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Evaluation of Hepatoprotective Effect of Methanol Extract of *Solanum melongena* on Carbon Tetrachloride Induced Hepatotoxic Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Authors RUH and MBU designed the study. Author ARA performed the statistical analysis. Authors MBB and ANA wrote the protocol and wrote the first draft of the manuscript. Authors MBB and EHO managed the analyses of the study and the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To evaluate the effect of methanol extract of *Solanum melongena* on carbon tetrachloride-induced liver damage in albino rats.
Methodology: The qualitative and quantitative phytochemicals screening of the plant extract were done using standard method. Liver function test such as alanine aminotransferase (ALT), aspartate aminotransferase (AST) and Alkaline phosphatase (ALP) were determined in the serum of the rats at the end of the experiment. Likewise, the level of malondialdehyde (MDA) and the liver antioxidant enzymes such as superoxide dismutase (SOD) and Catalase were also determined.
Results: Phytochemical screening revealed the presence of bioactive compounds such as tannins (0.005±0.00 mg/g), saponins (0.64±0.03 mg/g), flavonoids (0.36±0.01mg/g) and alkaloids

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(0.036±0.00 mg/g). The activities of ALT, AST and ALP significantly increased (p>0.05) in CCl_4 induced groups when compared to all treated groups. The administration of methanol extract of *Solanum melongena* at 500 mg/kg body weight (bw) and 1500 mg/kgbw decreased (p<0.05) the activity of ALT in the treated groups. The activity of SOD and CAT in the CCl_4- induced group was decreased and this was significantly increased on treatment with the extracts at 500 mg/kg and 1500 mg/kgbw. However, methanolic extract of *Solanum melongena* at 1500 mg/kgbw showed a more significant increase in the activity of these enzymes. Also, increase in the level of MDA in CCl_4 treated group was observed when compared with the normal group and this was decreased (p<0.05) on administration with methanol extract of *Solanum melongena* at 500 mg/kg and 1500 mg/kgbw.

Conclusions: It can be concluded that methanol extract of *S. melongena* possess important phytoconstituents and hepatoprotective activity. Thus, the extract possess potent therapeutic agent for the management of liver damage.

Keywords: Liver enzymes; Solanum melongena; hepatotoxic; phytochemical; liver damage.

1. INTRODUCTION

The liver is a vital organ which assumes an essential position in the body [1]. It plays a vital role in xenobiotics metabolism and responsible for protection of human body against adverse effects of drugs, chemicals toxin, bacteria, virus and parasite. While carrying out the aforementioned activities, the liver itself is under threat and obviously needs protection. If any damage occurred to the liver, it results into many disorders ranging from transient elevation in liver enzymes to life threatening cirrhosis and hepatic failure [2]. So far, no effective measures are available for the treatment of liver diseases. The different medical, surgical and therapeutic methods available for the treatment of liver diseases are inadequate with generally poor result. Also, some of the drugs may themselves cause liver damage, for example, several compounds such as paracetamol, carbon tetrachloride (CCl_4), bromobenzene, ethanol and polycyclic aromatic hydrocarbon have been implicated in the aetiology of liver injury [3]. Hence, these chemicals have been widely employed in hepatotoxicity studies. It is therefore essential to search for alternative drugs for the treatment of liver disease to replace the existing drugs of doubtful efficacy and safety.

Plants are important sources of potentially useful compounds for the development of new chemotherapeutic agent [4]. There is a growing interest in natural antioxidants of plants origins in recent years. Several studies have indicated that medicinal plants contain a wide variety of natural antioxidant such as phenolic acids, flavonoids and tannins which possess antioxidant activity [5]. An example of medicinal plant with hepatoprotective activity is *Solanum melongena* as it has been proven from this study.

