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RESEARCH PAPER

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Toxicopathological Evaluation of *Citrullus colocynthis* Seed and Pulp Aqueous Extracts on Albino Rats

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ABSTRACT

Citrullus colocynthis belong to the family Cucurbitaeae, and is a perennial herb that is usually trailing. Its seeds and pulp are ethno-medicinally useful in the treatment of various diseases. The toxicity of the extracts was evaluated. The effects of aqueous seed and pulp extracts on kidney function parameters (creatinine, urea and bilirubin), electrolyte concentrations (K+, Na^+, Cl^- and HCO^-_3) as well as liver function indices such as albumin, globulin and total protein, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) of albino rats were investigated using standard methods. Acute toxicity studies of the extracts showed that the lethal dose (LD_{50}) was indeterminate. Oral administration of different concentrations of Citrullus colocynthis seed extract to groups 2-4 and pulp extract to groups 5-7 respectively for 28 days indicated that creatinine and urea concentrations increased significantly ($p<0.05$) as the concentrations of both the seed and pulp extracts increased, no significant ($p>0.05$) difference was observed in bilirubin concentrations when compared with the control which received water and feed without the extracts. Also, the activities of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) increased significantly ($p>0.05$) when compared with the control.

There were no significant (p>0.05) difference in the concentrations of albumin, globulin and total protein. There was no significant (p>0.05) difference in the concentration of the electrolytes (K+, Na^+, Cl^- and HCO^-_3) when compared with the control. The study revealed that Citrullus colocynthis seed and pulp extracts do not have detrimental toxicological effects on the studied organs/tissues function indices of the experimental rats. However, Citrullus colocynthis seed and pulp extracts may not be completely "safe" considering the observed significant changes in the liver function indices.

Key words: Citrullus colocynthis, Toxicity, Kidney, Liver Function and Electrolytes.

INTRODUCTION

Medicinal plants have been used for decades before the advent of orthodox medicine for the treatment of many illnesses. Various plants parts such as (leaves, flowers, stem barks, roots, seeds, fruits) have all been used as constituents of herbal medicines. The medicinal values of these plant parts lie in their phytochemical compositions, which produce definite physiological action on human body (Afolabi et al., 2007).

Rapid industrialization and urbanization has led to the over exploitation and loss of valuable natural resources, including medicinally important herbaceous plants. Medicinal plants are important source of compound for the pharmaceutical industries (Chand and Sahrawat, 2002). However, nearly 25% of modern medicine were described from plants that were first used traditionally (Hudson, 1989). It is imperative to provide information on the effects associated with the use of these plants for the treatment of ailments.

Citrullus colocynthis, a member of the *Cucurbitaceae* family is known as Bitter Apple in the English language, Hindal in Arabic, and Abujahl melon in Persian. The extract of the fruit is called *colocynthine* due to its extreme bitter taste. The known compounds found in *Citrullus colocynthis* include glycosides, alkaloids, and flavonoids (Chen et al., 2005; Yoshikawa et al., 2007). A number of plant secondary metabolites including Cucurbitacins, flavonoids, caffeic acid derivatives and terpenoids, phenalic compounds have previously been reported from this plant (Chen et al., 2005; Yoshikawa et al., 2007). However, unknown compounds may also contribute to its therapeutic reputation. The Curcurbitacins (highly oxygenated tetracyclic glycosides) have a broad range of applications due to their wide spectrum of biological activities (Tannin-Spitz et al., 2007). They are found mainly in plants belonging to the *Cucurbitaceae* family, but have also been found in several other families of the plant kingdom (Tannin-Spitz et al., 2007).

In folk medicine, *Citrullus colocynthis* is widely used by rural inhabitants as a purgative, anti-diabetic, anti-neoplastic, anti-rheumatic, and anti-allergic agent (Tannin-Spitz et al., 2007). Although, the whole fruit is often used for the treatment of the aforementioned diseases, but some particular parts of the fruit are also used for specific purposes. One of such example is the traditional application of the dried pulp and seed extract of *Citrullus colocynthis* for the treatment of constipation and diabetes (Kumar et al., 2008; Nmila et al., 2000). Despite the wide therapeutic potentials attributed to this fruit, the development of complications during treatment is not uncommon. Some adverse effects include bloody diarrhea and toxic colitis, and are responsible for *Citrullus colocynthis* classification as a toxic plant, where it is considered among the top 10 (Nmila et al., 2000). Interestingly, most of the studies on the toxic effects of this medicinal plant are evaluated on the whole fruit extract (colocynthine). Since different parts of the fruit, such as the pulp or the seed, are claimed to exert different therapeutic effects, it is reasonable to suggest that a particular therapeutic benefit, or toxic side effect could be attributed to one part of the fruit, and not

another. *Citrullus colocynthis* is known to increase peristaltic movement of the gut, and cause diarrhea when taken in excess.

