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The Effect of Methanol Extract of *Citrullus lanatus* Seed on Liver Function Indices in Wistar Albino Rats

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Abstract
This study examined the effect of methanol extract of Citrullus lanatus (watermelon) seed on the integrity of liver cells of Wistar rats. Thirty five (35) adult male rats were used. Various concentrations of the extract ranging from 10 to 5000 mg/kg b.wt were administered to the test rats. The normal control rats received distilled water, while the tween 80 control rats received tween 80 only. The treatment lasted for thirty-five days, after which plasma liver function indices were assessed. The results showed that body weight was significantly increased ($p < 0.05$) in the test rats compared to normal control. Liver to body weight ratio of test and control rats were not significantly different ($p > 0.05$), except in group VI where it was significantly increased ($p < 0.05$) compared to the other groups. The activities of plasma alanine aminotransferase (ALT) of groups IV, V, VI and VII were significantly increased ($p < 0.05$) compared to normal and tween 80 controls. Aspartate aminotransferase (AST) activities of test rats in groups III, VI and VII were significantly reduced ($p < 0.05$) compared to the two controls, while group IV (100 mg/kg b.wt. treatment) showed no significant difference ($p > 0.05$). Alkaline phosphatase (ALP) activities of rats in groups III and VI were significantly increased ($p < 0.05$) compared to the other groups. The total protein concentrations of rats in the treatment groups did not show any significant difference ($p > 0.05$) when compared to the normal control. The levels of albumin in groups IV, V, VI and VII were significantly decreased ($p < 0.05$) in comparison with both controls. The extract did not cause any significant difference ($p > 0.05$) in the concentrations of total, direct and indirect bilirubin.
Key words: *Citrullus lanatus,* Liver, hepatotoxicity, Liver function indices, Enzymes

Introduction
Consumption of fruits is no longer a mere result of personal preference and taste, but a concern of health as they contain important macronutrients and considerable amounts of micronutrients, such as minerals, fibers, vitamins and phytochemical compounds. Increasing evidence shows the importance of these micronutrients for human health [1] [2].. Tropical fruit consumption is increasing on domestic and international markets due to growing recognition of its nutritional and therapeutic values.

These fruits represent an opportunity for local growers to gain access to special markets where consumers lay emphasis on exotic character and the presence of nutrients capable of preventing degenerative diseases [3]. There is the potential use of tropical fruit pulps and their by-products in isolating specific phytochemicals for application in food supplements, dietary additives, new foods and pharmaceutical products [4].

The juice or pulp from *Citrullus lanatus* is considered as the edible portion but rind and seeds are discarded as major solid wastes [5]. As a member of the cucurbitaceae family, it is related to the cantaloupe, squash and pumpkin and other plants that grow on vines on the ground. *Citrullus lanatus* is a good source of carotenoid and lycopene. Lycopene has been found to be protective against a growing list of cancer [6]; it helps quench the free radicals that contribute to conditions like asthma, atherosclerosis, diabetes, colon cancer and arthritis. It is also high in fibre, citrulline and arginine [7, 8]. *Citrullus lanatus* seeds are known to be highly nutritional; they are rich sources of protein, B-group of vitamins , minerals (such as magnesium, potassium, phosphorous, sodium, iron, zinc, manganese and copper) and fat among others as well as phytochemicals [9]. The seeds are for instance used to prepare snacks, milled into flour and used for sauces. Oil from the seeds are used in cooking and incorporated into the production of cosmetics [10].

The liver is a major body organ involved in the detoxification and distribution of nutrients hence could be used to assess and establish the safety of a substance [11].

In this study, the effect of methanol extract of *Citrullus lanatus* seeds on the liver integrity of Wistar rats were investigated.

Materials and Methods
Sample Collection and Preparation
Citrullus lanatus fruits were purchased from a major market in Benin City, Edo State, Nigeria. Only healthy looking fruits were collected. The seeds obtained from their pods were shade dried, until a constant weight was

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obtained. The dried seeds were pulverized into fine powder using a mechanical blender. The powder was weighed and kept away from light before extraction.

Preparation of the Extract

Exactly, 2 kg of the powdered seeds was extracted with 5 litres of methanol (95 %) for 96 hours with constant stirring at intervals. The extract was filtered using muslin cloth and concentrated using a rotary evaporator, freeze-dried and kept refrigerated.

