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Original research

Comparison of biochemical, haematological and pathological effects of *Citrulllus lanatus* rind fractions in liver of male wistar rats

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ABSTRACT
Medicinal plants are rich in phytochemicals compounds that can be beneficial for livestock production and for treatment of human diseases. This study was designed to compare biochemical, haematological and histopathological changes in rats treated with different fractions and doses of *Citrullus lanatus* rind (CLR). The powdered CLR was extracted with ethanol, later fractionated with different solvents (n-hexane, chloroform, ethanol) and crude ethanol extract subsequently labelled as CLRH, CLRC, CLREF and CLRCE respectively. This study was done with forty male Wistar rats randomly assigned to thirteen groups. Group A received 10ml/kg distilled water; groups B1, B2 and B3 received 100,800 and 160mg/kg CLRH respectively; groups C1, C2 and C3 received CLRC; groups D1, D2 and D3 received CLREF; groups E1, E2 and E3 received CLRCE dosages were administered similar to group B, these were done daily for 14 days. Blood and liver samples were subjected to haematological, serum biochemistry and histopathological analysis. Data were evaluated with differences between groups by analysis of variance, P<0.05 considered significant using Graphpad prism. The PCV (50.00±1.16% versus 48.50±0.65%) and leucocytes (9.37±0.71x10^{3}cell/μl versus 9.11±0.02 x10^{3}cell/μl) of rats treated with 1600mg/kg CLRCE were significantly high, while AST (18.00±1.16iu/Lversus 13.50±0.65iu/L) of rats treated with 1600mg/kg CLRH were significantly high when compared with rats treated with distilled water. Meanwhile there were congested central vein, hepatic atrophy and sinusoidal dilatations in liver of rats treated with 1600mg/kg of CLRC. It was concluded that CLR in ethanol is safe and improves haematological and biochemical parameters.

KeyWords: Biochemical, *Citrullus lanatus*, haematological, liver, pathological, rats.

INTRODUCTION
The use of medicinal plants is a common, indigenous and modern practice for therapeutics as lifestyle and complementary medical purposes (King *et al.,* 2006). Medicinal plants are naturally rich in phytochemicals such as flavonoids, tannins, glycosides, alkaloids and saponins (Bruneton 1999; De-Monte *et al.,* 2014). These bioactive metabolites initiate therapeutic properties for treatment of many diseases such as hypertension, bacterial infection, inflammation, and hepatic damage (Zlotek *et al.,* 2016). Medicinal plants can be in different preparations such as herbs, decoctions, extracts, and infusions (Kim *et al.,* 2002). The medium or solvent in which medicinal plants are stored can determine the pharmaceutical benefits or health importance of such medicinal plants.

The bioactive constituents of medicinal plants such as polyphenol compounds, flavonoids, isoflavonoids, coumarins, alkaloids, terpenoids and phytosterol had been shown to reduce liver enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase, (AST) and alkaline phosphatatase, (ALP) activities in liver (Stockham & Scott, 2002). These liver enzymes (AST, ALT and ALP) are primarily in hepatocellular cytoplasm and in a lesser value in kidney, heart and the skeletal muscles. This makes them indicators of acute hepatocellular damage when they are released from the cytoplasm into blood stream leading to high serum levels (Uduak *et al.,* 2013). These enzymes are good biochemical markers for liver necrosis in small animals (Cornelius, 1989; Ruethalo, 2008). High serum activity of AST, ALT and ALP may be noticed in cases like liver injury, cancers and cardiac infection (Jaroslaw *et al*., 2009).

Albumin is mostly synthesized in liver. So, the level can reflect liver function (Friedman & Fadem 2010). Albumin has a variety of physiological functions, such as maintaining osmotic pressure, transporting nutrients (fatty acids, bile acids, and cholesterol), scavenging free oxygen radicals and

enhancing hemostasis in the body (Wada *et al.,* 2017, Li *et al.,* 2019).

Haematology is study of the presence of metabolites and morphology of blood constituents in circulatory system of animals and the observations play vital roles in prediction of physiological, nutritional, clinical and pathological status of an animal patient (Etim *et al.,* 2014). Changes in haematological indices could be used to determine oxidative stress due to environmental, nutritional and/or pathological factors (Etim *et al.,* 2014). The cellular parts of blood consist of Red blood cells (RBCs; erythrocytes) which transport oxygen and carbon dioxide; White blood cells (WBCs; .leucocytes) which are the mobile elements of the body defence system (Waugh *et al.,* 2001).

Watermelon also known as *Citrullus lanatus* belongs to the family *Cucurbitaceae,* is cultivated worldwide and throughout the year (Levi and Thomas 2005; Aruna *et al.,* 2012).

