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RESEARCH ARTICLE

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## Effects of aqueous *Cucurbita pepo* Linn seed extract on some haematological parameters and serum electrolytes of lactating albino rats

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### ABSTRACT

The present study investigated the effects of aqueous *Cucurbita pepo* Linn seed extract on some haematological parameters and serum electrolyte concentration of lactating albino rats. A total of twenty five (25) female apparently healthy albino rats were used for the study. They were assigned into the following groups: Group 1: Served as control group and received 1ml of distilled water, Group 2: Received metoclopramide 5mg/kg body weight, Group 3: Received 250 mg/kg b w of extract, Group 4: Received 500 mg/kg b w of extract and Group 5: Received 1000 mg/kg b w of extract respectively. 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 obtained from this study showed that the extract significantly decreased ( $p < 0.05$ ) the level of red cell count in the group treated with 1000 mg/kg b w when compared with the control group. However, total white blood cell count and neutrophil counts were statistically significantly decreased ( $p < 0.05$ ) in the groups treated with 1000 and 250 mg/kg b w respectively when compared to the control group. However, there was a significantly decreased ( $p < 0.05$ ) serum sodium ion and potassium ion level in the groups administered with 500 and 250 mg/kg b w respectively when compared to the control group. The result of acute toxicity studies obtained 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. It can be concluded that the plant extract did not produce a significant change on haematological parameters and serum electrolyte profile. However, the red cell count and serum sodium and potassium ions were significantly altered when compared to the control group.

**Key words:** Calcium ion, *cucurbita pepo* Linn, lactation, packed cell volume, red cell, leucocytes, sodium ion, potassium ion

### INTRODUCTION

Milk is a food that is of great biological significance for people from the moment they are born. It is accredited by the quantity and quality of nutrient. It contains high biological values proteins and significant quantities of inorganic salts—phosphorus and calcium which are structural materials for the skeleton [1]. The prevalence of lactation insufficiency may be as high as 15% in newly lactating women [2]. The rate of lactation insufficiency is

even higher in mothers of premature infants [3]. The causes of lactation insufficiency are multi-factorial and include poor suckling, structural breast abnormalities and infrequent feedings by the infants. They also include poor milk production and poor letdown [2]. According to the World Health Organization (WHO) a medicinal plant is any plant which in one or more of its organ contains substances that can be used for the synthesis of useful drugs [4]. Medicinal plants are believed to be important sources of new chemical substances with potential therapeutic effects [5]. Many medicinal plants have been found and put into use by traditional healers in the management of many ailments for many years. Medicinal plants contain biologically active chemical substances such as saponins, tannins, essential oils, flavonoids, alkaloids and other chemical compounds [6], which have curative properties. These complex chemical substances of different compositions are found as secondary plant metabolites in one or more of these plants [7]. *Cucurbita pepo* belongs to the family cucurbitaceae 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), Gooji (Hausa), Ukoro (Igbo), Famfan (Kanuri), Agbadu (Tiv), Elegede (Yoruba) [8]. 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, *curcubita pepo L.* seed has demonstrated anti-androgenic and anti-inflammatory activity and recent studies also reported that pumpkin seed inhibit 5 $\alpha$ -reductase in vitro. Men traditionally consumed pumpkin seeds to reduce prostate enlargement [9]. 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 [10]. The seed are claimed to be useful in the management of benign prostatic hyperplasia [11]. Anti-ulcer cucurbitane type triterpenoid has been isolated from the seeds of *cucurbita pepo* [12]. The assessment of haematological parameters could be used to reveal the deleterious effect of foreign compounds toxins, chemicals and plant extracts on the blood constituents of animals. They are also used to determine possible alterations in the levels of biomolecules such as enzymes, metabolic products, haematology, normal functioning and histomorphology of the organs [13]-[14]. Serum electrolytes are one of the markers of damage to renal function [15]. Electrolytes play an important role in many body processes, such as controlling fluid levels, acid-base balance (pH), nerve conduction, blood clotting and muscle contraction [16]. Therefore, this study was designed to evaluate the effects of aqueous *Cucurbita pepo Linn* seed extract on some haematological parameters and serum electrolytes of lactating albino rats.