Given the varied ethno-medicinal uses of the plant when used singly or in combination with other plants, there is the need to evaluate its toxicity and effects their aqueous extracts on kidney and liver function indices on albino rats at varied concentrations and this prompted the bases of the study.

MATERIAL AND METHODS

Collection, Identification and Preparation of Plant Extract

The fruit of *Citrullus colocynthis* used for this work was bought at Ekeonuwa market, in Owerri Municipal Council, Imo State. The plant was identified by Mr Francis Iwueze of the Department of Forestry and Wildlife, School of Agricultural Technology, Federal University of Technology, Owerri. The seeds of *Citrullus colocynthis* were carefully removed from the fruit rinds, and air-dried. After drying to constant weight for few days, they were ground into fine powder using a mechanical homogenizer. After the seeds were removed, the pulps were carefully removed from the epicarp. The pulp was treated as the seed above. Extracts of the seed and pulp were made. Rats in Group 1 (Control) were orally administered with 0.5ml distilled water (the vehicle) while those in Groups 2 to 4 and Groups 5 to 7 were administered with the same volume of *Citrullus colocynthis* seed and pulp extracts at 100, 200 and 400 mg/kg body weight/day, respectively and the treatments continued for 28 days.

Acute Toxicity Test

Lethal Dose (LD_{50}) Determination:

The lethality of *Citrullus colocynthis* seed and pulp extracts were estimated using 36 healthy female albino mice divided into 6 groups with 6 mice in each group. Each group received a dose of the extracts (ranging from 100-400 mg/kg body weight/day,) orally. The number of deaths in each group within 24 hours was recorded. The LD_{50} was then estimated from the graph of percentage mortality (converted to probit) against log-doae of the extract using the probit method.

Animal Handling

Thirty-five (35) male albino rats, weighing between 156-186 grammes were used for the experiments were purchased from Animal Unit, Abia State University, Uturu, Nigeria. The animals were treated and handled humanly in accordance with the standard principle of the laboratory animal care of the National Institute of Health (NIH, 1995). They were supplied with feed and water *ad libitum*. The animals were divided into seven groups of four (5) in each cage according to their relative body weights. The weights of the rats before the administration of feeds were recorded. The animals were allowed to acclimatize to the environment for one (1) week on a regular water and feed. After acclimatization, each group was administered with their respective concentrations.

Animal Sacrifice and Collection of Blood Samples

After 28 days, the rats were anesthetized by exposure to dichloromethane vapor in covered transparent plastic container. Incisions were then made into the thoracic regions and were terminally bled by cardiac puncture using 5mL hypodermic syringes and needles. The blood samples were collected with needle and syringe and introduced into sterile sample bottles. The blood samples were allowed to clot and centrifuged at 3000 rpm for 10mins. The serum was separated using micropipette and used for the determination and assay of the various parameters.

Determination of kidney function indices/Assay of enzymes activities

Serum potassium and sodium concentrations were determined by the use of ion selective electrode method using humalyte machine (Human Germany described by Tietz (1985). Serum chloride concentration was determined by the use of a CO_2 gas electrode (ASTRA CO_2 apparatus Beckem Instrument, USA). Serum creatinine and urea concentrations were determined by Jaffe's reaction and urease method respectively as described by Tietz (1985). Serum total bilirubin concentration was determined by Balistreri and Shaw (1987). Serum protein concentration was determined according to the method ot Allain et al (1974). The concentration of serum albumin and globulin were determined according to Doumas et al (1971). The activities of the liver enzymes ALT and AST were assayed using the method of Reitman and Frankel (1957) and that of ALP according to Tietz (1991).

