Original Research Article

HEPATOTHERAPEUTIC TENDENCY OF METHANOLIC EXTRACT OF *CITRULLUS LANATUS* RIND ON LIVER FUNCTION MARKERS IN NORMAL MALE WISTAR RATS.

ABSTRACT

It is a common practice to discard the peel or rinds of fruits. Interestingly, some parts of fruit humans find inedible actually possess bioactive nutrients that may be used for medicinal purposes. The effect of methanolic extract of *Citrullus lanatus* rind on liver function in normal male wistar rats was studied. 24 wistar rats with body weight of 150-250g were used for this research. The animals were simple randomly divided into four groups, 6 rats in each. Group 1 contained the control given normal saline and feed; group 2, a low dose, 50mg/kg of methanolic extract of *Citrullus lanatus* rind was administered, group 3 and 4 were administered medium and high dose of *Citrullus lanatus* rind extract 100mg/kg and 200mg/kg respectively. The *Citrullus lanatus* rind extract was administered via orogastric route and the experiment lasted for a period of 56 days. Blood samples were collected by left ventricular cardiac puncture for liver function test at the last day of the experiment. The outcome of this research revealed that medium and high dose administration of *citrullus lanatus* rind significantly ($p \leq 0.05$) reduced the serum level of liver enzymes alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) and also the liver substrate, total protein (TP).There was a non-significant ($p \leq 0.05$) change in serum total bilirubin and albumin when all doses were compared to the control. Prolonged and moderate ingestion of *Citrullus lanatus* rind may be of benefit in regulating blood level of liver enzymes, hence, its ingestion should be encouraged.

KEY WORDS: *Citrullus lanatus*, Liver function, Methanolic extract, Transaminase

INTRODUCTION

The use of plants as source of medicines can never be underestimated [1]. The discovery of the bioactive phytoconstituents derived from various plants have provided indispensible knowledge concerning the therapeutic effect of various types of plants and their varying species [3]. *Citrullus lanatus* is commonly called watermelon [2]. It is a popular vegetable that is consumed globally for diverse reasons due to its nutritional equivalent. The rind of watermelon is usually green in color but may vary depending on the specie [5]. In countries like Nigeria and some other parts of western Africa, the only part regarded as edible is the reddish inner fleshy part of the fruit [3]. Despite reports from other studies carried out generally, that revealed that the phytonutrient composition of the outer part or peel and seeds of most fruits are more abundant than the edible fleshy part [4], most individuals still dispose the rind of watermelon because they believe it has no nutritionally importance or better still it may be poisonous if ingested. Some tribes in Asia have already adopted it as a practice to prepare the rind as a special delicacy [6]. The rind can be fried, boiled, roasted or consumed in raw form by some natives in Asia and Europe [4]. The liver is a vital abdominal organ. Survival without the liver is very much impossible [13]. Liver function tests are used to help detect, monitor or evaluate liver diseases or damages and recovery from such changes in response to various therapeutic methods or agents [11]. Liver function tests include test for

liver enzymes like alanine transaminase, alkaline phosphatase, aspartate transaminase[10] and test for substrates like bilirubin and albumin [14].

MATERIALS AND METHODS

Plant and extract preparation

Large, fresh and healthy watermelons were purchased from Creek road market in Port Harcourt. The fruits were properly washed and the rinds were collected and extracted by maceration process for 48 hours using methanol. Qualitative phytochemistry and phytochemical analysis was carried out using standard laboratory techniques [21] to determine the phytoconstituents or phytoactive agents present in the rind. The preparation of the plant extract was carried out in the Department of Phytochemistry and Pharmacognosy, Faculty of Pharmaceutical Sciences, Madonna University.

Extract preparation

The watermelon rind was collected. It was ensured that it was well peeled out, separating it from the fleshy red part of the fruit using a knife. The rinds were dried for 2 days in an incubator at 70°C. The rind appeared smaller in size after drying due to heat induced shrinkage. The dried rind was then grinded using a manual grinder. The powdered rind was then measured with a weighing balance.50gram of the extract was introduced into 250ml of methanol using a measuring cylinder. The mixture containing 250ml methanol and 50gram of powdered extract was allowed to stay for about 2 days. After 2 days, the mixture was filtered using a filter paper and surgical gloves. The extract (filtrate) derived after the filtration process was dried for about 4 days in an incubator. The shaft (residue) derived from the filtration process was disposed. After about 4 days, the extract reduced in quantity due to the evaporation of methanol when exposed to heat in an incubator (Heat induced evaporation). The extract had a dark brown coloration. Concentration of the extract was 0.2mg/ml.

