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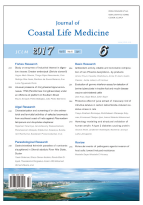

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Protective effect of juice extract of mesocarp rind of *Citrullus lanatus* in carbon tetrachloride-induced oxidative stress in rats

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ABSTRACT

Objective: To investigate the protective roles of juice extract of mesocarp rind of *C. lanatus* in carbon tetrachloride-induced oxidative stress in rats.
Methods: Thirty albino rats were divided into six groups of 5 animals each, namely Groups A–F. The oxidant (CCl_4) was used to induce oxidative stress in rats. It was administered as a single subcutaneous injection (2.0 mL/kg body weight) diluted 1:1 in paraffin oil on the 21st day. Animals in Group A (control) received 1 mL of distilled water; Group B received 1 mL of distilled water + CCl_4; Group C received 100 mg/kg body weight ascorbic acid + CCl_4; while Groups D, E and F were administered with 50, 100 and 200 mg/kg body weight of *C. lanatus* extract + CCl_4 respectively. The treatments were given once a day and lasted for 21 days. The levels of antioxidant enzymes: superoxide dismutase (SOD), catalase (CAT), glutathione-s-transferase (GST), reduced glutathione (GSH) and malondialdehyde (MDA) in the liver and serum were evaluated.
Results: The assessment of antioxidant parameters revealed a significant ($P < 0.05$) hepatic oxidative damage in CCl_4 treated albino rats, and this was considerably reversed to almost normal level in rats co-administered with juice extract of mesocarp rind of *C. lanatus* at the dose level of 200 mg/kg body weight/day for 21 consecutive days.
Conclusions: It can be concluded from this present study that the mesocarp rind of *C. lanatus* is rich in antioxidants and thus suggesting the possibility of utilizing it as nutraceutical or functional foods to prevent or manage some critical complications in living cells.

1. Introduction

Reactive oxygen species (ROS) are main free radicals generated as a result of oxidative stress, and these ROS may induce oxidative damage to essential biomolecules[1]. Over-production of ROS is termed as oxidative stress. Oxidative stress represents a disproportion between the systemic manifestation of ROS and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage[2]. There are various neurodegenerative diseases that can occur as a result of oxidative stress caused by generation of reactive oxygen species. These diseases include cancer, cardiovascular diseases, diabetes, tumors, rheumatoid arthritis and epilepsy[3,4]. Human body system is endowed with endogenous protective antioxidant enzymes such as superoxide dismutase that acts as first line of defense, and catalase that mop-ups free radicals which may be produced[5]. However, if the endogenous protective enzymes fall below normal level or become insufficient, dietary antioxidants are needed to counter the effect of excess free radicals production[6].

A number of synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylhydroxytoluene (BHT) have been shown to be carcinogenic[7,8]. Therefore, it is very necessary to find alternative antioxidants of natural origin which are cheap and safe. It has been reported that consumption of certain plant materials may prevent the risk of degenerative diseases caused by oxidative stress[9]. The protective effect of these plant materials against oxidative damage may be connected to the antioxidant compounds including carotenoids, vitamins, flavonoids and phenolics present in the plant[10]. Therefore, the search for antioxidants of natural origin as well as isolation of these antioxidant active components now become imperative[11-13]. Certain plants have been reported to be of medicinal importance, and the decoctions of local medicinal plants as well as vegetables consumed by people are believed to have a meaningful contribution to human health improvement, in terms

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of prophylaxis, or total cure of diseases[14]. One of such plants is *Citrullus lanatus* (*C. lanatus*) (family Cucurbitaceae).