Statistical Analysis

Each reading was taken in triplicate. All data were expressed as mean ±standard deviation and analyzed for statistical significance by one way Analysis of Variance (ANOVA). Values were considered significance at $p \leq 0.05$.

RESULTS AND DISCUSSION

One major problem associated with the use of plants for ethno-pharmacological purpose is the choice of dose as most of them are administered without any standard dosage which may have serious toxicological implications on vital organs in human body.

The results of the acute toxicity study showed an indeterminate LD_{50}, even at the highest dose of extract administration. The rats slightly reduced agility and were occasionally clustered together at one end of their cages.

Table 1. Effect of *Citrullus colocynthis* Aqueous Seed and Pulp Extracts on Serum Electrolyte Concentrations of Rats.

	Concentrations (mmol/L)			
Groups	K^+	Na^+	Cl^-	HCO^-_3
1	5.13±0.23	147.0±2.98	81.60±0.81	29.50±1.00
2	5.10±0.20	150.0±1.08	82.70±0.20	30.70±2.10
3	5.20±0.10	154.5±1.50	82.80±0.22	31.60±2.15
4	5.30±0.15	156.0±1.70	82.90±0.15	27.50±1.70
5	5.05±0.10	152.2±1.10	83.20±0.16	25.70±1.80
6	5.05±0,30	153.2±1.00	84.30±0.17	29.10±2.00
7	53.31±0.32	155.0±1.00	85.40±0.18	29.50±1.60

Values are means ± SD of triplicate determinations

The results of the effect of aqueous seed and pulp extracts of *Citrullus colocynthis* on the concentrations of creatinine and urea are presented in Figures (1-2). There were significant ($p<0.05$) increase in creatinine and urea concentrations as the concentrations of *Citrullus colocynthis* seed and pulp aqueous extracts increased. Creatinine concentration test measure the level of waste product, creatine in the blood. It reflects the glomerular rate (GFT). The GFR is clinically important because it is a measure of renal function (Harita et al., 2009). Urea concentrations followed the same trend, as creatinine when compared to the control. A significant increase in blood creatinine concentration is observed only with marked damage to the nephrons, while urea concentration above normal range indicate renal imparment associated with kidney function and occasional nephritic syndrome (Sood,

2009). This indicated that the extracts might have elicited slight renal dysfunction in the experimental rats.

Table 2. Effect of *Citrullus colocynthis* Aqueous Seed and Pulp Exracts on Albumin, Globulin and Total Protein Concentrations.

Concentrations (mg/ml)			
	Albumin	Globulin	Total Protein
1	5.12±1.02	1.64±0.07	6.76±1.50
2	5.08±1.05	1.62±0.08	6.70±1.40
3	4.05±1.03	1.60±0.05	5.65±1.60
4	4.06±1.04	1.62±0.09	5.80±1.30
5	4.05±1.06	1.54±0.04	5.60±1.70
6	3.98±1.05	1.52±0.02	5.51±1.50
7	3.96±1.03	1.50±0.06	5.46±1.40