The extracts of *citrullus lanatus* seed had been confirmed to possess pharmacological actions such as anti-inflammatory, hepatoprotective and antioxidant activity (Alka *et al.,*2008)

The *citrullus lanatus* fruit had been shown to possess significant anti-hepatotoxic properties (Altas & Kizil, 2011), with high anti-hyperlipidemic (Aruna *et al.,* 2012), anti-giardia, anti-inflammatory, anti-secretory, laxative, gastroprotective, anti-ulcerative, antibacterial, antioxidant, antimicrobial and analgesic activities in man and animals (Deshmukh *et al.,* 2015). *Citrullus lanatus* rind possesses analgesic activity (Kumari *et al.,* 2013); cardioprotective effects (Akintunde *et al.,* 2017). The *citrullus lanatus* fruit has haematinic effects in rats (Akintunde *et al.,* 2020). These medicinal potentials of *Citrullus lanatus* may be due to the presence of phytochemical constituents found therein.

The *Citrullus lanatus* rind (CLR) or watermelon outer cover is regularly discarded after it is removed from the fruit but it is edible and is sometimes used as a vegetable in meals (Zafar *et al.,* 2016). With presence of high phytochemical compounds in CLR-rind and its good antioxidant potentials (Akintunde *et al.,* 2021) there is need to preserve it in solvents or other media so that it can be used for livestock production instead of regularly discarding it. This study was designed to compare effects of different fractions of CLR on blood biochemistry, haematology and liver-pathology in male Wistar rats.

MATERIALS AND METHODS

ETHICAL APPROVAL

This study was approved by College of Veterinary Medicine, research ethics committee (CREC), Federal University of Agriculture, Abeokuta with ethical number FUNAAB/COLVET/CREC/2020/10/04. A sample of *Citrullus lanatus* fruit was deposited at herbarium unit of Botany Department, University of Ibadan, Ibadan where it was authenticated and a voucher number UIH 22872 was assigned to it.

COLLECTION OF PLANT AND PREPARATION OF EXTRACTS

Citrullus lanatus fruits were purchased from a local fruit market in Abeokuta Southwest Nigeria, with the geographical coordinates 7^{0}13’47’N 3^{0}26’21’E.

Each *Citrullus lanatus* fruits was rinsed and sliced while the rinds were carefully removed with a sharp knife. The rinds were chopped into small pieces and air-dried at room temperature. This was done until it attained a constant dry weight. The dried rinds were grinded to powdery form using an electric-powered grinder (Euro premium blender^{R} Ultima made in China).

PREPARATION OF CRUDE ETHANOL CLR EXTRACT

One hundred and ninety-two grammes (192g) of grinded CLR was soaked in 95% ethanol. The mixture was stirred at intervals of 12hours for 3days. The mixture was filtered using a clean and sterilized muslin bag. The filterate obtained was further re-filtered with a clean, sterile filter paper (whatman 2.0) and concentrated using a rotary evaporator (Heidolph laboaratory efficient model 517-01002-002 Germany) at 40^{0}C (Kil *et. al.,* 2009). The crude extract was labelled CLRCE and kept in refrigerator (4^{0}C) until used.

FRACTIONATION PROCESS OF CRUDE ETHANOL EXTRACTS OF CLR.

The crude ethanol CLR extract (CLRCE) was fractionated using the following solvents n-hexane, chloroform and ethanol according to their polarity index (Abdullahi & Mainul 2020).

The fractionation was initiated by pre-absorption process done by mixing 105g of silica gel added to 130g of crude ethanol CLR extract and air-dried overnight. Afterwards, about 50g of silica gel was poured into a vacuum liquid chromatograph (VLC) chamber or distillation tube under pressure by using a pump. This was followed with the addition of 100ml of n-hexane solution into the tube. These brought about a wine coloured filterate initially but later became colourless as the volume of the n- hexane increased. It was later stopped after a clear n-hexane filterate was obtained which was labelled CLRH. The procedure was repeated with chloroform and ethanol using same sample of *Citrullus lanatus* rinds inside the tube. The filterate were concentrated again using a rotary evaporator to obtain fractions that were weighed and labelled as CLRH, CLRC and CLREF (n-hexane, chloroform, and ethanol fractions) respectively.

EXPERIMENTAL DESIGN

The dosages used were according to the guidelines of Organization for Economic Cooperation and Development (OECD) number 130 (2010). This was used to determine the effectiveness in different fractions of CLR (CLRH, CLRC, CLREF) and CLRCE in vivo study. Forty (40) male Wistar rats weighing between 160-180g body weight. The rats were housed in experimental animal unit, Department of Veterinary Physiology and Biochemistry, University of Ibadan. They were kept in plastic cages, fed with feed (Vital Feed Nig. Ltd^R) and allowed access to clean water *ad libitum for* 7days. The rats were later randomly assigned into thirteen (13) groups. The group A (n=4) were given 10ml/kg distilled water (Control group); groups B_1, B_2 and B_3 (n=3 per group) were given 100mg/kg, 800mg/kg and 1,600mg/kg CLRCE respectively, however groups C_1, C_2 and C_3 (n=3 per group) were given 100mg/kg, 800mg/kg and 1,600mg/kg CLREF respectively. Also, groups D_1, D_2 and D_3 (n=3 per group) were given 100mg/kg, 800mg/kg and 1,600mg/kg CLRH respectively. Finally, groups E_1, E_2 and E_3 (n=3 per group) were given 100mg/kg, 800mg/kg and 1,600mg/kg CLRC respectively. The Wistar rats in different groups were orally administered daily for 14 days with different doses of extracts according to OECD (2010) guidelines. They were observed for toxicity signs, behavioural changes and mortality.