Solanum melongena belongs to the family solanaceae, a vegetable crop widely cultivated in many tropical, Asian, and some European countries [6]; it is popularly known as eggplant in English. It is known as Aubergine (UK) [7]. Melanzana, Garden egg, Brinjal (India). In Nigeria, it is traditionally called “Gauta” (Hausa) “Afufu” or “Anara” (Igbo) and Igba (Yoruba). The name eggplant derived from the shape of the fruit which are similar to chicken’s egg, the colour, size shape of the fruit vary significantly with the type of eggplant cultivar [8].

The Eggplant is widely used in traditional medicines, with various parts of the plant used in decoction as powder or ash for curing ailment such as diabetes, cholera, chronchitis, dysuria, dysentery, otitis, toothache, skin infection, haemorrhoid and asthma [9,10]. It has also been reported that this plant has various pharmacologic properties including anti-hypelipidemic [11] and analgesic properties [12], *Solanum melongena* has also been shown to suppress the development of blood vessels required for tumor growth and metastasis [13]. Nasunin isolated from the skin of purple eggplant is one phenolic compound implicated in both inhibitions of hydroxyl radical generation and super oxide scavenging activities [14].

Studies have shown that S. *melogena* possess important phytochemicals such as phenolics and flavonoids that provide definite physiological effect, against tumour [13], inflammation [15] and oxidative stress [16]. However, to the best of our knowledge scientific information are lacking on the hepatoprotective potentials of this plant using

a carbon tetrachloride induced hepatotoxic rats, hence the reason for this study.

2. MATERIALS AND METHODS

2.1 Sample Extraction

Ripe fruits of *Solanum melongena* were purchased in June 2013 from Kure market, Minna, Niger State, Nigeria and were authenticated at the National Institute of Pharmaceutical Research and Development (NIPRD) Herbarium, Idu-Abuja. The *Solanum melongena* fruits were rinsed in clean water, cut into pieces and air dried at room temperature. The dried fruit were grounded into powder and stored at room temperature in air tight container prior to use. The powdered sample of *Solanum melongena* (100 g) was extracted with methanol (400 ml) for 2 hours at 64℃ and the mixture was filtered using muslin cloth after which the filtrate was concentrated in water bath. Studies were carried out in the department of Biochemistry of Federal University of Technology Minna, Niger state.

2.2 Methods

2.2.1 Quantative phytochemical test

2.2.1.1 Estimation of alkaloids

Methanol extract (0.5 g) was dissolved in 96% ethanol and 20% H_2SO_4 (1:1) mixture. 1 ml of the filtrate was added to 5 ml of 60% tetraoxosulphate (VI) acid and allowed to stand for 5 minutes. Then, 5 ml of 0.5% formaldehyde was added and allowed to stand for 3 hours. The reading was taken at absorbance of 565 nm [17].

2.2.1.2 Total flavonoids determination

Aluminium chloride colorimetric method was used for flavonoids determination [18]. Each plant extracts (0.5 ml) was separately mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminium chloride, 0.1 ml of 1 M sodium acetate and 2.8 ml of distilled water. lt remained at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm with a double beam spectrophotometer (USA). The calibration curve was prepared by preparing quercetin solutions at concentrations 12.5 to 100 mg ml-l in methanol [17].

2.2.1.3 Estimation of saponins

Methanol extract (0.5 g) was added to 20 ml of 1 N HCl and boiled for 4 hours. After cooling, it was filtered and 50 ml of petroleum ether was added to the filtrate for ether layer and evaporated to dryness. Five millilitres (5 ml) of acetone ethanol was added to the residue. 0.4 ml of each was taken into 3 different test tubes. Six millilitres (6 ml) of ferrous sulphate reagent was added into them followed by 2 ml of concentrated H_2SO_4 which was thoroughly mixed after 10 minutes and the absorbance was taken at 490 nm [18].

