Effect of aqueous extract of *Acacia nilotica* ssp *adansonii* on milk production and prolactin release in the rat

Zourata Lompo-Ouedraogo^{1,2}, Daan van der Heide¹,
Eline M van der Beek¹, Hans J M Swarts¹, John A M Mattheij¹
and Laya Sawadogo²

¹Human and Animal Physiology Group, Department of Animal Sciences, Wageningen University, Wageningen, The Netherlands

²Unité de Formation et de Recherche en Sciences de la Vie et de la Terre, Université de Ouagadougou, Burkina Faso

(Requests for offprints should be addressed to D van der Heide; Email: daan.vanderheide@wur.nl)

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 of aqueous extract of this plant during their first lactation produced about 59% more milk than controls ($P < 0.01$). Pup weight gain was also significantly higher than that in the control group. A lower dose, comparable to that used by women to improve their milk yield, led to about 33% more milk with the same growth rate for pups as that in the high-dose

group. The extract of AN was found to stimulate the synthesis and release of prolactin (PRL) significantly ($P < 0.05$). In addition, the mammary glands of oestrogen-primed rats treated with the extract showed clear lobulo-alveolar development with milk secretion. This study demonstrates that the aqueous extract of AN can stimulate milk production and PRL release in the female rat and could consequently have the properties claimed for lactating women.

Journal of Endocrinology (2004) **182**, 257–266

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, breastfeeding is essential for the newborn's survival. 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 (Bognounou *et al.* 1974, Adjanohoun *et al.* 1979a,b, Nacoulma-Ouedraogo 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 (prolactin (PRL), growth hormone, cortisol) and/or β -endorphin and β -casein accumulation in the mammary gland *in vivo* and *in vitro* (Sawadogo 1987, Sawadogo & Houdebine 1988, Sawadogo *et al.* 1988a,b). Indeed, PRL is known to play a key role in mammogenesis and lactogenesis (Djiane *et al.* 1982, Hennighausen *et al.* 1997, Briskin *et al.* 1999, Horseman 1999, Llovera *et al.* 2000) in all species, including the rat. However, for PRL to exert its

mitotic effect, the tissue must be exposed to oestrogen (Silberstein *et al.* 1994). In rodents, the 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 the synthesis of milk proteins in the epithelial cells and indirect stimulation of the 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 which stimulate PRL release (Sawadogo 1987), 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 Burkina Faso, *Acacia nilotica* ssp *adansonii* (AN) is one of the plants widely used in folk medicine, in particular during lactation.

A. nilotica species have been reported to have anti-hyperglycaemic (Akhtar & Khan 1985), antimicrobial (Sotohy *et al.* 1997), antiparasitic (El-Tahir *et al.* 1999), anti-inflammatory, analgesic and antipyretic (Dafallah & al-Mustapha 1996) properties. The methanol extract of the pods of *A. nilotica* ssp shows antihypertensive activities (Gilani *et al.* 1999), and the aqueous extract of the seeds exhibits spasmogenic and vasoconstrictor actions

(Amos *et al.* 1999). *A. nilotica* subspecies are traditionally used in Pakistan for the treatment of diarrhoea, and Shah *et al.* (1997) have recently found antiplatelet aggregatory activities. Methanol (bark and pods) and aqueous (pods) extract of *A. nilotica* shows considerable inhibitory effects against HIV-1 protease (Hussein *et al.* 1999) and hepatitis C virus protease (Hussein *et al.* 2000). However, despite its widespread use in folk medicine for the treatment of many diseases, little is known about its biological effects in induction and stimulation of lactation.

In view of the claim that AN extract is frequently used traditionally to improve lactation, experiments were performed to determine whether AN extract can stimulate milk production, PRL release and mammary gland development in the rat.

Materials and Methods

Preparation of the plant extract

Traditionally, a nursing woman (± 65 kg in body weight) drinks the extract of about 100 g dry AN leaves per day. For our experiments, AN fresh leaves were air-dried in the shade and pulverised, and 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% of dry leaves. 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, centrifuged at 3000 g for 10 min and stored at 4 °C.

Animals

For all experiments, mature Wistar rats were purchased from Charles River (Lyon, France), and maintained on a 12-h light–dark cycle (lights on from 0600 to 1800 h). They were housed individually in standard plastic cages with wood chips on the floor and were allowed access to food and water *ad libitum*. All experiments were seen and approved by the animal experimental committee of the University of Wageningen and the University of Ouagadougou.

