

Research Article

Lactogenic Study of the Effect of Ethyl-acetate Fraction of *Hibiscus sabdariffa* Linn (*Malvaceae*) Seed on Serum Prolactin Level in Lactating Albino Rats

¹I.G. Bako, ²M.S. Abubakar, ³M.A. Mabrouk and ¹A. Mohammed

¹Department of Human Physiology, Faculty of Medicine,

²Department of Pharmacognosy and Drug Development, Faculty of Pharmaceutical Sciences, Ahmadu Bello University Zaria, Nigeria

³Department of Medical Physiology, Faculty of Medicine, Al-Azhar University Cairo, Egypt

Abstract: Lactogenic effect of ethyl acetate fraction of *Hibiscus sabdariffa* seed was evaluated on serum prolactin and milk production in lactating albino rats. Twenty four lactating rats were grouped randomly at parturition into control, metoclopramide-treated and Ethyl-acetate-treated group consist of six rats in each group (n = 6). The lactating rats were administered control (normal saline), metoclopramide (5 mg/kg) and ethyl-acetate fraction (100 and 200 mg/kg) respectively from day 3-17 of lactation. Milk yield for rats were estimated by pup weight and weight gain. The animals were then euthanized on the day 18 and serum prolactin was analyzed using prolactin kit. The prolactin level of ethyl-acetate fraction of *Hibiscus sabdariffa* showed a significant increase (p<0.01) when compared to control group. Pup weight gain was also significantly higher (p<0.05) than the control group. This can be deduced that ethyl-acetate fraction of *Hibiscus sabdariffa* L. seed has lactogenic activity because it increases serum prolactin level and milk production in lactating female albino rats. *Hibiscus sabdariffa* L. seed stimulate prolactin synthesis; release and milk production in albino rats and it is affordable and safe for consumption.

Keywords: Dam, ethyl acetate, *Hibiscus sabdariffa* L., lactation, milk, prolactin, pups

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 breastfed 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; Olaleye, 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 IQS, 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

Corresponding Author: I.G. Bako, Department of Human Physiology, Faculty of Medicine, Ahmadu Bello University Zaria, Nigeria, Tel.: +234 803 698 2739

This work is licensed under a Creative Commons Attribution 4.0 International License (URL: <http://creativecommons.org/licenses/by/4.0/>).

in November 2010. The plant was identified in the Department of Biological Sciences, Ahmadu Bello University, Zaria by a taxonomist authenticated with a voucher number 1056 and deposited in the Herbarium section of the Department of Biological Sciences, Ahmadu Bello University Zaria, Nigeria. Extraction and Fractionation was conducted in Department of Pharmacognosy and drug development, Ahmadu Bello University Zaria. Extraction was done using maceration method while the crude aqueous extracts were fractionated by using Ethyl acetate and n-Butanol reagents.

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 (Dombrowicz *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 LD₅₀ 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 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

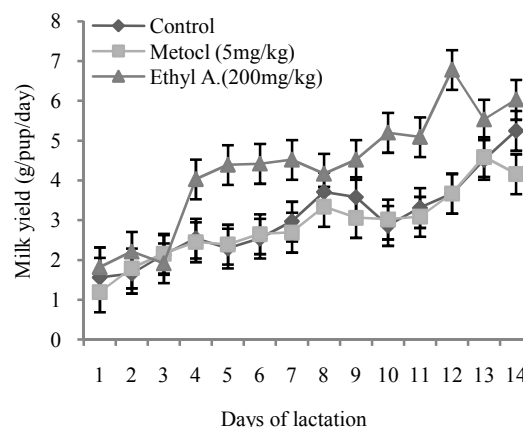


Fig. 1: Effect of ethyl acetate fraction *Hibiscus sabdariffa* L. on milk production 18 h after administration, there was significant difference ($p < 0.01$) throughout the period of administration for 200 mg/mL, while significant differences was not recorded throughout the period of administration for 100 mg/mL

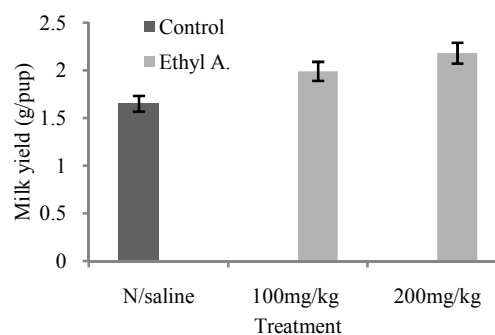


Fig. 2: Mean milk production per day was significant for 100 mg/mL ($p < 0.05$) and 200 mg/mL ($p < 0.01$)