Values are means ± SD of triplicate determinations

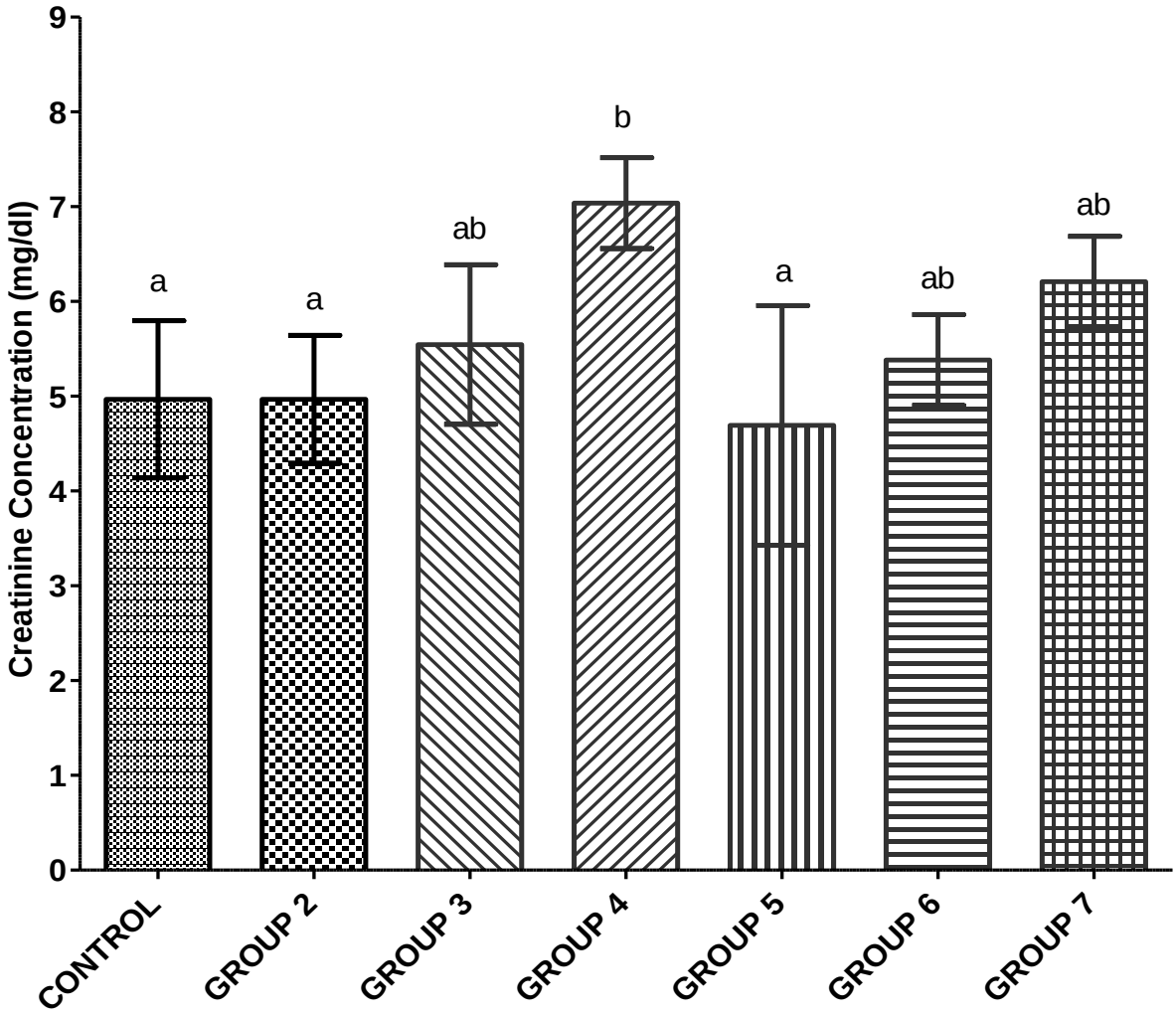

Figure 1. Creatinine concentration (mg/dl) of albino rats administered aqueous extracts of *Citrullus colocynthis*. Bars are mean ± standard deviation. Bars with different letters are statistically significant (p<0.05).

