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ANTIOXIDANT, ANTI-INFLAMMATORY AND HEPATOPROTECTIVE ACTIVITIES OF *Citrullus LANATUS* RIND ON PARACETAMOL-INDUCED HEPATOTOXIC MALE WISTAR RATS

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ABSTRACT

Hepatotoxicity is a silent killing disease which may arise due to lifestyle and drug abuse. Paracetamol (PCM) is a common drug that is regularly used with and without physician prescription. An overdose of PCM can cause hepatotoxicity. *Citrullus lanatus* rind (CLR) is known to possess various beneficial properties such as antioxidant and anti-inflammatory effects. This study evaluated antioxidant, anti-inflammatory and hepatoprotective activities of CLR on PCM-induced hepatotoxic Wistar rats. Thirty male Wistar rats were distributed into six groups, namely: normal saline (NS), PCM, Silymarin (SLY), 100, 200 and 400 mg/kg CLR, respectively. Rats were pre-treated with SLY, 100, 200 and 400 mg/kg CLR for fourteen days prior to a single dose of 2000 mg/kg of PCM on fifteenth day. Antioxidant and inflammatory markers were evaluated in the liver. It was observed that 400 mg/kg CLR-treated rats had significantly increased antioxidant parameters such as catalase and glutathione –s- transferase in rat liver. There were observable hepatic atrophies in liver of rats treated with PCM which were prevented in CLR-treated rats. It was seen in the liver of CLR-treated rats that there was increased level of Cyptochrome p450, but reduced levels of Interleukin -6 and Tumour Necrotic factor- α in CLR-treated rats. It may be concluded that CLR demonstrated antioxidant, anti-inflammatory and hepatoprotective effects on PCM-induced hepatotoxic rats which may be suggestive of the potential to safeguard against hepatotoxicity.

Keywords: Watermelon; acetaminophen; liver damage; oxidation inhibitors; swelling reducers

INTRODUCTION

The Liver is an essential organ with many important functions in the body, including digestion, storage of glucose as glycogen, synthesis of proteins involved in the coagulation cascade, synthesis and storage of cholesterol, apoproteins, and vitamins. These diverse actions cause injury to the liver, which may include numerous etiologies such as drugs, chemicals, heavy metals, and plant toxins (Akharaiyi *et al.,* 2023). The liver plays a vital role in immunity development through the action of Kupffer cells (KC), besides being involved in xenobiotic detoxification, through cytochrome P450 family enzymes (Cornu *et al., 2020*). This exposes the liver to various oxidative stresses that cause accumulation of damage to cellu-

lar macromolecules, which may lead to excessive cell apoptosis, mainly via the mitochondrial pathway during liver injury (Chen *et al.,* 2020; Yang *et al., 2021*). Drugs or toxic substances can cause production of free radicals, and reactive oxygen species (ROS), which in excess can cause oxidative damage to cell constituents and macromolecules, such as membrane lipids, proteins, DNA, and enzymes. These pathogenic mechanisms lead to inflammation and invariably be cancerous at high dose of the toxicants, (Juan *et al.,* 2021). Paracetamol also known as Acetaminophen (APAP) is one of the most widely used analgesic and antipyretic over-the-counter drugs in the world (McCracken, 2015). The therapeutic concentrations, approximately 60–90% of APAP is metabolized in the liver by glucuronidation and sulfation, with a small part (approximately 5–15%) being metabolized by Cytochrome P_{450} pathway (CYP_{450}), (Marto *et al.,* 2021). Overdose of APAP depletes glutathione reservoir, leading to the rise of N-acetyl-p-benzoquineimine (NAPQI) level. The NAPQI will then bind to the cellular macromolecules, including proteins, lipids, and nucleic acids, resulting in centrilobular liver injury and hepatocyte death (Guengerich, 2020). Natural products have long been considered important sources of novel medicines and therapeutics due to the presence of phytochemicals such as triterpenoid, saponins, polysaccharides, flavonoids and quinones have been shown to have protective activity against APAP-induced hepatotoxicity, (Wang *et al.,* 2018; Liao *et al.*, 2023). There are numerous phytoconstituents taken from several plant species that have been proved to possess hepatoprotective activity (Ali *et al.,* 2019). Despite developments in modern medicine system, there are just a few effective natural medicines in the market that can be used to boost the liver function, protect the damage or help to regenerate hepatic cells (Bansal *et al.,* 2014). Antioxidant enzymes like catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), and non-enzymic antioxidants, such as ascorbic acid (AA) and reduced glutathione (GSH) are valuable indicators to relief oxidative damages and toxicities from toxicants. Phytochemicals from plants such as flavonoids, polyphenol, isoflavonoids, coumarins, alkaloids, terpenoids, phytosterol and saponin found in fruits and vegetables have been shown to avert risk of chronic degenerative diseases such as liver diseases and some cancers as well acting as a good antioxidants, (Zhu *et al.,* 2019). This is done with the assistance of Acetylcysteine. Acetylcysteine (NAC) is an effective antidote in cases of hepatotoxicity, (Chidiac *et al.,* 2023). The main function of NAC is to restore levels of glutathione (GSH), which is the primary antioxidant in all tissues. NAC can also decrease the levels of pro-inflammatory markers such as tumor necrosis factor-alpha (TNF-α), interleukins 6 (IL-6), and interleukin 1 beta (IL-1β) (Tieu *et al.,* 2023). Antioxidant *N*-acetylcysteine (NAC) has been reported to aid in shielding the liver against various injuries incurred through *medications,* toxins, and alcohol intake (Licata *et al.,* 2022). A number of herbal medicines and natural substances have been documented for their hepatoprotective properties, inferring that these act by preventing or repairing liver damage (Alkandahri *et al.,* 2023; Li *et al.*, 2022). Hepatotoxicity may be caused by drugs that can lead to acute reactions (such as hepatocellular necrosis), cholestasis (with or without inflammation), and miscellaneous reactions (Soren *et al.,* 2022). A wide range of studies revealed that production of free radicals such as reactive oxygen/nitrogen species (ROS/RNS) exert oxidative stress which is a major cause of hepatic abnormali-

