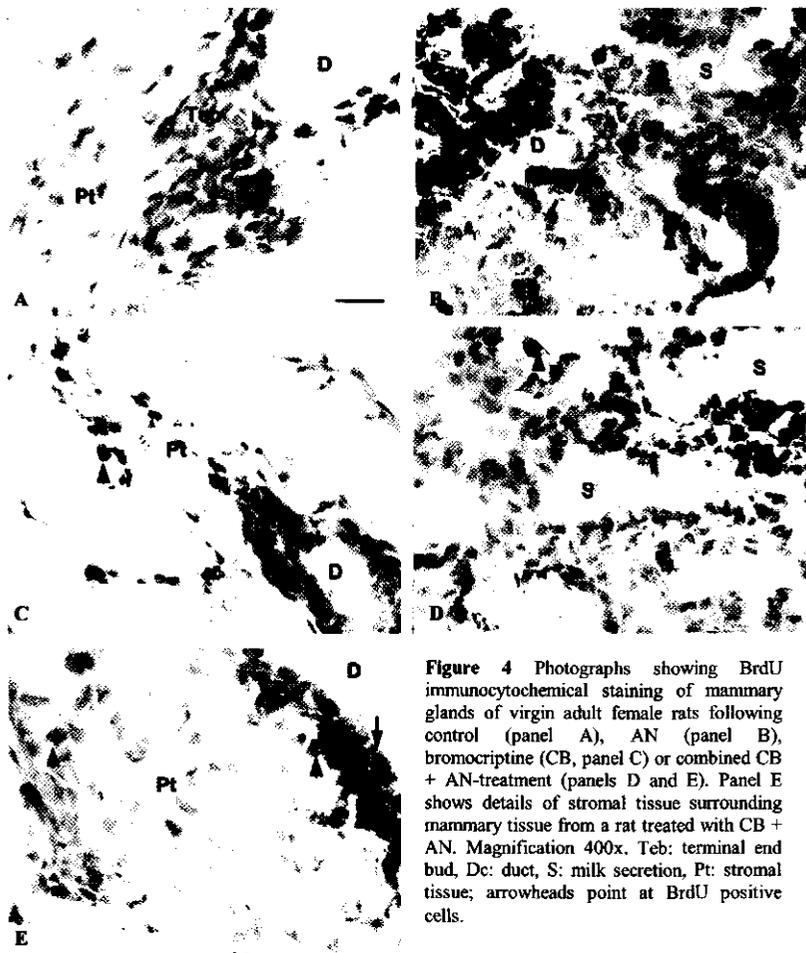
Figure 4 Photographs showing BrdU immunocytochemical staining of mammary glands of virgin adult female rats following control (panel A), AN (panel B), bromocriptine (CB, panel C) or combined CB + AN-treatment (panels D and E). Panel E shows details of stromal tissue surrounding mammary tissue from a rat treated with CB + AN. Magnification 400x. Teb: terminal end bud, Dc: duct, S: milk secretion, Pt: stromal tissue; arrowheads point at BrdU positive cells.

reported previously (Chapter 5). Treatment with CB154 significantly lowered plasma and pituitary PRL compared to that of control animals. A significant increase of pituitary PRL content compared to CB154 treatment alone was observed in the group treated with 3 g AN + CB154 although no significant increase was noticed in plasma PRL concentrations. Oral administration of a low dose of 560 mg AN after CB154 was not efficient in increasing either plasma or pituitary PRL. This suggests that the dose of 4.5 mg CB154/kg BW was sufficient to block PRL synthesis and release after 2 days of treatment, and that the increase observed after the high dose of AN extract may likely be due to an effect of the extract. These findings suggest that AN extract acts, at least in part, through interaction with the hypothalamo-pituitary system to stimulate PRL synthesis and release. Yet, also other factors are likely to be involved, as suggested by the higher incidence of BrdU positive cells in the surrounding stroma of mammary epithelial cells.
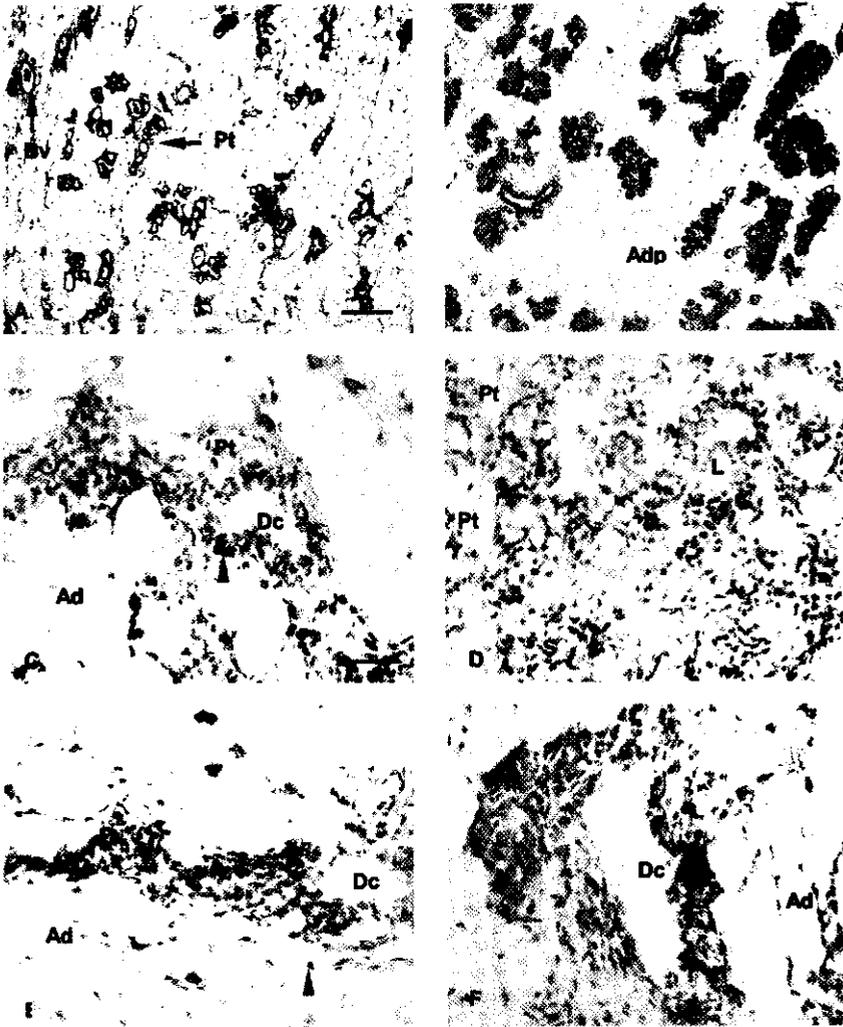
Figure 5 Photographs showing BrdU immunocytochemical staining of mammary glands of parous female rats after weaning of their pups of control animals (panels A, C and E) and following continuous treatment with AN extract (panels B, D and F). Panels A and B show the presence of lobulo-alveolar structures in haematoxylin-eosin stained sections of the mammary gland at 7 days after pup weaning at a low magnification (100x). Panels B and D show representative examples of alveolar structures from material following BrdU staining at 14 days after pup weaning, magnification 400x. Note the abundance of BrdU positive cells in the lobuli of AN-treated animals. Panels E and F show comparable degrees of differentiation of mammary glands in control and AN-treated animals at 21 days after pup weaning in BrdU stained sections, magnification 400x. Arrowheads point at BrdU positive cells in the tissue, L: lobule, Adp: adipose tissue, Pt: stromal tissue, Dc: ducts, S: milk secretion in the lumen.