BLOOD AND LIVER SAMPLES COLLECTION

At the end of treatments, the Wistar rats were placed on light anesthesia with diethyl ether in a desiccator. 5ml of blood sample was collected through retro-orbital plexus (eye) from each rat. About 2ml was collected into EDTA coated bottles for haematological analysis and 3ml into plain sample bottle for serum analysis. The rats were humanely sacrificed while the liver was collected into 10% formalin solution for histopathological studies.

DETERMINATION OF HAEMATOLOGICAL PARAMETERS

The Packed cell volume (PCV) analysis was done using micohaematocrit method, total and differential leucocytes (WBC) count by haemocytometer methods (Schalm *et al.,* 1975).

SERUM ANALYSIS FOR LIVER FUNCTION MARKERS

The blood was left to clot in plain bottles, then centrifuged at 5000 rpm for 5 minutes afterwards, the serum was collected into plain serum bottles. The serum total protein was estimated using Biuret test (Lowry *et. al.,* 1951). The total protein concentration was calculated with the formula below:

$$\text{Total Protein (g/dl)} = \frac{\text{Sample}}{\text{Standard}} \times \text{standard concentration}$$

The albumin was determined with bromocresol green method (Doumas *et. al.,* 1971), albumin concentration was calculated with the formula below:

$$\text{ALB conc. (g/dl)} = \frac{\text{Sample}}{\text{Standard}} \times \text{Concentration of Standard}$$

Alanine transaminase (ALT) and Aspartate transaminase (AST) were assayed by method of Reitman and Frankel 1957, while Alkanine phosphatase (ALP) was determined by Rec. GSCC (1972) method. The total protein, albumin, AST, ALT and ALP were evaluated using Randox kits while the manufacturer's procedures were strictly followed.

HISTOPATHOLOGICAL TECHNIQUES

The liver was preserved in 10% formalin solution for histopathology. The tissues were dehydrated in 70%, 95%, absolute ethanol and another absolute ethanol for two hours in each solution. The sections were stained with haematoxylin and eosin (H and E) and mounted on permanent slides and observed under high power (x 40) microscope lens.

STATISTICAL ANALYSIS

The data generated from this study were presented as mean ± SD. The difference between the means in the treated groups and in the untreated groups were compared by one way analysis of variance (ANOVA) at 95% confidence interval using the Prism 7.0 Graphpad Statistic software.

RESULTS

HAEMATOLOGICAL RESULTS OF WISTAR RATS TREATED WITH DIFFERENT DOSES AND FRACTIONS OF CLR

PACKED CELL VOLUME, PCV (%)

The PCV value of rats treated with 1600mg/kg CLREF (50.33 ±0.88%) and 1600mg/kg of CLRCE (50.00±1.16%) were significantly ($P<0.05$) higher when compared with rats treated with normal saline (48.50±0.65%). While the PCV value of rats treated with 800mg/kg CLRH (46.00±1.00%), 1600mg/kg CLRH (46.33±0.88%) and 100, 800 and 1600mg/kg CLRC (43.67±2.40%, 44.67±0.88% and 42.33±2.33%) respectively were significantly ($P<0.05$) lower than rats in the control group treated with normal saline (48.50±0.65%).

PLATELETS ($X10^3$CELL/ μL)

The platelets conc. of rats treated with 800 mg/kg CLRH ($3.73 \pm 0.35 x10^3$cell/μL), 1600mg/kg CLRH ($4.23 \pm 0.23 x10^3$cell/μL) and 1600mg/kg CLRC (4.43 ± 0.33 $x10^3$cell/μL) was significantly ($P<0.05$) higher than rats treated with normal saline (2.67 ± 0.20 $x10^3$cell/ μL). However, the platelets conc. of rats treated with 100 mg/kg CLRCE ($2.56 \pm 0.30 x10^3$cell/μL) and 800mg/kg CLRCE ($2.98 \pm 0.51 x10^3$cell/ μL) and 1600mg/kg CLEF (3.11 ± 0.46 $x10^3$cell/μL) were increased but not significantly ($P>0.05$)

different from the platelets conc. of rats treated with normal saline(2.67±0.20 x10^3cell/ µL).