ties like degeneration, necrosis apoptosis, swelling (Kumar *et al.,* 2019). Watermelon **(***Citrullus lanatus)* belongs to the family *Cucurbitaceae*. The pharmacological effects of *Citrullus lanatus* fruits have been enumerated to have antiemetic, diuretic and blood pressure lowering effects, (Zafar *et al.,* 2016). Also, anti-hepatotoxic (Altas and Kizil, 2011), anti-inflammatory, anti-secretory, laxative, gastroprotective, antiulcerative, antibacterial, antioxidant, antimicrobial and analgesic activities in man and animals (Deshmukh *et al.,* 2015) and hematinic effects in rats (Akintunde *et al.,* 2020) have been documented. These confirm that Watermelon possesses high antioxidant potentials that make it perform great functions as anti-inflammatory, antibiotics and antifungal roles (Abdul-azeez *et al.,* 2020). This may be due to the presence of numerous phytochemicals found in *Citrullus lanatus* such as flavonoids, steroids, phenolic, saponin and others. This study evaluated the antioxidant, anti-inflammatory and hepatoprotective activities of Watermelon (*Citrullus lanatus* Thumb) rind on paracetamol-induced hepatotoxic male Wistar rats.

MATERIALS AND METHODS

Collection and Authentication of *Citrullus lanatus* fruits.

The *Citrullus lanatus* fruits were purchased from a local fruit market near The Nigeria Police Force office, Eleweran Abeokuta Ogun State, Nigeria. A sample of *Citrullus lanatus* fruit was deposited at the Herbarium Unit, Department of Botany, University of Ibadan, Ibadan where it was authenticated and a voucher number UIH 22872 was assigned.

Reagents used

Gallic acid, 1,2-dichloro-4-nitrobenzene, thiobarbituric acid (TBA), trichloroacetic acid (TCA), sodium hydroxide, xylenol orange (XO), potassium hydroxide, reduced glutathione (GSH), O-dianisidine, and hydrogen peroxide (H_2O_2) were purchased from Sigma (St. Louis, MO, USA). Normal goat serum, biotinylated antibody, and horse radish peroxidase (HRP) system were purchased from KPL, Inc. (Gaithersburg, MD, USA). All other reagents were of analytical grade, unless otherwise stated. Deionized water was used to prepare all reagents and solutions. The antibodies were purchased from the following manufacturers: Cytochrome p450 from LSBio; TNF-α from Thermofischer and IL-6 from Origene while the DAB Chromogen and Polymer Detection were both from Biocare medical, all in USA.

Animals, grouping and dosing:

Thirty-five male Wistar rats weighing 160-180 g were used. The rats were given standard feed and water ad *libitum*, housed in controlled conditions (24±1°C temperature, 45 ± 5% humidity, and natural photoperiod of 12 h light and 12-h dark throughout the study period. The rats were acclimatized for one week before the dosing. This study was approved by FUNAAB Research Ethical Committee with approval number: FUNAAB/COLVET/CREC/2020/10/04. All animals received humane care according to the criteria outlined in the 'Guide for the Care and Use of Laboratory Animals' prepared by the National Academy of Science and published by the National Institute of Health. Five rats were used to confirm induction of hepatotoxicity by single oral administration of PCM 2000 mg/kg after fasting for seven hours; only rats with elevated level of AST higher than 12IU and creatinine value higher than 60 mg/dl were considered hepatotoxic, according to Enemali *et al.,* (2020).

The remaining thirty rats were randomly distributed into six groups containing 5 rats per group (n=5) as shown below:

Group I: administered with 1.0 ml/kg body weight normal saline (NS), for 14 days.

Group II: administered with 2000 mg/kg of PCM only, for 24 hours.

Group III: administered with 200 mg/kg of silymarin (SLY), for 14 days.

Group IV: administered with 100 mg/kg of CLR.

Group V: administered with 200 mg/kg of CLR.

Group VI: administered with 400 mg/kg of CLR.

Groups IV to VI were given CLR for 14 days, followed by 2000 mg/kg of PCM 24 hours later and the study was terminated on the 15th day.

Liver samples collection.
The rats were humanely euthanized, dissected to harvest liver sample. The harvested livers were divided into 2 parts (A and B). Part A was immediately frozen for antioxidant assay and Part B was fixed in 10% formalin for histopathological and immunohistochemistry analysis.

Preparation of liver homogenates
The liver homogenates were prepared for oxidative stress and antioxidant marker analysis.

Frozen livers were rinsed with homogenized buffer (50 mM Tris- HCl, pH 7.4) having 1.15% KCl. The homogenates were opened to cold centrifugation at -20°C with a speed of 10,000 revolutions per 15 mins to yield post-mitochondrial fractions (PMFs) of hepatic homogenates.