Basically, treatment with E_2 stimulates both PRL and GH release; PRL either alone or with E_2 has been found to stimulate progesterone production. All these hormones are known to stimulate mammary growth and differentiation. Indeed, CB154 may act on the D2 receptors of the pituitary gland to inhibit PRL secretion but it does not alter levels of extra-pituitary PRL and PRL of mammary gland origin. The biological effects of PRL are presumably mediated by the PRL receptor (PRL-R) that is a member of the cytokine receptor super family (Kelly *et al.* 1993). Therefore, it is feasible to suggest that AN extract could also act directly on the mammary gland by stimulating either PRL-R or by activating mammary cell activity. The latter may be achieved by increasing amino acid transport since a decrease of milk yield, with a concomitant decrease in milk protein and lactose, has been reported in lactating rats treated with CB154. Most likely, all these events may occur concurrently as evidenced by the presence of basophilic secretions in the lumen of alveoli of mammary glands from rats treated with CB154 + extract.

Likewise, *Euphorbia hirta* has also been found to stimulate B-casein secretion in rat mammary glands following CB154 treatment (Sawadogo *et al.* 1988).

These results strongly suggest that AN extract stimulates milk production, and mammary gland development and differentiation by inducing PRL synthesis and release at the level of the pituitary, or via inhibition of stimulation of other pituitary hormones. These in turn stimulate mammary growth and differentiation. In addition, AN extract may also act directly on the mammary gland itself by stimulating the synthesis of milk components directly or indirectly, although further research will be necessary in order to elucidate the mechanism of action of AN extract on mammary gland tissue directly.

References

Anderson E, Clarke RB & Howell A 1998 Estrogen responsiveness and control of normal human breast proliferation *Journal of Mammary Gland Biology and Neoplasia* 3 37-48.

Brisken C, Kaur S, Chavarria TE, Binart N, Sutherland RL, Weinberg RA, Kelly PA & Ormandy CJ 1999 Prolactin controls mammary gland development via direct and indirect mechanisms *Developmental Biology* 210 96-106.

Couse JF & Korach KS 1999 Estrogen receptor null mice: what have we learned and where will they lead us? *Endocrine Reviews* 20 358-417.

Daniel CW, Silberstein GB, & Strickland P 1987 Direct action of 17-beta estradiol on mouse mammary ducts analyzed by sustained release implants and steroid autoradiography. *Cancer Research* 47 6052-6057.

Deeks S, Richards J & Nandi S 1988 Maintenance of normal rat mammary epithelial cells by insulin and insulin-like growth factor I. *Experimental Cell Research* 174 448-460.

Fendrick JL, Raafat AM & Haslam SZ 1998 Mammary gland growth and development from the postnatal period to postmenopause: ovarian steroid receptor ontogeny and regulation in the mouse. *Journal of Mammary Gland Biology and Neoplasia* 3 7-22.

Feng Z, Marti A, Jehn B, Altermatt HJ, Chicaiza G, & Jaggi R 1995 Glucocorticoids and progesterone inhibit involution and programmed cell death in the mouse mammary gland *Endocrinology* 138 5101-5107.