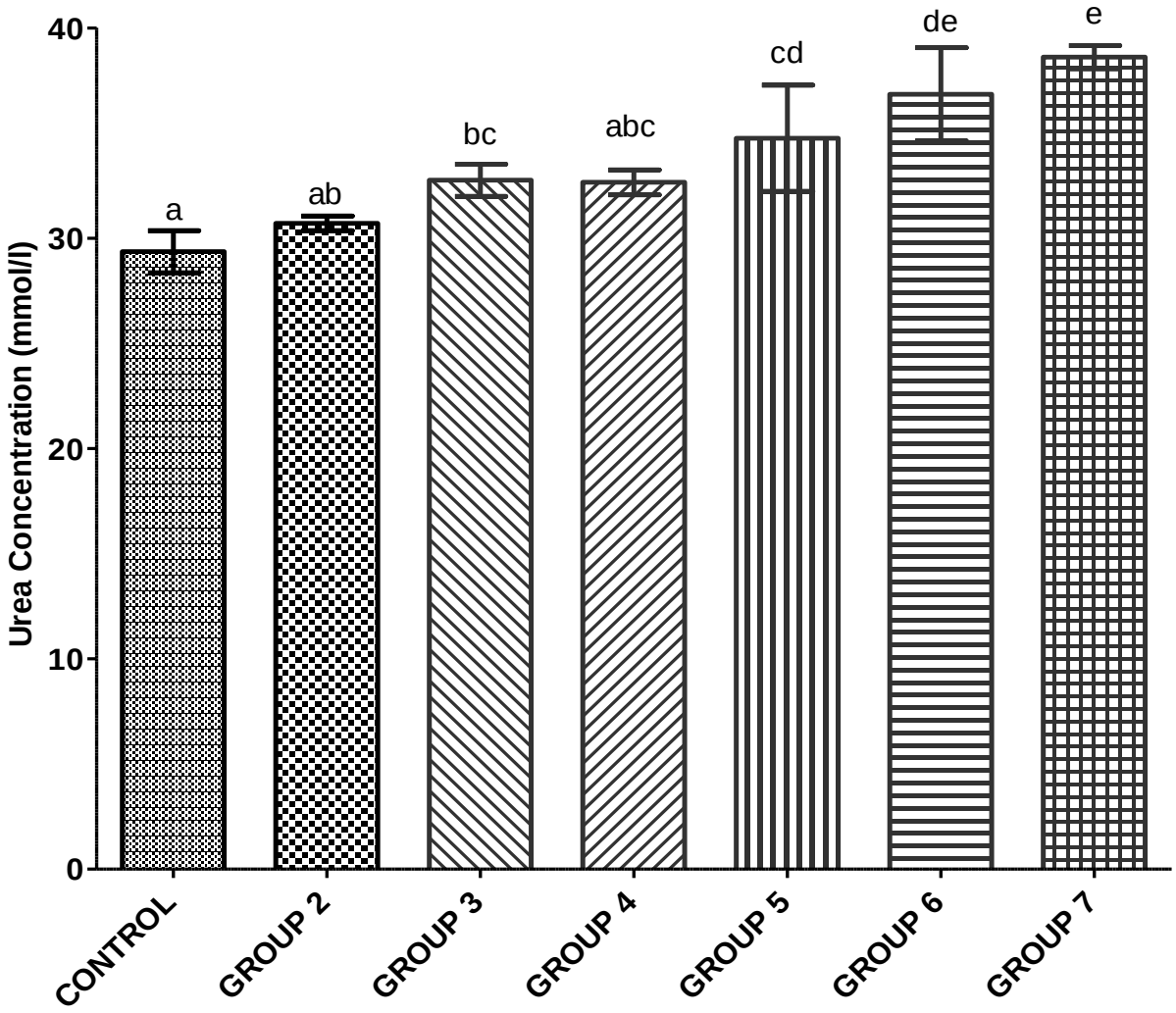

Figure 2. Urea concentration (mmol/l) of albino rats administered aqueous extracts of *Citrullus colocynthis*. Bars are mean ± standard deviation. Bars with different letters are statistically significant (p<0.05).

The functional capacity of the kidney can be measured by the dye excretory tests, clearance test and determination of serum concentration of excretory and electrolyte compositions (Panda, 1989). From our results, the extracts had no significant effect on all the concentrations of kidney function indices (K+, Na^+, Cl^- and HCO^-_3) investigated (Table 1). This suggests that the excretory ability and normal functioning of the kidney in relation to these parameters were not compromised, but with the results of the concentrations of creatinine and urea above, it indicates that the extent of damage done by the extracts did not cause the kidney to lose its normal functions within the experimental period.

The effect of *Citrullus colocynthis* seed and pulp aqueous extracts increased the activities of these liver function enzymes (ALT, AST and ALP) Figures 4-6. The aqueous seed and pulp extracts of *Citrullus colocynthis* caused significant (p>0.05) increase in activities of these enzymes. The liver of the rats in groups 3-4 and 6-7 which received high doses (200 and 400 mg/kg body weight/day) of the extracts might have been compromised when compared to control, and this caused the enzymes to leak into the blood stream thereby increasing their activities.