Oxidative stress Assay
Estimation of Nitric oxide (NO) assay
Nitric oxide (NO) assay in liver homogenates was determined using the Griess method as described by Sreejayan and Rao, (1997). The method utilizes conversion of Nitrite (NO_2^-) and Nitrate (NO_3^-) to a colored azo compound in acidic conditions. 1 mL of liver homogenate was incubated with equal volume of 1% sulfanilamide in 5% phosphoric acid for 15 minutes in the dark. Then, 1 mL of 0.1% N-(1-naphthyl) ethylenediamine (NED) was added, followed by thorough mixing and later incubated for 10-minute at room temperature in the dark. Absorbance was measured at 520 nm after 15 minutes of reaction. A standard curve generated with sodium nitrite ($NaNO_2$) was used to quantify NO levels in the samples. NO concentration (mol/L) was calculated as y - 0.03955)/0.1237, where y represents the sample absorbance, followed by calculations of NO concentration in g/g tissue.

Estimation of Lipid peroxidation (MDA).
The Lipid peroxidation was determined by measuring levels of Malondialdehyde (MDA) produced during lipid peroxidation. MDA generation, which was an index of lipid peroxidation was estimated from liver homogenates, quantified from post mitochondria fraction (PMFs) of liver tissues according to the method described by Oyagbemi *et al.*

(2015). The Thiobarbituric acid (TBA) reacted with Malondialdehyde (MDA) to form MDA-TBA solution which is a (Pink –coloured complex). This was determined with 400 µl of sample mixed with 1.6 ml of Tris-KCl buffer solution and 500 µl of 30% TCA was added. Then 500 µl of 0.75% TBA was added and placed in a water bath for 45 minutes at 80^0C. This was then cooled in ice and centrifuged at 3000 g for 5 minutes. The clear supernatant was collected and absorbance was measured against a reference blank of distilled water at 532 nm. The lipid peroxidation was expressed as MDA formed/mg protein or gram tissue was computed with a molar extinction coefficient of $1.56 \times 10^5 M^{-1} cm^{-1}$.

$$\text{LPO (MDA formed/mg protein)} = \frac{\text{Absorbance x volume of a mixture}}{\text{E532 nm x volume of sample x mg protein}}$$

Where: E532nm, molar extinction coefficient = $1.56 \times 10^5 M^{-1} cm^{-1}$.

Determination of Myeloperoxidase (MPO) activity

The MPO utilizes H_2O_2 produced by neutrophils to oxidize variety of aromatic compounds to give substrate radicals for bacterial activity. The HOCI is the most powerful bactericidal produced by neutrophils while excessive production of these radicals can cause oxidative stress leading to oxidative tissue injury. The MPO activity was measured spectrophotometrically using o-dianisidine (Sigma-Aldrich) and hydrogen peroxide. In presence of H_2O_2 as an oxidizing agent, MPO catalyzes oxidation of o-dianisidine yielding a brown-colored product, oxidized o-dianisidine, with a maximum absorbance at 470 nm. 200 µl of o-dianisidine (16.7 mg in 100 ml phosphate buffer) and 50 µl of diluted H_2O_2 (14 µl of H_2O_2 + 96 µl of distilled H_20) was added to 7 µl liver homogenate in duplicate. One unit of MPO is defined as that solution giving an increase in absorbance of 0.001 per min and specific activity is given as IU/mg protein.

Antioxidant Assay

Determination of Superoxide Dismutase (SOD) Activity

The SOD activity was evaluated from liver homogenates using method described by Oyagbemi *et al.* (2015). This was done with 10 µl of liver homogenates added to 2.5 ml of 0.05M carbonate buffer (pH 10.2) to equilibrate the spectrophotometer (i.e allow the reading to be stable in spectrophotometer before adding the epinephrine) while the reaction was initiated by adding 300 µl of freshly prepared 0.3 mM epinephrine to the mixture which was quickly mixed by inversion. The reference glass curvette contained 2.5 ml buffer, 300 µl of epinephrine, and 10 µl of distilled water was used as blank. The increase in absorbance at 480 nm was monitored at every 30 seconds from 0 and 150 seconds (2.5 mins).

Calculation of SOD:

$$\text{Increase in absorbance per minute} = \frac{A_3 - A_0}{2.5}$$

Where Ao = absorbance at 0 seconds; A_3 = absorbance at 150 seconds.

$$\% \text{ inhibition} = \frac{100 - 100 \text{ x increase in absorbance for substrate}}{\text{Increase in absorbance for blank}}$$

Reduced glutathione (GSH) assay

The method of Jollow *et al.,* (1974) modified by Oyagbemi *et al.* (2015) was used in estimating the concentration of reduced glutathione (GSH). Liver homogenates were deproteinized with addition of 0.15 M sulphosalicyclic acid (1:1, v/v). Protein precipitate was centrifuged at 4000 × g for 5 min. Thereafter, 0.5 ml of supernatant was added to 4.5 ml of DTNB (0.001 M). At 412 nm, absorbance of mixture was read against a blank consisting of 0.5 ml of deproteinizing agent diluted with water (1:1) and 4.5 ml of DTNB. The concentration of reduced glutathione is proportional to the absorbance. GSH concentration was inferred from calibration curve prepared with GSH standards.

Estimation of catalase (CAT) activity

Catalase (CAT) activity was determined by the method of Sinha (1972) modified by Oyagbemi *et al.* (2015) based on the reduction of dichromate (in acetic acid) to chromic acetate in the presence of H_2O_2. The assay mixture contained 4 ml of H_2O_2 solution (800 mol) and 5 ml of phosphate buffer (0.01 M, pH 7.0). 1 ml of diluted sample (1:10) was rapidly mixed with the reaction mixture at room temperature. 1 ml portion of the reaction mixture was withdrawn and blown into 2 ml dichromate/acetic acid reagent (1:3 by volume) at 60 s intervals. The chromic acetate then produced was measured colorimetrically at 570 nm, 3 min at 60 s intervals after heating reaction mixture in boiling water bath for 10 min. Catalase activity expressed as mol H_2O_2consumed/min/mg protein.