Figure 1: *Cucurbita pepo Linn* plant seeds

## 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.

### Materials

The materials used in this study include: Dissecting kit ,Anaesthesia box, Pasteur pipette, 5ml syring, 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

### Chemicals

The chemicals used include: Prolactin ELISA, Microwells – Accubrd 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 while Chloroform was obtained from the Human Physiology Department, Ahmadu Bello University, Zaria, Nigeria.

### Animals

A total of twenty five (25) female healthy albino wistar rats weighing 150 to 200 g 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*.

### Collection of plant material and extraction

The samples of *cucurbita pepo L.* seed were collected from Basawa village of Kaduna State in the Month of October 2011. The plant was identified at Department of Biological Sciences, Ahmadu Bello University, Zaria, by Mallam Musa. The extraction of *Cucurbita pepo linn* seed was done in the Department of Pharmacognosy and Drug Development, Ahmadu Bello University, Zaria, Nigeria. 100g of powdered seed was soaked with 2.5 L of distilled water and the mixture was shaken for ten hours with mechanical shaker. 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 40-45%.

### Acute toxicity study of the extract

Acute toxicity study of the extract was carried out by the methods of analysis described by Lorke [17].

### Phytochemical screening of the extract

Phytochemical screening of the extracts was carried out according to the methods described by Trease and Evans [18] to identify the presence or absence of chemical constituents such as alkaloids, tannins, phenolics, glycosides, saponins, flavonoids, steroids and triterpenes.

### Experimental protocol

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 the control group and received 1ml of distilled water orally.
- Group 2: Received metoclopramide 5mg/kg body weight
- Group 3: Received 250mg/kg b w of *C. pepo L.*
- Group 4: Received 500mg/kg b w of *C. pepo L.*
- Group 5: Received 1000mg/kg b w of *C. 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. The research work was carried out according to the specification of the Ahmadu Bello University Animal Research Committee and in accordance with Helsinki Declaration.

### Collection of blood and serum sample preparation

At the end of the 11<sup>th</sup> day of treatment period, all animals from each group were euthanized on the 12<sup>th</sup> day and the blood samples collected in labeled sample bottles with drops of Ethylenediaminetetraacetic acid (EDTA) for determination of haematological parameters. For evaluation serum electrolytes, blood sample from each animal was collected into plain tubes and allowed to clot and centrifuged at 3500 rpm for 10 minutes. The sera was separated and stored at -4 °C for serum electrolytes analysis.

### Determination of Haematological parameters

Determination of haematological parameters such as haemoglobin (Hb), haematocrit (PCV), red cell count, total white blood cell count (TWBC) and its differentials was done using standard operative procedures according to Dacie and Lewis [19].

**Evaluation of serum electrolytes**

Serum sodium and potassium ions were measured by the flame photometry method of Vogel [20] and bicarbonate ion was determined using the titration method of Segal [21]. Chloride ion was analyzed using the method of Schales and Schales [22]. Calcium and phosphate ions were determined according to laboratory procedures of Randox Laboratories Limited kits, United Kingdom.

**Statistical analysis**

Data obtained were expressed as mean  $\pm$  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 version 17.0 software. The value of  $P < 0.05$  were considered significant.

**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.

There was no statistically significant change ( $p > 0.05$ ) on the levels of packed cell volume, Haemoglobin concentration and red cell count in all groups administered with graded doses (250, 500 and 1000mg/kg b w) of the extract when compared to the control group. However, there was significantly decreased ( $p < 0.05$ ) level of red cell count in the group treated with 1000 mg/kg b w when compared to the control group as shown in table 4. The study also revealed that total white blood cell count, neutrophils count, lymphocyte count and monocytes count did not significantly differ ( $p > 0.05$ ) in all groups treated with various doses of the extract (250, 500 and 1000 mg/kg b w) when compared to the control group. However, total white blood cell count was statistically significantly decreased ( $p < 0.05$ ) in the group treated with 1000 mg/kg b w and the neutrophils count was also significantly ( $p < 0.05$ ) reduced in group administered with 250 mg/kg b w respectively when compared to the control group as shown in table 4.