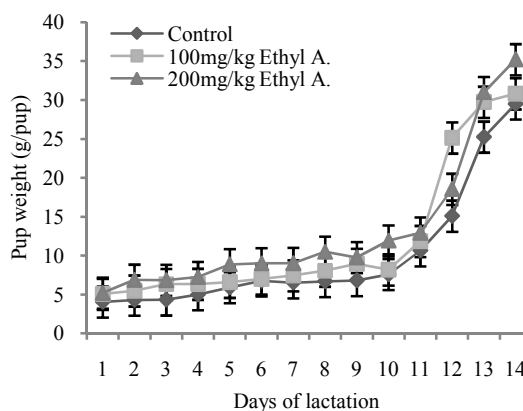


Fig. 3: Effect of ethyl acetate fraction *Hibiscus sabdariffa* L. on pup weight 18 h after administration. There was significant difference ($p < 0.05$) between control and treated group

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

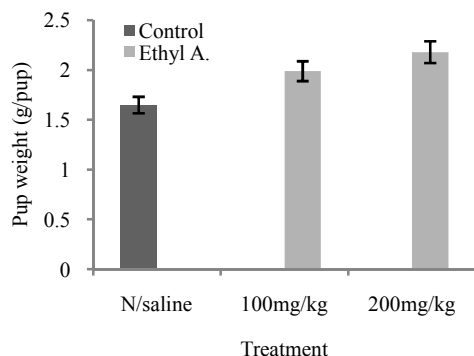


Fig. 4: Mean weight gain of pup was very significant ($p < 0.01$) when control compared to treated group

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

Groups	Prolactin ng/mL
Control	
Norma saline	18.55±0.5
Metoclopramide 5 mg/kg	24.83±0.7*
Ethyl acetate	
100 mg/kg	24.15±0.2**
200 mg/kg	28.37±0.7**

*: ($p < 0.05$); **: ($p < 0.01$)

for receiving 200 mg of ethyl acetate fraction of *Hibiscus sabdariffa* L. The daily average weight gain was 1.57 ± 0.05 , 1.96 ± 0.06 and 2.25 ± 0.08 g/pup, respectively, as shown in Fig. 4. There was a significant difference between all ethyl acetate fraction groups and the control ($p < 0.05$). While no significant effect on the body of the dams was seen.

Prolactin: The results obtained in this experiment showed that the Ethyl acetate seed fractions of *Hibiscus sabdariffa* L. have increased serum prolactin level significantly ($p < 0.01$) in lactating albino rats as shown in Table 1. Ethyl acetate fraction for 100 and 200 mg increased the serum prolactin significantly to 24.15 ± 0.2 and 28.37 ± 0.7 ng/mL, respectively, while the control that received normal saline have the prolactin value of 18.55 ± 0.5 ng/mL. In regard to metoclopramide effect on serum prolactin level, it also produced an appreciable (24.82 ± 0.7 ng/mL) increase in prolactin level when compared to the normal saline treated group. This means that both of metoclopramide and the ethyl acetate fraction had similar effect on serum prolactin level of lactating female rats as shown in Table 1 this implies that the potency of the ethyl acetate fraction is almost the same or higher than the standard drug metoclopramide.

DISCUSSION

The accurate estimate of milk yield in the rat 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; Oketch-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.

