THE EFFECTS OF METHANOLIC LEAF EXTRACT OF GONGRONEMA LATIFOLIUM ON MILK YIELD AND SOME LACTOGENIC HORMONES IN LACTATING WISTAR RATS

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ABSTRACT
Proper and exclusive breastfeeding is recommended during the early infancy stage of a child. Lactation insufficiency serves as a hinderance to this. Gongronema latifolium is an important medicinal plant known for its various therapeutic effects. The lactogenic effects of Gongronema latifolium used locally to boost lactation was investigated. Twenty Wistar dams were grouped into five groups (n=4); consisting of control group (1 ml/kg distilled water), standard drug group (metoclopramide 5 mg/kg), 200 mg/kg and 800 mg/kg Gongronema latifolium methanolic extract groups. The pups were weighed 18 hours after the administration of Gongronema latifolium orally, to assess milk yield while the serum was gotten from the dams on the ninth day of administration. Toxicity study showed that the plant extract was non-toxic (LD<sub>50</sub> > 5000 mg/kg) when administered orally. The leaf extract significantly increased the serum prolactin (p< 0.05). There was also significant increase in the milk yield in the group administered with Gongronema latifolium group versus the control group (7.28 ± 1.15 g/pup, 7.33 ± 1.08 g/pup and 9.68 ± 0.97 g/pup vs 5.5 ± 1.01 g/pup respectively). These lactogenic effects were also dose-dependent. The leaf extract had no significant effect on the oxytocin serum concentration of the lactating Wistar rats. The lactogenic effect Gongronema latifolium may be ascribed to the presence of phytochemicals such as Saponins (e.g., diosgenin, kaempferol, quercetin and silybin) that have phytoestrogenic effects that could promote milk synthesis, secretory differentiation and mammary epithelial cells proliferations.

Keywords: Galactagogue, Gongronema latifolium, lactation, lactogenic, prolactin

INTRODUCTION
Breast milk provides the best nutrition, immune protection, and regulation of growth, development, and metabolism for the human infant (Goldman, 2012). One in eight women experience early, undesired weaning that they attribute to difficulties with the physiology of breastfeeding (Stuebe et al., 2014). Insufficient lactation experienced by some women is due to the presence of factors that interfere and interrupt normal breast morphology and production of milk (Pillay and Davis, 2021). Some of these factors include glandular tissue reduction due to breast surgeries, postpartum haemorrhage, prematurity/ birth weight (Sultana et al., 2013). The use of galactagogues is often considered where there is declined milk production. Galactagogues are medications that induce lactation generally from exerting its effects through oxytocin or and prolactin enhancement (Gabay, 2002). Orthodox galactagogue medications such as metoclopramide and domperidone are known to possess side effects and adverse reactions that are at times detrimental to health (Baliki and Balibey, 2012). Certain hormones in the human body play their roles in the control and regulation of breast milk synthesis and release. These hormones include prolactin and oxytocin. Prolactin is synthesized and secreted by specialized cells of the anterior pituitary gland, known as the lactotrophs; and is necessary for the final preparation of mammary glands for the production and secretion of milk (Hartmann et al., 1996). Oxytocin is produced in neurons that originate mainly in the paraventricular and also supraoptic nuclei of the hypothalamus, and is transported to the posterior pituitary through the hypothalamo-hypophysial tract (Wakerley, 1978). Oxytocin acts on mammary glands by activating G-protein coupled oxytocin receptor which causes the contraction of myoepithelial cells that lines the mammary gland. This leads to the ejection of milk from alveoli of the mammary gland (Gu et al., 2016). Gongronema latifolium also known as Bush buck leaf, is a climbing perennial shrub that belongs to the family of plants known as Asclepiadaceae and it is widespread in tropical rainforest of West African countries, such as Nigeria, Guinea-Bissau, Côte d’Ivoire, Cameroon, Sierra Leone, Ghana and Senegal, etc (Dutta, 2005). In Nigeria, it is locally called ‘Utasi’ by the Efikis, Ibibios and Quas, ‘Utazi’ by the Igbos and ‘Arokeke’ or ‘Madunmaro’ by the Yorubas (Eleyinmi, 2008). In the southern part of Nigeria especially, the leaves of Gongronema latifolium are used commonly for nutritional purposes. The phytochemical screening of the leaves of G. latifolium shows that it contained tannins, phytosterols, glycosides, flavonoids, anthocyanidins and saponins. Several researches have reported therapeutic the actions of this plant (Ugochukwu et al., 2003; Owu et al., 2012; Morebise et al., 2005; Iweala and Obidoa, 2009). The leaves have been used locally by women from certain ethnic groups to improve lactation in lactating mothers, therefore scientific investigations to assess its lactogenic properties is necessary. The aim of this study was to evaluate the effect of Gongronema latifolium on milk yield, serum prolactin concentration and oxytocin concentration.