Experimental protocols

Experiment 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 eight to nine pups were used for this experiment. Females were divided into three experimental groups and received 2 ml of 0.9% NaCl ($n=6$), and 280 mg ($n=6$) and 560 mg of plant extract/kg body weight per 2 ml 0.9% NaCl ($n=6$) respectively. All animals were treated daily, starting on the

evening of day 2 of lactation. The extract was administered orally with a gavage syringe each day at 1800 h. Milk production was estimated 18 h after gavage. 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 with an electronic balance (Sartorius Basic Plus) accurate to 0.01 g.

Every day during the study period, the pups were weighed at 0700 h (w1) and subsequently isolated from their mother for 4 h (Sampson & Jansen 1984). At 1100 h, the pups were weighed (w2), returned to their mother and allowed to feed for 1 h. At 1200 h, they were weighed (w3). Milk yield 18 h after the gavage was estimated as $w3 - w2$. 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 $(w2 - w1)/4$. 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.

Experiment B Fifteen lactating dams weighing 225–250 g at the beginning of lactation and suckling eight to nine pups were divided into two experimental groups and received 2 ml 0.9% NaCl ($n=7$) and 280 mg of plant extract/kg body weight per 2 ml 0.9% NaCl ($n=8$), respectively.

All animals were treated daily, starting on the evening of day 2 of lactation. The extract was administered orally with a gavage syringe each day at 1800 h. Milk production was estimated at 18 and 23 h after gavage. For the measurement of milk yield 18 h after gavage, the same procedure as described above for experiment A was followed. For measurement of milk yield 23 h after gavage, the pups were subsequently isolated between 1200 and 1600 h. After weighing at 1600 h (w4), they were reunited with their mother for 1 h of feeding and, finally, they were weighed (w5). They were subsequently left with their mother during the night. Milk yield 23 h after gavage was estimated as $w5 - w4$. Daily milk yield was corrected for weight loss due to metabolic processes in the pup (respiration, urination and defecation) during suckling. The value used for correction for weight loss was $[(w2 - w1) + (w4 - w3)]/8$. 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 again calculated from the pup weight at w2.

Experiment II: effect of 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 three groups and

received either 0.2 ml 0.9% NaCl, or 45 or 110 mg plant extract/kg body weight per 0.2 ml 0.9% NaCl. All doses were given as a single injection each day via the intravenous cannula during a period of 6 days.

Animals were adjusted to blood-sampling procedures 2 days before the start of the experiment. On day 1 (at dioestrous) and day 3 (at oestrus) 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 day 7 of the experiment, the animals were killed by decapitation under ether anaesthesia. 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 were 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, USA). PRL values were expressed in terms of the reference standards RP-3. The intra-assay variation are 7.92% and 9.95% for plasma PRL and pituitary PRL respectively.

Experiment III: effect of i.m. 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 two groups. The first group first received a subcutaneous injection of oestradiol (E2) in a dose of 10 μg /0.1 ml sesame oil twice daily for 2 days. Subsequently they were divided into three subgroups and received an i.m. injection of either 0.9% NaCl, or 200 or 400 mg plant extract/kg body weight twice daily for 5 days. The second group first received an injection of 0.9% NaCl and was then divided into three subgroups receiving the same injections as the first group. On day 6, all animals were anaesthetised with ether and killed by decapitation. Pituitaries were collected for PRL extraction, as previously described. The two inguinal mammary glands were removed, immediately fixed in alcoholic Bouin and then embedded in paraffin wax after dehydration in a graded series of ethanol and xylene. Paraffin sections (5 μm) of the mammary glands were sliced and stained with Harris' haematoxylin and eosin. Mammary gland structures were identified by the criteria of Russo and Russo (1978, 1996) and Masso-Welch *et al.* (2000), using a Zeiss microscope coupled to an image analysis system.

Experiment IV: effect of the treatment with AN extract after bromocriptine injection

Twenty-four female rats at 90 days old were divided into four groups. Two groups were treated orally with 2 ml NaCl 0.9% ($n=6$) and 550 mg plant extract/kg body weight per 2 ml NaCl 0.9% ($n=6$) twice daily during 5 days respectively.