Flint DJ & Knight CH 1997 Interactions of prolactin and growth hormone (GH) in the regulation of mammary gland function and epithelial cell survival *Journal of Mammary Gland Biology and Neoplasia* 2 41-48.

Gallego MI, Binart N, Robinson GW, Okagaki R, Coschigano KT, Perry J, Kopchick JJ, Oka T, Kelly PA & Hennighausen L 2001 Prolactin, growth hormone, and epidermal growth factor activate Stat5 in different compartments of mammary tissue and exert different and overlapping developmental effects. *Developmental Biology* **229** 163-175.

Hadsell DL & Bonnette SG 2000 IGF and insulin action in the mammary gland: lessons from transgenic and knockout models. *Journal of Mammary Gland Biology and Neoplasia* **5** 19-30.

Haslam SZ 1988 Local versus systematically mediated effects of estrogen on normal mammary epithelial cell deoxyribonucleic acid synthesis. *Endocrinology* **122** 860-867.

Horseman ND 1999 Prolactin and mammary gland development *Journal of Mammary Gland Biology and Neoplasia* **4** 79-88.

Kelly PA, Ali S, Rozakis M, Goujon L, Nagano M, Pellegrini I, Gould D, Djiane J, Edery M, Finidori J & Postel Vinay MC 1993 The growth hormone/prolactin receptor family. *Recent Progress in Hormone Research* **48** 123-164.

Nandy S 1959 Hormonal control of mammogenesis and lactogenesis in the C3H/He Crgl mouse. *University of California Publications in Zoology* **65** 1-128.

Robinson GW, McKnight RA, Smith GH & Hennighausen L 1995 Mammary epithelial cells undergo secretory differentiation in cycling virgins but require pregnancy for the establishment of terminal differentiation *Development* **121** 2079-2090.

Sawadogo L and Houdebine LM 1988 Induction de la synthèse de caséine-*B* dans la glande mammaire de rates traitées par des extraits de plantes, *Comptes Rendus de l'Academie des Sciences Paris* **306** 167-172.

Shyamala G 1997 Roles of estrogen and progesterone in normal mammary gland development: insights from progesterone receptor null mutant mice and in situ localization of receptor. *Trends in Endocrinology and Metabolism* **8** 34-39.

Shyamala G 1999 Progesterone signaling and mammary gland morphogenesis *Journal of Mammary Gland Biology and Neoplasia* **4** 89-104.

Silberstein GB, Van Horn K, Shyamala G & Daniel CW 1994 Essential role of endogenous estrogen in directly stimulating mammary growth demonstrated by implants containing pure antiestrogens *Endocrinology* **134** 84-90.

Sivaraman L, Hilsenbeck SG, Zhong L, Gay J, Conneely OM, Medina D & O'Malley BW 2001 Early exposure of the rat mammary gland to estrogen and progesterone blocks co-localization of estrogen receptor expression and proliferation *Journal of Endocrinology* **171** 75-83.

Sejrsen K, Purup S, Vestergaard M, Weber MS & Knight CH 1999 Growth hormone and mammary development *Domestic Animal Endocrinology* **17** 117-129.

Travers MT, Barber MC, Tonner E, Quarrie LH, Wilde CJ & Flint DJ 1996 The role of prolactin and growth hormone in the regulation of casein gene expression and mammary cell survival: relationships to milk synthesis and secretion *Endocrinology* **137** 1530-1539.

Vonderhaar BK & Greco AE 1979 Lobulo-alveolar development of mouse mammary glands is regulated by thyroid hormones *Endocrinology* **104** 409-418.

The aim of this study was to document plants used by women for the treatment of lactation-related defects, to investigate the biological activity of the plants used for induction and stimulation of lactation, and finally to assess the underlying mechanism of action in a rat model.

Lactation is an essential phase during development since young are very immature at birth and totally dependent on their mother's milk for a varying time thereafter. Milk is synthesised within the cell lining of the mammary gland alveoli. Milk is a complex product consisting mainly of water, sugars, lipids (mainly triglycerides), proteins (e.g. casein, lactalbumin) as well as monovalent and divalent cations and, most interesting, antibodies. In the Western world, survival of newborns does not rely solely on the capacity of the mother to lactate because of the tremendous availability of artificial milk. In developing countriesmother's milk is even more important for survival of the newborn because artificial milk is not available or out of reach for the majority of women. Although 99% of the women in Burkina Faso that gave birth are breast-feeding, infant morbidity and mortality are still high. Next to their nutrients, colostrum and milk contain maternal antibodies for the newborn, serving as important protective agents against infections. Deviations in maternal milk quantity and/or quality early after birth might therefore affect infant well-being and development, which later determines the infant's resistance to infections. With limited access to modern milk alternatives, African women with milk production deficiencies traditionally use plant extracts to induce milk production or to increase milk yield.

In chapter 1, a definition of African Traditional Medicine and its implications for the practice of breast-feeding in African communities are reported. In addition, a review of mammogenesis, lactogenesis, and related hormonal regulation is reported. Finally, the possible causes of disruption of lactation are briefly discussed.
From this review, it appears as if lactation is a result of long physiological processes occurring in the mammary gland from pregnancy until the day of parturition, and that its success depends on mammary epithelial cell proliferation and differentiation, and on PRL levels for stimulation of synthesis of milk components. The effect of the nutritional state of the mother on milk yield, however, is still unclear.