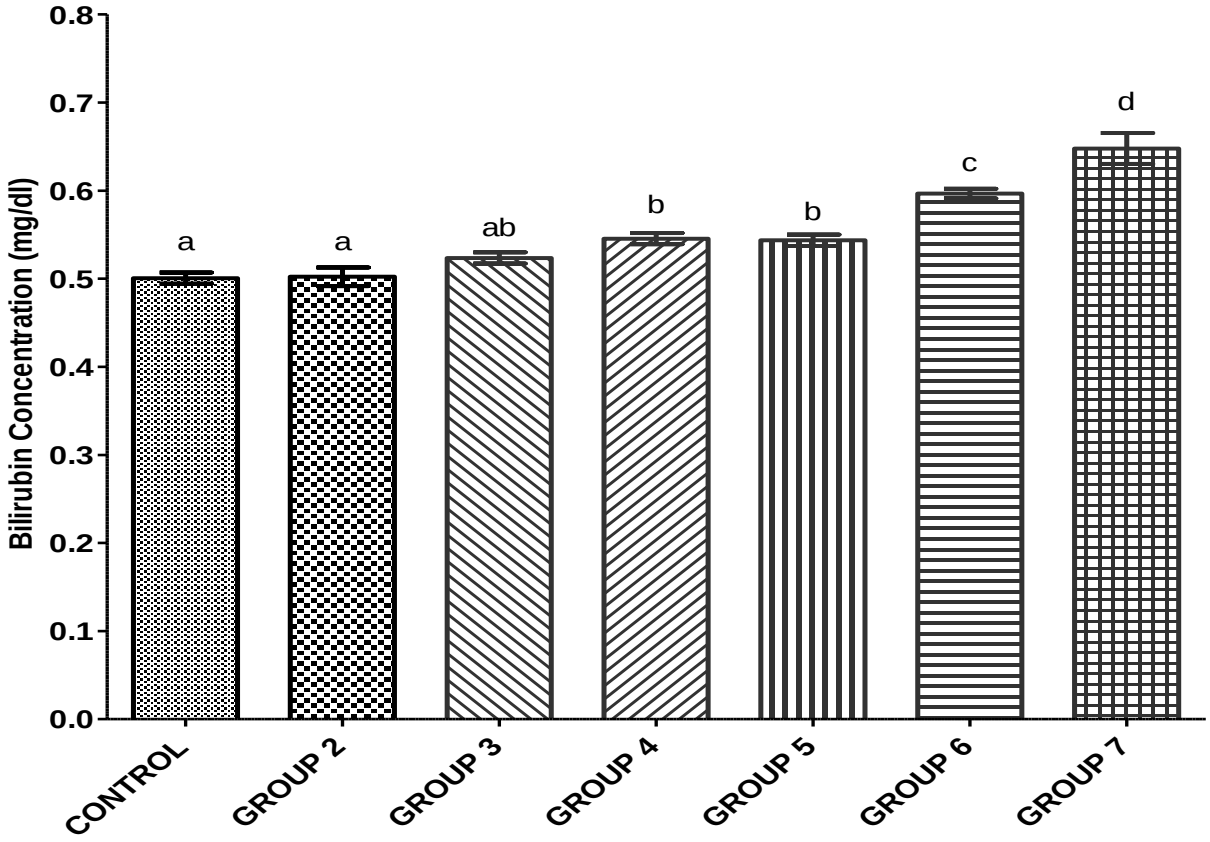

Figure 3. Bilirubin concentration (mg/dl) of albino rats administered aqueous extracts of *Citrullus colocynthis*. Bars are mean ± standard deviation. Bars with different letters are statistically significant (p<0.05).