C. lanatus is an annual plant which is originated from southern part of Africa[15]. The plant is up to 3 m long and the older part of the plant becomes hairless[16]. *C. lanatus* is an important vegetable crop which can adapt to different environmental and ecological conditions[17]. Morphologically, the leaves are simple, alternate on long petioles, cordate with seven shallow lobes and variously serrated margins, very hairy on the abaxial surface, acute, deep green, and about 7–15 cm in diameter[16]. The antioxidant activities, anti-inflammatory effects and several essential nutrients of *C. lanatus* fruits have been reported[18,19]. The juice or pulp of *C. lanatus* is consumed by people, while the rind and the seeds are usually discarded as agro-wastes[20-22]. Pectin can be extracted from the rind and the rind can also be used for products like pickles and preserves[23,24]. The rind is edible and sometimes used as vegetable. Therefore, this study was carried out to investigate the protective roles of juice extract of mesocarp rind of *C. lanatus* in carbon tetrachloride-induced oxidative stress in rats.

2. Materials and methods

2.1. Materials

2.1.1. Plant materials and authentication

The fresh *C. lanatus* was obtained from Ipata Market in Ilorin, Kwara State, Nigeria. It was identified and authenticated at the Herbarium Unit of Department of Botany, Faculty of Life Sciences, University of Ilorin, Ilorin, Nigeria where a voucher specimen (UILH/001/1252) was deposited.

2.1.2. Experimental animals

Thirty healthy albino rats (*Rattus norvegicus*) with an average weight of (147.21 ± 1.56) g were obtained from the animal house of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria. The animals were housed in clean iron cages, under well-ventilated housing conditions. The rats were acclimatized for one week and fed with rat pellet (Guinea growers mash) and tap water *ad libitum*. The present research adhered strictly and conformed to the Principles of Laboratory Animal Care (NIH Publication, No. 85-23).

2.1.3. Chemicals and reagents

Chloroform, ethanol, distilled water, soya oil, sucrose, hydrogen peroxide (H_2O_2), disodium carbonate (Na_2CO_3), sodium hydrogen carbonate ($NaHCO_3$), ascorbic acid, adrenaline (epinephrine), 1-chloro–2,4–dinitrobenzene (CDNB), sodium phosphate, glutathione, 5'5'-Dithiobis-(2-nitrobenzoate) (DTNB), sulfosalicylic acid ($C_7H_6O_6S{\cdot}2H_2O$), trichloroacetic acid (TCA), thiobarbituric acid (TBA), Tris base and potassium chloride (KCl) were purchased from SCBT, Heidelberg, Germany. Other chemicals used were of analytical grades.

2.2. Methods

2.2.1. Extract preparation

The back of *C. lanatus* was washed with distilled water to remove dirt. The fruit was cut with knife and the mesocarp rind was carefully separated from the epicarp and seed (endocarp). The white thick rinds (mesocarp) (200 g) of *C. lanatus* were homogenized in a blender and 1 100 mL of juice extract from *C. lanatus* was obtained and later lyophilized to give a final yield of 7.2g representing 3.6%.

2.2.2. Characterization of chemical constituents

The Trace GC Ultra and DSQII model MS from Thermo Fisher Scientific Limited, were used for analysis. The parameters of the instrument were set as follows: injector port temperature 250 ℃, interface temperature 250 ℃, and source was kept at 200 ℃. The oven temperature was programmed as a variable: 70 ℃ for 2 min, and increased to 150 ℃ at 8 ℃/min, then to 260 ℃ at 10 ℃/min. Split ratio was set as 1:50 and the injector used was in splitless mode. The DB-35 MS non-polar column (0.25 mm OD × 0.25 μm ID × 30 m length) procured from Agilent Co., USA was used. Helium was used as the carrier gas at 1 mL/min. The MS was set to scan from 50 to 650 Da. The source was maintained at 200 ℃ and < 40 motor vacuum pressure. The ionization energy was –70 eV. The MS also had inbuilt pre-filter which reduced the neutral particles. The data system had two inbuilt libraries for searching and matching the spectrum. NIST4 and WILEY9 each contained more than five million references. Only those compounds with spectral fit values equal to or greater than 700 were considered positive identification.

