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Study of the Effect of Aqueous *Hibiscus Sabdariffa Linn* Seed Extract on Serum Prolactin Level of Lactating Female Albino Rats

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Abstract

The effects of different doses of aqueous *H. sabdariffa l.* seed extract were examined on serum prolactin level in four groups of lactating female rats. Control, metoclopramide-treated and extract plus dopamine-treated groups consisted of five rats each (n=5). The extract-treated group was sub-divided into five sub-groups of five rats each and accordingly they were administered the extract in different five dose concentrations, one group for each dose. Female lactating rats were administered the extract (100, 200, 400, 800 and 1600 $mgkg^{-1}$ orally), metoclopramide (5 $mgkg^{-1}$orally), extract (1600 $mgkg^{-1}$) + dopamine (5 μgkg^{-1} intraperitoneally), while control received normal saline (orally) from day 4-9 of lactation. The animals were then euthanized on the day 10 and serum prolactin levels were analyzed using prolactin kit. The serum prolactin level of the extract-treated rats showed a dose – dependent significant increase ($P<0.01$) when compared to control group. The extract plus dopamine-treated group did not show any change in serum prolactin level when compared to the control group. The LD_{50} of *Hibiscus sabdariffa l.* extract was found to be above $5000mgkg^{-1}$. Administration of the extract in a dose of 1g for four weeks did not show any significant changes in liver or renal functions of the treated rats.

Keywords: *H. sabdariffa*, extract, acute study, lactation, prolactin, dopamine.

1. Introduction

For ages plants have been a good source of food and they provide essential nutritional values, medicinal properties and notable physiological effect to life (Dalziel, 1973). *Hibiscus sabdariffa linn* (family: Malvaceae) is a herb that is cultivated for leaf, fleshy calyx, seed or fiber (Dalziel, 1973). *Hibiscus sabdariffa l.* is growing in all parts of the world and it is taken as a common local drink popularly known as zobo in Nigeria. It is a medicinal herb, used in folk medicine in treatment of hypertension (Wang *et al.,* 2000; Odigie *et al.,* 2003). *Hibiscus* anthocyanin, a group of phenolic natural pigments present in the dried flower of *Hibiscus sabdariffa* and *Hibiscus rosasinensis*, have been found to have cardioprotective (Jonadet,1990; Olaleye, 2007), hypocholesterolemic (Chen *et al.*, 2003; Powers, 1999; Olaleye, 2007), anti-oxidative and hepatoprotective (Wang *et al.,* 2000; Amin and Hamza, 2005) effects in animals.

Delphinidin 3-sambubioside, a *Hibiscus* anthocyanin, induces apoptosis in human leukemia cells through oxygen reactive species-mediated mitochondrial pathway (Hou *et al*., 2005). Polysaccharides from *Hibiscus sabdariffa* flowers stimulate proliferation and differentiation of human keratinocytes (Brunold *et al.,* 2004). *Hibiscus* protocatechuic acid has inhibitory and inductive effect on tumour promotion in mouse skin and in human leukemia cells respectively (Tseng *et al.*, 1998). *Hibiscus sabdariffa l.* has been reported to be antiseptic, aphrodisiac, astringent, cholagogue, demulcent, digestive, diuretic, emollient, purgative, refrigerant, sedative, stomachic and tonic (Morton, 1987; Olalelye, 2007). Lactating effect of herbs, seeds and micronuttrients has been reported in many plants (Asparagus racemosus, fennel seed, Grape sap, milk thistle and goat's rue) (Garcia and Adams, 2005; Winyo *et al.*, 2004; Oketch-Rabah, 1998; Narendranath *et al.,* 1986; Sholapurkar, 1986). In Nigeria, a decoction of the seeds is given to enhance or induce lactation in cases of poor milk production, poor letdown and maternal mortality.

However, there is dearth of literature supporting the use of seeds decoction in enhancement and induction of milk during lactation. Breastfeeding in the first six months of life stimulates babies' immune systems and protects them from diarrhea and acute respiratory infections (UNICEF, 2006). Based on the overwhelming advantage of breast milk as an infant's source of nutrition coupled with a number of women who have lactation insufficiency due to prolactin deficiency, additional medication to augment lactation without side effects is needed (Freeman *et al.,* 2000). In lights of this, the study is designed to evaluate the effect of *Hibiscus sabdariffa* seed extract on serum prolactin level in animal models.

