INTRODUCTION
Prolactin secretion is affected by a large variety of stimuli provided by the environment and internal milieu like the effect of the chemistry of lactation. There have been reports that some of the African women with lactation insufficiency particularly those living in villages depend largely on herbal supplements for milk yield increase or milk induction despite the modern alternative bottle feeding (Okasha et al., 2008). With a serious constraints to modern alternative breast feeding, rural women use herbal decoction to boost (Sholapurkar, 1986; Morton, 1987) lactation so that they can breastfeed their newborn babies very well. Prolactin has a multiple biological activities, however its principal role is in reproduction during lactation and the synthesis and secretion is from anterior pituitary gland (Bern and Nicoll, 1968; Freeman et al., 2000). The best-known physiological stimulus affecting prolactin secretion is the suckling stimulus applied by the nursing young. This has been characterized as a classical neuro-endocrine reflex, just as muscle contraction evoked by an electrochemical stimulus is described as a stimulus-contraction reflex (Freeman et al., 2000). Hibiscus sabdariffa L. is also used in folk medicine against many complaints that include high blood pressure, liver diseases and fever (Dalziel, 1973; Morag et al., 1975; Wang et al., 2000; Chen et al., 2003) and it has been reported to be lactogenic, stomachic and tonic (Ben-Jonathan, 1985; Ben-Jonathan and Liu, 1992; Olaleyé, 2007; Okasha et al., 2008). Hibiscus sabdariffa being a potential herb used as source of many foods and beverages in especially local community in Africa and parts of the World, so its practical benefits needs to be established. In light of this, the study is designed to evaluate the lactogenic effect of the ethyl acetate fraction of Hibiscus sabdariffa seed.

MATERIALS AND METHODS

Chemicals and drugs: Ethyl acetate puriss Reg. No 27227 Sigma-Aldrich®, 1-Butanol puriss Reg. No 33065 Sigma-Aldrich®, Prolactin ELISA 96 test kits (Fortress® Diagnostics Limited, BT41 I QS, UK), Metoclopramide (NAFDAC Reg. no. 04-5946) and Chloroform Poole, BH15 1TD England.

Preparation of the plant extract: The samples of Hibiscus sabdariffa L. seed were collected in Gaya Hong Local Government in Adamawa state of Nigeria...
Hibiscus sabdariffa production was estimated 18 h after gavage. The pups body weight of dams and weight gain of pups were prolactin kit (Dombrwicz phosphate buffer pH 8 and then finally analyzed using and ethyl acetate fraction of respectively and 200 mg/kg ethyl acetate fraction of and metoclopramide) and Groups III and IV (100 each group (n = 6). Group I (normal saline), Group II (5 were weighed every day during the study period at 07:00 h (w1) and then isolated from their dams for 4 h (Sampson and Jansen, 1984; Ann and Linzell, 2003). At 11:00 h, the pups were weighed (w2), returned to their dams 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 with a correction for weight loss due to metabolic processes in the pups as (w2-w1)/4. For Group IV that received 200 mg/kg ethyl acetate fraction H. sabdariffa. 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 Group III was followed. For measurement of milk yield 23 h after gavage, the pups were subsequently isolated between 12:00 and 16:00 h. After weighing at 1600 h (w4), they were reunited with their dams for 1 h of feeding and, finally, they were weighed (w5) (Sampson and Jansen, 1984; Ouedraogo et al., 2004). They were subsequently left with their dams during the night. Milk yield 23 h after gavage was estimated as w5-w4 with a correction for weight loss due to metabolic processes in the pups as [(w2 - w1) + (w4 - w3)]/8.

Experimental protocol: Twenty Four female albino rats weighing 180-240 g were obtained from the Animal house of Department of Human Physiology Ahmadu Bello University, Zaria. The animals were housed and mated with the male rats in a stainless steel metal cage under standard laboratory condition with 12 h dark/light cycle. They were fed with commercial feeds and tap water ad libitum. Following birth, the litters' weights were recorded and culled to 6 L/dam. The twenty four lactating rats were randomly divided at parturition into four groups (control, metoclopramide-treated (Alexandre et al., 2002), Ethyl acetate-treated) groups consist of six rats each (n = 6). All groups received control (normal saline), metoclopramide-treated (5 mg/kg metoclopramide) and Ethyl acetate-treated groups (100 and 200 mg/kg of ethyl acetate fraction) orally for fourteen days starting from day 3 to day 17 of lactation (Vogel and Vogel, 1997). The animals were then euthanized on day 18 and pituitary gland was removed, weighed and homogenized with phosphate buffer pH 8 and then finally analyzed using prolactin kit (Dombrwicz et al., 1992).