Chapter 2 describes a field study carried out between 1997 and 1999 to document plant use for the treatment of lactation-related defects within five provinces of Burkina Faso. From the literature, floristic and ethnobotanical aspects of African plants with therapeutic properties have been extensively studied. However, much information regarding the number of cases reported, the number of people who have used this medication as well as the biological activities is missing.
During this study, questions were asked and discussions were carried out with 296 people, including traditional healers (34), village elders (45) and women (217) supposedly using those prescriptions. Transmission of knowledge is essentially oral, from father to son or from mother to daughter: 89% of the informants had received their knowledge from their parents. Ninety species of plants with medical use belonging to forty families are listed together with their local names, the plant parts used for treatments, the number of times

reported by the informant, and the number of women that used this prescription. It appears that all women that were interviewed during this inventory actually used plant medication during their lactation period. This indicates a real existence of lactation-related defects and/or more cultural practices. The latter, however, seems quite unlikely since 40% of the women have used plant medication for the treatment of agalactia or hypogalactia, and at least 10 % have stopped an ongoing lactation because of mastitis or death of the infant.

The number of species reported gives an overview of the widespread use of plants in basic health care as well as of the prevalence of a given disorder; the number of people that used this medication may be of great interest for statistics on the state of health among the general population. It should be noted that not all the plants reported by the informants are used by the women. This discrepancy could be due to the fact that, for the greater part, plants used for induction or stimulation of lactation were given in a form of mixture of at least two plants. This survey pointed out seven species including *Pennisetum americanum*, *Vitellaria paradoxa, Cadaba farinosa, Acacia nilotica* var *adansonii, Leptadenia hastata*, *Capsicum frutescens*, and *Crateva adansonii* widely used by women in such a variety of recipes. However, the unsolved question is which plant or plants contain active components? It seems likely that some of the plants in a given treatment mixture actually induce or stimulate lactation, while other plants in the same mixture may be added to inhibit the possible toxicity of active plants.
Pennisetum americanum, Vitellaria paradoxa, Cadaba farinosa, Capsicum frutescens, Crateva adansonii, and *Leptadenia hastata* are commonly used in human alimentation, albeit that the latter is forbidden for pregnant women, what might be related to its abortive effects as reported by Noumi and Tchkonang (2001).

In contrast, *Acacia nilotica* var *adansonii* appeared to be widely used and frequently associated with other plants in other parts of Burkina Faso as well as in some other countries, for the treatment of a wide variety of diseases. Studies concerning the ethnopharmacology, phytochemistry and toxicology of *Acacia nilotica* species as reported in **chapter 3**, do not clearly specify the variety used. From Kerharo and Adams (1974), it appears as if *Acacia nilotica* var. *adansonii* (Guill. et Perr.) O. Ktze is a thorny tree of up to 10-12 m of length, with a dark grey bark colour, green-grey leaves and golden flowers and that three species that resemble it in foliage and inflorescence have nearly the same systematic characteristics. Consequently, this makes the systematic characterization of *A. nilotica* species rather confusing. Nevertheless, people in the villages are able to identify these 3 species very reliably. The literature review confirmed *A. nilotica*'s multiple therapeutic properties, as all parts of the tree are used in folk medicine. Despite its widespread therapeutic properties reported, little is known about its actual pharmacological mechanisms. In view of the traditional information that AN could induce and/or stimulate lactation in women, experiments were performed as described as in chapters 4 and 5.

In chapter 4, the effects of an aqueous extract of AN on milk production and PRL synthesis and release were investigated. Lactating female rats were given a low or high dose of AN extract orally during 13 consecutive days starting on day 2 of lactation, as

compared to a control group treated with saline. Milk yield was measured daily through pup weight and pup weight gain (Morag *et al.* 1975, Sampson *et al.* 1984, Kamani *et al.* 1987, Kim *et al.* 1998). This study demonstrated that animals treated with either a low or a high dose of AN extract produced significantly more milk associated with an increase in pup growth, as compared to that in control animals, and that milk yield appeared to be significantly stimulated approximately 24 hrs after start of the treatment. AN extract induced milk production in a dose-dependent manner, although the pups' growth rates of the low dose AN-treated animals were similar to that of the high dose group. This discrepancy may be explained by the fact that milk yield is governed by suckling frequencies, which depend on the energy content of the mother's milk (Perez-Escamilla *et al.* 1995, Prentice *et al.* 1986, Villalpando *et al.* 1999).

To investigate the effect of AN on PRL synthesis and release, experiments were performed in peri-pubertal and adult virgin female rats. PRL is known to vary during the estrus cycle as well as during lactation and weaning. A strong relation between extract administration and PRL release after "acute" as well as "chronic" treatment has been observed in the cycling female treated intravenously. PRL synthesis was also stimulated by AN extract in both adult and pre-pubertal animals, albeit that in the latter group, E_2-priming was needed for an efficient effect. This strongly suggests that the extract had a specific effect on PRL-synthesis/secretion under certain physiological conditions, which is consistent with its traditional use during lactation.

