

# Toxicological And Lactogenic Studies On The Seeds Of Hibiscus Sabdariffa Linn (Malvaceae) Extract On Serum Prolactin Levels Of Albino Wistar Rats

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## Abstract

Preliminary phytochemical screening of the ethanolic seed extract of Hibiscus sabdariffa L. revealed the presence of alkaloids, saponins, tannins, anthraquinones, steroids, cardiac glycosides, flavonoids and phlobatanins. The acute toxicity LD<sub>50</sub> of Hibiscus sabdariffa L. seedextract in albino rats was found to be above 5000 mgkg<sup>-1</sup>. The lactogenic effect of ethanolic seed extract of Hibiscus sabdariffa L. was investigated by administering extract and metoclopramide in albino rats. The extracts were administered at varying doses (200, 400, 800 and 1600mgkg<sup>-1</sup>) and drug (5mgkg<sup>-1</sup>) for six days orally. There was significant increase (p<.01) in serum prolactin levels in a dose dependent manner. The doses of 800mgkg<sup>-1</sup> and 1600mgkg<sup>-1</sup> seems to be more effective with serum prolactin levels of 15.74±0.8 and 17±0.6 respectively. The serum prolactin level of metoclopramide (17.50ng/ml) when compared to the effective dose showed no significant difference (P>0.05). The ethanolic seed extract of Hibiscus sabdariffa L. possess lactogenic effect.

## INTRODUCTION

Hibiscus sabdariffa Linn is a herb belonging to the malvaceae family and it is cultivated for leaf, fleshy calyx, seed or fibre (7). Hibiscus sabdariffa L. is grown 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 (29, 19). 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 (14, 20), hypocholesterolemic (6, 20); anti-oxidative and hepatoprotective (29, 2) effects in animals.

Delphinidin 3-sambubioside, a Hibiscus anthocyanin, induces apoptosis in human leukemia cells through oxygen reactive species-mediated mitochondrial pathway (12). Polysaccharides from Hibiscus sabdariffa flowers stimulate proliferation and differentiation of human keratinocytes (5). Hibiscus protocatechuic acid has inhibitory and inductive effect on tumour promotion in mouse skin and in human leukemia cells respectively (26). Hibiscus sabdariffa has been reported to be antiseptic, aphrodisiac, astringent, cholagogue, demulcent, digestive, diuretic, emollient, purgative, refrigerant, sedative, stomachic and tonic (16, 20).

In Nigeria, a decoction of the seeds is used traditionally to enhance or induce lactation in cases of poor milk production, poor letdown and maternal mortality.

However, there is dearth of literature supporting its use in enhancing and induction of milk during lactation. Coupled with the importance of exclusive breastfeeding in the first six months of life stimulates babies' immune systems and protects them from diarrhea and acute respiratory infections (27). Based on the overwhelming advantage of breast milk as an infant's source of nutrition, and a number of women who have lactation insufficiency due to prolactin deficiency, additional medication to augment lactation without side effects is needed (25). In light of this, the study is designed to evaluate the prolactin effect of Hibiscus sabdariffa seed extract.

## MATERIALS AND METHODS

### CHEMICALS USED

All chemicals and drugs used were of analytical grade. Prolactin ELISA 96 test kits were obtained from Fortress Diagnostics Limited, BT41 1QS, UK. (BX0671A), while Metoclopramide was bought commercially with NAFDAC Reg. no.04-5946. It will be used to induce

hyperprolactinemia in experimental animals (1). Chloroform anaesthesia and Dopamine (Aldrich Chemical company, Gillingham England) were obtained from Department of Human Physiology, Ahmadu Bello University, Zaria.

### **PLANT MATERIALS**

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 (Taxonomist) and authenticated voucher samples were deposited in the Herbarium section (code number 1056).

### **EXTRACT PREPARATION**

The Hibiscus sabdariffa l. seeds were washed thoroughly, sun dried and ground into powder. The extraction of Hibiscus sabdariffa l. seed was done in Department of Pharmacognosy and Drug Development, Ahmadu Bello University Zaria. The powder (100 g) was macerated with ethanol for 48 h with occasional shaking. The extract was concentrated under reduced pressure to yield a dark green mass which weight 18 g and kept in dessicator until use.