2.2.3. Animal grouping and treatment

Thirty healthy albino rats were randomly divided into six groups (A–F) of five animals each. The oxidant (CCl_4) was used to induce oxidative stress in rats. It was administered as a single subcutaneous injection (2.0 mL/kg body weight) diluted 1:1 in paraffin oil on the 21st day. The juice extract of mesocarp rind of *C. lanatus* was administered at 50, 100 and 200 mg/kg body weight, respectively. The experiment was designed as follow: animals in Group A served as control and received 1 mL of distilled water; Group B was administered with 1 mL of distilled water plus CCl_4; Group C was administered with 100 mg/kg body weight ascorbic acid plus CCl_4; Groups D, E and F were administrated with 50, 100 and 200 mg/kg body weight of *C. lanatus* extract plus CCl_4 respectively. The administration was done once a day (10:00 AM) and lasted for 21 days.

2.2.4. Preparation of serum and liver homogenate

At the end of the experiment, the rats were made unconscious using diethyl ether fume, sacrificed by simply incising the jugular vein and then quickly dissected, and their blood was collected. Their livers were excised, and the liver with a known weight was rinsed and then homogenized in ice cold 50 mmol/L Tris/HCl buffer (pH 7.5)[25] containing Triton X-100[26] at a final concentration of 0.5% to maintain the integrity of the tissues. The blood collected was left for 30 min to clot, and after which it was centrifuged at 10 000 r/min for 10 min using a Uniscope Laboratory Centrifuge and the supernatant (serum) was collected into sample bottles with the aid of a Pasteur pipette. All operations were carried out at 0–4 ℃ and the homogenate was stored in the freezer (each in a labeled specimen bottle) and used for analysis within 24 h[27].

2.2.5. Estimation of antioxidant parameters

The levels of catalase and superoxide dismutase activity were assayed following the previously described methods[28,29]. Glutathione-s-transferase was assessed following the reported method[30]. The levels of reduced glutathione (GSH) and malondialdehyde (MDA) were determined based on the described methods[31,32].

2.3. Data analysis

All data are expressed as the mean of five replicates ± SE. One-way ANOVA and Dunnett's *post hoc* test were used for multiple comparisons of treatment groups using GraphPad prism version 5.02. Values were considered statistically significant at the 95% confidence level.

3. Results

3.1. Phytochemical profile of juice extracts of mesocarp rind of C. lanatus

GC-MS analysis of juice extract of mesocarp rind of *C. lanatus* revealed the presence of 10 different phytoconstituents. The percentage of identified constituents is presented in Table 1.

Table 1
Chemical composition of juice extracts of mesocarp rind of *C. lanatus*.

S/N	RT (min)	Compounds	% Composition
1	10.14	6-Tetradecene	2.42
2	12.09	Phenol, 2,4-bis(1,1-dimethylethyl)	7.42
3	12.89	Cetene	2.98
4	15.28	1-Octadecene	3.07
5	17.35	Hexadecanoic acid	5.02
6	17.12	Octacosanol	6.81
7	19.36	1-Docosene	11.12
8	19.89	Octadecanoic acid	16.88
9	20.07	Octadecanoic acid	4.06
10	23.50	Bis(2-ethylhexyl) phthalate	40.22

RT: Retention time.

3.2. Antioxidants enzymes

Figures 1–3 show the effects of juice extract of *C. lanatus* on the activities of SOD, CAT and GST in the liver and serum of CCl_4 induced-oxidative stressed rats. The induction of oxidative stress by CCl_4 significantly decreased the activities of these enzymes (superoxide dismutase, catalase and glutathione-s-transferase) in the liver and serum of the animals (Figures 1–3). These declines in the activities of these enzymes by CCl_4 in the present study were significantly reversed by administrations of all the doses of the extract in a dose dependent manner. However, the SOD, CAT and GST activities of the animals administered with 200 mg/kg body weight of the extract were high and in all case significantly lower than the rats that received distilled water only (Figures 1–3).

Also, there was a significant reduction ($P < 0.05$) in the concentration of GSH, a non-enzymatic antioxidant in the animals that received distilled water + CCl_4 when compared with animals that received distilled water only (Figure 4). This reduction was reversed following the administration of juice extract of *C. lanatus*.