MATERIALS AND METHODS
Fresh leaves were shade dried and pounded. The pulverized sample (100 g) was macerated with 1000ml of methanol/water and allowed to stand for 72 hours and filtered. The filtrate was concentrated using rotary evaporator and evaporated to dryness on a water bath at 50°C. The dried extracts were properly stored in a container before further experiments and analysis. The lethal dose (LD<sub>50</sub>) of the plant...
extract was determined by the method of Lorke (1983) using 12 rats and the median lethal dose (LD₅₀) was calculated. Twenty (20) Wistar rats were divided into five (5) groups containing four (4) Wistar rats each (n=4) as follows: Group 1 were administered 1 ml/kg distilled water; group 2 were administered 5 mg/kg metoclopramide for a period of nine days; group 3 were administered 200 mg/kg methanolic extract of Gongronema latifolium for a period of nine days; group 4 were administered 400 mg/kg methanolic extract of Gongronema latifolium for a period of nine days; group 5 was administered 800 mg/kg methanolic extract of Gongronema latifolium for a period of nine days.

During the periods of treatment, milk yield was estimated after 18 hours gavage. The dams were administered the drugs at 6pm and then at 7a.m of the next day, were weighed; also, the pups were weighed at this time which was recorded as weight 1 (W1). The pups were then isolated from their dams for a period of 4 hours (Samson and Jensen, 1984). After which the pups were then weighed as (w2) and returned to their dams and were allowed to feed for one hour and then were weighed again as (w3). Milk yield 18 hours after gavage was estimated as w3 – w2 (Lompo-Ouedraogo, et al., 2004). These procedures were carried out for nine days.

At the end of the nine days’ treatment period, the animals were anaesthetized using ketamine at 75 mg/kg (Veilleux-Lemieux et al., 2013). Blood sample were collected via cardiac puncture using syringes, and was then centrifuged at 1000rpm for 10 minutes to obtain serum which was used for biochemical assays. The assays for serum prolactin and oxytocin hormones concentrations were carried out using the appropriate rat ELISA kits respectively, according to the manufacturer’s manual. Ethical approval was obtained from the ethical committee on animal research of the Ahmadu Bello University, Zaria; with approval number ABUCAUC/2021/004.

### Table 1: The acute toxicity (LD₅₀) results

<table>
<thead>
<tr>
<th>Experimental phase</th>
<th>Dose (mg/kg)</th>
<th>No. of Morbid rats after 24 hours</th>
<th>No. of dead rats after 24 hours</th>
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<tbody>
<tr>
<td>Phase I</td>
<td></td>
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<tr>
<td>control</td>
<td>10</td>
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<td></td>
<td>100</td>
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<td>1,000</td>
<td>0/3</td>
<td>0/3</td>
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<td>Phase II</td>
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<tr>
<td>control</td>
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<tr>
<td></td>
<td>5,000</td>
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</table>

Figure 1: The milk yield 18 hours after administration of G. latifolium.

![Graph showing milk yield with legend]

* = significant difference (p < 0.05) compared to control, ** = significant difference compared to the metoclopramide group; GTG = G. latifolium Treated Group.

### Statistical Analysis

Data collected were expressed as mean ± Standard Error of Mean (SEM). It was analysed using one-way analysis of variance, ANOVA. SPSS version 22 was used for the analysis. Values of p < 0.05 were considered statistically significant.