PRL is known to stimulate milk synthesis and secretion. However, in the last decades, research has also shown a relationship with breast carcinogenesis; i.e. an increase in PRL synthesis and release may increase susceptibility to mammary carcinomas (Venugopal *et al.* 1999). In order to provide more insight into the effects of AN extract on mammary gland development and differentiation, experiments were performed as reported in **chapter 5**. In that chapter, we investigate the effect of AN extract on cell proliferation using bromodeoxyuridin (BrdU). BrdU is an analogue of thymidin that is incorporated into proliferating cells.
To this end, adult as well as virgin female rats were treated with AN extract, either oral or i.m., with or without prior E_2-priming. Perphenazine and sulpiride were used as positive controls. Before sacrifice, a subcutaneous injection of BrdU was given. Pituitaries were collected for PRL content determination. Mammary gland tissue was removed and processed for BrdU immunohistochemical staining. The BrdU labelling index was calculated from the total number of epithelial cell per unit of surface area.

The treatment with AN extract resulted in a significant increase of PRL synthesis, associated with increased lobulo-alveolar structure development, filled with basophilic secretions and lipids globules compared to control groups. Mammary gland from either perphenazine or sulpiride-treated groups showed a significant increase in pituitary PRL content, associated with highly developed alveolae filled with basophilic secretions and lipids globules, very similar to what has been reported for the lactating mammary gland (Pitelka 1980, Jeffers 1935).

Terminal end buds (TEBs) were completely suppressed in the E_2-primed, high dose AN-treated groups when treatment was given intramuscularly. Mammary cell proliferation was found to be significantly higher in all AN-treated groups compared to that of control animals. This study shows that the BrdU labelling index was significantly higher for the lobular structures than for the ductal structures in all AN-treated animals, indicating more proliferative activity in the lobular unit, which indeed is the working unit of the mammary gland (Stringer *et al.* 1990). In this chapter, we have shown that AN extract stimulates mammary gland growth and differentiation from the least differentiated structures (TEBs) to the highly differentiated ones (alveoli), and may thus have a stimulating effect on milk production.

Mammary gland development is the primary determinant of milk yield and persistency of lactation (Kim *et al.* 1998), as said highly proliferative mammary gland may promote development of mammary carcinoma (Harwell *et* al. 2002, Snyderwine 1999, Thompson *et* al. 1998). On the other hand, reduction of the highly proliferative terminal end bud (TEB) structures in a developing mammary gland by differentiation into alveolar buds and lobules as reported in this study, has been suggested to be protective of mammary carcinoma. Moreover, it has been reported that women who have carried out at least one pregnancy to term have lower circulating levels of PRL and a blunted PRL response to a secretagogue (Musey *et al.* 1987). Based on the above we believe that the risk of mammary carcinogenesis related to the use of AN extract is minimal, also since we have previously (Chapter 2) reported that AN extract is traditionally used for induction and stimulation of lactation in African women who will certainly undergo more than one pregnancy. Although the variability in the degree of lobulo-alveolar development in non-pregnant rats has been found to be strain-dependent (Imagawa *et al.* 1990), the overall pattern of growth and differentiation and generation of various morphological structures shows striking similarities to that of humans. It has been reported that alveolar differentiation is still incomplete during lactation in the rat (Masso-Welch *et al.* 2000), and that epithelial cells are undergoing proliferation during that period (Joshi *et al.* 1986). Based on the fact that AN extract has been found to stimulate milk production and PRL release in rats (Chapter 4), we hypothesise that AN extract can indeed induce milk production in women.

Chapter 6 deals with the effects of AN extract on mammary gland growth and differentiation. Are these processes only attributable to a response to the sustained elevation of PRL synthesis and release or rather, stemming from a direct effect on the mammary gland? As dopamine (DA) is the main inhibiting factor of PRL synthesis and release from the pituitary, bromocriptine (CB154), an agonist of DA was used to suppress PRL synthesis and release in cycling female rats. Plasma and pituitary PRL, mammary gland development and differentiation were examined after AN-treatment. Animals treated with sulpiride were used as positive controls. Plasma and pituitary PRL levels were significantly increased in both AN extract and sulpiride-treated animals with a concomitant lobulo-aveolar development with basophilic secretions in the lumen of the alveoli. Treatment with CB154 indeed significantly lowered plasma and pituitary PRL levels, compared to control but did not suppress PRL synthesis and release completely. A significant increase of pituitary PRL was observed in the group treated with 3 g AN +

CB154 as compared to CB154 alone, although no significant change was found in plasma PRL. Yet, oral administration of a low dose of 560 mg AN was not insufficient to increase either plasma or pituitary PRL levels. This suggests that the dose of 4.5 mg CB154/kg BW was sufficient in blocking PRL synthesis and release after 2 days of treatment, and that the increase observed after the high dose of AN is likely to be an effect of the extract.