2. Materials and Methods

2.1. Chemicals and drugs

Prolactin Elisa 96 test kits (Fortress Diagnostics Limited, BT41 IQS, UK), Metoclopramide (NAFDAC Reg. no. 04-5946) and Dopamine (Aldrich Chemical Co.).

2.2. Plant material

The samples of *Hibiscus sabdariffa l.* seed were collected in Gaya Hong Local Government in Adamawa state of Nigeria in November 2005. The plant was identified in the Department of Biological Sciences, Ahmadu Bello University, Zaria by Mallam Musa Ibrahim and authenticated with a voucher number **1056** and deposited in the Herbarium section of the Department of Biological Sciences, Ahmadu Bello University Zaria, Nigeria. Extraction was conducted using maceration method in Department of Pharmacognosy and drug development, Ahmadu Bello University Zaria.

2.3. Preparation of the plant extract

100g of the powdered seeds were soaked with 2.5L of distilled water and the mixture was then shaken for ten hours with mechanical shaker. The mixture (extract) was filtered through a plug of cotton or

glass wool. The process was repeated exhaustively for complete extraction. The extract filtrate was then concentrated over the water bath at the temperature of 40ºC-45ºC and an amber solid extract weighing 8.4g was obtained.

2.4. Animals

Forty female albino rats weighing 120-160g 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 12h dark/light cycle so that they can become pregnant. They were fed with commercial feeds and tap water ad libitum. Following birth, the litters' weights were recorded and culled to 6 litters per dam. The forty lactating rats were randomly divided into four main groups (control, metoclopramide-treated, extract-treated and extract plus dopamine-treated groups). Control, metoclopramide-treated and extract plus dopamine-treated groups consisted of five rats each (n=5), while the extract-treated group was sub-divided into five sub-groups of five rats each (n=5). Accordingly, the extract was administered in five different doses: 100, 200, 400, 800 and 1600 mgkg-1. All groups had received the extract and the drug for six days starting from day 4 to day 9 of lactation (Vogel and Vogel, 1997). The extract and the drugs were administered orally except for dopamine which was injected intraperitoneally. The animals were then euthanized on day 10 and heart blood samples were analyzed using prolactin kit.

2.5. Measurement of Prolactin Concentration

Serum prolactin levels in heart blood samples were analyzed using ELISA kit at the time the animals were euthanized.

2.6. Statistical analysis

All data are expressed as mean ± standard of error mean (Mean ± S.E.M.). The data obtained were analyzed using one way analysis of variance (ANOVA) and student-Newman Keul's test (Betty and Jonathan, 2003) *post hoc* test for multiple comparisons. The ($P<0.05$) will be accepted as significant.

3. Results

3.1. Phytochemical analysis

The preliminary phytochemical analysis of the aqueous seed extract of *Hibiscus sabdariffa l.* revealed the presence of varying amount of alkaloids, saponins, tannins, anthraquinones, cardiac glycosides, cardenoledes, flavonoids and phlobatanins in the concentrations shown in table (4).

Table 4: Phytochemical screening of aqueous extract of Hibiscus sabdariffa l. seeds.

Extract constituents	Concentrations
Alkaloids	++
Anthraquinones	+
Cardenoledes	+
Cardiac glycosides	++
Deoxy sugar	+
Flavonoids	+
Phlobatannins	+
Saponins	+++
Steroidal ring	+ +
Tannins	+

+++ = High concentration was recorded if a definite heavy precipitate or flocculation observed;
++ = Moderate concentration was recorded if a definite turbidity, but no flocculation observed.
+ = Low concentration was recorded if the reagent produced only a slight opaqueness.
- = Not detected (Faraz *et al.*, 2003).

3.2. Toxicity studies

The plant extracts are characterized by a very low degree of toxicity. The acute toxicity LD_{50} of *Hibiscus sabdariffa l.* seed extract in albino rats was found to be above 5000 $mgkg^{-1}$ according to the method of Lorke (1983). In sub-chronic toxicity of *Hibiscus sabdariffa l.* the index of chronic studies was done by accessing the liver enzymes and kidney metabolites as indicators for liver and renal function tests (Sandow, 1979). In liver function tests the mean values of the following enzymes Aspartate aminotransferase (AST; SGOT), Alanine aminotransferase (ALT; SGPT), Alkaline phosphatase, Bilirubin and Albumin were within the normal ranges as shown in table (5). Also, the levels of urea and creatinine, as indicators for renal function were within the normal range as shown in table (6).