Phytochemical analysis

Test for alkaloids

0.2ml dilution of the extract was measured into a 250ml beaker and 50ml of 10% acetic acid in ethanol was added and allowed to stand for some minutes. This was filtered and the extract was concentrated on a water bath for one quarter of the original volume. Concentrated ammonium hydroxide was added drop wise to the extract until the precipitation was complete. The whole solution was allowed to settle and the precipitate was collected and washed with dilute ammonium hydroxide and then filtered. The residue is the alkaloid which was dried and weighed.

Test for saponins

0.2ml of the extract was added to warm water. The tube containing the extract and warm water was mixed properly and the presence of soapy lather signified the presence of saponins.

Test for flavonoids

The total flavonoid content was determined using a slightly modified method reported by Minotti and Aust, the extract was measured into three test tubes in the range of 50, 100 and 200 µL and each was mixed with 500 µL of methanol. Water was added to mark up to 200 µml. 50µml 10% $AlCl_3$ followed by 50µL of 1M potassium acetate and 1400µL water was

added and allowed to incubate at room temperature for 30 minutes. The absorbance of the reaction mixture was subsequently measured at 415nm; the total flavonoid content was subsequently calculated. The non-flavonoid polyphenols were taken as the difference between the total phenol and total flavonoid content.

Test for tannins

About 1ml of the methanol extract was added in 2ml of water in a test tube. 2 to 3 drops of diluted ferric chloride solution was added and observed for green to blue-green (Cathechic tannins) or a blue-black (Gallic tannins) coloration.

Test for phenols

The total phenol content was determined according to the method of singleton [20]. Briefly, appropriate dilution of the extracts were oxidized with 2.5ml of 10% Foli-Ciocalteau's reagent (v/v) and neutralized by 2.0ml of 7.5% sodium carbonate to pH 7.4. The reaction mixture was incubated for 40 minutes at 45^0C and the absorbance was read at 765nm in the spectrophotometer. The total phenol content was subsequently calculated as Gallic acid equivalent.

Test for steroids

Sterols and Steroids were sought by the reaction of Liebermann [24]. Ten (10ml) ml of methanolic extract was evaporated. The residue was dissolved in 0.5ml of hot acetic anhydride; we added 0.5ml of the filtrate chloroform Treated with the reagent of Libermann Burchardt. The appearance, at the interphase, a ring of blue-green, showed a positive reaction.

Test for terpenoids

With CHCl3 (3ml), dissolve the extract (same 3ml), include H_2SO_4 (conc. 2ml) after drying. For 2 minutes, allow to heat. Terpenoids indicted by solution that is gray.

Test for cardiac glycosides

Added about 2ml of HCL (dilute), to the extract (same 2ml) then pyridine (containing sodium-nitroprusside) and NaOH were included in the initial solution. Glycosides indicated by the appearance of scarlet red or pinkish color solution[21].

Experimental animals and protocols

Twenty four (24) adult male wistar rats weighing 150 to 250 grams were obtained from the experimental animal unit, Department of Human Physiology, Madonna University. All animals were physically healthy. Using simple random technique of sampling, the animals were divided into four (4) groups containing six (6) rats per group. The animals were allowed to acclimatize for 2 weeks before the start of the experiment which lasted for 42 days. All animals had access to food and water *ad libitium*. The cages were properly cleaned twice daily to avoid coprophagy.

Group 1: Control

Group 2: Low dose of *citrullus lanatus* rind

Group 3: Medium dose of *citrullus lanatus* rind

Group 4: High dose of *citrullus lanatus* rind

Extract treatment

The LD_{50} of methanolic extract was 1500mg/kg. Methanolic extract of watermelon rind was administered in 3 doses;

Low dose : 50mg/kg

Medium dose : 100mg/kg

High dose : 200mg/kg

The route of administration was the orogastric route.

Sacrifice and collection of blood samples

Several hours after treatment on the last day (day 42) of the experimental period, the animals were anesthetized using diethyl ether from sigma chemicals® and then they were placed in a supine position after which 5ml of blood was collected from the left ventricular chamber using a syringe. The blood samples were collected into well labeled heparinized bottles. All samples were taken to the laboratory for hematology, Madonna University Teaching Hospital, for analysis of liver function enzymes and substrates.

LIVER FUNCTION TESTS

Experiment to determine the liver function biomarkers were carried out using the standard laboratory procedures [14]. The biomarkers tested for include; alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), total proteins (TP), bilirubin and albumin[19]. This test was carried out on blood samples collected on day 42 of the experimental period.