Table 5 showed the mean values of serum electrolyte concentration of the control and experimental animals. The study showed that the mean values of serum sodium and potassium ions were not significantly different ( $p > 0.05$ ) in the groups administered with 250 and 1000 mg/kg b w for sodium ion and 500 and 1000 mg/kg b w for potassium when compared to the control group. However, there was a significantly decreased ( $p < 0.05$ ) serum sodium ion and potassium ion level in the groups administered with 500 and 250 mg/kg b w respectively when compared to the control group. There was also no statistically significant change ( $p > 0.05$ ) in the level of serum chloride ion, calcium ion and phosphate ion in the groups administered with various doses of the extract when compared to the control group (table 5)

**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: Effects of aqueous *Cucurbita pepo Linn* seed extract on some haematological parameters of lactating albino rats.**

Treatment Given	PCV (%)	RBC( $10^{12}/L$ )	Hb (g/dl)	WBC ( $10^9/L$ )	Neutrophils (%)	Lymphocytes (%)	Monocytes (%)
Distilled water	42.4 ± 1.5	5.72 ± 0.12	14.12 ± 0.51	13.22 ± 0.50	31.6 ± 3.14	66 ± 3.32	4.60 ± 0.51
Metoclopramide (5mg/kg b w)	37.6 ± 1.2 <sup>a</sup>	3.28 ± 0.47 <sup>a</sup>	12.54 ± 0.41 <sup>ns</sup>	8.3 ± 1.61 <sup>a</sup>	50 ± 6.32 <sup>a</sup>	45 ± 6.32 <sup>a</sup>	5.00 ± 0.00 <sup>ns</sup>
Extract (250 mg/kg b w)	38.6 ± 3.5 <sup>ns</sup>	4.94 ± 0.04 <sup>ns</sup>	12.86 ± 1.13 <sup>ns</sup>	10.94 ± 0.80 <sup>ns</sup>	22 ± 1.14 <sup>a</sup>	73 ± 1.22 <sup>ns</sup>	5.40 ± 0.51 <sup>ns</sup>
Extract (500 mg/kg b w)	45.4 ± 1.9 <sup>ns</sup>	4.56 ± 0.37 <sup>ns</sup>	15.08 ± 0.68 <sup>ns</sup>	10.12 ± 0.75 <sup>ns</sup>	27.6 ± 2.15 <sup>ns</sup>	68.6 ± 1.57 <sup>ns</sup>	5.80 ± 0.49 <sup>ns</sup>
Extract (1000 mg/kg b w)	40.2 ± 2.6 <sup>ns</sup>	3.40 ± 0.52 <sup>a</sup>	13.4 ± 0.86 <sup>ns</sup>	9.54 ± 0.69 <sup>a</sup>	40 ± 0.0 <sup>ns</sup>	57 ± 1.53 <sup>ns</sup>	4.67 ± 0.33 <sup>ns</sup>

Values are statistically significant at <sup>a</sup>  $p < 0.05$  when compared to control group, while ns =not significant

**Table 5: Effect s of aqueous *Cucurbita pepo Linn* seed extract on serum electrolyte concentration of lactating albino rats**

Treatment Given	Sodium (mmol/L)	Potassium (mmol/L)	Chloride (mmol/L)	Calcium (mmol/L)	Phosphate (mmol/L)
Distilled water	141.0 ± 1.18	3.70 ± 0.45	101.6 ± 1.46	2.30 ± 0.02	1.08 ± 0.07
Metoclopramide (5mg/kg b w)	135.0 ± 1.81 <sup>a</sup>	4.56 ± 0.17 <sup>a</sup>	102.2 ± 1.49 <sup>ns</sup>	2.50 ± 0.04 <sup>ns</sup>	1.14 ± 0.04 <sup>ns</sup>
Extract (250 mg/kg b w)	140.8 ± 1.16 <sup>ns</sup>	4.18 ± 0.14 <sup>a</sup>	98.8 ± 1.01 <sup>ns</sup>	2.42 ± 0.07 <sup>ns</sup>	1.02 ± 0.16 <sup>ns</sup>
Extract (500 mg/kg b w)	135.0 ± 1.0 <sup>a</sup>	3.94 ± 0.18 <sup>ns</sup>	95.8 ± 1.69 <sup>ns</sup>	2.51 ± 0.07 <sup>ns</sup>	0.96 ± 0.05 <sup>ns</sup>
Extract (1000 mg/kg b w)	138.8 ± 2.28 <sup>ns</sup>	3.86 ± 0.09 <sup>ns</sup>	96.2 ± 0.49 <sup>ns</sup>	2.40 ± 0.05 <sup>ns</sup>	1.13 ± 0.03 <sup>ns</sup>