Table 5: Showing serum levels of Alanine aminotransfrease (**ALT**), Aspartate amino-transferase (**AST**), Alkaline phosphatase (**ALK**), Bilirubin and Albumin in control and extract-treated groups. Normal ranges of liver function tests were listed.

Groups / Parameters	Normal Range of Enzymes IUL^{-1}	Control Normal saline (5)	Extract – treated group			
			10$mgkg^{-1}$ (5)	100$mgkg^{-1}$ (5)	500$mgkg^{-1}$ (5)	1000$mgkg^{-1}$ (5)
ALT (SGPT)	16-40	28.25±1.7	24.75±0.8^{S}	25.75±0.7^{NS}	23.00±0.9^{S}	35.75±1.4^{S}
AST (SGOT)	6-25	23.50±2.2	19.00±0.9^{S}	23.00±1.4^{NS}	21.25±1.4^{NS}	22.00±0.9^{NS}
ALK	21-92	61.5±3.8	65.5±3.3^{NS}	63.5±3.8^{NS}	68.5±4.1^{S}	76.5±3.5^{S}
Bilirubin	4-17	7.2±1.1	8.5±2.1^{NS}	8.6±2.2^{NS}	9.1±1.2N^{S}	9.5±1.5^{NS}
Albumin	30-52	41.25±2.7	42.0±2.6^{NS}	40.75±2.3^{NS}	32.00±1.8^{S}	41.25±2.2^{NS}

- Number of animals is represented in between brackets.
- Not Significant=NS;
- Significant=S (P<0.05);

Table 6: Showing serum level of **urea** and **creatinine** in control and extract-treated groups. Normal ranges of renal function tests were listed.

Groups / Parameters	Normal Range of enzymes IUL^{-1}	Control Normal saline (5)	Extract – treated group			
			10$mgkg^{-1}$ (5)	100$mgkg^{-1}$ (5)	500$mgkg^{-1}$ (5)	1000$mgkg^{-1}$ (5)
Urea	2.5-6.5	4.33±0.2	4.10±0.4^{NS}	4.85±0.5^{NS}	4.98±0.2^{NS}	3.75±.2^{NS}
Creatinine	9-126	65.25±2.5	62.25±2.9^{NS}	75.75±2.1^{S}	82.00±4.3^{S}	71.00±4.5^{S}

- Number of animals is represented in between brackets.
- Not Significant=NS;
- Significant=S (P<0.05).

3.3. Evaluation of serum prolactin level

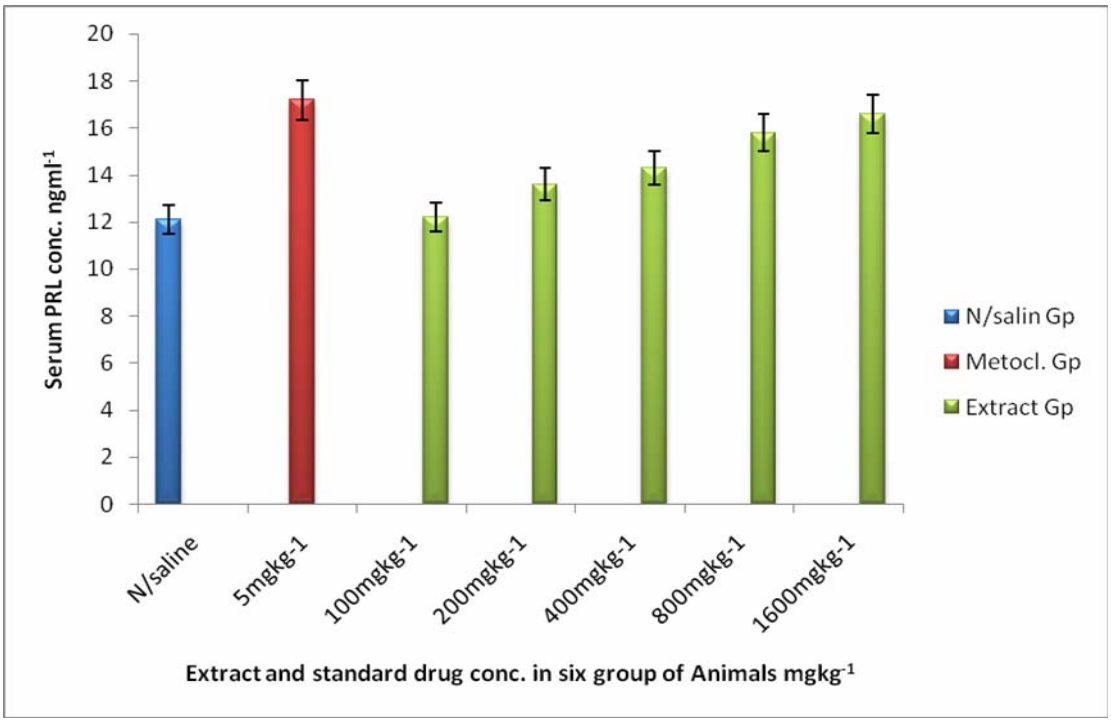
The results obtained in this experiment showed that the seed extract of *Hibiscus sabdariffa l.* have increased serum prolactin level significantly in lactating albino rats. The first dose concentration (100$mgkg^{-1}$) did not show any significant difference. In the meantime, the other dose concentrations of the extract (200, 400, 800 and 1600$mgkg^{-1}$) reported marked increase in prolactin level as compared to the control group. It is noticed that the increase is dose-dependent as shown in table (1) and fig. (1). In regard to metoclopramide effect, it reported an appreciable increase in serum prolactin level as compared to control group as illustrated in table (1) and fig. (1).