LEUCOCYTE (WBC) COUNT (X10^3CELL/µL)

There were leucophilia in rats treated with 100mg/kg CLRCE (9.50±0.04 x10^3cell//µL) and 1600 mg/kg CLREF (9.63±0.20 x10^3cell/µL) while there were leucopenia in rats treated with all doses of CLRH and CLRC fractions as well as 800mg/kg CLREF (9.37±0.11x10^3cell/ µL) when compared with rats treated with normal saline (9.11±0.02 x10^3cell/µL). Meanwhile, there were no significant (P>0.05) differences in leucocyte counts of rats treated with 1600mg/kg CLRCE (9.37±0.71x10^3cell/ µL) and 100m/kg CLREF (9.22±0.04x10^3cell/µL) when compared with rats treated with normal saline (9.11±0.02 x10^3cell/ µL)..

LYMPHOCYTE COUNT (X10^3CELL/µL)

There were lymphocytosis in rats treated with 1600mg/kg CLREF (8.15±0.12x10^3cell/ µL) while there were lymphopenia in rats treated with all doses of CLRH and CLRC fractions when compared with rats treated with normal saline in the control group (7.55±0.06 x10^3cell/ µL). Meanwhile there were no significant (P>0.05) differences in lymphocyte counts of rats treated with 1600mg/kg CLRCE (7.58±0.15 x10^3cell/ µL) and 100 and 800mg/kg CLREF (7.59 ±0.07x10^3cell/ µL; 7.60 ±0.03 x10^3cell/ µL) when compared with rats treated with normal saline (7.55±0.06 x10^3cell/ µL).

NEUTROPHIL COUNT (X10^3CELL/ µL

There were neutrophilia in rats treated with 100mg/kg and 800 mg/kg CLRCE, 800mg/kg CLREF (2.3±0.08x10^3cell/µL; 2.18±0.12x10^3cell/µL;1.71±0.04 x10^3cell/ µL) and all doses of CLRH and CLRC fractions when compared with rats treated with normal saline (1.39 ±0.02 x10^3cell/µL). Meanwhile there were no significant differences in neutrophil counts of rats treated with 1600mg/kg CLRCE (1.4±0.03 x10^3cell/µL) and 1600 mg/kg CLREF (1.36±0.01x10^3cell/µL) when compared with rats treated with normal saline (1.39 ±0.02 x10^3cell/µL).

EOSINOPHIL COUNT (X10^3CELL/ µL).

There were eosinopenia in rats treated with all doses of CLRCE, CLREF, CLRH and CLRC fractions when compared with rats treated with normal saline in the control group (Table I).

EFFECTS OF DIFFERENT FRACTIONS AND DOSES OF CLR ON SERUM PROTEIN IN RATS

TOTAL PROTEIN (G/DL)

There were slight hyperproteinaemia not significantly (P>0.05) different to rats treated with 1600 mg/kg CLREF (7.67±0.33 g/dl) and 1600 mg/kg CLRCE (7.67±0.33g/dl) when compared with rats treated with normal saline (7.50±0.29 g/dl g/dl). Meanwhile, there were significant (P<0.05) hypoproteinaemia in rats treated with 800mg/kg CLRCE (6.33±0.33 g/dl), 100, 800 mg/kg CLREF (6.33±0.33 g/dl, 6.00±0.00 g/dl); 100, 800 mg/kg CLRH (6.00±0.32 g/dl, 6.33± 0.33 g/dl); 800, 1600 mg/kg CLRC (6.00 ±0.00 g/dl) when compared with rats treated with normal saline (7.50±0.29 g/dl g/dl).

ALBUMIN (G/DL)

There were significant (P<0.05) hypoalbuminaemia in rats treated with 800mg/kg CLRCE (3.33±0.33g/dl)); 100, 800mg/kg CLREF (3.67±0.33g/dl)); 100, 800mg/kg CLRH (3.67±0.33g/dl)); 100, 800mg/kg CLRC (3.33±0.33g/dl), 3.67±0.33g/dl)) but slight hyperalbuminaemia not significantly (P>0.05) different in rats treated with 1600mg/kg CLREF (4.6±0.33 g/dl); 100mg/kg CLRC (3.3±0.33 g/dl) when compared with rats in control group treated with normal saline (4.50±0.29 g/dl).

ALANINE AMINOTRANSFERASE, ALT (IU/L)

The ALT concentration of rats treated with 1600mg/kg of CLRCE (7.00 ±0.58 iu/L), 100mg/kg of CLREF (7.00±1.73 iu/L) and 800mg/kg of CLRC (6.67±0.88 iu/L) were significantly (P<0.05) lower than ALT conc. of rats treated with normal saline (9.75±0.25 iu/L). While ALT concentration of rats treated with 800, 1600mg/kg of CLRH (11.33±2.19iu/L, 13.33±0.67iu/L), 100mg/kg, 1600mg/kg of CLRC (14.33±0.88 iu/L, 12.00±1.16 iu/L) were significantly (P<0.05) higher than ALT conc. of rats treated with normal saline (9.75±0.25iu/L).