Experimental Design

Thirty five (35) adult male Wistar rats were used and divided into seven groups of five rats each.

Group I served as normal control and was given distilled water in place of extract.

Group II animals were given tween 80 (tween 80 was used to dissolve the extract before administration).

Groups III to VII animals were given different doses of the crude extract (10, 100, 1000, 2900 and 5000 mg/kg b.wt. respectively), daily for thirty five days. The animals were allowed free access to feed and water.

Collection of blood sample

At the end of the administration, the animals were anaesthetized with chloroform vapour. Blood samples were drawn from the rats hearts through cardiac puncture into heparin containers. The plasma obtained after centrifuging the blood at 3000 rpm for 10min was used to assay for the activities and concentrations of the liver function indices.

Biochemical Analysis

Liver function tests were carried out to determine the activities of alkaline phosphatase (ALP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and the levels of total protein, albumin and bilirubin. Alkaline phosphatase was determined by the sodium thymolphthalein monophosphate method [12], AST and ALT by dinitrophenyl hydrazine method [13]; total protein by biuret method and albumin by bromocresol green method [14]. Bilirubin was determined by the method described by Jendrassik and Grof [15].

Statistical Analysis

Results are presented as mean ± standard error of mean. Analysis of Variance (ANOVA) was used to analyze the results with Graph Pad Prism Demo version 6.07.

Result

Table 1: Changes in body weight of rats administered methanol extract of *C. lanatus* seed.

Groups	Final weight (g)	Initial weight (g)	Increase in weight
I	167.2 ± 6.73	139.80 ± 4.58	27.40 ± 4.37[a]
II	228.60 ± 5.51	159.60 ± 2.01	69.00 ± 3.65[b]
III	221.40 ± 8.76	145.60 ± 7.55	75.80 ± 2.03[b]
IV	221.00 ± 10.04	130.80 ± 9.81	79.20 ± 2.71[b]
V	231.40 ± 9.34	153.20 ± 7.93	78.20 ± 2.75[b]
VI	235.20 ± 10.37	157.20 ± 11.6	78.00 ± 0.45[b]
VII	235.40 ± 11.41	148.40 ± 11.41	77.00 ± 3.92[b]

Data are reported as mean ± SEM (n = 5). The values with different superscript within the same row or column showed significant differences ($p < 0.05$).

Table 2: Relative organ to body weight of rats administered methanol extract of *C. lanatus* seed.

Parameters	Liver weight	Final body weight	Liver weight/body weight ratio
I	5.20 ± 0.20	167.20 ± 6.37	0.03 ± 0.00
II	8.40 ± 0.25[a]	228.60 ± 5.51[a]	0.04 ± 0.00
III	7.00 ± 0.32[a]	221.40 ± 8.76[a]	0.04 ± 0.00
IV	7.60 ± 0.32[a]	210.00 ± 10.04[a]	0.03 ± 0.00
V	7.60 ± 0.51[a]	231.40 ± 9.34[a]	0.03 ± 0.00
VI	8.2 ± 0.58[a]	235.20 ± 10.73[a]	0.09 ± 0.00[a]
VII	8.2 ± 0.58[a]	225.40 ± 11.41[a]	0.04 ± 0.00

Data are expressed as mean ± SEM (n=5). Values with superscript ([a]) differ significantly ($p < 0.05$) from the normal control value