Values are statistically significant at <sup>a</sup>  $p < 0.05$  when compared to control group, while ns =not significant

## DISCUSSION

The haematopoietic system is very sensitive to toxic compounds and serves as an important index of the physiological and pathological status for both animals and humans [23]. The study showed that the oral administration of aqueous *Cucurbita pepo Linn* seed extract at doses of (250 and 500 mg/kg b w) did not produce any significant change ( $p > 0.05$ ) on the levels of packed cell volume, Haemoglobin concentration and red cell count in the experimental animals when compared to the control group, suggesting that these doses of aqueous *Cucurbita pepo Linn* seed extract did not affect haematopoiesis in the animals, hence were not toxic and did not interfere with the synthesis of circulating red blood cells. This findings is contrary to the works of Adepoju and Adebajo [24], who reported a significantly decreased packed cell volume in animals treated with graded doses (100, 200, 300 and 400 mg/kg bw) of *Cucurbita pepo Linn* seed extract. However, there was significantly decreased ( $p < 0.05$ ) level of red cell count in the group treated with the highest dose of the extract (1000 mg/kg b w) when compared to the control group. The study also revealed that total white blood cell count, neutrophils count, lymphocyte count and monocytes count did not significantly differ ( $p > 0.05$ ) in all groups treated with various doses of the extract when compared to the control group, indicating that aqueous *Cucurbita pepo Linn* seed extract did not have any effect on leucopoiesis in rats. However, total white blood cell count was statistically significantly decreased ( $p < 0.05$ ) in the group treated with 1000 mg/kg b w and the neutrophils count was also significantly ( $p < 0.05$ ) reduced in group administered with 250 mg/kg b w respectively when compared to the control group. This findings is in consonance with the work of Adepoju and Adebajo [24], who showed that administration of *C. pepo Linn* seed extract produced a significantly reduction in total blood cells and neutrophils counts in rats. The entire white blood cells contribute to the host defense mechanism [23], hence, the observed overall reduction in white blood cells especially at higher doses, could compromise immunity and predispose to opportunistic infections in spite of the nutritional benefits of the seeds of *Cucurbita pepo Linn*. Serum electrolytes are one of the markers of damage to renal function [14]. The study showed that the serum sodium and potassium ions levels were not significantly different ( $p > 0.05$ ) in the groups administered with 250 and 1000 mg/kg b w for sodium ion and 500 and 1000 mg/kg b w for potassium when compared with the control group. However, there was a significantly decreased ( $p < 0.05$ ) serum sodium ion level in the group administered with 500 mg/kg b w of the extract and significantly elevated potassium ( $p < 0.05$ )

level in the group treated with 250 mg/kg b w of the plant extract when compared to the control group. The significant ( $P < 0.05$ ) decrease in sodium ion and increase in potassium ion in the group treated with 500 and 250 mg/kg body weight are also signs of renal failure [26]. The changes in biochemical indices of renal function may have been induced by the phytochemical constituents of the plant extract. This result also indicates that oral administration of *Cucurbita pepo* Linn seed extract at doses of 500 and 250 mg/kg b w could produce hyponatremic and hypokalaemic effects in the animals. This could be one of the possible ways of affecting the tonicity of body fluid, hence affecting blood pressure. Oral administration of *Cucurbita pepo* Linn seed extract at all doses produced no statistically significant change ( $p > 0.05$ ) in the level of serum chloride ion, calcium ion and phosphate ion concentration when compared with the control group.

### CONCLUSION

It can be concluded that the plant extract did not produce a significant change ( $p > 0.05$ ) on haematological parameters and serum electrolyte profile. However, the red cell count and serum sodium and potassium ions were significantly decreased ( $p < 0.05$ ) when compared to the control group.

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