ASPARTATE AMINOTRANSFERASE, AST (IU/L)

The AST concentration of rats treated with 1600mg/kg of CLRCE (11.00 ±0.58 iu/L) ; 100 and 1600mg/kg of CLREF (11.67±0.33iu/L) and 100mg/kg of CLRC (9.33±0.67 iu/L) were significantly (P<0.05) lower than AST conc. of rats treated with normal saline (13.50±0.65iu/L). While AST concentration of rats treated with 800, 1600mg/kg CLRH (15.67±2.33iu/L, 18.00±1.16iu/L) and 1600mg/kg of CLRC (16.67±1.76iu/L) were significantly (P<0.05) higher than ALT conc. of rats treated with normal saline (13.50±0.65iu/L).

ALKALINE PHOSPHATASE, ALP (IU/L)

The ALP concentration of rats treated with 100 and 1600mg/kg of CLRCE (37.67±2.40iu/L, 37.33±3.28iu/L); 800mg/kg CLRC (30.00±2.08iu/L) and 1600mg/kg of CLREF (41.00±1.16iu/L) were significantly (P<0.05) lower

Table I: Haematological values of Wistar rats treated with different fractions and doses of *Citrullus lanatus* rind (CLR)

Parameters	PCV (%)	Platelets (10^3cell/ µl)	WBC ($x10^3$ cell/ µl)	LYM ($x10^3$ cell/ µl)	Neutro. ($x10^3$ cell/ µl).	Eosino. ($x10^3$ cell/ µl)
A1(NS) Control	48.50 ±0.65	2.67 ±0.20	9.11± 0.02	7.55± 0.06	1.39 ±0.02	0.68 ±0.4
B1(100CLRCE)	49.33± 0.88	2.56±0.30	9.50 ±0.04^b	6.88 ±0.07 ^{a}	2.3 ±0.08^b	0.13 ±0.03^a
B2 (800CLRCE)	48.33 ±0.88	2.98± 0.51	8.86 ±0.05^a	6.45 ±0.05 ^{a}	2.18± 0.12^b	0.09±0.001^a
B3 (1600CLRCE)	50.00 1.16^b	3.11± 0.46^b	9.37± 0.71	7.58± 0.15	1.4± 0.03	0.3± 0.27^a
C1 (100CLREF)	47.67 ±0.88	3.46 ±0.37^b	9.22 ±0.04	7.59 ±0.07	1.41± 0.02	0.22±0.002^a
C2 (800CLREF)	48.67 ±1.20	3.00 ±0.58	9.37 ±0.11^a	7.60 ±0.03	1.71 ±0.04^b	0.06±0.008^a
C3 (16000CLREF)	50.33±0.88^b	2.59±0.28	9.63 ±0.20^b	8.15 ±0.12^b	1.36 0.01	0.11±0.008^a
D1 (100CLRH)	47.33 ±1.20	3.72±0.87^b	8.41 ±0.20^a	5.61 ±0.06^a	2.69± 0.04^b	0.12±0.009^a
D2 (800CLRH)	46.00±1.00^a	3.73±0.35^b	7.34 ±0.12^a	5.64 ±0.21^a	1.58 ±0.04	0.12±0.002^a
D3 (1600CLRH)	46.33 ±0.88	4.23±0.23^b	7.23 ±0.18^a	4.86±0.05^a	2.22± 0.04^b	0.12±0.002^a
E1 (100CLRC)	43.67 ±2.40^a	3.71±0.69^b	7.64 ±0.19^a	4.27±0.05^a	2.52 ±0.26^b	0.05 ±0.01^a
E2 (800CLRC)	44.67 ±0.88^a	4.82±0.84^b	7.28 ±0.07^a	5.29 ±0.08^a	2.8 ±0.04 ^{b}	0.08±0.008^a
E3 (1600CLRC)	42.33 ±2.33^a	4.43±0.33^b	6.99 ±0.01^a	4.77±0.08^a	2.13 ±0.06^b	0.087±0.003^a

a, b values with superscripts within the same column are significantly different (P<0.05) from control.

than ALP conc. of rats treated with normal saline (45.00±1.47iu/L). While ALP concentration of rats treated with 800, 1600mg/kg of CLRH (49.33±7.79iu/L, 54.00±4.33iu/L) and 1600mg/kg of CLRC (54.67±4.26iu/L) were significantly (P<0.05) higher than ALP conc. of rats treated with normal saline (45.00±1.47iu/L). However, there were no significant (P>0.05) differences in ALP conc. of rats treated with 800,1600mg/kg of CLRCE (46.00±4.16 iu/L, 37.33±3.28iu/L); 800mg/kg of CLREF (44.00±4.16iu/L) when compared with ALT conc. of rats treated with normal