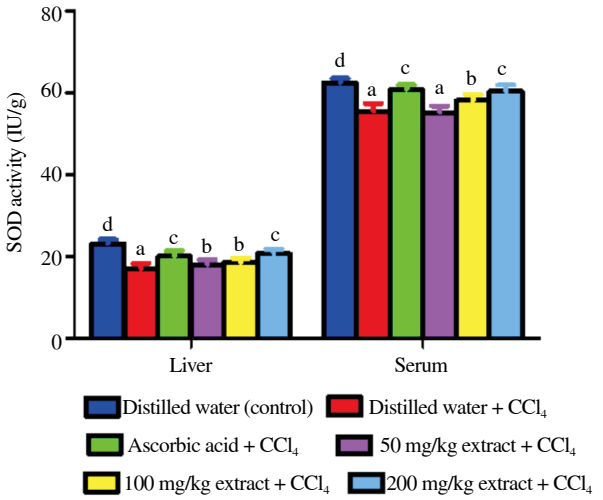

Figure 1. Effect of juice extract of *C. lanatus* on the specific activity of SOD in the liver and serum of CCl_4 induced-oxidative stressed rats. Bars with different superscripts for the parameter are significantly different ($P < 0.05$).

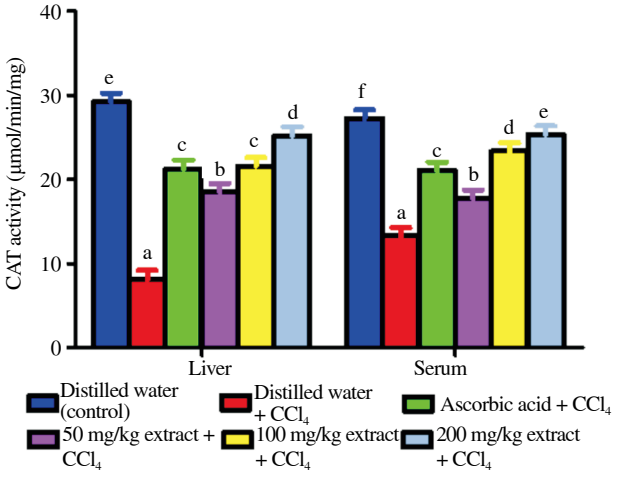

Figure 2. Effect of juice extract of *C. lanatus* on the specific activity of CAT in the liver and serum of CCl_4 induced-oxidative stressed rats. Bars with different superscripts for the parameter are significantly different ($P < 0.05$).

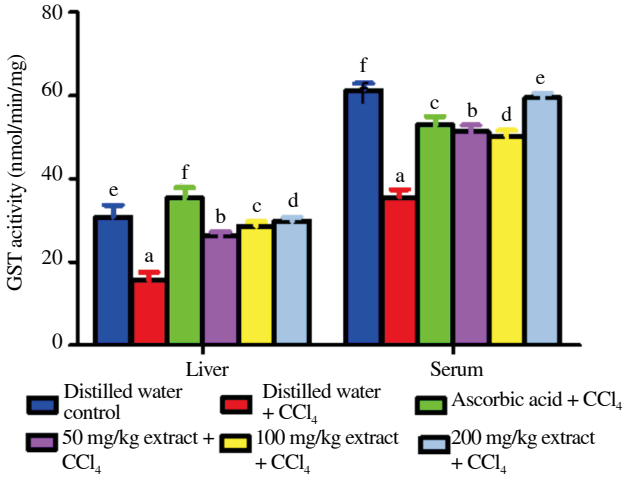

Figure 3. Effect of juice extract of *C. lanatus* on the specific activity of GST in the liver and serum of CCl_4 induced-oxidative stressed rats. Bars with different superscripts for the parameter are significantly different ($P < 0.05$).

