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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Citation

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 phlobatansins. The acute toxicity LD\textsubscript{50} of Hibiscus sabdariffa l. seedextract in albino rats was found to be above 5000 mgkg\textsuperscript{-1}. 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\textsuperscript{-1}) and drug (5mgkg\textsuperscript{-1}) for six days orally. There was significant increase (p<.01) in serum prolactin levels in a dose dependent manner. The doses of 800mgkg\textsuperscript{-1} and 1600mgkg\textsuperscript{-1} 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 antisepic, aphrodisiac, astringent, chalagogue, 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$_{50}$) 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 body weight (i.p). The median lethal
dose (LD$_{50}$) 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 ± 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$_{50}$) 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, Cardenoledes, 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±0.8 and 17.24±0.6ng/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.

<table>
<thead>
<tr>
<th>Doses</th>
<th>Control Normal saline (S)</th>
<th>Metoclopramide Treated group</th>
<th>Extract-treated group</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>6.68±0.5</td>
<td>17.42±0.6</td>
<td>17.28±0.9</td>
</tr>
<tr>
<td>200mg/kg</td>
<td>10.64±0.9</td>
<td>12.76±0.4</td>
<td>15.74±0.8</td>
</tr>
<tr>
<td>400mg/kg</td>
<td></td>
<td></td>
<td>16.31±0.5</td>
</tr>
<tr>
<td>800mg/kg</td>
<td></td>
<td></td>
<td>15.74±0.8</td>
</tr>
<tr>
<td>1600mg/kg</td>
<td></td>
<td></td>
<td>17.28±0.9</td>
</tr>
</tbody>
</table>

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

**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 LD₅₀ of Hibiscus sabdariffa l. seed extract in albino rats was found to be above 5000 mgkg⁻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 anerior 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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