2.2.1.4 Tannin determination

Methanol extract (0.5 g) was weighed into a 50 ml of distilled water and shaken for 1 hour using a mechanical shaker, it was then filtered into a 50 ml volumetric flask and made up to the mark. Five millilitre of the filtrate was the pipetted into another test tube and mixed with 2 ml of 0.1 M $FeCl_3$ in 0.1 M HCl solution and 0.008M potassium ferrocyanide. The absorbance was then taken at 395 nm [19].

2.2.2 Phytochemical analysis (qualitative)

All the qualitative phytochemical analysis were done as reported by [17].

2.2.2.1 Alkaloids

Methanol extract (2 ml) were mixed with 10 ml of dilute HCl and then filtered. The filtrate was treated with Mayer's regent and dragendorff's reagent respectively, the test was observed for colour changes.

2.2.2.2 Tannin

Methanol extract (0.25 g) was diluted with 10 ml of distilled water, few drops of ferric chloride was added to the mixture for the formation of blue-black precipitate.

2.2.2.3 Steroid

Methanol extract (0.2 g) was added to minimum quantity of chloroform and then 3-4 drops of acetic anhydride and 3 drops of concentrated sulphuric acid were added and observed for redish brown colour formation.

2.2.2.4 Saponin

Methanol extract (0.2 g) was added to 10 ml of water and was well shaken. It was observed for presence of a froth which does not break readily upon standing.

2.2.2.5 Phenol

Methanol extract (2 ml) was added to alcohol and then few drops of neutral ferric chlonde solution was added and observed for deep bluish green solution.

2.2.2.6 Flavonoids

Methanol extract (0.2 g) were added to dilute sodium hydroxide. Then few drops of concentrated H_2SO_4 acid was added and boiled and the result was observed for a yellow coloration.

2.2.2.7 Anthraquinone

Methanol extract (2 ml) was mixed with 2 ml of chloroform and 10% ammonium solution. The result was observed for the formation of brick red precipitate.

2.2.2.8 Glycoside

Methanol extract (0.5 g) was mixed with 2 ml of glacial acetate and 1 drop of ferric chloride solution and 1 ml of concentrated sulphuric acid were added. The reaction was observed for a brown ring formation.

2.2.2.9 Phlobatannins

Methanol extract (0.2 g) was boiled with 5 ml of 1%HCl. The reaction was observed for the formation of red precipitate.

2.3 Hepatoprotective Activity

The hepatoprotective activity was studied using a modification of CCl_4 induced hepatic damage model. Animals were divided into five groups of 5 rats each. Group I served as control and received 2 ml/kgbw of liquid paraffin, group II, group III, group IV and group V received 2 ml/kgbw of CCl_4 in liquid paraffin 1:1 ratio (ip) on the 9^{th} day and group II animals were maintained as CCl_4 groups. Group III and IV were treated with methanol extract of *S. melongena* at 500 and 1500 mg/kg respectively for 9 days (P.O) and group V was treated with standard drug vitamin E at 100 mg/kg for the same 9 days.

2.4 Blood Collection and Biochemical Analysis

Collection of blood samples was done according to the method described previously by Yakubu in [20]. Rats were anaesthetized in slight chloroform and blood samples collected into a clean, dry centrifuge tubes. The blood samples were allowed to stand for 10 minutes at room temperature and then centrifuged at 3000rpm for 10 minutes. The liver were excised, weighed and transferred into 0.25 M sucrose solution. One gram of the liver was homogenized in 4 ml of ice cold 0.25 M sucrose solution using ceramic mortar and pestle. Then transferred into clean centrifuge tube and centrifuged at 4000 rpm for 10 minute and the supernatant were also stored in the fridge.

2.5 Superoxide Dismutase

Superoxide dismutase (SOD) activity was assessed using a Xanthine oxidase system to generate superoxide radicals (O^{2-}) as described by [21]. Ten dilution of the tissue was prepared and 0.1ml of the diluted serum was added to 1.25 ml of 0.05 M phosphate buffer PH 7.8 to equilibrate the spectrophotometer. The reaction started by the addition of 0.15 ml of freshly prepared 0.3 M adrenaline to the mixture which was quickly mixed by inversion. The reference cuvette contained 1.25 ml of phosphate buffer 0.15 ml of adrenaline and 0.1 ml of distil water. The absorbance was then taken every 30 seconds.