Table II: Result of liver function tests of Wistar rats treated with different fractions and doses of *Citrullus lanatus* rind (CLR)

Parameters	TP(g/dl)	ALB. (g/dl)	ALT (iu/L)	AST (iu/L)	ALP (iu/L)
A1(NS)	7.50± 0.29	4.50± 0.29	9.75 ±0.25	13.50 ±0.65	45.00 ±1.47
B1 (100CLRCE)	7.50± 0.29	4.50± 0.29	9.75 ±0.25	13.50 ±0.65	45.00 ±1.47
B2 (800CLRCE)	7.00± 0.00	4.00 ±0.00	8.33 ±0.88	12.33 ±0.62	37.67 ±2.40^a
B3 (1600CLRCE)	6.33 ±0.33^a	3.33 ±0.33^a	11.00±0.58^b	14.67 ±0.88	46.00 ±4.16
C1 (100CLREF)	7.67 ±0.33	4.33 ±0.33	7.00 ±0.58^a	11.00 ±0.58 ^{a}	37.33 ±3.28^a
C2 (800CLREF)	6.33 ±0.33^a	3.67 ±0.33^a	7.00 ±1.73^a	11.67 ±1.20 ^{a}	42.67 ±3.28
C3 (16000CLREF)	6.00 ±0.00 ^{a}	3.67 ±0.33 ^{a}	10.00 ±1.53	13.33 ±1.45	44.00 ±4.16
D1 (100CLRH)	7.67 ±0.33	4.67 ±0.33	8.33 ±0.88	11.67 ±0.33 ^{a}	41.00 ±1.16^a
D2 (800CLRH)	6.00 ±0.32^a	3.67 ±0.33	10.67 ±0.67	14.33 ±0.88	44.67 ±5.90
D3 (1600CLRH)	6.33± 0.33^a	3.67 ±0.33	11.33± 2.19^b	15.67 ±2.33 ^{b}	49.33±7.79^b
E1 (100CLRC)	6.67± 0.33	4.00 ±0.00	13.33± 0.67^b	18.00 ±1.16 ^{b}	54.00 ±4.33^b
E2 (800CLRC)	7.00 ±0.00	3.33 ±0.33^a	14.33 ±0.88^b	9.33 ±0.67^a	40.67 ±1.20
E3 (1600CLRC)	6.00 ±0.00^a	3.67 ±0.33 ^{a}	6.67 ±0.88^a	13.33 ±2.40	30.00 ±2.08^a

a, b values with superscripts within the same column are significantly different (P<0.05) from control.

saline (45.00±1.47 iu/L) (Table II).

HISTOPATHOLOGY

The liver of rats treated with 1600mg/kg of CLRCE had no enlarged central vein (CV), no sinusoidal dilatation (SD) and without hepatic atrophy (HA). There were acinar formation (AF) in liver of rats treated with 100mg/kg CLREF. Meanwhile, there were moderated hepatic atrophy (HA), moderated sinusoidal dilatation (SD), and degeneration of central vein (CV) in liver of rats treated with 800mg/kg of CLREF. However, there were hepatic atrophy (HA) in liver of rats treated with 1600mg/kg of CLREF (Plate I).

There were progressive pathological damages in the liver of rats treated with CLRH as the doses increseases from slight central vein degeneration (CV) in liver of rats treated with 100mg/kg CLRH; while liver of rats treated with 800mg/kg of CLRH had severe sinusoidal dilatation (SD), hepatic atrophy with severe central vein degeneration (CV) seen in 800mg/kg of CLRH. Meanwhile the liver of rats treated with 1600mg/kg of CLRH had enlarged central vein (CV), severe sinusoidal dilatation (SD) and moderate hepatic atrophy (HA) seen in 1600mg/kg of CLRH (Plate II). There was slight enlarged central vein (CV) in liver of rats treated with 100mg/kg of CLRC. Meanwhile, there were severe enlarged central vein (CV), moderate sinusoidal dilatations, with moderate hepatic atrophy (HA) in liver of rats treated with 800mg/kg of CLRC. Whereas, there were congestion in central vein (CV), severe hepatic atrophy (HA) and moderate sinusoidal dilatations in liver of rats treated with 1600mg/kg of CLRC (Plate III).

DISCUSSION

The mean PCV value of rats treated with 1600mg/kg CLRCE and 1600mg/kg CLREF were significantly higher than mean PCV values compared with rats treated with 100mg/kg CLRH which were significantly lowered when compared with mean PCV of rats treated with normal saline

The results of oral sub-acute toxicity test with fractions of CLR and that of crude ethanol extract of *Citrullus lanatus* rinds showed that they were all safe since there was no mortality recorded with oral daily administration for 14 days even at a dose of as high as 1600mg/kg. Loubna *et al.,* (2020) had stated that any substance with a lethal dose (LD_{50}) above 1000mg/kg body weight is considered safe.