Table 1: Showing serum prolactin levels in the control, metoclopramide-treated and extract-treated groups.

Groups / Parameter	Control Normal saline (5)	Metoclopramide-treated group5$mgkg^{-1}$ (5)	Extract-treated group				
			100 $mgkg^{-1}$ (5)	200 $mgkg^{-1}$ (5)	400 $mgkg^{-1}$ (5)	800 $mgkg^{-1}$ (5)	1600 $mgkg^{-1}$ (5)
Prolactin ($ngml^{-1}$)	12.1 ±0.2	17.18 ±0.4	12.2 ±0.2	13.6 ±0.3	14.3 ±0.2	15.8 ±0.4	16.6 ±0.2
Level of significance		S**	NS	S*	S**	S**	S**

- Number of animals is represented in between brackets.
- Not Significant=NS;
- Significant=S* (P<0.05);
- Significant=S** (P<0.01).

Figure 1: Showing serum prolactin level in control, metoclopramide-treated and *Hibiscus sabdariffa l.* seed extract-treated groups in lactating Albino rats.



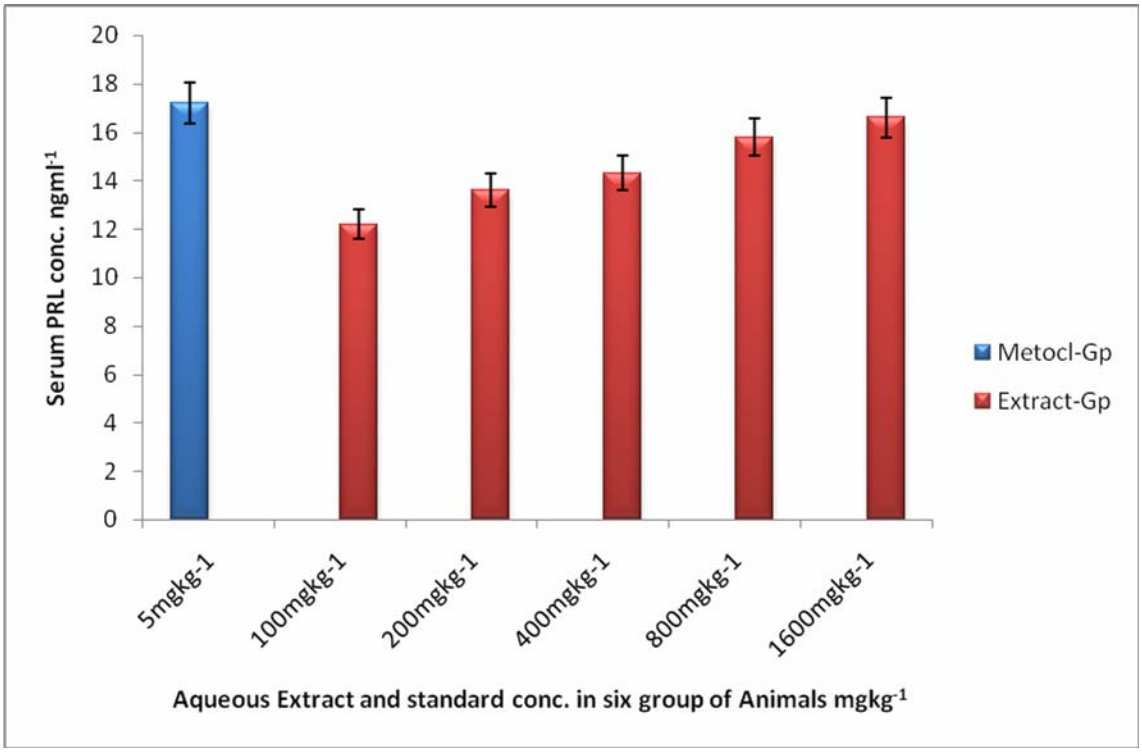
In regard to metoclopramide effect on serum prolactin level, it produced an appreciable increase in prolactin level as compared to the *Hibiscus sabdariffa l* extract-treated rats in the dose concentrations of 100, 200, 400 and 800mgkg^{-1} body weight. As regards the largest dose of the extract (1600mgkg^{-1}), it showed no significant difference in serum prolactin level when compared to metoclopramide-treated group. This means that both of metoclopramide and the largest dose of the extract had similar effect on serum prolactin level of lactating female rats as shown in table (2) and fig. (2). This implies that the potency of the extract at the largest dose is almost the same with metoclopramide.