Test for alanine transaminase (ALT) and aspartate transaminase (AST)

Activities of serum Aspartate transaminase (AST) and Alanine transaminase (ALT) were assayed by the reitman and frankel calorimetric method[17] in which 0.2 ml of serum reacted with 1ml of substrate (Aspartate and α-ketoglutarate for AST, while alanine and α-ketoglutarate for ALT, in phosphate buffer pH 7.4) and was incubated for an hour in the case of AST and 30 minutes for ALT. then 1ml of DNPH (Dinitrophenyl-hydrazine) solution was added to arrest the reaction and kept for 20 minutes in room temperature. After incubation, 1 ml of 0.4 N NaOH was added and absorbance was read at wavelength of 540nm.

Test for alkaline phosphatase (ALP)

Alkaline phosphatase in serum is determined by measuring the rate of hydrolysis of various phosphate esters under specified condition. The principle in the test includes;

$$\rho\text{- Nitro phenyl Phosphate} + H_20 \xrightarrow{\text{ALP}} \rho\text{-Nitro phenol} + H_3PO_4$$

P-Nitro phenyl Phosphate is hydrolyzed to ρ-Nitro phenol and inorganic phosphate. The rate at which the ρ-Nitro phenol Phosphate is hydrolyzed, measured at 405nm, is directly proportional to the alkaline phosphatase activity.

Test for total protein (TP)

The assay is based on a polypeptide chelation of cupric ion (colored chelate) in strong alkali. In general, biuret assays are useful for samples containing -1 to 10 mg protein/ml, which is diluted -5-fold by the added reagent to give a concentration of 0.2 to 2 mg/ml final assay volume (F.A.V.). Most proteins produce a deep purple color, with a maximum absorbance (λmax) at about 550nm.

Test for bilirubin

Method of estimation of bilirubin in serum was based on an indirect reaction method of Van den Berg: the bilirubin in serum reacted with a freshly prepared solution of Van den Berg's diazotized sulphonilic acid (0.5 ml). Afterwards, purple colored azobilirubin compound was formed which was measured at a wavelength of 540nm. This color was observed after the addition of methanol and serum was diluted with distilled water, (0.2 ml + 1.8 ml distilled water) (Klot, 2005).

Test for albumin

A bromocresol green (BCG) dye binding procedure was first proposed in 1964[16]. This procedure exhibited greater sensitivity and much lower susceptibility to interfering substances[15]. Albumin is bound by the BCG dye to produce an increase in the blue-green color measured at 630nm. The color increase is proportional to the concentration of albumin present.

Ethical Approval

This study was approved by Madonna University Research Ethics Committee. All experimental procedures were done strictly following the guidelines provided by the research ethics committee. The animals were sacrificed after exposure to diethyl ether according to EC directives 86/609/EEC. In addition, the laid down standards according to the 1964 declaration of Helsinki were strictly adhered to.

Statistical Analysis

Experimental data are presented in Mean±SEM. Percentage change was also calculated to make the data well translated. SPSS 20.0 was used for all calculations and statistical analysis such as One-way analysis of variance (ANOVA). Values are significant at $p \leq 0.05$ or at confidence interval of 95%.

RESULTS

Table 1: Phytochemical constituents of methanolic extract of *Citrullus lanatus* rind.

Phytochemicals	Indication
Saponin	−
Tannin	−
Flavonoids	++
Steroids	−
Alkaloids	++
Terpenoids	+
Phenol	+
Cardiac glycosides	+

Oils	+

+ = present - = Absent

Table 2:Effect of methanolic extract of *citrullus lanatus* rind on liver enzymes.

Treatments	ALT(U/L)	ALP(U/L)	AST (U/L)
Normal saline	1.1086±0.02	2.0874±0.17	39.3420±4.1
% Change	*-0.02*	*-0.03*	*-2*
50mg/kg of Extract	1.1083±0.01	2.0802±0.16	38.6000±33.5
% Change	*-60*	*-50*	*-50*
100mg/kg of Extract	0.476±0.02*	1.0498±0.41*	21.3350±148.3*
% Change	*-70*	*-50*	*-50*
200mg/kg of Extract	0.300±0.01*	1.0124±0.16*	21.0600±9.68*

*Values are expressed in Mean±SEM, n=6, * p≤0.05 compared to control*

From **table 1**, the Phytoconstituents of methanolic extract of *Citrullus lanatus* (watermelon) rind include flavonoids, alkaloids, terpenoids, phenols and cardiac glycosides.