It can then be deduced that the CLRCE and CLREF can provide more relief during oxidative stress as well as protect the body against nutritional and pathological derangement in the body (Bamishaye *et al.,* 2009). This study had shown that 1600mg/kg CLRCE has a high-value mean of leucocyte count when compared with other fractions of CLR considered in this study. This suggests that CLRCE can provide more phagocytic potentials when compared with other fractions of CLR considered which is also support the studies of Waugh *et al.,* (2001) and Douglas *et al.,* (2010) for phagocytic potentials or activity. This study revealed that ethanol extract of *Citrullus lanatus* rind has haematinic potentials as seen in *Citrullus lanatus* fruit (Akintunde *et al.,* 2020).

There was hyperproteinaemia in rats treated with 800 and 1600mg/kg CLREF and rats that received 800 and 1600mg/kg CLRCE which is an indication that the CLREF can initiate or enhance good affinity for transporting lipids, vitamins and minerals in the body with improved body immune system (Peng *et al.,* 2020). It can also be inferred that CLRCE will exhibit good affinity for effective cardiovascular system in the body as seen in the study of Akintunde *et al.,* (2017) on cardioprotective effects of *Citrullus lanatus* fruits in rats. It was observed in this study that there was marked hypoalbuminaemia in rats treated with 1600mg/kg CLRC in contrast to slight hypoalbuminaemia seen in rats treated with 1600mg/kg CLREF. This suggests that the CLRC at a high dose can cause hepatic malfunctioning if it is given for a longer period of time (Douglas *et al.,* 2010).

This study revealed that rats treated with 1600mg/kg CLRC had significantly higher mean AST, ALT and ALP values which suggest damage to the liver (Wu *et al.,* 2019). Thus the CLRH and CLRC at the studied doses caused increased AST, ALT and ALP values which could cause an impaired functional integrity of hepatic cells according to Peng *et al.,* (2020) who reported that increase in AST, ALT and ALP values indicate hepatocellular failure in the body. It was seen in this study that there was high level of AST, ALP and ALT values in rats treated with 1600mg/kg CLRH and 1600mg/kg CLRC. This suggests that there are possibilities of acute hepatocellular damage by these fractions of *Citrullus lanatus* rind when administered in the body (Uduak *et al.,* 2013).

The significantly low mean concentrations of AST, ALT and ALP values seen in rats treated with CLREF and CLRCE is a confirmation that they will be beneficial to the body. This hepatoprotective potentials of CLRCE in this study may be due to the presence of phytochemical compounds such as flavonoids, isoflavonoids, coumarins, alkaloids, terpenoids and phytosterol that are present in CLREF which can enhance liver integrity and functions of the liver (Li *et al.,* 2019). This could be due to the phytochemicals performing antioxidant roles by ensuring redox equilibrium in preserving the correct functionality of cellular vital functions (Valko *et al.,* 2007).

It was observed in liver of rats treated with CLRC and CLRH that there were enlarged central veins, sinusoidal dilatation and hepatic atrophy. This trend reveal the possibility of CLRC and CLRH causing acute liver failure leading to hepatotoxicity (Marwa *et al.,* 2019).

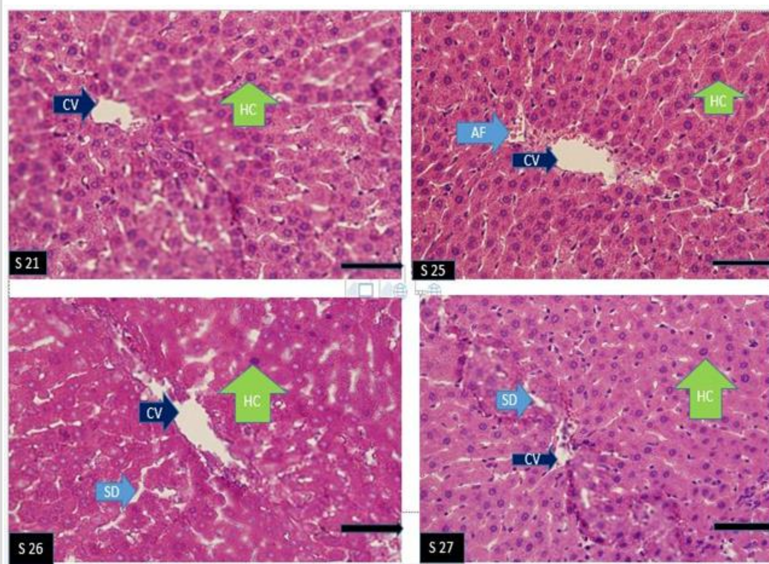

Plate I: Histopathology changes in liver of rats treated with normal saline (S21), 100mg/kg(S25), 800mg/kg (S26) and 1600mg/kg (S27) ethanol fraction of CLR (CLREF) (H and E stain Scale bar: 250µm). Details of acronyms: CV; central vein, HC; hepatic cell, AF; acinar formation, HA; hepatic atrophy, SD; sinusoidal dilatation, SD; sinusoidal dilatation.

