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Original article

The study of effect of aqueous *Cucurbita pepo linn* seed extract on serum prolactin level of lactating female albino rats

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ABSTRACT

Prolactin participation has been established for a growing number of physiological actions of these, the best studied is the stimulation of mammary gland development and induction of milk synthesis at the end of pregnancy and during lactation.The study investigated the effect of aqueous *Cucurbita pepo Linn* seed extract on serum prolactin level of lactating female albino rats. A total of twenty five (25) female healthy wistar albino rats were used for study. They were divided into the following groups as follows: Group 1: Served as control group and received 1ml of distilled water, Group 2: Received Metoclopramide 5mg/kg b w, Group 3: Received 250 mg/kg b w of *C. pepo L,*Group 4: Received 500 mg/kg b w of *C. pepo L.* and Group 5: Received 1000 mg/kg b w of C. *pepo L*. All regimen was given orally once daily for a period of eight (8) days, starting from day 3 to day 11 of lactation. The acute toxicity and phytochemical screening were carried out. The result of acute toxicity studies showed that the aqueous *Cucurbita pepo Linn* seed extract was safe up to 5000 mg/kg b w. Phytochemical screening of the extract revealed the presence of carbohydrates, glycosides, cardiac glycosides, saponins, tannins, flavanoids and alkaloids. From the results obtained in this study, it was evident that the administration of plant extract at doses of 250,500 and 1000mg/kg b w produced a significantly increased ($p<0.0001$) prolactin level from 4.90 ± 0.18 in the control group to 26.3 ± 1.60, 26.5 ± 1.60 and 24.9 ± 2.13 in the experimental groups respectively when compared to the control group. However, a maximum increased prolactin

concentration was observed in the group that received 500 mg/kg b w of the extract. The values of prolactin levels obtained in the extract treated groups are comparable to the metoclopramide (5mg/kg b w). It can be concluded that elevated level of prolactin as observed in this study justifies the folkloric use of this plant in stimulating lactation and as well as increasing milk production.

1. Introduction

The key hormone that initiates milk biosynthesis is prolactin, which exerts a direct effect on the mammary gland through prolactin receptors (Vonderhaar, 1987). When a mother has low prolactin level, milk supply may be affected. The drugs used for increasing milk supply works by blocking dopamine which is a major inhibitor of prolactin (Brown *et al.,* 2000).Agents that increase endogenous prolactin such as metoclopramide and domperidone, have been used in a 7-10 day course to increase breast milk supply in mothers with lactation insufficiency (Gabay, 2002). However, one concern about these medications is its ability to penetrate the blood-brain barrier, which can result in central nervous system side effects such as depression and involuntary body movements (dystonia), restlessness, drowsiness, fatigue especially with longer term use, The toxicity of the currently available drugs has stimulated the search for alternative medicine(Anfinson, 2002; Gabay, 2002).Plant-derived chemicals that influence endocrine activities in both humans and animals have received a great deal of attention due to their possible therapeutic effects (Gamache and Acworth, 1998).

Cucurbita pepo belongs to the family *Cucurbitacae* and has been described as a multipurpose plant which is used extensively both for its nutritional and medicinal properties. It is an annual, coarsely, herbaceous climbing, trailing or bushy, polymorphic plant known as pumpkin and a multitude of common names such as marrow and vegetable marrow (English) (Burkill, 1985). In Nigeria the plant is called Gooji (Hausa), Ukoro (Igbo), Famfan (Kanuri), Agbadu (Tiv), Elegede (Yoruba). The medicinal parts are seeds and pulp. Pumpkins are native to North and Central America but have since been cultivated around the world. The seed is cooling and of the nature of the melon. An annual creeper with stems up to 30ft (9m) long furnished with large clusters. The leaves are large and rough like melons. The fruit is very large and contains white flattish seeds. In vivo,C*curcubita pepo L.* seed has demonstrated anti-androgenic and anti-inflammatory activity and recent studies also reported that pumpkin seed inhibit 5α-reductase in vitro. Men traditionally consumed pumpkin seeds to reduce prostate enlargement (Tyler, 1993). The fruit is astringent to the bowels, increases appetite, cures leprosy and purifies the blood. Seeds cure sore chests, bronchitis and fever. The seed extracts modulate immune-biochemical pathways induced by interferon (Winkler *et al.,* 2005). The seed are claimed to be useful in the management of benign prostatic hyperplasia (Abdel-Rahman *et al.,* 2006). Anti-ulcer cucurbitane type triterpenoid has been isolated from the seeds of *cucurbita pepo* (Gile *et al.,* 2011). Many herbs are used to stimulate milk production. Most of them have long histories of traditional use, mainly in stimulating milk production in animals. *Hibiscus sabdariffa l.* has a lactogenic activity with favorable enhancement ability in increasing serum prolactin level. Mechanism of action of *Hibiscus sabdariffa l.* has been reported to be through dopamine receptors as dopamine antagonist (Okasha *et al*, 2008).*Acacia milotecasp adansonii* stimulate milk production in lactating woman by stimulating the synthesis and release of prolactin and enhancing lobuloalveolar development (Lompo-ovedraogo *et al.,* 2004). *Foeniculum vulgare (fenne)*and Raspberry leaf *(Rubus udaus L. (Rosaceae)* have been reported to stimulate lactation and increase milk production as well as enrich breast milk by restoring the body's vitamins and minerals (Bauley and Day, 2004). Therefore, the study aimed at investigating the effect of aqueous *Cucurbita pepo linn* seed extract on serum prolactin level of lactating female albino rats.