These findings suggest that AN extract acts through the hypothalamo-hypophysis-axis in stimulating PRL synthesis and release, although other factors are likely to be involved as well. Indeed, CB154 acts on the D2 receptors of the pituitary gland to inhibit PRL secretion. Secretion of PRL, however, is not limited to the pituitary as numerous extra-pituitary sites of PRL expression including the mammary gland have been reported recently. Apart from the possibility that AN extract might act through stimulating the release of other hormones (since treatment with E_2 stimulates both PRL and GH release; PRL either alone or with E_2 stimulates progesterone production, and these are all known to stimulate mammary growth and differentiation) it could be that AN extract acts directly on the mammary gland by stimulating either PRL-R or by activating mammary cell activity by increasing amino acid transport. Most likely, all of these events may occur simultaneously, as evidenced by the presence of basophilic secretion in the lumen of alveoli of mammary gland from rat treated with CB154 + extract. Likewise, *Euphorbia hirta* has noted to stimulate B-casein secretion in rat mammary glands after CB154-treatment (Sawadogo *et al.* 1988). Prolactin plays a key role in maintaining lactation in rat. Moreover, suppressing lactating in rat by the use of CB154 led to a decrease of 57% of milk yield with a concomitant decrease in milk protein and lactose.
These results indicate that the AN-extract indeed act by stimulating the synthesis and the release of PRL and probably other pituitary hormones. In turn, these hormones stimulate the mammary growth and differentiation. More likely, AN-extract may act directly on the mammary gland by stimulating the synthesis of milk components, although further research is needed to elucidate the possible mechanisms of action of AN extract.

More research on the effect of AN-extracts on milk secretion and yield is certainly needed before the findings from this research can be used to improve traditional practices related to the management of milk production in the human. Work may also have to be conducted on unveiled other possible positive or negative effects of AN extracts. However, from this study, the fact that a traditional practice has been demonstrated to be scientifically sound, improves the credibility of traditional medicine in general and the traditional practices during lactation in particular. It is also opens a tremendous array of possibilities for research on tradtional health care practices and traditional use of plants. As pointed out by our survey, there are a large number of plants used to solve various health-related problems. Thus, it seems reasonable to *(i)* suggest the development of specific research programs on the subject in the near future, and to *(ii)* promote the inclusion of the traditional medicine in national health care policies and programs.

References

Harwell DME, Strecker TE, Xie B, Pennington KL, McComb RD, and Shull JD 2002 Dietary energy restriction inhibits estrogen-induced mammary, but not pituitary, tumorigenesis in the ACI rat. *Carcinogenesis* **23** 161-169.

Horseman ND 1999 Prolactin and mammary gland development *Journal of Mammary Gland Biology and Neoplasia* **4** 79-88.

Imagawa W, Bandyopadhyay GK & Nandi S 1990 Regulation of mammary epithelial cell growth in mice and rats *Endocrine Reviews* **11** 494-523.

Jeffers KR 1935 Cytology of the mammary gland of the albino rat I. Pregnancy, lactation and involution *American Journal of Anatomy* **56** 257-278.

Joshi K, Ellis JTB, Hugues CM, Monaghan P and Neville AM 1986 Cellular proliferation in the rat mammary gland during pregnancy and lactation *Laboratory Investigation* **54** 52-61.

Kerharo J & Adam JC 1974 La Pharmacopée Sénégalaise Traditionnelle, Plantes Médicinales et Toxiques. *Edition Vigot-Freres*, Paris.

Kamani HT, Karunanayake EH & Mahindartna MPD 1987 Evaluation of galactoguic activity of *Asparagus falcatus. Ceylon Journal of Medical Sciences* **30** 63-67.

Kim SH, Moon YS, Keller WL and Park CS 1998 Compensatory nutrition-directed mammary cell proliferation and lactation in rats *British Journal of Nutrition* **79** 177-183.

Masso-Welch AP, Darcy MK, Stangle-Castor NC and Ip MM 2000 A developmental atlas of rat mammary histology *Journal of Mammary Gland Biology and Neoplasia* **5** 165-185.

Morag M, Popliker F, & Yagil R 1975 Effect of litter size on milk yield in the rat. *Laboratory Animals* **9** 43 - 47.

Noumi E & Tchkonang NYC 2001 Plants used as abortifacients in the Sangmelima region of Southern Cameroon. *Journal of Ethnopharmacology* **76** 263-268.

Perez-Escamillia R, Cohen RJ, Brown KH, Landa Rivera L, Canahuati J & Dewy KG 1995 Maternal anthropometric status and lactation performance in low income Honduran population: evidence for the role of the infant. *American Journal of Clinical Nutrition* **61** 528-534.

Prentice MA, Paul AA, Prentice A, and Black AE, Cole TJ & Whitehead RG 1986 Cross-cultural differences in lactational performance. In Hamosh M and Goldman AS (eds.), *Human Lactation 2*, Plenum Publishing Corp., New York, p. 13-44.

Pitelka DR 1980 General morphology and histology of the adult gland. In: *The Mammary Gland*, Plenum Press, New York, pp 944-965.

Musey VC, Collins DC, Musey PI, Martino-Saltzman D, & Preedy JRK 1987 Long-term effect of a first pregnancy on the secretion of prolactin *New England Journal of Medicine* **316** 229-234.

Sampson DA & Jansen GR 1984 Measurement of milk yield in the lactating rat from pup weight and weight gain *Journal of Pediatry, Gastroenterology and Nutrition* **3** 613-617.

Sawadogo L and Houdebine LM 1988 Induction de la synthèse de caséine-*B* dans la glande mammaire de rates traitées par des extraits de plantes, *Comptes Rendus de l'Academie des Sciences Paris* **306** 167-172.

Snyderwine EG 1999 Mammary gland carcinogenesis by 2-amino-1-methyl-6-phenylimidazo[4,5-b]pyridine in rats: possible mechanisms *Cancer Letters* **143** 211-215.

Stringer BM, Rowson J, Greer W, Wynford-Thomas D, Williams ED. (1990) Effect of sustained serum prolactin elevation on breast epithelial and myoepithelial cell proliferation. Cell Tissue Kinetics **23** 17-30.