Lactogenic effect of ethyl acetate fraction of Hibiscus sabdariffa L.: Twenty four lactating dams weighing 180-240 g at the beginning of lactation and suckling six pups were used for this experiment. They were divided into four groups of six lactating rats in each group (n = 6). Group I (normal saline), Group II (5 mg/kg metoclopramide) and Groups III and IV (100 and 200 mg/kg) ethyl acetate fraction of H. sabdariffa respectively. All animals were treated daily, starting from the evening of day 3 of lactation. Metoclopramide and ethyl acetate fraction of H. sabdariffa was orally administered each day at 18:00 h. Milk production was measured from day 4 to 17 of lactation. Milk yield and body weight of dams and weight gain of pups were measured each day with an electronic balance (Mettler P3) accurate to 0.01 g. For Group III that received 100 mg/kg ethyl acetate fraction of H. sabdariffa, Milk production was estimated 18 h after gavage. The pups were weighed every day during the study period at 07:00 h (w1) and then isolated from their dams for 4 h (Sampson and Jansen, 1984; Ann and Linzell, 2003). At 11:00 h, the pups were weighed (w2), returned to their dams 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 with a correction for weight loss due to metabolic processes in the pups as (w2-w1)/4. For Group IV that received 200 mg/kg ethyl acetate fraction H. sabdariffa. 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 Group III was followed. For measurement of milk yield 23 h after gavage, the pups were subsequently isolated between 12:00 and 16:00 h. After weighing at 1600 h (w4), they were reunited with their dams for 1 h of feeding and, finally, they were weighed (w5) (Sampson and Jansen, 1984; Ouedraogo et al., 2004). They were subsequently left with their dams during the night. Milk yield 23 h after gavage was estimated as w5-w4 with a correction for weight loss due to metabolic processes in the pups as [(w2 - w1) + (w4 - w3)]/8.

Prolactin analysis: The animals were then euthanized on day 18, at the termination of the experiment; they were anaesthetized by chloroform inhalation in a closed chamber and thereafter sacrificed. The thorax of each anaesthetized animal was cut open and with the aid of 5 mL syringe with 21 Gauge needle, the pulsating heart of the rat was pierced at the left ventricle and blood was aspirated and immediately centrifuged to obtain serum for the determination of prolactin. The analysis was conducted in the Department of Chemical Pathology Ahmadu Bello University Teaching Hospital, Shika-Zaria. The prolactin species specific Enzyme-Linked Immunosorbent Assay (ELISA) kit ALPCO Diagnostics® Limited, 29-AH-R011 US in micro-plate was designed for the quantitative evaluation of rat prolactin. The micro-plate is coated with a first monoclonal antibody specific for rat prolactin. Calibrators and samples are pipetted into the antibody coated micro-plate. During 2 h incubation endogenous rat prolactin in the sample bind to the antibodies fixed on the inner surface of the wells. Non-reactive sample components are removed by a washing step. Afterwards, a second polyclonal horseradish peroxidase-labeled antibody, directed against another epitope of the prolactin molecule, was added. During 1 h incubation, a sandwich complex consisting of the two antibodies and the rat prolactin is formed. An excess of enzyme conjugate is washed out. A chromogenic substrate, TMB (3, 3', 5, 5'-Tetra-Methyl-Benzidine), was added to all the wells. During 30 min incubation, the substrate is converted to a colored end product (blue) by the fixed enzyme. Enzyme reaction is stopped by dispensing of hydrochloric acid as stop solution. The color intensity is direct proportional to the concentration of rat prolactin present in the sample. The optical density of the color solution is measured with a micro-plate reader at 450 nm. A standard curve was
obtained by plotting the concentration of the standard versus the absorbance. The PRL concentration of the specimens and controls run concurrently with standards were calculated from standard curve. The lowest detectable level of prolactin with this test was 0.6 ng/mL and a range of 6.64-23.26 ng/mL for male, 13.7-23.3 ng/mL for female (Dombrwicz et al., 1992).

**Data analysis:** All data are expressed as mean±standard of error mean (Mean±S.E.M.). The data obtained were analyzed using t-test student-Newman Keul’s test (Betty and Jonathan, 2003), one way Analysis of Variance (ANOVA), SPSS package version 20.0 and post hoc test for multiple comparisons. The (p<0.05) was accepted as significant.

**RESULTS**

**Toxicity studies:** The plant seed fractions are characterized by a very low degree of toxicity. The acute toxicity LD$_{50}$ of ethyl acetate fraction of *Hibiscus sabdariffa* L. seed in albino rats was found to be above 5000 mg/kg according to the method of Lorke (1983).