The remaining two groups were treated subcutaneously with bromocriptine (CB154) at a dose of 4.5 mg/kg body weight per 0.25 ml 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 plant extract/kg body weight per 2 ml NaCl 0.9% ($n=6$) twice daily during 3 days. On day 6, all the animals were anaesthetised with ether and killed by decapitation. Pituitaries were collected for PRL extraction and determined as described above.

Statistical analysis

Data were analysed by Student's *t*-test or one-way ANOVA followed by the Bonferroni or Scheffe test or least square difference (LSD), using the statistical package SPSS (version 7.5 for Windows). Data that were not normally distributed (plasma PRL concentrations in experiment II) were analysed using the non-parametric Kruskal–Wallis test. $P<0.05$ was considered significant.

Results

Milk production

Milk production of both groups receiving 280 and 560 mg of plant extract was higher than that of the control group, as illustrated in Fig. 1A (experiment A). Milk yield increased from 1.38 ± 0.16 , 1.44 ± 0.15 and 1.52 ± 0.05 g/pup per day to about 3.07 ± 0.29 , 4.46 ± 0.06 and 5.27 ± 0.15 g/pup per 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 per day over the experimental period respectively (Student's *t*-test with Bonferroni correction at least $P<0.05$) (Fig. 1B).

Milk production data 18 and 23 h after gavage indicated that milk production was significantly increased in all groups receiving the extract, at both time points (ANOVA followed by the Scheffe test, $P<0.05$) (Fig. 2). The mean milk yield for the control group was 0.37 ± 0.02 and 0.38 ± 0.03 g/pup at 5, 18 and 23 h after gavage with saline respectively. For the group receiving the extract, the mean milk yield was 0.51 ± 0.02 and 0.57 ± 0.03 g/pup at 5, 18 and 23 h after treatment respectively.

Body weight

All pups gained weight during the study period (Fig. 3A) 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 per day for the controls, from 8.17 ± 0.66 to 22.94 ± 0.57 g/pup per day for those receiving 280 mg, and from 8.72 ± 0.39

Figure 1 (a) Effect of aqueous extract of AN on milk production 18 h after gavage. Values are presented as means $\pm$ S.E.M. A statistically significant difference was observed for the whole period for the group receiving 560 mg ($P < 0.01$). In addition, a significant difference between days 8–11 and days 14–15 was observed for the group receiving 280 mg ($P < 0.05$). Statistically significant differences are given compared to the control treatment (ANOVA followed by Bonferroni). (b) Mean milk production per day. Values are means $\pm$ S.E.M. ** $P < 0.01$; * $P < 0.05$ (Student's *t*-test with Bonferroni correction). C: control group treated with 0.9% NaCl; T280 mg: group receiving 280 mg of AN extract/kg body weight; T560 mg: group receiving 560 mg of AN extract/kg body weight.

to 25.20 ± 1.51 g/pup per day for those receiving 560 mg extract. The daily weight gain was 0.86 ± 0.08 , 1.42 ± 0.12 and 1.43 ± 0.11 g/pup respectively (Fig. 3B). 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 the body weights of the dams was seen.

Figure 2 Mean milk production 18 and 23 h after gavage. Values are means $\pm$ S.E.M. and have been given per 5 h (4-h separation plus 1-h suckling). C18 h: milk yield 18 h after gavage with saline ($n = 7$); T18 h: milk yield 18 h after gavage with AN extract (280 mg/kg body weight) ($n = 8$); C23 h: milk yield 23 h after gavage with saline ($n = 7$); T23 h: milk yield 23 h after gavage with AN extract (280 mg/kg body weight) ($n = 8$). The Scheffe test was used to assess differences between groups. * $P < 0.05$.

PRL content

To evaluate the effect of the extract on PRL secretion and release, PRL concentrations were measured in both plasma and the pituitary. In experiment II, the effect of an acute (Fig. 4A) and a 'chronic' (Fig. 4B) i.v. administration of the extract on the plasma PRL concentration was studied. A wide variation in individual responses to treatment was observed. However, the plasma PRL concentration was significantly higher in treated animals than controls; in the latter, the PRL level remained constant (non-parametric Kruskal–Wallis, at least $P < 0.05$). On day 1 of treatment (acute treatment), the highest levels were observed 1 h after the injection. They remained relatively high until day 3 ('chronic' treatment) of treatment, where the highest levels were observed earlier (40 min). PRL levels 40 min after the 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, the plasma levels were, at the same time, 4.02 ± 0.76 , 10.53 ± 2.44 and 43.93 ± 16.24 ng/ml for controls, and the groups receiving 45 and 110 mg respectively. Furthermore, the pituitary PRL content for the same animals (Fig. 5) 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.