Table 2: Showing serum prolactin level in metoclopramide-treated and extract-treated groups.

Groups / Parameter	Metoclopramide-treated group 5mgkg^{-1} (5)	Extract-treated group				
		100 mgkg^{-1} (5)	200 mgkg^{-1} (5)	400 mgkg^{-1} (5)	800 mgkg^{-1} (5)	1600 mgkg^{-1} (5)
Prolactin (ngml^{-1})	17.18±0.4	12.2±0.2	13.6±0.3	14.6±0.2	15.8±0.4	16.6±0.2
Level of significance		S**	S**	S**	S*	NS

- Number of animals is represented in between the brackets.
- Not Significant=NS;
- Significant=S* (P<0.05);
- Significant=S** (P<0.01).

Figure 2: Showing serum prolactin level of metoclopramide-treated and extract-treated group in lactating Albino rats.



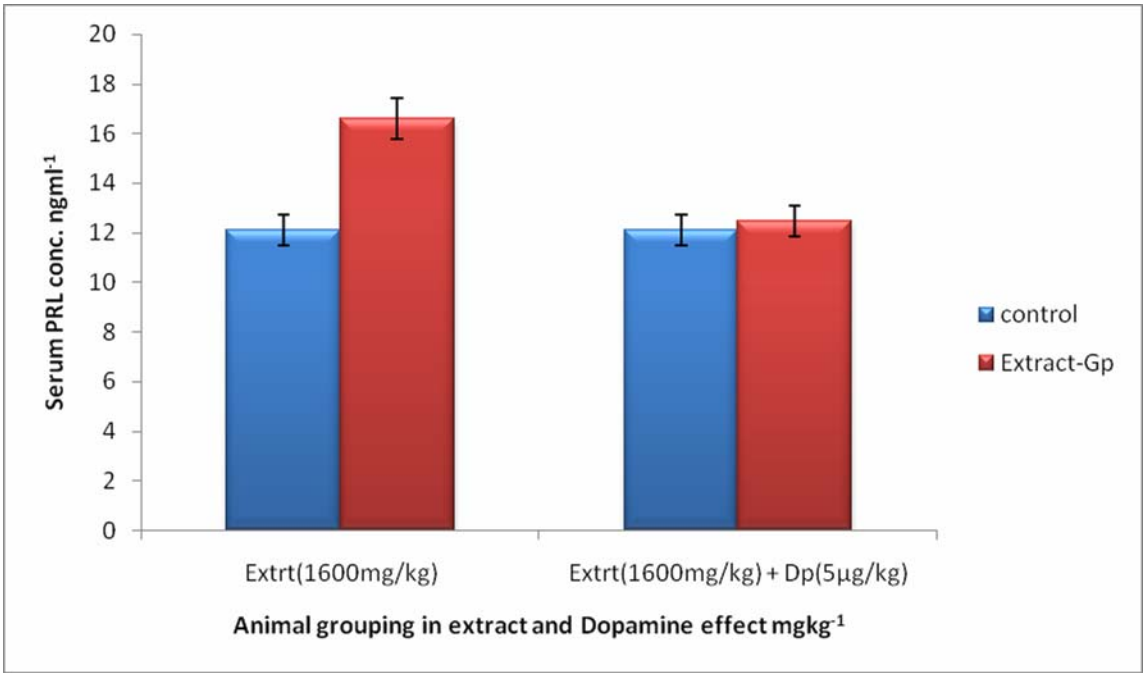
The largest dose of the extract (1600mg/kg) plus dopamine reported no change (P>0.05) in serum prolactin level when compared to the control group, while without combination with dopamine, it showed marked increase (P<0.01). This implies that dopamine has almost inhibited completely the effect of the extract as shown in table (3) and fig. (3), and that dopamine and its agonists have blocked the seed extract effect mostly back to the control level.