**Milk production:** Milk production of both groups receiving 100 and 200 mg/kg of ethyl acetate fraction of *Hibiscus sabdariffa* L. seed was higher than that of the control group as shown Fig. 1. Milk yield increased from 1.56±0.2, 1.92±0.3 and 2.08±0.2 g/pup/day to about 5.26±0.1, 5.92±0.3 and 6.78±0.2 g/pup/day for the control and ethyl acetate group respectively. The significant differences observed started from day 4 until the end of treatment day 17 (p<0.01). The mean milk yield for the control group was 3.02±0.4, 3.83±0.3 and 4.36±0.4 g/pup/day throughout the experimental period respectively (p<0.05) as shown in Fig. 2. Milk production data at 18 and 23 h after gavage showed that milk production was significantly in all groups receiving ethyl acetate fraction at both time points. The mean milk yield for the control group was 0.42±0.02 and 0.52±0.03 g/pup at 18 and 23 h after gavage with normal saline respectively. For the group receiving the ethyl acetate fraction, the milk yield was 0.64±0.04 and 0.72±0.03 g/pup at 18 and 23 h after treatment, respectively. In rats, blood prolactin concentrations begin to rise within 1-3 min of initiation of nursing, peak within 10 min, are sustained at a constant level as long as nursing continues and fall when nursing is terminated (Freeman et al., 2000).

**Body weight:** All pups gained weight during the study period and the rate of weight gain for the ethyl acetate fraction groups was significantly higher than the control as shown in Fig. 3. Body weight increased from 6.8±0.13 to 30.79±1.23 g/pup/day for the control, from 7.82±0.21 to 32.29±1.41 g/pup/day for those receiving 100 mg and from 9.64±0.14 to 35.24±1.23 g/pup/day for those receiving 200 mg.
Prolactin level of lactating female rats as shown in Hibiscus sabdariffa body of the dams was seen. Hibiscus is an important component in lactation research. The measurement of milk production rates in rats is difficult, (Morag et al., 1975; Sampson and Jansen, 1984) however a more feasible direct method is to use the pups to remove the milk from the dam and to determine the amount of milk sucked by the litter (Morag et al., 1975). Milk yield estimations for rats by means of pup weight and weight gains have been used in several studies (Morag et al., 1975; Sampson and Jansen, 1984; Kamani et al., 1987; Kim et al., 1998).

The purpose of this study was essentially to determine whether ethyl acetate fraction of Hibiscus sabdariffa seed is the lactogenic components responsible for milk production. Milk production was significantly higher in the ethyl acetate-treated group than in the controls. In addition, milk yield appears to be significantly stimulated about 23 h after administration of the extract and the pup growth rate was significantly improved. The ethyl acetate fraction of Hibiscus sabdariffa L. seed produced an appreciable increase in serum prolactin level when compared to the control with potency higher than the standard drug metoclopramide. The ethyl acetate fraction of seed of Hibiscus sabdariffa L. exhibited a lactogenic activity by increasing the serum prolactin in lactating rats. The effect of Ethyl acetate fraction may be responsible for the lactogenic activity displayed by the aqueous seed extract of Hibiscus sabdariffa L. (Okasha et al., 2008). Human breast milk is widely accepted to be the optimal source of nutrition for the newborn infant, containing all the proteins, lipids, carbohydrates, micronutrients and trace elements required for growth, development and immune protection (Ostrom, 1990; Taffetani et al., 2007). Herbs and seeds has been reported in other plants (Asparagus racemosus, fennel seed, Grape sap, milk thistle and goat’s rue) (Joglekar et al., 1967; Narendranath et al., 1986; Sholapurkar, 1986; Oketcho-Rabah, 1998; Goyal et al., 2003) to have lactogenic effect. The presence of Cardiac glycosides, Saponins and Steroidal ring in higher concentration in seed extract of Hibiscus sabdariffa L. may be responsible for the lactogenic effect of ethyl acetate fraction. The mechanism through which Hibiscus sabdariffa L. exerted its effect might be by dopaminergic influence, as dopamine receptor antagonist (Ben-Jonathan, 1985), since dopamine blocked the largest dose. It can therefore be inferred that ethyl acetate fraction of Hibiscus sabdariffa L. effectively stimulates milk production as well as prolactin synthesis and release in the rat. Milk production at 23 h after gavage and milk yield of pup per day for 200 mg/kg is more appreciably higher than control. Therefore, the traditional belief that Hibiscus sabdariffa L. seed decoction can improve milk production in lactating women may be valid. Hibiscus sabdariffa L. seed is essential to the synthesis and secretion of prolactin and milk production.

![Fig. 4: Mean weight gain of pup was very significant (p<0.01) when control compared to treated group](image)

Table 1: Serum prolactin levels in the control, metoclopramide-treated and ethyl acetate-treated groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Prolactin ng/mL</th>
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<tbody>
<tr>
<td>Control</td>
<td></td>
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<tr>
<td>Norma saline</td>
<td>18.55±0.5</td>
</tr>
<tr>
<td>Metoclopramide</td>
<td></td>
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<tr>
<td>5 mg/kg</td>
<td>24.83±0.7*</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td></td>
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<tr>
<td>100 mg/kg</td>
<td>24.15±0.2**</td>
</tr>
<tr>
<td>200 mg/kg</td>
<td>28.37±0.7**</td>
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*: (p<0.05); **: (p<0.01)

The accurate estimate of milk yield in the rat is an important component in lactation research. The
REFERENCES