Thompson HJ, McGinley JN, Wolfe P, Singh M, Steele EV and Kellof GJ 1998 Temporal sequence of mammary intraductal proliferations, ductal carcinomas in situ and adenocarcinomas induced by 1-methyl-1-nitrosourea in rats *Carcinogenesis* **19** 2181-2185.

Venugopal M, Callaway A & Snyderwine EG 1999 2-Amino-1-methyl-6-phenylimidazo [4,5-*b*] pyridine (PhIP) retards mammary gland involution in lactating Sprague-Dawley rats. *Carcinogenesis* **20** 1309-1314.

Villalpando S & Del Prado M 1999 Interrelation among dietary energy and fat intakes, maternal body fatness and total lipid in humans. *Journal of Mammary Gland Biology and Neoplasia* **4** 285-295.

NEDERLANDSE SAMENVATTING

In dit proefschrift worden extracten van planten beschreven, welke worden gebruikt door vrouwen in Burkina Faso voor de behandeling van problemen met de lactatie. De biologische activiteit van planten voor de inductie en stimulatie van de lactatie is onderzocht en met name het onderliggende mechanisme van de activiteit is van één extract is nader onderzocht in de rat.

De lactatie-periode is een essentiële fase omdat een baby erg immatuur is direct na de geboorte en daarom totaal afhankelijk van moedermelk in de periode daarna. Melk wordt gesynthetiseerd in de cellen die rondom de alveoli van de melkklier liggen. Melk is een complex product bestaande uit voornamelijk water, melksuikers, vetten (voornamelijk triglyceriden), melkeiwitten (caseinen, lactalbumine), mineralen en immunoglobulinen. In de westerse wereld is de overleving van de nieuwgeborene niet direct afhankelijk van het vermogen van de moeder om melk te produceren, aangezien er een groot aanbod is van commerciële kunstmelk. In ontwikkelingslanden daarentegen, is moedermelk essentieel voor de overleving van de nieuwgeborene, omdat kunstmelk óf niet beschikbaar is óf buiten de financiële vermogens van de familie ligt. Ondanks dat 99% van de vrouwen in Burkina Faso borstvoeding geven, is de morbiditeit en mortaliteit van de jonge kinderen nog steeds erg hoog. Colostrum en melk hebben een heel belangrijke functie bij de immunologische bescherming tegen infecties. Een snel optredend tekort aan moedermelkkwantiteit en –kwaliteit kan het welzijn en de ontwikkeling van een nieuwgeborene schaden, waardoor later in het leven problemen met de weerstand tegen infecties optreden. Met de gelimiteerde beschikbaarheid van moderne moedermelksubstituten gebruiken Afrikaanse vrouwen in geval van problemen met de lactatie liever traditioneele plantenextracten om de melkproductie te induceren of te verhogen.

In **hoofdstuk 1** zijn de implicaties van de traditionële gebruiken van het borstvoeden in deAfrikaanse leefgemeenschap beschreven. Daarnaast wordt een breed overzicht van de regulatie van mammogenese, lactogenese en de daartoe benodigde hormonale regulatie gegeven. Uiteindelijk worden de mogelijk oorzaken van problemen met de lactatie bediscussieerd.
Met de huidige kennis is het duidelijk dat de lactatie het resultaat is van een lange serie fysiologische processen die zich in de melkklier voltrekken van zwangerschap tot aan de dag van geboorte en de eerste dagen daarna. De mate van succes van de lactatie is afhankelijk van de mate van proliferatie en differentiatie van het melkklierepitheel en van het vrijkomen van het hormoon prolactine (PRL) uit de hypofyse, nodig voor de stimulatie van de melksynthese.

In **hoofdstuk 2** is een veldstudie in 5 provincies van Burkina Faso beschreven, uitgevoerd tussen 1997 en 1999, om te documenteren welke plantenextracten worden gebruikt om problemen met de lactatie te behandelen. De ethnobotanische aspecten van deze Afrikaanse planten met therapeutische eigenschappen zijn beschreven. Echter het aantal vrouwen dat deze medicatie gebruikt en de mate van biologische activiteit van deze extracten, zijn onbekend.

In Burkina Faso, en ook in verschillende andere omliggende landen, wordt *Acacia nilotica* var *adansonii* veelvuldig gebruikt, vaak in combinatie met andere planten in de behandeling van verschillende ziekten. Echter, uit ethnofarmacologisch, phytochemisch en toxicologisch onderzoek is, zoals beschreven in **hoofdstuk 3**, niet duidelijk welke specifieke varieteiten gebruikt worden. Er zijn drie speciës beschreven met bijna identieke systematische karakteristieken, hetgeen de systematiek van *Acacia nilotica* erg verwarrend maakt. Echter, de locale bevolking in de dorpen hebben hier geen enkel probleem mee. Gebaseerd op de traditioneele informatie dat een extract van *Acacia nilotica* var *adansonii* (AN) de lactatie kan induceren en/of stimuleren, zijn experimenten in ratten, zoals beschreven in de hoofdstukken 4, 5 en 6, uitgevoerd.