REFERENCES

- Alexandre, G.Z.R., M.S. Jose Jr, L.A.M. Eduardo, J.S. Manuel, M.O. Richardo, A.H. Mauro, R.D.L. Geraldo and C.B. Edmund, 2002. Metoclopramide- induced hyperprolactinemia affects mouse endometrial morphology. *Gynecol. Obstet. Inves.*, 54: 185-190.
- Ann, H. and J.L. Linzell, 2003. A simple technique for measuring the rate of milk secretion in the rat. *Comp. Biochem. Phys. A*, 43(2): 259-270.
- Ben-Jonathan, N., 1985. Dopamine: A prolactin-inhibiting hormone. *Endocr. Rev.*, 6: 564-589.
- Ben-Jonathan, N. and J.W. Liu, 1992. Pituitary lactotrophs: Endocrine, paracrine, juxtacrine and autocrine interactions. *Trends Endocrin. Met.*, 3(7): 254-258.
- Bern, H.A. and C.A. Nicoll, 1968. The comparative endocrinology of prolactin. *Recent Prog. Horm. Res.*, 24: 681-720.
- Betty, R.K. and A.C. Jonathan, 2003. *Essential Medical Statistics*. 2nd Edn., Blackwell Science, USA, pp: 15-409.
- Chen, C.C., J.D. Hsu, H.C. Wang, M.Y. Yang, E.S. Kao, Y.O. Ho and C.J. Wang, 2003. *Hibiscus sabdariffa* extract inhibit the development of atherosclerosis in cholesterol-fed rabbits. *J. Agric. Food Chem.*, 51(18): 5472-5477.
- Dalziel, T.M., 1973. *The Useful Plants of West Tropical Africa*. 3rd Edn., Watmough Ltd., Idle Bradford and London, pp: 526-530.
- Dombrowicz, D., B. Sente, J. Closset and G. Hennen, 1992. Dose-dependent effects of human prolactin on immature hypox rats testis. *Endocrinology*, 130: 695-700.
- Freeman, M.E., K. Bela, A. Lerant and G. Nagy, 2000. Prolactin: Structure, Function and regulation of secretion. *Physiol. Rev.*, 80: 1523-1631.
- Goyal, R.K., J. Singh and L. Harbans, 2003. *Asparagus racemosus* an update, review. *Indian J. Med. Sci.*, 57(9): 408-414.
- Joglekar, G.V., R.H. Ahuja and J.H. Balwani, 1967. Galactogogue effect of *Asparagus racemosus*. *Indian Med. J.*, 61: 165.
- Kamani, H.T., E.H. Karunanayake and M.P.D. Mahindartna, 1987. Evaluation of galactoguc activity of *Asparagus falcatus*. *Ceylon J. Med. Sci.*, 30: 63-67.
- Kim, S.H., Y.S. Moon, W.L. Keller and C.S. Park, 1998. Compensatory nutrition-directed mammary cell proliferation and lactation in rats. *Brit. J. Nutr.*, 79: 177-183.
- Lorke, D., 1983. A new approach to practical acute toxicity testing. *Arch. Toxicol.*, 54: 275-287.
- Morag, M., F. Popliker and R. Yagil, 1975. Effect of litter size on milk yield in the rat. *Lab. Anim.*, 9: 43-47.
- Morton, J.F., 1987. Roselle. In: Dowling, C.F. (Ed.), *Fruits of Warm Climate*. Media, Inc., Greensboro, NCP, pp: 281-286.
- Narendranath, K.A., S. Mahalingam, V. Anuradha and I.S. Rao, 1986. Effect of herbal galactogogue (Lactare) a pharmacological and clinical observation. *Med. Surg.*, 26: 19-22.
- Okasha, M.A.M., M.S. Abubakar and I.G. Bako, 2008. Study of the effect aqueous *Hibiscus sabdariffa* L. seed extract on serum prolactin level in lactating albino rats. *Eur. J. Sci. Res.*, 22(4): 575-583.
- Oketch-Rabah, H.A., 1998. Phytochemical constituents of the genus asparagus and their biological activities. *Hamdard*, 41: 33-43.
- Olaleye, M.T., 2007. Cytotoxicity and antibacterial activity of methanolic extract of *Hibiscus sabdariffa*. *J. Med. Plants Res.*, 1(1): 009-013.
- Ostrom, K.M., 1990. A review of the hormone prolactin during lactation. *Prog. Food Nutr. Sci.*, 14(1): 1-43.
- Ouedraogo, Z.L., D.V. Heide, E.M.V. Beek, H.J.M. Swarts, J.A.M. Mattheij and L. Sawadogo, 2004. Effect of aqueous *Acacia nilotica* spp *adansonii* on milk production and prolactin release in the rat. *J. Endocrinol.*, 182: 257-266.
- Sampson, D.A. and G.R. Jansen, 1984. Measurement of milk yield in lactating rat from pup weight and weight gain. *J. Pediatr. Gastr. Nutr.*, 3: 613-617.
- Sholapurkar, M.L., 1986. Lactare-for improving lactation. *Indian Practitioner.*, 39: 1023-1026.
- Taffetani, S., G. Shannon, H. Francis, D. Sharon, U. Yoshiyuki, D. Alvaro, M. Luca, M. Marco, G. Fava, J. Venter, V. Shelley, V. Bradley, P. Ian, H.L. Vien, G. Eugenio, C. Guido, B. Antonio and G. Alpini, 2007. Prolactin stimulates the proliferation of normal female cholangiocytes by differential regulation Ca^{2+} -dependent PKC isoforms. *BMC Physiol.*, 7: 6.
- Vogel, H.G. and H.W. Vogel, 1997. *Drug Discovery and Evaluation: Pharmacological Assays*. Springer, Berlin, pp: 645-670.
- Wang, C.J., J.M. Wang, W.L. Lin, C.Y. Chu, F.P. Chou and T.H. Tseng, 2000. Protective effect of *Hibiscus anthocyanins* against tert-butyl hydroperoxide-induced hepatic toxicity in rats. *Food Chem. Toxicol.*, 38(5): 411-416.