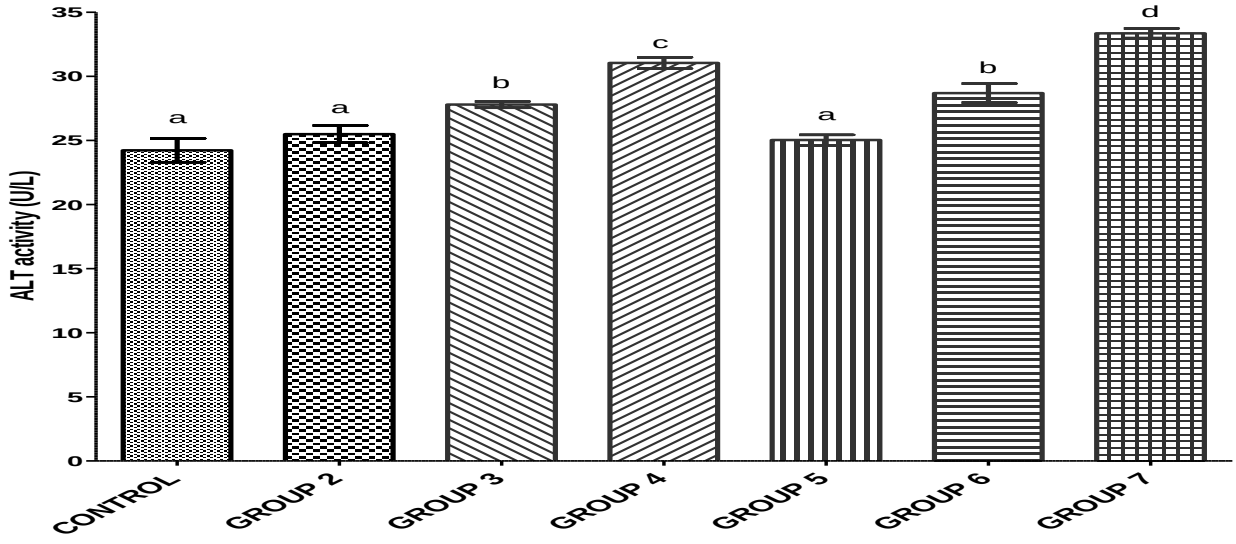

Figure 4. Alanine aminotransferase activity (U/l) of albino rats administered aqueous extracts of *Citrullus colocynthis*. Bars are mean ± standard deviation. Bars with different letters are statistically significant (p<0.05).

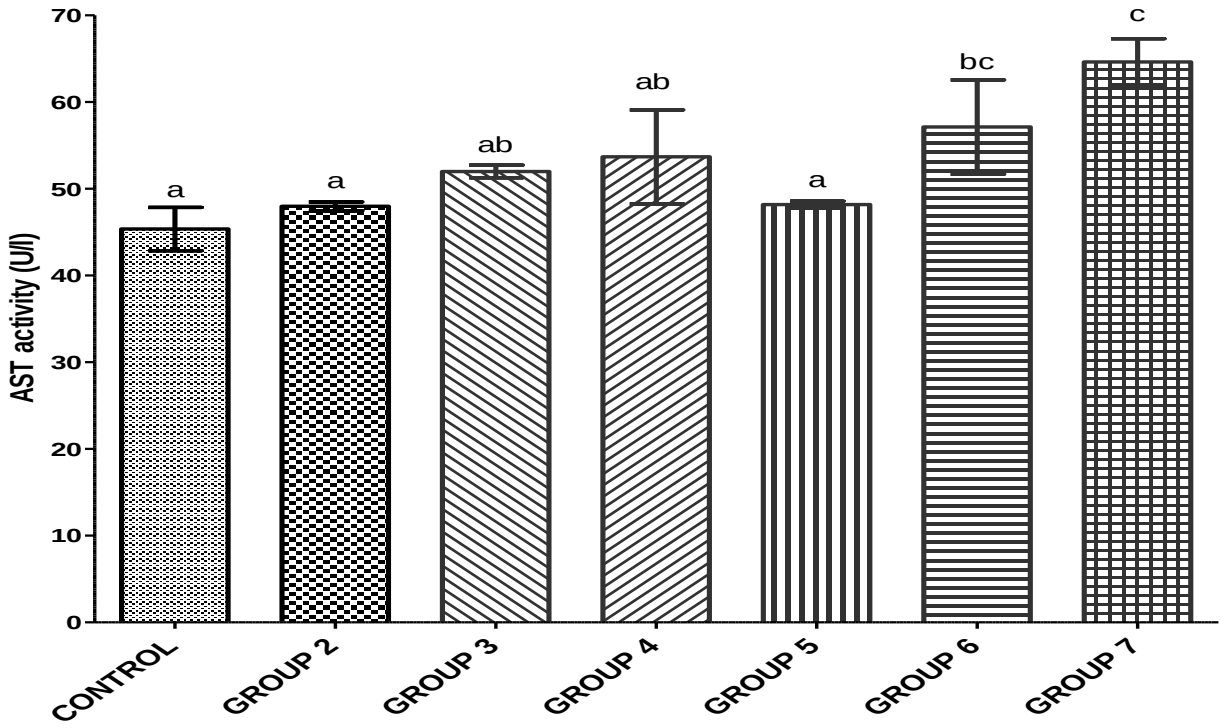

Figure 5. Aspartic aminotransferase activity (U/l) of albino rats administered aqueous extracts of *Citrullus colocynthis*. Bars are mean ± standard deviation. Bars with different letters are statistically significant (p<0.05).