With regard to pituitary PRL in animals receiving the extract intramuscularly (experiment III), significantly higher PRL concentrations were observed in the

Figure 3 (A) Effect of aqueous extract of AN on pup weight after 18-h gavage. Values are means $\pm$ S.E.M. A significant difference was observed between the treated group and the controls (at least $P < 0.05$). (B) Mean weight gain of pup. Values are means $\pm$ S.E.M.

** indicates statistical significance ($P < 0.01$) (Student's *t*-test followed by Bonferroni correction). C, T280 mg, and T560 mg: see Fig. 1 legend for explanation.

E2-primed animals treated with the extract than in the non-primed ones (ANOVA, followed by Bonferroni, at least $P < 0.05$) (Fig. 6). Moreover, a significant increase was found for the E2-primed animals receiving the extract compared to the E2-primed control group.

In both CB154-treated groups (Fig. 7), pituitary PRL content was significantly lower than the control value ($P < 0.05$). AN treatment significantly increased PRL content compared to that in both animals treated with either CB154 alone or CB154 plus AN extract ($P < 0.05$), albeit the treatment with the extract after CB154 injection had no effect on PRL content.

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 (Fig. 8). The mammary glands of non-E2-primed rats treated with saline showed a bare duct system and terminal end buds (TEBs) within an important fat pad (A). In the mammary gland of the E2-primed animals, ducts branching into ductule (B), with less alveolar bud (Alvbs) development, were observed. In all of E2-primed and non-primed animals receiving AN extract (C–F), ductule branching with alveolar development, and lipid droplets in the alveoli, was observed. However, there was no evidence of secretion in the ducts of the non-primed group receiving 400 mg AN extract/kg body weight (E). The largest alveolar structures with basophilic secretions in

the lumen of alveoli and ducts were observed in the oestrogen-primed group receiving 400 mg AN extract.

Discussion

The measurement of milk production rates in rats 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 extract is lactogenic. As expected, milk production was significantly higher in the treated animals than in the controls. In addition, milk yield appears to be significantly stimulated about 24 h after administration of the extract and the pup growth rate was significantly improved. Moreover, the pup growth rate in the group receiving the lower dose (that is, T280, in the same range as that used by the native women) was similar to that in the group receiving the higher dose, whereas the dams produced 20% less milk. This suggests a possible effect of the extract on milk components. However, the suggestion can be confirmed only by studying the composition of the milk. Likewise, extract of *Asparagus racemosus* has been observed to stimulate milk production in buffaloes (Patel & Kanitkar 1969). On the other hand, treatment with some of these plants did not improve the pup growth rate in rats (Kamani *et al.* 1987) or milk production in ewes (Sawadogo *et al.* 1989) but did stimulate PRL secretion in

Figure 4 Plasma PRL profiles after acute (A) and 'chronic' (B) treatment (i.v. injection). Values are means $\pm$ S.E.M. *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 (i.v.); T45 mg: group receiving 45 mg of AN extract/kg body weight (i.v.); T110 mg: group receiving 110 mg of AN extract/kg body weight (i.v.). '0' time represents the time of injection of AN extract with samples taken at 20-min intervals after injection.

Figure 5 Pituitary PRL content after i.v. injection, treatment during 6 days. Values are means $\pm$ S.E.M. **Statistically significant ($P < 0.01$) (ANOVA followed by Bonferroni). C: control group receiving 0.9% NaCl (i.v., 6 days); T45 mg: group receiving 45 mg of AN extract/kg body weight (i.v., 6 days); T110 mg: group receiving 110 mg of AN extract/kg body weight (i.v., 6 days).

Figure 7 Pituitary PRL content after oral administration of the extract and after subcutaneous injection of CB154. Values are means $\pm$ S.E.M. *Statistically significant compared to the bromocriptine-treated group receiving AN 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) plus T550 mg (oral, 3 days); T550 mg: group receiving AN extract (oral, 5 days).

ewes (Sawadogo *et al.* 1989). Interestingly, the results obtained in this study showed not only that milk production was increased but also that plasma and pituitary PRL levels were increased. The mechanism of action is still unknown as are the active components in the

Figure 6 Pituitary PRL content after i.m. injection. Values are means $\pm$ S.E.M. *Statistically significant compared to the control; #statistically significant compared to the E2-primed control group. (ANOVA followed by Bonferroni, at least $P < 0.05$). Without E2: not previously treated with oestradiol; with E2: previously treated with oestradiol. C: control group receiving 0.9% NaCl (i.m., 5 days); T200 mg: group receiving 200 mg of AN extract/kg body weight (i.m., 5 days); T400 mg: group receiving 400 mg of AN extract/kg BW (i.m., 5 days).