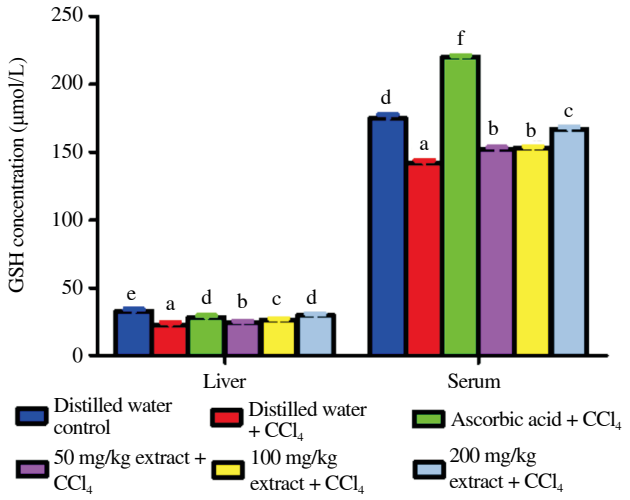

Figure 4. Effect of juice extract of *C. lanatus* on the concentration of GSH in the liver and serum of CCl_4 induced-oxidative stressed rats. Bars with different superscripts for the parameter are significantly different ($P < 0.05$).

Figure 5 presents the effect of juice extract of *C. lanatus* on the concentration of MDA in the liver and serum of CCl_4 induced-oxidative stressed rats. The result obtained from this study shows that there was a significant increase ($P < 0.05$) in the concentration of MDA in liver and serum in the animals that received distilled water + CCl_4 when compared with the rats administered with distilled water only. Upon treatment with the juice extract of *C. lanatus* and ascorbic acids, there was a significant reduction ($P < 0.05$) when compared with untreated groups. However, the activities of SOD, GST and GSH in the serum of extract-treated animals were higher compared with the liver (Figures 1, 3 and 4).

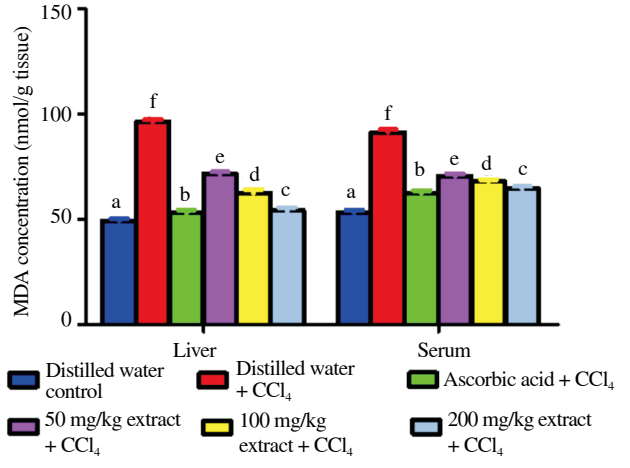

Figure 5. Effect of juice extract of *C. lanatus* on the concentration of MDA in the liver and serum of CCl_4 induced-oxidative stressed rats. Bars with different superscripts for the parameter are significantly different ($P < 0.05$).

4. Discussion

Researchers have carried out lots of researches and come up with the presence of varieties of secondary metabolites in medicinal plants[33,34]. These secondary metabolites are useful and have a wide range of effects on the biological systems[35]. The activities including antimicrobial, anti-inflammatory, antioxidant, hypocholesterolemic, cancer preventive, hepatoprotective, antiarthritic, antihistimic, antieczemic and anticoronary activities of some phytoconstituents such as palmitic acid (hexadecanoic acid, ethyl ester and n-hexadecaonoic acid), unsaturated fatty acid and linolenic (docosatetraenoic acid and octadecatrienoic acid) have also been reported[36]. Dietary antioxidants play a crucial role in delaying or thwarting the oxidation of lipids or other cellular compounds by hindering the initiation or prolongation of oxidative chain reactions[37]. The phytoconstituents present in extracts of medicinal plants are progressively used as a natural source of antioxidants in the manufacture of food or are taken directly as raw ingredients[38].