Estimation of Glutathione – S- Transferase activity (GST).

The GST activity was evaluated from liver homogenates using the method described by Habig *et al.* (1974) and modified by Oyagbemi *et al.* (2015). The principle was based on the fact that all known GSTs demonstrate a relatively high activity with 1-chloro-2, 4-dinitrobenzene (CDNB) as the second substrate. Consequently, the conventional assay for GST activity utilizes CDNB as substrate. There will be conjugation of this substrate with reduced glutathione which will lead to a shift at maximum absorption to a longer wavelength. The increase in absorption at new wavelength of 340 nm provides a direct measurement of enzymatic reaction. Each cuvette contained 0.15 mL CDNB, 0.03 mL reduced glutathione, and 2.82 mL phosphate buffer [0.1 M, pH 7.4, (blank)] or 2.79 mL phosphate buffer and 0.03 mL sample (liver homogenates). The reaction was initiated by adding the sample to the test cuvette, and the absorbance at 340 nm (yellow chromophore of CDNB-SG conjugate) was measured against the blank every 60 seconds intervals for 3 minutes.

Calculation:

ABS, Change in Absorbance = $Abs_{180} – Abs_0$

Where: Abs_0 = Absorbance at 0seconds; Abs_{180} = Absorbance at 180 seconds (3 minutes).

GST activity = ABS x 3.47/mg protein

= μmole/min/mg protein.

GST specific activity (μmole of CDNB – SG conjugate/min) mg protein

$$\frac{\text{Change Absorbance at 340 per min X reaction volume x dilution factor}}{\text{b x ε x sample volume x mg protein/mL}}$$

The molar extinction coefficient (Ɛ) at 340 nm = 9.6 mM^{-1} cm^{-1} and the cuvette length (b) is typically 1 cm.

Processing of Immunohistochemistry

The immunohistochemistry analysis was done for Cyptchrome P450 (Cypto p450), Interleukin-6 (Inter-6) and Tumor Necrotic factor (TNF)- α activities in rats liver homogenates were done in Department of Pathology, College of Veterinary Medicine, University of Georgia, U.S.A using MACH 43, Rabbit AP polymer detection and Warp Red chromogen kits according to the manufacturer (Thermo Fischer^{R}) procedure. Cytochrome p450 was done with the dilution rate 1:200, Interleukin-6 with the dilution rate of 1: 40 while Tumour Necrotic Factor - α was done with the dilution rate of 1:100.

Statistical analysis: Data were analyzed using one-way analysis of variance and expressed as mean ± standard deviation. Statistical analyses were performed using Graph Pad Prism version 6.02, for windows (Graph Pad software, San Diego, CA). Differences between mean values of different groups were considered statistically significant at $P < 0.05$ and marginally significant at $P < 0.1$.

RESULTS

Antioxidant parameters in liver of PCM – induced hepatotoxic rats treated with and without ethanol extract of CLR.

a. Superoxide dismutase SOD, µmol/mg

The concentration of SOD in liver of rats treated with 400 mg/kg of CLR (5.89±0.29) was significantly higher than concentration of SOD in liver of rats treated with PCM (4.31±0.08) -Table 1.

b. Catalase CAT, µmol/mg

The concentration of CAT in liver of rats treated with 100 and 400 mg/kg of CLR (2.73±0.02 2.71±0.19), respectively were significantly higher than concentration of CAT in liver of rats treated with PCM (1.95±0.14), Table 1.

c. Glutathionine GSH, µmol/mg

The concentration of GSH in liver of rats treated with 200 mg/kg of CLR (2.91± 0.49) and 400 mg/kg of CLR (2.94±0.96) were significantly higher than concentration of GSH in liver of rats treated with PCM (1.71± 0.11) – Table 1.

d. Glutathionine –S- transferase GST, µmol/mg

The concentration of GST in liver of rats treated with SLY (18.19±0.08), 200 mg/kg of CLR (18.09± 0.11) and 400 mg/kg of CLR 17.43± 0.07 were significantly higher than concentration of GST in liver of rats treated with PCM (17.09±0.10), Table 1.

Table 1: Antioxidant parameters in liver of PCM –induced hepatotoxic rats treated with and without ethanol extract of CLR.

Markers	NS (I)	PCM (II)	SLY (III)	100E+PCM (IV)	200E+PCM (V)	400E+PCM (VI)
SOD	7.37 ±0.17	4.31±0.08[a]	6.31± 0.10	5.14± 0.13[a]	4.33±0.17[a]	5.89±0.29
CAT	3.17 ±0.04	1.95±0.14[a]	2.78 ±0.02	2.73± 0.02	2.40±0.16[a]	2.71±0.19
GST	19.23±0.12	17.09±0.10[a]	18.19±0.08	16.58±0.3[a]	18.09±0.11	17.43±0.07[a]
GSH	2.44 ±0.38	1.71± .11[a]	3.66±0.09[b]	1.93 ±0.06[a]	2.91±0.49[b]	2.94 ±0.96[b]

a,b values superscripts within the same column are significantly different ($P<0.05$) from Groups I and II respectively.
SOD= superoxide dismutase, μmol/mg; CAT= Catalase, μmol/mg; GSH= Glutathionine, μmol/mg; GST= Glutathionine –S- transferase, μmol/mg.
NS: Normal saline; PCM: Paracetamol; SLY: Silymarin; 100 CLR: 100 mg/kg of CLR; 200 CLR: 200 mg/kg of CLR; 400 CLR: 400 mg/kg of CLR.