Calculation:

$$\text{Increase in absorbance per minute} = \frac{A3 - A0}{2.5}$$

Where

A_o = Absorbance after 30 s
A_3 = Absorbance after 150 s

SOD = A/min x VT

Where:

A = Change in absorbance,
VT=Total Volume,
Vs = Sample Volume.

2.6 Catalase Activity Determination

Catalase activity was determined according to [22]. It was assayed colorimetrically at 620 mm and expressed as µmoles of H_2O_2 consumed/min/mg protein at 25℃. The reaction mixture (1.5 ml contained 1.0 ml of 0.01 M phosphate buffer (pH 7.0), 0.1 ml of tissue

homogenate and 0.4 ml of 2 M H_2O_2. The reaction was stopped by the addition of 2.0 ml of dichromate-acetic acid reagent (5% potassium dichromate and glacial acetic acid were mixed in 1:3 ratio).

CAT = A/min x VT
A = Change in absorbance
VT = Total volume
Vs = Sample volume

2.7 Lipid Peroxidation

Malondialdehyde (MDA) an index of lipid peroxidation was determined using the method of of (23). The supernatant was added to 2 ml of (1:1:1 ratio) TCA-TBA, HCI reagent (thiobarbituric acid 0.37%, 0.24 N HCI and 15% TCA) tricarboxylic acid- thiobarbituric acid-hydrochloric acid reagent boiled at 100℃ for 15 min, and allowed to cool. Flocculent materials were removed by centrifuging at 3000 rpm for 10 min. The supernatant was removed and the absorbance read at 532 nm against a blank. MDA was calculated using the molar extinction coefficient for MDATBA – complex of 1.56 x 10^5 M^{-1} CM^{-1}.

$MDA = A \times V_T$.

2.8 Total Protein

Total protein was determined using the Biuret method as described by [23]. Protein standards and the sample were prepared with saline solution (8.5 g/L). Three millilitre (3 ml) of Biuret reagent was added to each standard and samples. The solution was mixed well and incubated at room temperature.

3. RESULTS AND DISCUSSION

This study revealed the presence of various important medicinal phytochemical including terpenes, saponins, alkaloids, steroids, flavonoids, phenols and anthraquinones in the methanolic fruit extract of *Solanum melongena.* Although, this finding is in agreement with the finding of Anushree [8] who worked on qualitative phytochemical screening of *Solanum melongena* but glycoside was not present in his finding.

Quantitative screening revealed that Saponins is the most abundant (0.32±0.03 mg/g) phytoconstituents of the extract. This value is lower than the value (11.63±0.29 mg/g) reported for *Solanum melongena* by Agoreyo [24]. Saponins are used as adjuvant in the production of vaccines. They are known for their antihypentension potential and it accounts for the use of vegetable in meals by those that are recovering from stroke and other heart diseases [25]. Saponins and tannins also exhibit cytotoxic effects and growth inhibition making them suitable as tumour inhibiting agents [26]. The next abundant phytochemical in this plant was found to be flavonoids (0.18±0.11 mg/g). Flavonoids are strong antioxidant and are active in reducing high blood pressure [27]. It also possesses anti–carcinogenic, anti-mutagenic effect [28] and anti- inflammatory properties [29].