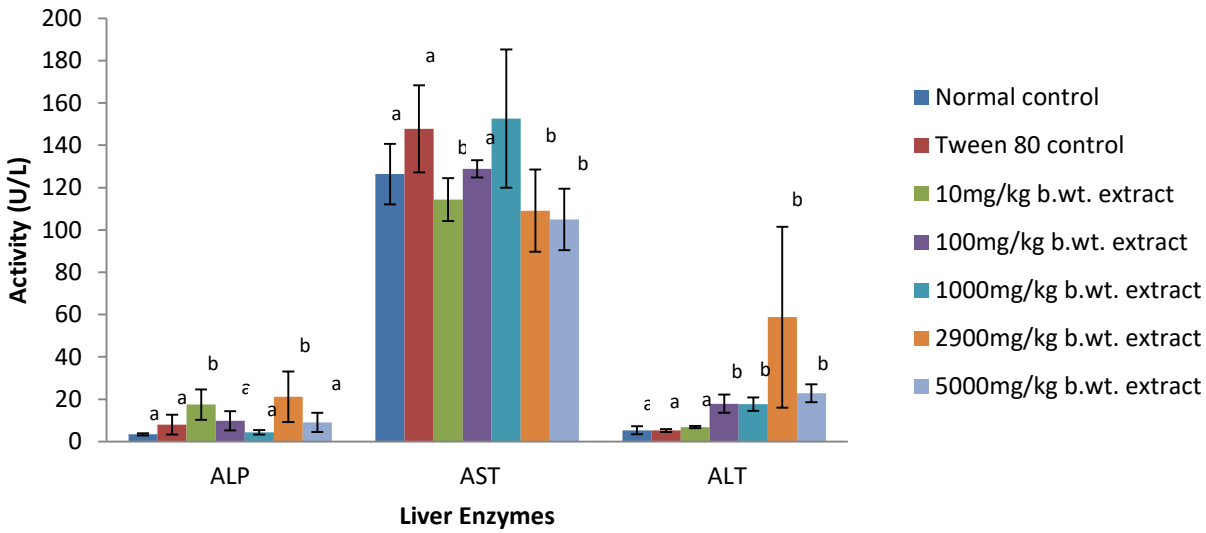

Figure 1: Effects of methanol extract of *C. lanatus* seed on liver function enzymes. Data are expressed as mean ± SEM (n = 5). The activities of plasma ALT of groups IV, V, VI and VII were significantly increased ($p < 0.05$) compared to normal and tween 80 controls. The activities of AST of test rats in groups III, VI and VII were significantly reduced ($p < 0.05$) compared to the two controls, while group IV (100 mg/kg b.wt. treatment) showed no significant difference ($p > 0.05$). The activities of ALP of rats in groups III and VI were significantly increased ($p < 0.05$) compared to the other groups.
ALP = alkaline phosphatase, AST = aspartate aminotransferase, ALT = alanine aminotransferase

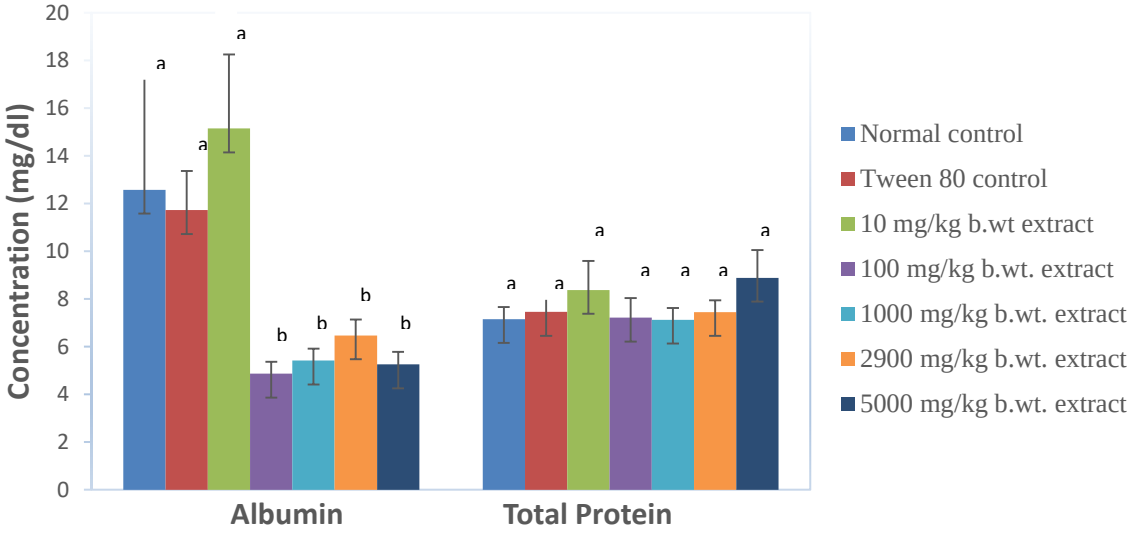

Figure 2: Plasma proteins and total bilirubin levels of rats administered methanol extract of *C. lanatus* seed. Data are reported as mean ± S.E.M. (n = 5). The total protein concentrations of rats in the treatment groups did not show any significant difference ($p > 0.05$) when compared to the normal control. The levels of albumin in groups IV, V, VI and VII were significantly decreased ($p < 0.05$) in comparison with both controls.