Reactive oxygen species parameters in Liver of PCM–induced hepatotoxic rats treated with and without ethanol extract of CLR.

a. Hydrogen peroxide H_2O_2, μmol/mg
The concentration of H_2O_2 in liver of rats treated with SLY (34.28±1.36); 200 and 400 mg/kg of CLR (34.28±1.36 and 41.07±1.84), respectively were significantly lower than concentration of H_2O_2 in liver of rats treated with PCM (53.68 ±3.04), Table 2.

b. Malondiahydide MDA, μmol/mg
The concentration of MDA in liver of rats treated with 100, 200 and 400 mg/kg of CLR (0.58 ±0.21, 0.36±0.11 and 0.40±0.17, respectively) were only significantly lower than concentration of MDA in liver of rats treated with PCM (1.92±0.67), Table 2.

c. Nitric oxide NO, μmol/mg
The concentration of NO in liver of rats treated with 400 mg/kg of CLR (0.15±0.47) was significantly lower than the concentration of NO in liver of rats treated with PCM (0.32±0.01), Table 2.

d. Myeloperoxidase MPO, umol/mg
The concentration of MPO in liver of rats treated with SLY (13.91±1.47) and 400 mg/kg of CLR (13.91±1.47) were significantly lower than the concentration of MPO in liver of rats treated with PCM (24.99±0.75), Table 2.

Table 2: Oxidative stress marker parameters in liver of PCM–induced hepatotoxic rats treated with and without extract of CLR

	NS (I)	PCM (II)	SLY (III)	100E+PCM (IV)	200E+PCM (V)	400E+PCM (VI)
H_2O_2	46.37± 1.93	53.68± 3.04[b]	34.28± 1.36[a]	48.90 ±3.48[b]	34.28 ±1.36[a]	41.07 ± 1.84[a]
MDA	0.42± 0.22	1.92± 0.67[b]	0.20± 0.01[a]	0.58 ±0.21	0.36 ±0.11	0.40 ±0.17
NO	0.038± 0.00	0.32± 0.01[a]	0.076± 0.01	0.16 ±0.07[b]	0.16 ±023[b]	0.15 ± 0.47[b]
MPO	13.12± 1.05	24.99± 0.75 [b]	13.91± 1.47	19.82 ±1.57[b]	15.10 ±1.12[b]	13.91 ±1.47

a,b values superscripts within the same column are significantly different (P<0.05) from Groups NS and PCM respectively. H_2O_2, = Hydrogen peroxide, μmol/mg; MDA= Malondiahydide, μmol/mg; NO=Nitric oxide, μmol/mg; MPO= Myeloperoxidase, μmol/mg.

NS: Normal saline; PCM: Paracetamol; SLY: Silymarin; 100 CLR: 100 mg/kg of CLR; 200 CLR: 200 mg/kg of CLR; 400 CLR: 400 mg/kg of CLR.

a,b values superscripts within the same column are significantly different (P<0.05) from Groups NS and PCM respectively. H_2O_2, = Hydrogen peroxide, μmol/mg; MDA= Malondiahydide, μmol/mg; NO=Nitric oxide, μmol/mg; MPO= Myeloperoxidase, μmol/mg.

NS: Normal saline; PCM: Paracetamol; SLY: Silymarin; 100 CLR: 100 mg/kg of CLR; 200 CLR: 200 mg/kg of CLR; 400 CLR: 400 mg/kg of CLR.