Figure 8 Sections of mammary glands from virgin oestrogen (E2) primed and non-primed rats 60–70 days old treated with saline or AN extract. (A) Mammary gland from a non-primed rat receiving 0.9% NaCl, showing ductules and terminal end buds, but no alveolar development. Notice the abundance of mammary fat pad. (B) Mammary gland from an oestrogen-primed rat receiving 0.9% NaCl, showing ducts branching into ductule, with fewer alveolar buds (Alvbs). (C) Mammary gland from a non-primed rat receiving 400 mg AN extract/kg body weight, showing a well-defined alveolar structure (compare panels A and B) with fewer lipid droplets within the alveoli (compare panel D). (D) Mammary gland from an oestrogen-primed rat receiving 400 mg AN extract/kg body weight, showing a well-defined alveolar structure with lumens filled with basophilic secretions (S) (see panel F) (compare panels A and B). Mitotic cells are also observed (panel E). Paraffin-wax sections of 5 μ m were stained with Harris' haematoxylin and eosin. Magnification: panels A–D $\times$ 100; panels E and F $\times$ 200. Teb, terminal end bud; Adp, adipose tissue; Bv, blood vessel; L, lobule; Dc, ductus; Alvbs, alveolar buds; S, basophilic secretion; Pt, proliferating tissue.

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 1987, 1989, Sepehri *et al.* 1990, 1992). Moreover, the pituitary PRL content in the peripubertal animals treated just with the extract did not significantly differ from the control, compared to that in the cycling female treated intravenously with the extract. This indicates that the extract has a specific PRL-secretion activity under certain physiological conditions. A strong relation between treatment with the extract and PRL release after ‘acute’ as well as ‘chronic’ treatment has been observed. From this, it can be expected that the mechanism of the elevation of PRL will be related to neuroendocrine regulation. Our results may suggest involvement of dopaminergic activity, since the treatment with the extract after CB154 injection 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 pathological effects being reported. Further investigations are in progress and will provide more 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.
- 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* seeds. *Phytotherapy Research* **13** 683–685.
- 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.
- Briskin 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.
- 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.
- Djiane J, Houdebine LM & Kelly PA 1982 Correlation between prolactin-receptor interaction, down-regulation of receptors, and stimulation of casein and deoxyribonucleic acid biosynthesis in rabbit mammary gland explants. *Endocrinology* **110** 791–795.
- 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.
- Flint DJ, Tonner E, Beattie J & Pantou D 1992 Investigation of the mechanism of action of growth hormone in stimulating lactation in the rat. *Journal of Endocrinology* **134** 377–383.
- 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.
- Hennighausen 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.
- 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.
- 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.
- Kamani HT, Karunanayake EH & Mahindartna MPD 1987 Evaluation of galactogogue 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.
- 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, vol 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 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 Pediatrics, 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.
- Sawadogo L & 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'Académie Sciences Paris* **306** 167–172.
- Sawadogo L, Houdebine LM, Thibault JF & Rouau X 1988a Mise en évidence d'extraits de plantes possédant une activité galactogène. *Bulletin Médecine Traditionnelle* **2** 133–146.
- Sawadogo L, Houdebine LM, Thibault JF, Rouau X & Olivier-Bousquet M 1988b 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 chèvre de brasserie. *Reproduction, Nutrition, Development* **29** 139–146.
- Sepehri H, Renard C & Houdebine LM 1990 β -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.
- 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.
- 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.
- Sotohy SA, Sayed AN & Ahmed MM 1997 Effect of tannin-rich plant *Acacia nilotica* on some nutritional and bacteriological parameters in goats. *Deutsche Tierärztliche Wochenschrift* **104** 432–435.
- Van Dongen JJ, Remie R, Rensema JW & Van Wunnik GHJ 1990 *Manual of Microsurgery on the Laboratory Rat*. Amsterdam: Elsevier.
- 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.

Received in final form 24 March 2004

Accepted 14 May 2004