In **hoofdstuk 4** is het effect van een waterig extract van AN op de melkproductie en de PRL synthese en afgifte bestudeerd. Lacterende vrouwelijke ratten kregen dit extract in een lage respectievelijk hoge dosis oraal toegediend gedurende 13 dagen, te beginnen op de tweede dag van de lactatie. De melkgift werd gemeten door de jonge ratjes te wegen en de toename in gewicht te berekenen. Uit dit onderzoek werd duidelijk dat de moederdieren, behandeld zowel met de lage als met de hoge dosis, significant meer melk produceerden en dat hun jongen sneller groeiden dan die van controle moederdieren. De toename in melkgift was reeds 24 uur na het begin van de behandeling waar te nemen.
Het effect van het AN-extract werd ook bestudeerd in peri-pubertale en volwassen maagdelijke ratten. Hierbij bleek dat de PRL-synthese en –afgifte bij deze ratten eveneens werd gestimuleerd. Bij de peri-pubertale ratten was een korte voorbehandeling met oestradiol nodig om het vereiste effect efficiënt te laten plaatsvinden. Dit suggereert dat het extract de PRL-synthese en –afgifte stimuleert tijdens condities die aanwezig zijn tijdens de lactatieperiode.

In **hoofdstuk 5** is het effect van het AN-extract op de proliferatie van cellen in de melkklier bestudeerd door gebruik te maken van bromodeoxyuridine (BrdU). BrdU is een analoog van thymidine dat wordt geïncorporeerd in cellen in de S-fase van de celcyclys vlak voordat ze celdeling ondergaan. BrdU wordt immunohistochemisch aangekleurd voor detectie in histologisch materiaal.
Volwassen, maagdelijke vrouwelijke ratten werden behandeld met het AN-extract zowel oraal als intramusculair, met of zonder voorbehandeling met oestradiol. Een uur voordat de dieren werden gedood werd een subcutane injectie met BrdU toegediend. De proliferatie van cellen in de melkklier was in alle groepen met AN-extract verhoogd. De labelingsindex van BrdU was significant hoger in de lobulaire structuren in vergelijking tot in de ductus structuren. Dit suggereert dat de proliferatieve activiteit met name gestimuleerd wordt in de lobuli, het melkproducerende deel van de melkklier.

In **hoofdstuk 6** is onderzocht of de effecten van het AN-extract op de groei en differentiatie van de melkklier alleen dient te worden toegeschreven aan de gestimuleerde PRL-synthese en –afgifte, of dat er ook een direct effect op de melkklier aanwezig is. Aangezien dopamine (DA) de belangrijkste remmende factor op de synthese en afgifte van PRL in de hypofyse is, werd in de volgende experimenten bromocryptine (CB 154), een agonist van DA. Vrouwelijke ratten, behandeld met CB 154, vertoonden inderdaad een

verlaging van de gehaltes PRL in plasma en hypofyse. Toediening van een hoge dosis AN-extract veroorzaakte een stijging van het hypofysaire PRL gehalte maar geen stijging in het plasma.

Deze bevindingen suggereren dat de mogelijkheid aanwezig is dat het AN-extract werkzaam is op de hypothalamus-hypofyse as via stimulatie van PRL synthese en –afgifte maar dat andere regulerende factoren zeker niet kunnen worden uitgesloten.

Meer onderzoek naar de effecten van het AN-extract op de melkkwaliteit en –kwantiteit is nodig alvorens de beschreven bevindingen kunnen worden toegepast om de traditionele gebruiken in de lacterende Afrikaanse vrouw te verbeteren. Ook is verder onderzoek nodig om andere eventueel positieve dan wel negatieve effecten van AN-extract te bestuderen. Het in dit proefschrift beschreven onderzoek toont aan dat een traditioneel gebruik van een plantenextract inderdaad werkt en het beoogde doel heeft, te weten de lactatie te op gang te brengen, c.q. te stimuleren. Dit opent perspectieven om onderzoek te verrichten op het gebied van het gebruik van traditionele planten in de traditionele gezondheidszorg. Het lijkt daarom redelijk om (i) speciale onderzoeksprogramma's te ontwikkelen op deze onderwerpen, en (ii) traditionele gebruiken in nationale gezondheidszorg programma's op te nemen.

Curriculum Vitae

Zourata Ouedraogo was born on August 24th 1964 in Ouahigouya, Burkina Faso. After her High School training in Mathematics and Natural Sciences in 1985, she joined the Institute of Natural Sciences and Rural Development at the University of Ouagadougou, Burkina Faso where she obtained the degree of engineer in Zootechnology and Animal Nutrition in 1990. In 1991 Zourata joined the Department of Animal Physiology where she completed her degree of DEA (Diplome d'Etude Approfondie) in Applied Biology in 1992. After this degree, she worked at this department as a part-time lecturer (1994-2001) while doing research in biology to complete the degree of Third Cycle Doctorate in 1998 in Animal Physiology/Applied Biology. In 1996, Zourata joined Wageningen University as part of a linkage programme between University of Ouagadougou and Wageningen University, to pursue a Sandwich PhD in Plants and Lactation: from tradition to the mechanism of action. In 2001, she joined the National Centre for Scientific Research and Technology, at the Research Institute of Health, where she is involved in research on the use of Traditional Medicine/Pharmacopoea for the prevention of mother-to-child HIV transmission during breastfeeding. Since June 2003, she is the National coordinator of IAEA Regional Project RAF/7/006 on Nutrition and HIV. She is married and mother of a daugther and a son.

Cover design by Zourata Lompo-Ouedraogo