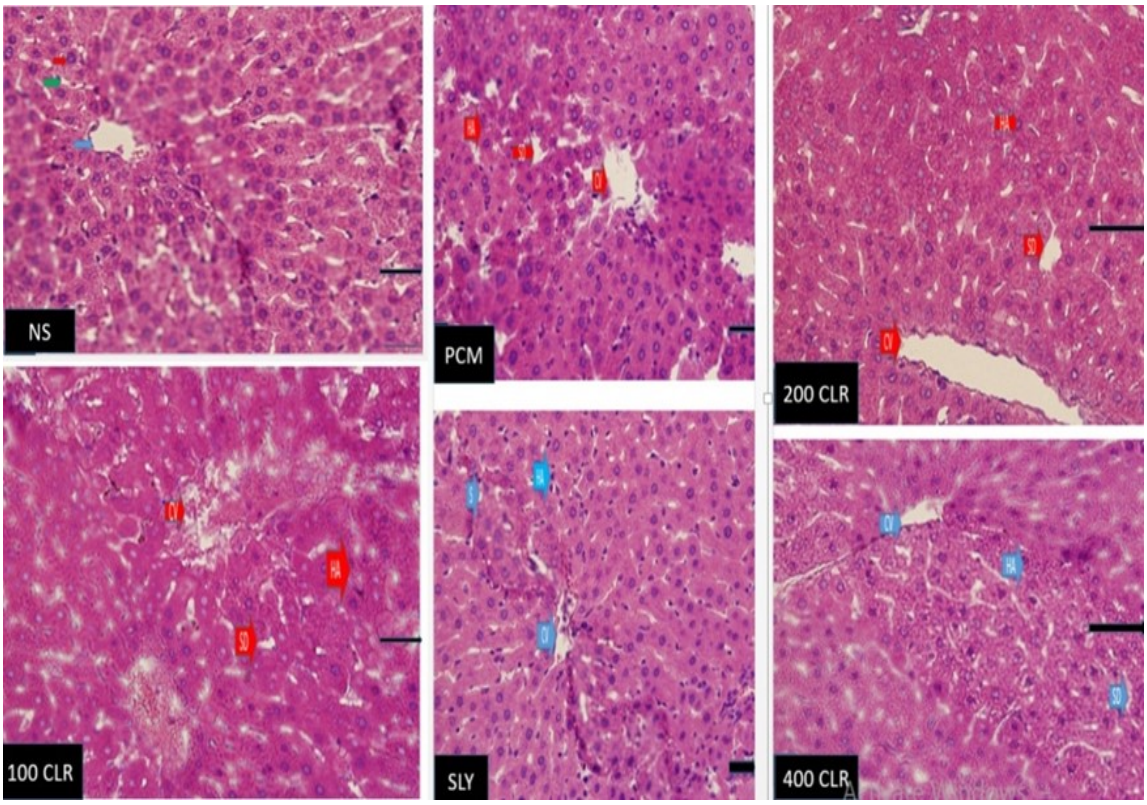

Plate 1: Histopathological results of liver in PCM-induced hepatotoxic rats treated with and without ethanol extract of CLR. (H and E stain Scale bar: 250 µm).
NS: Normal saline; PCM: Paracetamol; SLY: Silymarin; 100 CLR: 100 mg/kg of CLR; 200 CLR: 200 mg/kg of CLR; 400 CLR: 400 mg/kg of CLR.

Immunohistochemical expressions of Cyptochrome (P450) in Liver of PCM-induced hepatotoxic Wistar rats treated with and without ethanol extract of CLR.

There were severe Cyptochrome P450 antigen-antibody reactions in the hepatocytes marked with red arrow in liver of rats treated with PCM while Cyptochrome P450 antigen and antibody reactions were moderately expressed on hepatocytes in liver of rats treated with 100 mg/kg CLR but mildly expressed in hepatocytes of rats treated with SLY; 200 and 400 mg/kg of CLR (Plate 2).

Plate 2: Immunohistochemical expressions of Cyptochrome (P450) in Liver of PCM-induced hepatotoxic Wistar rats treated with and without ethanol extract of CLR.
(IHC stain, Scale bar: 250 µm). NS =Normal saline; PCM=Paracetamol; SLY= Silymarin; 100 CLR=100 mg/kg of CLR; 200 CLR=200 mg/kg of CLR; 400 CLR= 400 mg/kg of CLR; CV means Central Vein; HP means Hepatocyte

Immunohistochemical expressions of Interleukin-6 in Liver of PCM- induced toxic Wistar rats treated with and without ethanol extract of CLR.

There were no Interleukin-6 antigen – antibody reactions seen on liver of rats treated with normal saline and silymarin, but there were severe Interleukin-6 antigen-antibody reactions around kupkffer cell marked with red arrow in liver of rats treated with PCM while the Interleukin-6 antigen and antibody were moderately expressed around the kupkffer cell in liver of rats treated with 100 mg/kg of CLRCE + PCM but it was mildly expressed around the kupkffer cell marked with red arrow in liver of rats treated with 200 and 400 mg/kg of CLR (Plate 3).

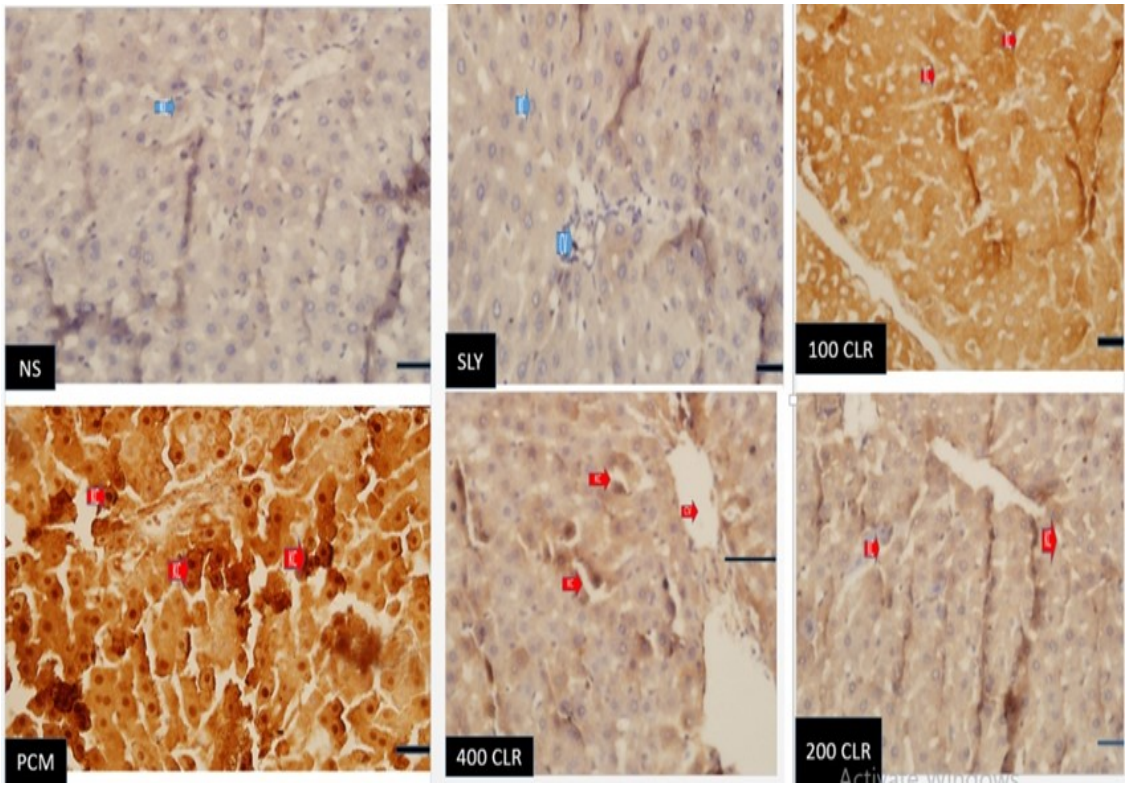

Plate 3: Immunohistochemical expressions of IL-6 reactions in Liver of PCM-induced hepatotoxic Wistar rats treated with and without ethanol extract of CLR. (IHC stain, Scale bar: 250 µm). NS: Normal saline; PCM: Paracetamol; SLY: Silymarin; 100 CLR: 100 mg/kg of CLR; 200 CLR: 200 mg/kg of CLR; 400 CLR: 400 mg/kg of CLR; CV means Central Vein; HP means Hepatocyte.