### **ANIMALS**

Forty three Albino rats of both sexes weighed between 120-150 grams were used for both toxicity studies as well as testing the effect of Hibiscus sabdariffa l. seed extract on serum prolactin level in rats. They were kept in standard cages and 12 hours light/dark cycle condition in the animal room of the Department of Human Physiology, Ahmadu Bello University Zaria. They were fed on commercial feeds with tap water ad libitum.

### **PHYTOCHEMICAL SCREENING**

The ethanolic seed extract of Hibiscus sabdariffa l. were subjected to preliminary phytochemical screening, to identify the chemical constituents. The methods of analysis employed were those described by Brain and Turner (4).

### **ACUTE TOXICITY STUDY**

The lethal doses (LD<sub>50</sub>) of the plant extract was determined by method of Lorke (15, 23) using 13 rats. In the first phase rats were divided into 3 groups of 3 rats each and were treated with the ethanol extract of the seed at doses of 10, 100 and 1000 mg/kg body weight intraperitoneal. They were observed for 24 h for signs of toxicity. In the second phase 4 rats were divided into 4 groups of 1 rat each and were also

treated with the ethanolic extract at doses of 1000, 1600, 2900 and 5000 mg/kg bodyweight (i.p). The median lethal dose (LD<sub>50</sub>) was calculated using the second phase.

### **EXPERIMENTAL DESIGN**

In the experiment, a total of 30 rats were used which were divided into 6 groups of 5 rats in each. The first group served as control, group 2 was given 5mg/kg metoclopramide (1, 28) while group 3, 4, 5 and 6 received the extract at the doses of 200, 400 and 800 1600mg/kg (orally) respectively.

### **DETERMINATION OF SERUM PROLACTIN LEVES**

Prolactin levels in heart blood samples at the time the animals were euthanized were analyzed using ELISA kit (8). The analysis was conducted in Department of Chemical pathology Ahmadu Bello University Teaching Hospital, Zaria.

### **STATISTICAL ANALYSIS**

All data are expressed as Mean  $\pm$  S.E.M. The data obtained were analyzed using one way analysis of variance (ANOVA) and Turkey-Kramer post hoc test for multiple comparisons (3). The (P<0.05) will be accepted as significant.

### **RESULTS**

#### **ACUTE TOXICITY STUDY (LD)**

The sign of toxicity were first noticed after 4 - 8 h of extract administration. There was decreased locomotor activity and decreased in sensitivity to touch. Also there was decreased feed intake, and prostration after 16 h of extract administration. There is no any mortality rate after administered 5000 mg/kg. The median lethal dose (LD<sub>50</sub>) in rats was calculated to be greater than 5000 mg/kg body weight.

The preliminary phytochemical screening of the aqueous seed extract of Hibiscus sabdariffa l. found the presence of alkaloids, saponins, Cardenolides, Deoxy sugar tannins, anthraquinones, steroidal ring, cardiac glycosides, flavonoids and phlobatanins in the following concentrations in table.II below.

Table I: and fig.I. showed the results of the control, extract treated and metoclopramide-treated groups in Wistar rats. All the extract-treated and metoclopramide-treated groups showed a significant increase in serum prolactin level (p<0.01) when compared to control normal saline group

except the extract-treated dose of 200mg/kg which has serum prolactin level ( $P < 0.05$ ). The highest activity resides at the doses 800mg/kg and 1600mg/kg which have prolactin level of ( $15.74 \pm 0.8$  and  $17.24 \pm 0.6$  ng/ml) respectively. Also in fig. 1: the graph represents the activity of varying dose of extract and metoclopramide in the dose dependent manner.