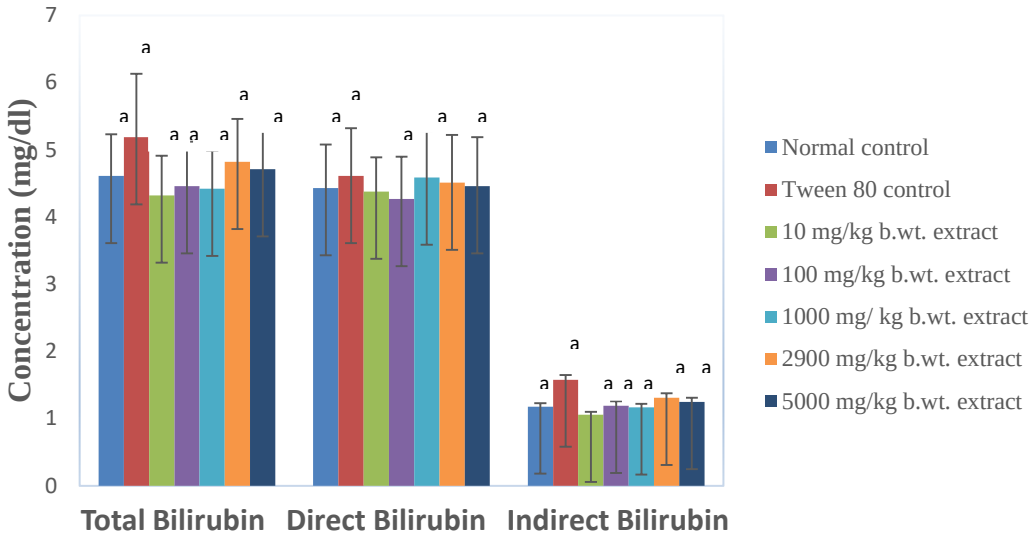

Figure 3: Direct and indirect bilirubin levels of rats administered methanol extract of *C. lanatus* seed. Data are reported as mean ± S.E.M. (n = 5). The extract did not cause any significant difference ($p > 0.05$) in the concentrations of total, direct and indirect bilirubin.

Discussion

In this study, liver function indices of rats treated with sub-chronic doses of methanol extract of *Citrullus lanatus* seed were assessed. There were significant increases in the weights of test rats compared to normal control. Liver to body weight ratio of test and control rats were not significantly different, except group VI (2900 mg/kg b.wt treatment) which was significantly increased compared to the other groups. Plasma ALT and AST are useful indices for identifying inflammation and necrosis of the liver. The activity of ALT is highest in the liver and lower in the kidneys and skeletal muscles. The activity of AST is located in the microsomal and mitochondrial portions of the liver cells as well as in the skin, skeletal muscles, pancreas and kidneys [16]. In this study, the activities of ALT of groups IV, V, VI and VII were significantly increased compared to normal and tween 80 controls. The activities of AST of test rats in groups III, VI and VII were significantly reduced compared to normal control, while group IV (100 mg/kg b.wt treatment) showed no significant difference when compared to the normal control. The activities of ALP of rats in groups III and VI were significantly increased compared to the other groups. The ability of the liver to synthesize albumin is reduced when the synthetic function of the liver is affected. The evaluation of plasma total protein alone may not tell the actual picture of the metabolic state of an individual, since the concentration of the various proteins are not affected by each other. An elevated level of total protein may be due to dehydration or infection. Plasma concentration may decrease due to impaired synthesis that can result from malnutrition, malabsorption, over-hydration and some forms of liver diseases [17]. The total protein concentrations of rats in the treatment groups did not show any significant difference when compared to the normal control. The significant decrease in albumin level may be due to an increase in the dose of extract given to rats in groups IV, V, VI and VII in comparison with both controls. This could be an indication that the changes may not necessarily be related to the extract. Bilirubin is a useful index of the excretory function of the liver and a marker for hemolytic anemia. In this study, the methanol extract did not cause any significant difference in the concentrations of total, direct and indirect bilirubin in the rats treated with sub-chronic doses of the extract. It may be stated that the excretory function of the rats liver were not significantly affected by the extract.

Conclusion

Considering the biochemical parameters measured, it appears that sub-chronic doses of methanol extract of *C. lanatus* seed may have no obvious effect on the integrity of the liver cells.

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