Immunohistochemical expressions of TNF-α in Liver of PCM- induced toxic Wistar rats treated with and without ethanol extract of CLR

The TNF-α antigen and antibody reactions were expressed around kupffer cells in liver of rats treated with PCM marked with red arrow. There were slight TNF-α antigen – antibody reactions marked with red arrow seen in liver of rats treated with 200 and 400 mg/kg of CLR (Plate 4).

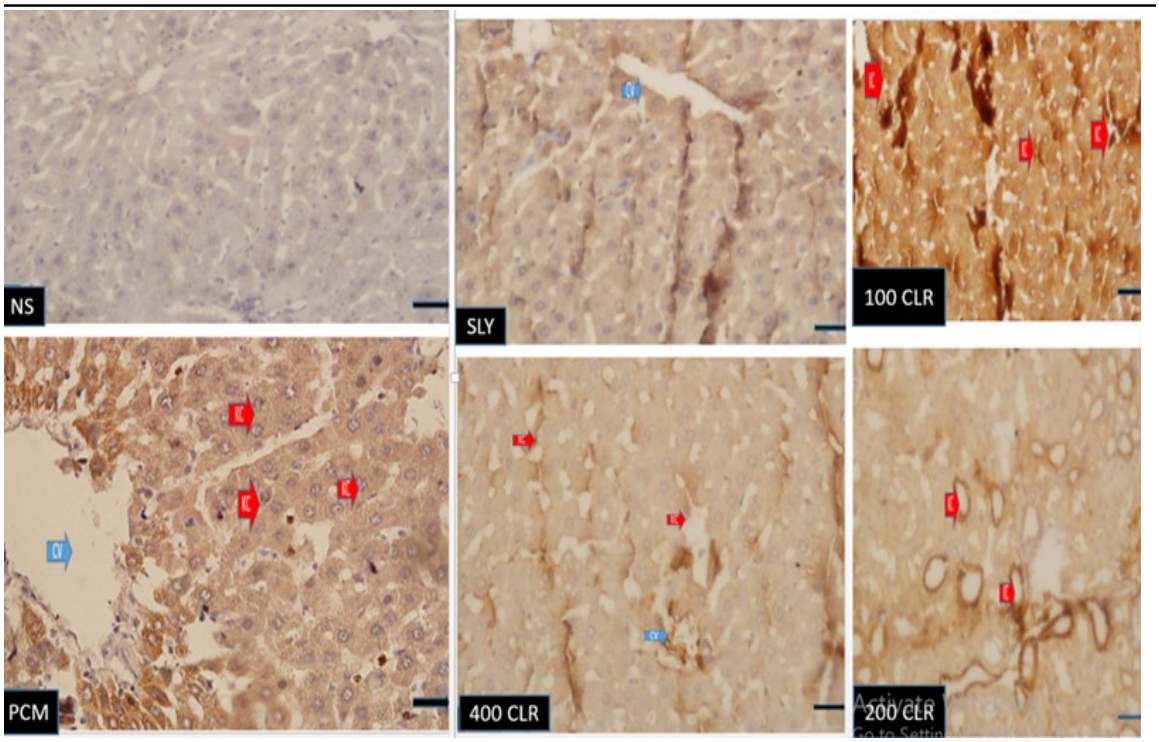

Plate 4: Immunohistochemical expressions of TNF-α reactions in Liver of PCM-induced hepatotoxic Wistar rats treated with and without ethanol extract of CLR. (IHC stain, Scale bar: 250 µm). NS: Normal saline; PCM: Paracetamol; SLY: Silymarin; 100 CLR:100 mg/kg of CLR; 200 CLR: 200 mg/kg of CLR; 400 CLR: 400 mg/kg of CLR; CV means Central Vein; HP means Hepatocyte

DISCUSSION

The non-significantly different concentrations of SOD and catalase seen in liver of rats treated with PCM-induction only but increased with Silymarin and 400 mg/kg CLR treated rats may be due to antioxidant defenses by phenolics substances, flavonoids, polyphenol and saponin found in Silymarin and CLR, (Zhu *et al.,* 2019). The SLY and 400 mg/kg CLR-treated rats exhibited protective effects with significant increase in GSH and GST in their liver which may be due to the antioxidants potentials seen in rats treated with SLY and CLR. This could have been due to the presence of enzymatic and non-enzymatic antioxidants found in SLY and CLR that defended the tissues against deleterious effects of reactive oxygen species caused by PCM induction, (Gillessen and Schmidt 2020). The observed reduced antioxidants such as low SOD, CAT and GST levels seen in PCM-induced rats liver in this study may be due to GSH depletion causing covalent binding of metabolites to macromolecules (Ritter *et al.,* 2008). This was a regular remarkable metabolism in acetaminophen-protein adducts formation (Gajendrer *et al.,* 2023), but this condition was reversed in Silymarin and 400 mg/kg CLR treated rats, suggesting APAP toxicity with nitric oxide production that scavenged superoxide to produce peroxyni-

trite due to protein nitration seen in tissue injury which is usually detoxified in conjugation with reduced GSH (Zhu *et al.,* 2019). It can be inferred that Silymarin and 400 mg/kg CLR protected the metabolism to achieve hepatoprotective effects. It can also be inferred that increased concentration of Glutathione-S-transferases (GST) which is a crucial Phase II enzyme in drug metabolism catalyzed the conjugation of –SH moiety of glutathione thereby neutralizing the electrophilic sites and rendering the products more water-soluble. This is a common occurrence in glutathione conjugation to produce a mercapturic acid for excretion (Gajendrer *et al.,* 2023).