Table 1. Qualitative phytochemical screening of *Solanum melongena*

Phytochemical	Inference
Phlobatanins	–
Saponins	+
Glycosides	+
Alkaloids	+
Steroids	+
Flavonoids	+
Phenols	+
Anthraquinones	+

Note: + = presence, - =absence

Table 2. Quantitative phytochemical screening of *Solanum melongena*

Phytochemicals	Composition (mg/g)
Tannins	0.005±0.05
Saponins	0.64±0.03
Alkaloids	0.036±0.00
Flavonoids	0.36±0.01

Values are expressed as mean of triplicate ± SEM

Tannins have also shown to be a potential antiviral, antibacterial and antiparasitic agent due to their ability to react with protein to form stable water- soluble compound there by killing the microorganism by directly damaging it cell membrane [30]. Alkaloids have pharmacological effect and are used as local anaesthetic, stimulant cocaine and caffeine nicotine. The analgesic morphine, the antibacterial berberine and antimalarial drugs are all alkaloids [31]. Some bitter alkaloids in plant are metabolized in the liver into dimethyl xanthine and finally methyl uric acid by $CytP_{450}$ oxygenase system [32]. Methyl uric acid in the liver stimulate the expression of tumour necrosis factors which modulate the immune system [32].

Steroids according to [33] are used in growth and bone marrow stimulation and play a vital role in prevention of bone loss in elderly man [33]. Anthraquinones also have wide application as immunosuppressive, immune stimulant, anti-ulcer and antioxidant [34].

Phenolics are known for their antihyperglycemic properties [35]. Phenolic also possess antiviral [30], antibacterial [36] and antiparasitic effect [37].

The presence of all this phytochemcials in the fruits of *Solanum melongena* is an indication that this plant if properly screened could yield a drug of pharmacological significance. However, the absence of anthranoid, emodol and phlobatannin correspond with the fact that different part of the

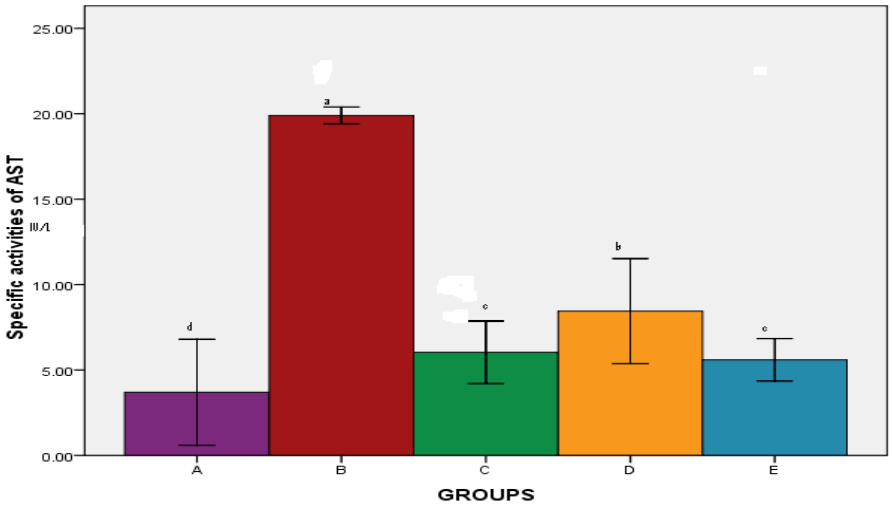

Fig. 1. Specific activity of aspartate transaminase in hepatotoxic rat treated with methanolic extract of *Solanum melongena*

Key; A: Normal, B: Normal +CCl_4, C: CCl_4 + Vitamin E, D: CCl_4 +500 mg/kgbw S. melogena, D: CCl_4 +1500 mg/kgbw S. Melogena.
Values are expressed as Mean ±SEM