Table 3: Showing serum prolactin level in control, extract-treated and extract plus dopamine-treated groups.

Groups Parameter	**Control Normal saline (5)**	**Extract-treated group 1600mgkg^{-1} (5)**	**Extract-treated group (1600mgkg^{-1}) plus Dopamine (5µgkg^{-1}). (5)**
Serum Prolactin level (ngml^{-1})	12.1±0.2	16.6±0.2	12.48±0.1
Level of significance		S**	NS

- Number of animals is represented in between the brackets.
- Not Significant=NS;
- Significant=S* (P<0.05);
- Significant=S** (P<0.01).

Figure 3: Showing serum prolactin level of control, extract-treated and extract plus dopamine-treated groups in lactating Albino rats.



4. Discussion

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). The results of the present study reported that, the aqueous seed extract of *Hibiscus sabdariffa l.* produced an appreciable increase in serum prolactin level when compared to the control in a dose-dependent manner. The seed extract of *H. sabdariffa l.* exhibited a lactogenic activity by increasing the serum prolactin level in lactating rats. The lactogenic activity displayed by the largest dose of aqueous seed extract (1600mgkg^{-1}) when compared with that of metoclopramide (commercial hyperprolactinemia-inducing agent) showed no significant statistical difference in serum prolactin level. This means that both of metoclopramide and the largest extract dose have similar effect on serum prolactin level in lactating female rats as shown in table (2) and fig. (2). This implies that the potency of the extract at largest dose is almost the same with metoclopramide. Lactogenic effect of herbs and seed has been reported in other plants (Asparagus racemosus, fennel seed, Grape sap, milk thistle and goat's rue) (Garcia and Adams, 2005; Goyal *et al.*, 2003; Oketch-Rabah, 1998; Narendranath *et al.*, 1986; Sholapurkar, 1986). The presence of steroidal saponins and sapogenins constituents contributes in the lactogenic effect of Asparagus racemosus (Goyal *et al.*, 2003; Oketch-Rabah, 1998). In the same vein the presences of saponins, tannins, alkaloids and flavonoids in *Hibiscus sabdariffa l.* may be responsible for the increase in serum prolactin level. The mechanism through which *Hibiscus sabdariffa l.* exerted its effect might be by dopaminergic influence, as dopamine receptor antagonist, since dopamine blocked the largest dose effect as shown in table (3) and fig. (3). Besides, we could not find any previous studies on *Hibiscus sabdariffa l.* extract to be tested as galactogogue which can contrast or confirm the results of our studies in this field of research work. The plant extract has characterized by a very low degree of toxicity. The acute toxicity LD_{50} of *Hibiscus sabdariffa l.* seed extract in albino rats was found to be above 5000 mgkg^{-1} according to the method of Lorke (1983). Sub-chronic toxicity studies of *Hibiscus sabdariffa l.* extract have been done by accessing the liver enzymes and kidney metabolites as indicators for liver and renal function tests. The liver enzymes and kidney metabolites were found to be within the normal ranges when compared to control.

Thus, it can be concluded from this study that the aqueous seed extract of *Hibiscus sabdariffa l.* possess a lactogenic activity with a favourable enhancement ability in increasing serum prolactin level which is the principal lactogenic hormone secreted by anterior pituitary. This lactogenic activity establishes a rationale for the ethnomedicinal use of these seeds as galactogogue. Mechanism of action of *Hibiscus sabdariffa l.* may be through dopamine receptors as dopamine antagonist, because dopamine blocked the extract effect. The acute and sub-chronic toxicity studies characterize the plant to have low toxicity which makes it safe for human consumption even when taken for long duration.

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