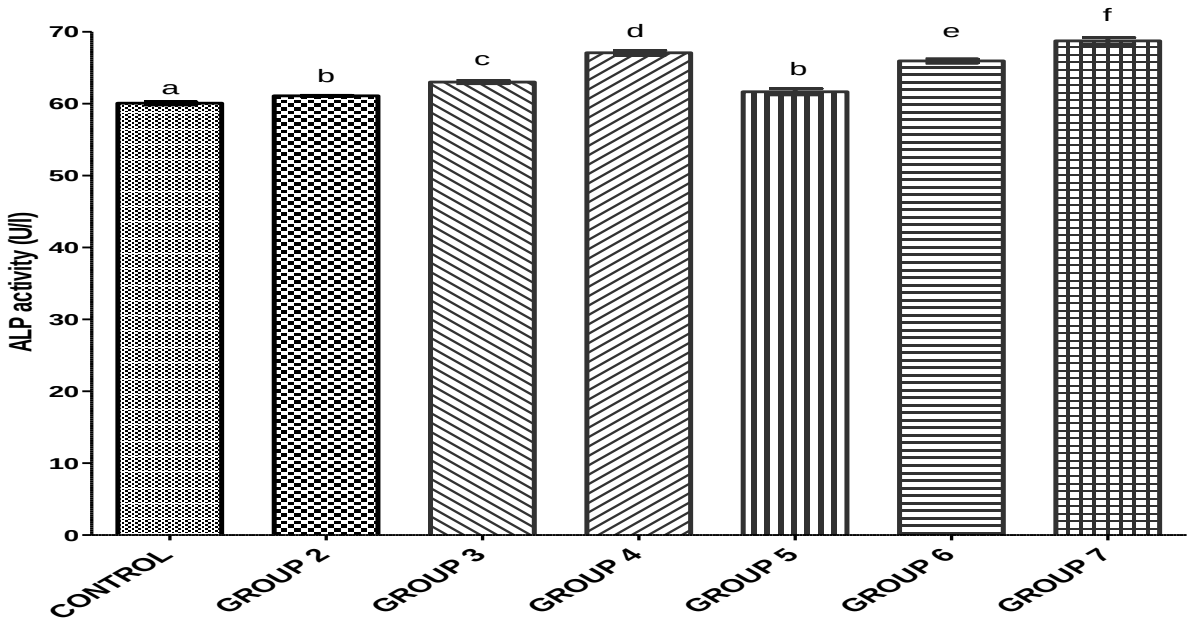

Figure 6. Alkaline phosphatase activity (U/l) of albino rats administered aqueous extracts of *Citrullus colocynthis*. Bars are mean ± standard deviation. Bars with different letters are statistically significant (p<0.05).

Furthermore, our results in Table 2 show the results of the concentrations of bilirubin and Figure 3 that of the concentrations of albumin, globulin and total protein. These concentrations of albumin, globulin, total protein and bilirubin in the serum can be used to assess the health status of the liver and can also be used to ascertain different type of liver damage (Yakubu et al., 2003). The liver is the sole site for the synthesis of albumin, which makes up to approximately 60% of serum protein concentration. This observation indicates that the extracts impaired the synthesis of albumin and subsequently serum total protein, hence causing no significant ($p>0.05$) difference in the concentrations of these parameters when compared with the control. Some of the data obtained with respect to liver function indices indicate a slight cellular toxicity of the aqueous extracts of *Citrullus colocynthis* on the liver of the experimental rats. This result agrees with the reports of Alkofahi et al (2007) who worked on combined toxicity of *Cassia senna* and *Citrullus colocynthis* of albino rats. The damage is however not total as the extracts did not affect significantly ($p>0.05$) the concentrations of albumin, globulin, total protein and bilirubin.

CONCLUSION

The available results from this study reveal that aqueous seed and pulp extracts of *Citrullus colocynthis* exhibited mild and selective toxicity with the liver as the target organ. Therefore, ethno-medicinal preparations from *Citrullus colocynthis* seed and pulp extracts may not be completely "safe" as an oral remedy; and long term administration and at high concentration should be avoided.

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