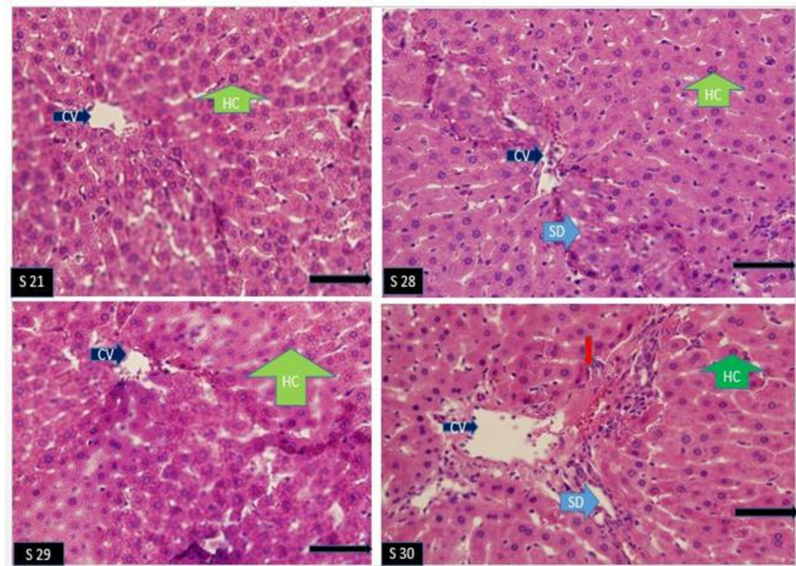

Plate II: Histopathology changes in liver of rats treated with normal saline (S21), 100mg/kg(S28), 800mg/kg (S29) and 1600mg/kg (S30) ethanol fraction of CLR (CLRH) (H and E stain Scale bar: 250µm). Details of acronyms: CV; central vein, HC; hepatic cell, HA; hepatic atrophy, SD; sinusoidal dilatation.

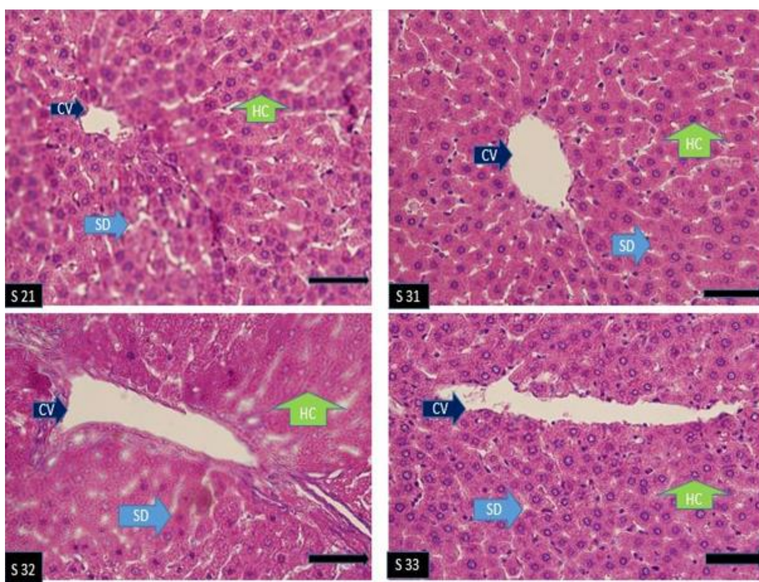

Plate III: Histopathology changes in liver of rats treated with normal saline (S21), 100mg/kg(S31), 800mg/kg (S32) and 1600mg/kg (S33) ethanol fraction of CLR (CLRC) (H and E stain Scale bar: 250µm). Details of acronyms: CV; central vein, HC; hepatic cell, HA; hepatic atrophy, SD; sinusoidal dilatation.

Meanwhile the liver of rats treated with CLRCE had no pathological changes. This is to confirm that CLRCE may not be injurious to liver. Thus, it is suggestive that CLRCE may possess some plethora phytoconstituents as found in *Citrullus lanatus* fruits (Kumari *et al.,* 2013) that may have been involved in protective effect.

CONCLUSION

It can be concluded from this study that *Citrullus lanatus* rind has some beneficial properties similar to observations seen in *Citrullus lanatus* fruits and seeds from previous studies (Shapiro, 2003; Aruna *et al.,* 2012; Kumari *et al.,* 2013 and Marwa *et al.,* 2019) considering the haematological parameters, liver function tests and histopathological changes in the liver. This may be due to presence of some phytochemicals found in CLRCE.

It can be inferred from this study that for high pharmaceutical activity, the CLR can be stored in ethanol medium or solvent before it is served to livestock for production when CLR is found in abundance which will invariably reduce the environment pollution that may arise from disposing it indiscriminately.

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