**Figure 1**

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

Groups	Control Normal saline (5)	Metoclopramide Treated group 5mgkg <sup>-1</sup> (5)	Extract-treated group			
			200 mgkg <sup>-1</sup> (5)	400 mgkg <sup>-1</sup> (5)	800 mgkg <sup>-1</sup> (5)	1600 mgkg <sup>-1</sup> (5)
Parameter						
Prolactin (ngml <sup>-1</sup> )	6.68 ± 0.5	17.42 ± 0.6	10.64 ± 0.9	12.76 ± 0.4	15.74 ± 0.8	17.24 ± 0.6
Level of significant		S <sup>-</sup>	S <sup>-</sup>	S <sup>**</sup>	S <sup>**</sup>	S <sup>**</sup>

- Number of animals is represented in between brackets.
- Not Significant=NS,
- Significant=S<sup>-</sup> (P<0.05)
- Significant=S<sup>\*\*</sup> (P<0.01).

**Figure 2**

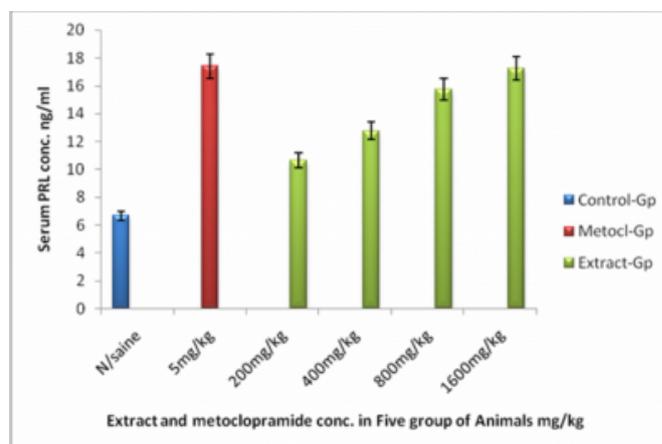
Table II Phytochemical screening of ethanolic seed extract of

Seed constituents of <i>Hibiscus sabdariffa</i> l.	Concentrations
Alkaloids	++
Anthraquinones	+
Cardenolides	+
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.

**Figure 3**

Fig.1: Showing serum prolactin level in control, metoclopramide-treated and Hibiscus sabdariffa l. extract-treated groups in albino rats



## DISCUSSION

Medicinal plants are widely used by the populations of underdeveloped countries as alternative therapy. In Africa, hundreds of plants are used traditionally for the management and/or for enhancing lactation in cases of poor letdown or maternal mortality. Traditionally, the interest in induced lactation was motivated to provide nourishment for an infant whose mother either had died in childbirth or was unable to breast-feed. Unfortunately only a few of such African medicinal plants have received scientific scrutiny. The results of the present study reported that, the seed extract of *Hibiscus sabdariffa* l. produced an appreciable increase in serum prolactin level ( $P < 0.01$ ) when compared to control in a dose-dependent manner as shown in table I and fig.I. The seed extract of *H. sabdariffa* exhibited a lactating activity by increasing the serum prolactin level in lactating rats. The principal lactogenic hormone, prolactin, secreted by the anterior pituitary is critical to the establishment of lactation, milk macronutrient content and milk production (22). Prolactin is a polypeptide hormone that is synthesized in and secreted from specialized cells of the anterior pituitary gland, the lactotrophs. It has many separate biological activities and subserve multiple roles in reproduction other than lactation (9).

Lactogenic effect of herbs and seed has been reported in other plants (*Asparagus racemosus*, fennel seed, Grape sap, milk thistle and goat's rue) (10, 11, 21, 18, 13, 24). The presence of steroidal saponins and sapogenins constituents contributes in the lactogenic effect of *Asparagus racemosus* (11, 21, 17). 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 is not ascertained, 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 extracts are characterized by a very low degree of toxicity. The acute toxicity LD<sub>50</sub> of *Hibiscus sabdariffa* l. seed extract in albino rats was found to be above 5000 mg/kg-1 according to the method of Lorke (15, 23).

This study demonstrated that seed extract of *Hibiscus sabdariffa* l. possess lactogenic activity with a favourable enhancing 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. is not ascertained, however the acute 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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