2. Materials and methods

This study was carried out from the Month of June to October, 2012 in the Department of Human Physiology, Ahmadu Bello University, Zaria, Kaduna State, Nigeria.The materials used in this study include: Dissecting kit

,Anaesthesia box, Pasteur pipette, 5ml syringe, 25 standard bottles, 25 sample bottles, Bench centrifuge Hawksley Reg. No. 891481 Ser. No. 07.4.75, Weighing balance mettler P3 and H80 Gallenkamp, Bcorad machine registration number PR 3100, Dissecting board, Microwell reader, Audicom

2.1. Drugs and chemicals used

The chemicals used include: Prolactin ELISA, Microwells – Accubrnd product (code 725-300), Momobinaline, Lorke Forest CA 90630 USA, Metoclopramide (NAFDAC Reg. No. 04-5946) was procured from a reputable pharmaceutical shop in Zaria, Kaduna state whileChloroform was obtained from the Human Physiology Department, Ahmadu Bello University, Zaria, Nigeria.

2.2. Collection of plant material and extraction

The samples of *cucurbita pepo L.* seed were collected from Basawa village of Kaduna State, Nigeria in the Month of October 2011.The plant was then taken to the herbarium unit of the Biological Science Department, Ahmadu Bello University, Zaria, Kaduna state, where the plant was identified by Mal. M. Musa and a voucher specimen number deposited. The extraction of *Cucurbita pepo linn* seed was done in the Department of Pharmacognosy and Drug Development, Ahmadu Bello University, Zaria, Nigeria. Hundred (100 g) of powdered seed was soaked with 2.5 L of distilled water for 24 h and the mixture was continuously shaken at interval. The mixture (extract) was then filtered through a plug of cotton or glass wool. The process was repeated exhaustively for complete extraction. The extract filtrate was then concentrated over a water bath temperature of 30 ^{0}C. A brownish residue weighing 20 g was obtained and kept in a sealed container until it was reconstituted.

2.3. Animals

A total of twenty five (25) female healthy albino wistar rats were used for study. The animals were housed in stainless steel metal cages under standard laboratory condition with 12hr dark/light cycle condition in the Animal House of the Department of Human Physiology, Ahmadu Bello University, and Zaria. They were fed on standard commercial feeds (Vital feeds) with water *ad libitum.*

2.4. Experimental design

Twenty five (25) lactating female albino rats were used. They were randomly divided into five groups of five rats each as follows:

Group 1: Served as control group and received 1ml of distilled water.
Group 2: Received metoclopramide 5mg/kg b w
Group 3: Received 250mg/kg b w of *Cucurbita pepo L.*
Group 4: Received 500mg/kg b w of *Cucurbita pepo L.*
Group 5: Received 1000mg/kg b w of *Cucurbita pepo L*.

All animals were treated with the extract, drug or distilled water for 8 days orally, starting from day 3 to day 11 of lactation.

2.5. Acute toxicity study of the extract

Acute toxicity study of the extract was carried out by the methods of analysis described by Lorke, (1983).

2.6. Phytochemical screening of the extract

Phytochemical screening of the extracts was carried out according to the methods described by Trease and Evans, (1989) to identify the presence or absence of chemical constituents such as alkaloids, tannins, phenolics, glycosides, saponins, flavonoids, steroids and triterpenes.

2.7. Collection of blood and preparation of serum samples

At the end of the 11th day of treatment period, all animals from each group were euthanized on the 12th day and the blood samples collected into plain tubes and allowed to clot and centrifuged at 3500 rpm for 15 minutes. The sera was separated and stored at -4 ^{0}C for serum prolactin analysis.

2.8. Evaluation of serum prolactin concentration

Serum prolactin levels were determined using prolactin assay kits according to the procedures described by Tietz, (1995). The analysis was carried out in the Department of Chemical Pathology, Ahmadu Bello University Teaching Hospital Zaria with Biovad-machine registration number (3100).

2.9. Statistical analysis

Data obtained were expressed as mean ± SEM. The data were analysis using one-way analysis of variance (ANOVA) and Tukey's post hoc test was used to determine the level of significance between control and the experimental groups. All statistical analysis was done using SPSS version17.0 software. The value of $P<0.05$ were considered significant.

3. Results

The study revealed that all the graded doses of aqueous *Cucurbita pepo L.* seed extract administered to the animals both in the first and the second phase of the acute toxicity test produced no signs of toxicity, and no deaths were recorded. Therefore, the median lethal dose (LD_{50}) of aqueous *Cucurbita pepo L.* seed extract was found to be safe up to 5000 mg/kg body weight as presented in tables (1 and 2) respectively. The preliminary phytochemical screening of aqueous *Cucurbita pepo L.* seeds extract revealed the presence of carbohydrates, glycosides, cardiac glycosides, saponins, steroids and triterpenes, tannins, flavanoids and alkaloids as shown in table 3.