From **table 2**, the data showed that there was a significant decrease (p≤0.05) in the alanine transaminase (ALT) 100mg/kg medium dose (0.476±0.02*) with a % change of -60 and 200mg/kg high dose (0.300±0.01*) groups with a % change of -70 when compared with the control (1.1086±0.02). There was no significant difference in the 50mg/kg low dose (1.1083±0.01) with a % change of -0.2, compared to the control.

The result for alkaline phosphatase (ALP) showed that there was a significant decrease (p≤0.05) in the group 3 (1.0498±0.41*) with a % change of -0.5 and group 4 (1.0124±0.16*) with a % change of -50 when compared with the control group (2.0874±0.17). There was no significant difference in the group 2 (2.0802±0.16) with a % change of -0.3, compared to the control..

The result for aspartate transaminase (AST) showed that there was a significant decrease (p≤0.05) in medium dose (21.3350±148.3*) with a % change of -50 and in high dose (21.0600±9.68*) with % change of -50 when compared to the control group (39.3420±4.1). Low dose group (38.6000±33.5) with a % change of -0.2 had no significant difference compared to the control.

Table 3: Effect of methanolic extract of *Citrullus lanatus* rind on liver substrates.

Treatments	TP(µmol/L)	ALB(µmol/L)	TB (µmol/L)
Normal saline	238.0468±25.0	59.2660±4.1	1.1370±0.06
% Change	*30*	*-0.1*	*20*
50mg/kg of Exract	298.4138±4.7*	58.4980±3.8	1.3800±0.13
% Change	*50*	*-10*	*40*
100mg/kg of Extract	361.2048±23.3*	54.1712±2.6	1.6177±0.02
% Change	*50*	*-10*	*-0.03*
200mg/kg of Extract	362.7020±5.2*	55.5514±1.8	1.1340±0.03

*Values are expressed in Mean±SEM, n=6, * p≤0.05 compared to control*

From **table 3**, the result for total protein (TP) showed that there was a significant increase ($p \leq 0.05$) in low dose (298.4138±4.7*) with % change 30 , medium dose (361.2048±23.3*) with % change 50 and high dose (362.7020±5.2*) with % change 50, when compared to the control group (238.0468±25.0*).

The result for albumin (ALB) showed that there was no significant change ($p \leq 0.05$) in all doses; low dose (58.4980±3.8), medium dose (54.1712±2.6) and high dose (55.5514±1.8)when compared to the control group (59.2660±4.1), at a % change of -0.1,-10 and -10 respectively.

The result for total bilirubin showed that there was no significant change ($p \leq 0.05$) in all doses; low dose (1.3800±0.13), medium dose (1.6177±0.02) and high dose (1.1340±0.03)when compared to the control group (1.1370±0.06), at a % change of 20,40and -0.03 respectively.

DISCUSSIONS

The phytoconstituents of methanolic extract of *Citrullus lanatus* rind is in correspondence with earlier studies [4][5][7]. Methanolic form of extraction yielded better concentration of the phytochemicals probably due to the non-polar biochemical nature of the various agents extracted for [4].The ALT, ALP, AST, Total protein, Albumin, Total Bilirubin are the most sensitive biochemical markers employed in the diagnosis of hepatic dysfunction [16].Treatment with moderate and high doses of *citrullus lanatus* rind methanolic extract resulted in significant reduction in serum levels of ALT, ALP and AST.Low dose treatment of the extract caused no significant change in serum ALT, ALP and AST in comparison with the control.The findings of this study is in agreement with earlier reports [3][7]. Watermelon rind contains significant quantity of antioxidant phytochemicals [4]. Alkaloids, flavonoids and phenols possess antioxidant properties [18]. Several studies have revealed the positive correlation between oxidative stress and cellular damage [4] [18].The extract also significantly increased serum level of total proteins (TP). This increase further reflects the ability for the extract to enhance the synthetic function of the liver as well as its hepatoprotective function. It is believed that flavonoid content in the rind extract may promote the synthesis of immunocompetent proteins by the hepatocytes [3]. This may be the reason behind the significant increase in total proteins after treatment with all doses of the extract. The total bilirubin and albumin showed no significant change in all treatment doses. The extract may, at the level of this study, be of low potency in affecting the rate of synthesis of albumin and secretion of bilirubin. Also the absence of saponins, a reported hemolytic phytoagent, may probably reduce or maintain the blood level of bilirubin[4].

Conclusion

Citrullus lanatus rind should be ingested as part of the fruit due to its therapeutic phytoconstituents. It has the tendency of being effective in management of defects in liver function.

Recommendation

This research work should be replicated on human subjects.

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