Antioxidant *N*-acetylcysteine (NAC) has been reported to aid in shielding the liver against various injuries incurred through medications, toxins, and alcohol intake (Licata *et al.,* 2022). The oxidative damage caused by free radicals and reactive oxygen species (ROS) generated by toxicants are highly reactive which can predispose tissues to lipid peroxidation and tissue damage, thus causing increased values of H_2O_2, MDA, NO and MPO. This can exert oxidative stress which is a major cause of hepatic abnormalities like degeneration, necrosis apoptosis, swelling (Kumar *et al.,* 2019). This is similar to what was observed in liver of rats treated with PCM only but was significantly different from those observed in rats treated with 400 mg/kg CLR and Silymarin, thus emphasizing the protective effects of CLR and Silymarin. This is due to reduction of Fe^{3+} to Fe^{2+}, as well as generation of OH^- from H_20_2, and interaction with nitric acids (NO^-) to generate nitrite ($ONOO^-$) in the hepatocytes. These pathogenic mechanisms lead to inflammation and be cancerous at high dose of the toxicants, (Juan *et al.,* 2021). Drugs or toxic substances can cause free radicals, and reactive oxygen species (ROS) production, which in excess can cause oxidative damage to cell constituents and macromolecules, such as membrane lipids, proteins, DNA, and enzymes. This was exhibited in this study by the rats treated with PCM only.

The histopathological changes observe in liver of rats treated with PCM-induction and rats treated with 200 mg/kg CLR revealed that there were hepatic atrophy and mild sinusoidal dilatation with slight enlarged central vein in liver. There were improvement in histopathological changes in liver of rats treated with Silymarin and 400 mg/kg of CLR-treated rats prevented with pathological changes. Since hepatotoxicity caused by drugs can lead to acute reactions such as hepatocellular necrosis, cholestasis (with or without inflammation), and miscellaneous reactions (Soren *et al.,* 2022) thus still emphasizing the hepatoprotective effects of silymarin and 400 mg/kg of CLR.

Considering the inflammatory markers (Cytochrome p450, TNF-α and Interleukin), it has been established that high dose of acetaminophen administration can possibly lead to Cytochrome P450 and glutathione depletion (Garcia-Cortes *et al.,* 2020). This same trend was observed in this study with low Cytochrome P450 antigen-antibody reactions in rats treated with PCM-treated group but high Cytochrome P450 antigen-antibody reactions in rats treated with 200 and 400mg/kg CLR groups. It may be inferred that PCM-induced hepatotoxic rats showed initiated lipid peroxidation and protein carbonylation with formation of free radicals, (Tsukamoto and Lu 2001). These may have been resolved in the liver of rats treated with Silymarin and CLR. Thus it can be inferred that Silymarin and CLR can re-

duce or prevent lipid peroxidation and protein carbonylation as well as reduction of free radicals formation. This is also suggestive that Sliymarin and CLR can prevent hepatocellular damage by inhibiting the increase production of free radicals and other polyunsaturated fatty acids (PUFAs) within the hepatic that CLR could have prevented inflammatory reactions in the body. This may be prevented by the presence of phytochemicals such as triterpenoid, saponins, polysaccharides, flavonoids and quinones found in medicinal plants such as *Citrullus lanatus* that have shown protective activity against APAP-induced hepatotoxicity (Liao *et al*., 2023). Thus it may be inferred that the *Citrullus lanatus* had exhibited antioxidant properties with free radical scavenging ability in this study.

The high interleukin– 6 antigen – antibody reactions in PCM-treated rats but low interleukin antigen – antibody reactions in liver of rats treated with 200 mg/kg and 400 mg/kg groups is suggestive that Silymarin and CLR can reduce inflammatory changes in the liver. This may be due to generation of antioxidant *N*-acetylcysteine (NAC) in liver that restored the concentration of glutathione (GSH) with *Citrullus lanatus* which was generated by the PCM that caused initiated inflammatory reactions with production of pro-inflammatory markers such as tumor necrosis factor-alpha (TNF-α), interleukins 6 (IL-6), and interleukin 1 beta (IL-1β) (Tieu *et al.,* 2023). This study revealed that *Citrullus lanatus* resolved inflammatory reactions such as tumor necrosis factor-alpha (TNF-α) and interleukins 6 (IL-6). This confirms the assertion that medicinal plants always shown hepatoprotective properties with generation of NAC that lead to repairing liver damage (Alkandahri *et al.,* 2023).

CONCLUSION

It may be inferred from this study that:
Silymarin and 400 mg/kg *Citrullus lanatus* rind (CLR) prevented liver injury caused by paracetamol poisoning.

Oral doses of 200 and 400 mg/kg CLR protect inflammatory reactions caused by PCM-induced hepatotoxicity.

Citrullus lanatus rind (CLR) has antioxidant, anti-inflammatory and hepatoprotective effects on PCM-induced hepatotoxic rats which may be suggestive of the potential to safeguard against hepatotoxicity.

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