### RESULTS

In the acute toxicity test, there was no morbidity or death amongst all rats in all groups at both phases when orally administered Gongronema latifolium (Table 1). Milk yield was significantly higher in all the treated groups compared to the control viz; GTG 200 mg/kg, 400 mg/kg and GTG 800 mg/kg vs control (7.28 ± 1.15 g/pup, 7.33 ± 1.08 g/pup and 9.68 ± 0.97 g/pup vs 5.5 ± 1.01 g/pup respectively. There was significant (P < 0.05) increase in milk yield in the group administered 5mg/kg metoclopramide (11.02 ± 1.5g/pup) compared to the control (5.5 ± 1.01 g/pup) group and all the three G. latifolium treated groups. There was significant (p < 0.05) increase in the serum prolactin concentration of the GTG treated groups; 200 mg/kg, 400 mg/kg and 800 mg/kg [44.60 ± 1.51 ng/ml, 45.03 ± 1.95 ng/ml, 46.51 ± 1.39 ng/ml] respectively, compared to the control group (40.95 ± 1.46 ng/ml) (figure 2). Serum prolactin was significantly higher (p< 0.05) in the metoclopramide treated group compared to the control group and all GTG treated groups. There was a dose-dependent increase in milk yield within the Gongronema latifolium treated groups although it was not statistically significant. As shown in figure 3, there was no significant increase oxytocin serum concentration of the three G. latifolium treated group compared with the control group. Also, there was not a significant increase in oxytocin concentration between all the groups that were administered the G. latifolium leaf extract.

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DISCUSSION

The acute toxicity test of the methanolic extract of Gongronema latifolium leaf, showed that the LD<sub>50</sub> when orally administered was greater than 5000 mg/kg. Substances are considered non-toxic when LD<sub>50</sub> is greater than 5,000 mg/kg (Lorke, 1983). This indicates that the methanolic extract of Gongronema latifolium leaf is non-toxic and is safe for consumption.

The increased prolactin serum concentration that was observed in the Gongronema latifolium treated group compared to the control could be as a result of steroidal saponins and flavonoids such as diosgenin, kaempferol, quercetin and silybin that act as phytoestrogens (Sharma and Bhatnagar, 2011) which are present in Gongronema latifolium leaf. These phytoestrogens stimulate pS2 expression in MCF-7 cell lines. In assessing the estrogenicity of a compound, pS2 is usually used as a marker (Sreeja et al., 2010).

Phytoestrogens have also been demonstrated to increase milk flow (Mortel and Mehta, 2013), probably via potentiating prolactin synthesis and secretion from the anterior pituitary gland; as phytoestrogenic compounds possess 17β-estradiol-like actions (Tabares et al., 2014). The increase in prolactin concentration was observed to be dose dependent among the Gongronema latifolium treated group. This suggests that the effect of Gongronema latifolium leaf on prolactin secretion increases with increasing doses. This is similar to the work done by Igwe et al., (2015), who reported that Vernonia amygdalina increased serum prolactin concentration in a dose dependent manner. In contrast the study on humans carried out by Özalkaya et al., (2018) in which the galactagogue effect of herbal tea that contained mixture of stinging nettle, Melissa, caraway, anise, fennel, goat rue and lemon grass; on the milk production and prolactin secretion showed that there was increased milk yield but not significant concurrent
increase in serum prolactin levels of the lactating mothers. Some studies on galactagogue have shown that there is poor correlation between serum prolactin level and milk production. (Sharma et al., 1996; Gupta and Shaw 2011). In this study, there was an increase in milk yield with the group administered Gongronema latifolium compared to the control group. The boost in milk production could be attributed to the increased prolactin hormone secretions. It was also observed that the milk yield increased with increasing doses of Gongronema latifolium extract. This suggests that the galactagogue effect of Gongronema latifolium will be more effective with increasing doses. Gongronema latifolium had no significant effect on the serum oxytocin level during lactation in Wistar rats. The reason could be that the Gongronema latifolium leaf extract does not affect the synthesis and the secretion of oxytocin hormone from the hypothalamus, and does not play a role its mechanism of action. Similar to a study carried out by Ushioyama et al., (2007), it was observed that the Chinese herbal medicine (Xiong-gui-tiao-xue-yin) used, stimulated lactation with increase in prolactin levels and had no effect on oxytocin level, in postpartum mothers. Another possible explanation may be that oxytocin secretion is mostly based on psychophysiological stimulation dependent mostly on neural responses; and Gongronema latifolium probably had an influence on it. This finding is in contrast to the research carried out by Servin, et al., (2020) where fenugreek was found to increase oxytocin secretions in the pituitary gland.

CONCLUSION

Based on the findings of this study, Gongronema latifolium administration increased milk yield and serum prolactin concentration. This could be associated with the certain phytochemicals (saponins and flavonoids) that are present in the leaf extract; which possess phytoestrogenic properties that stimulate the synthesis and secretion of prolactin hormone. This indicates that Gongronema latifolium has potential lactogenic properties which may be explored in lactating mothers with low milk production.

REFERENCES