Table 1
Percentage mortality of different doses of *Cucurbita pepo L.* seed extract administered orally to wistar rats in the first phase of acute toxicity study.

Group	Dose (mg/kg)	Death	% Mortality
1	10	0/3	0
2	100	0/3	0
3	1000	0/3	0

Table 2
Percentage mortality of different doses of aqueous *Cucurbita pepo L.* seed extract administered orally to wistar rats in the second phase of acute toxicity study.

Group	Dose (mg/kg)	Death	% Mortality
1	1600	0/1	0
2	2900	0/1	0
3	5000	0/1	0

Table 3
Phytochemical screening of aqueous *Cucurbita pepo L.* seed extract**.**

Phytochemicals	Inference
Carbohydrate	+
Cardiac glycosides	+
Saponins	+
Steroids	+
Triterpenes	+
Tannins	+
Flavanoids	+
Alkaloids	+

Key: [+] = Present
[-] = Absent

Table 4 showed the results of the effect of graded doses of *Cucurbita pepo linn* seed extract administration on serum prolactin level of lactating female rats. From the results obtained, it was evident that administration of plant extract at doses of 250,500 and 1000 mg/kg b w produced a significantly increased ($p<0.0001$) prolactin level from 4.90 ± 0.18 in the control group to 26.3 ± 1.60, 26.5 ±1.60 and 24.9 ± 2.13 in the experimental groups respectively when compared to the control group. However, a maximum increased prolactin concentration was observed in the group that received 500 mg/kg b w of the extract. The values of prolactin levels obtained in the extract treated groups are comparable to the metoclopramide (5mg/kg b w), which is a standard drug used in this present study.

Table 4
Effect of aqueous *Cucurbita pepo Linn* seed extract on mean (± SEM) prolactin level of lactating female albino rat.

Treated Groups	Mean ± SEM
Distilled water	4.90 ± 0.18
Metoclopramide (5mg/kg b w)	25.9 ± 0.87[a]
Extract (250 mg/kg b w)	26.3 ± 1.60[a]
Extract (500 mg/kg b w)	26.5 ± 1.60[a]
Extract (1000 mg/kg b w)	24.9 ± 2.13[a]

Values are statistically significant when compared to control group at [a] $p < 0.05$ while ns =not significant.

4. Discussion

Prolactin in man appears to form an essentialpart ofthe complex of hormones necessary for milk secretionand lactation.The role of prolactin in the control of milk secretionin animals is now reasonably well understood.It is apparent that prolactin forms part of a complexof hormones (including growth hormone and insulin)necessary to maintain milk secretion (McNeilly, 1965). Lactation is an important component of the reproductive process (Diazi et al., 1995). The results obtained, in this present study showed that administration of aqueous *Cucurbita pepo L.* seeds extract at doses of 250,500 and 1000 mg/kg b w produced a significantly increased prolactin level from 4.90 ± 0.18 in the control group to 26.3 ± 1.60, 26.5 ±1.60 and 24.9 ± 2.13 in the experimental groups respectively when compared to the control group. However, a maximum increased prolactin concentration was observed in the group that received 500 mg/kg b w of the extract.

Prolactin helps to initiate breast development by inducing lobuloalveolar growth of the mammary gland. It also stimulates lactogenesis (Fitzgerald*et al.,* 2008). Dopamine serves as the major-inhibiting factor or break on prolactin secretion. The enhanced level of prolactin observed in this study may be attributed to the effect of the extract probably acting as a dopamine antagonist. High prolactin levels tend to suppress the ovulatory cycle by inhibiting the secretion of both follicle-stimulating and gonadotropic-releasing hormones (GnRH) which are necessary for ovulation.Such increase in prolactin may inhibit ovulation and promote the loss of menstrual periods which will hinder conception (Fitzgerald*et al.,* 2008). The preliminary phytochemical screening of aqueous *Cucurbita pepo L.* seeds extract revealed the presence of carbohydrates, glycosides, cardiac glycosides, saponins, steroids and triterpenes, tannins, flavanoids and alkaloids. Alkaloids and flavonoidshave been reported to reduce plasma concentrations of LH, estradiol and FSH which were not assayed in this present study (Lauritzen *et al.,* 1997; Browning*et al.,* 1998; Bianco*et al.,* 2006).This suggest that thepresence of these phytochemicals may account forthe increased circulating level of prolactin observed in this study. This is in agreement with the study of Okasha *et al.,* (2008), who reported increased serum prolactin concentration of Lactating female albinorats administered with aqueous *Hibiscus abdariffa linn* seed.

5. Conclusion

In conclusion, the findings in this study showed that administration of aqueous *Cucurbita pepo L.* seeds extractat all doses produced a significantly increased serum prolactin level. The elevated level of prolactin in this study justifies the folkloric use of the plant in stimulating lactation.

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