To the best of our knowledge, researchers have focused on the antioxidant and phytochemical components of *C. lanatus* fruit seeds and peels[19] with scanty information on the juice extract of mesocarp rind. The fruit of *C. lanatus* is consumed universally with the mesocarp rind always being discarded. Consequently, this study reported the protective roles of juice extract of mesocarp rind of *C. lanatus* in carbon tetrachloride-induced oxidative stress in rats. The results of the GC-MS analysis identified various compounds present in the juice extract of mesocarp rind of *C. lanatus* and showed 10 distinct peaks. The major compound present in the juice extract of mesocarp rind of *C. lanatus* identified by GC-MS was Bis(2-ethylhexyl) phthalate (40.22%) with RT of 23.50 min. The phenolic components[39] have been shown to possess antioxidant properties that mop-up free radicals, reduce singlet and triplet oxygen and/or decompose peroxides[40,41]. These phytochemical constituents have shown an important antioxidant activity, with protective effects against the free radicals, and may be beneficial for diseases such as diabetes[40] and other cardiovascular complications.

The CCl_4 taken by experimental animals is a toxic substance causing an alteration in the metabolism of liver through oxidative damage which can lead to degeneration of fats, fibrosis, hepatocellular death and cancer[42,43]. These alterations lead to generation of free radicals which in turn bring about loss of antioxidant defense enzymes in the biological system. The antioxidant enzymes have been reported to play a vital role in maintaining the physiological levels of oxygen and hydrogen peroxide and removing peroxides generated from inadvertent exposure to foreign compounds[44]. Any naturally occurring compounds with antioxidant properties when continuously taken as components of dietary food, spices or drugs may help in quenching free radicals that may be generated as a result of oxidative damage[44]. The CCl_4 caused free radical generation in form of reactive oxygen species and this was evident with the significant decrease ($P < 0.05$) observed in activities of superoxide dismutase (SOD), catalase (CAT) and glutathione s-transferase (GST) in the animals administered with distilled water + CCl_4. The increase in the levels of antioxidant enzymes *i.e.* SOD, CAT, and GST by juice extracted from mesocarp rind of *C. lanatus* may be attributed to the presence of phenol, 2,4-bis(1,1-dimethylethyl). The antioxidant potentials of phenol, 2,4-bis(1,1-dimethylethyl) had already been reported by Ajayi *et al.*[45]. GST being a soluble protein located in the cytoplasm plays a vital role in the cleansing

and excretion of foreign compounds[46,47]. GST binds GSH and this binding increases the solubility of hydrophobic substances which also play an important role in the storage and excretion of xenobiotics. Results from this study suggested that juice extracted from mesocarp rind of *C. lanatus* increased the GST activity and converted toxic compounds to less toxic compounds and thereby protected the membrane from oxidative damage[44,47].

Also, results obtained from this study revealed a total depletion in the concentration of GSH, a non-enzymic antioxidants, following the exposure of the rats to CCl_4. This was later increased following the administration of juice extracted from mesocarp rind of *C. lanatus* and ascorbic acid. GSH has been shown to play an important role in the detoxification of toxic reactive oxygen species. Previous studies on the mechanism of CCl_4-induced hepatotoxicity have shown that GSH is very important in detoxifying the reactive toxic metabolites generated as a results of CCl_4 induction, and liver necrosis begins when the GSH stores are markedly depleted[48].

Lipid peroxidation is the biological damage caused by free radicals, which are formed during oxidative stress. It is also used to investigate the oxidative damage of proteins and lipid peroxidation of the membrane and lipoproteins as possible pathogenic mechanisms for liver injury[49]. The increase in the level of lipid peroxidation in the liver and serum of the animals treated with water + CCl_4 suggests excessive formation of free radicals and activation of lipid peroxidation system. The reduction in the level of lipid peroxidation in the liver and serum of the animals following the administration of juice extract of mesocarp rind of *C. lanatus* suggests an anti-lipid peroxidative effect of the plant's juice extract. It is therefore not improbable that the active chemical compositions found in juice extract of mesocarb rind of *C. lanatus* may be responsible for the reported biological activities and pharmacological properties of the plant.

Researches are ongoing in our laboratory to isolate and investigate antioxidant effects of individual constituents of the extract from *C. lanatus* in order to ascertain which of the compound(s) is/are actually responsible for the observed antioxidant properties.

It can be concluded from this present study that the mesocarp rind of *C. lanatus* is rich in antioxidants and thus suggesting the possibility of utilizing it as nutraceutical or functional food to prevent or manage some critical complications.

Conflict of interest statement

We declare that we have no conflict of interest.

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