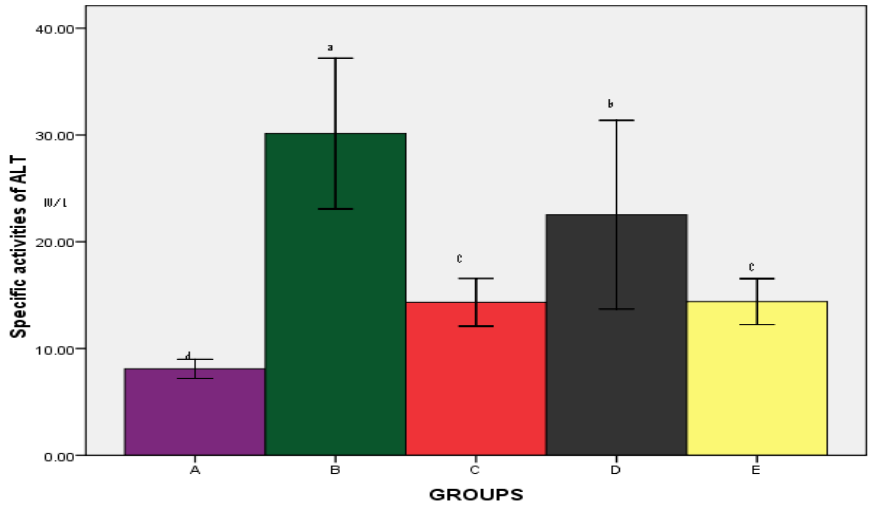

Fig. 2. Specific activity of alanine transaminase in hepatotoxic rats treated with methanolic extract of *Solanum melongena*

Key; A: Normal, B: Normal +CCl_4, C:CCl_4 + Vitamin E, D: CCl_4 +500 mg/kgbw S. melogena, D: CCl_4 +1500 mg/kgbw S. Melogena.
Values are expressed as Mean ±SEM

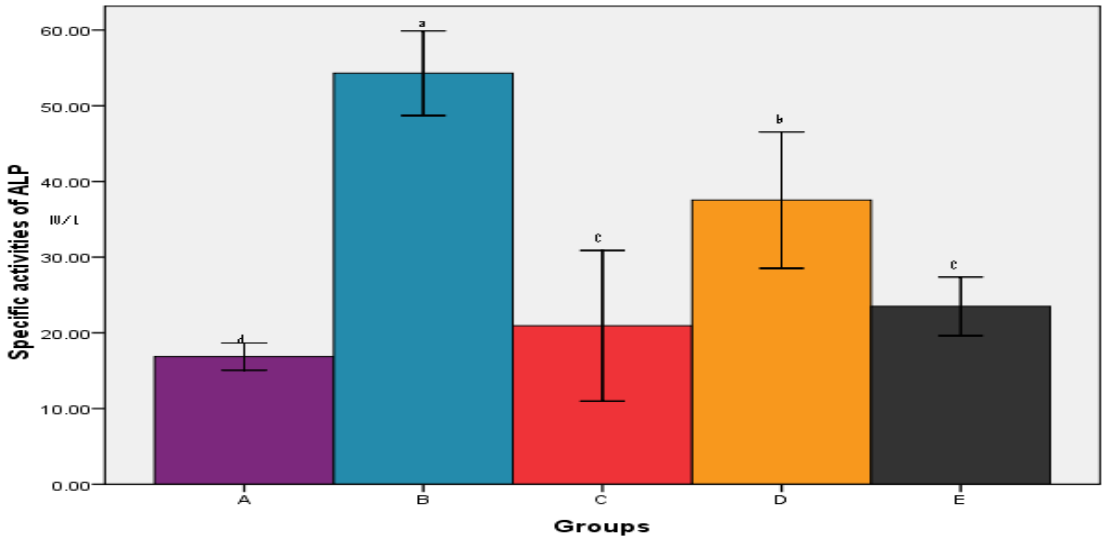

Fig. 3. Specific activity of alkaline phosphatase in hepatotoxic rat treated with methanolic extract of *Solanum melongena*

Key; A: Normal, B: Normal +CCl_4, C:CCl_4 + Vitamin E, D: CCl_4 +500 mg/kgbw S. melogena, D: CCl_4 +1500 mg/kgbw S. Melogena.
Values are expressed as Mean ±SEM

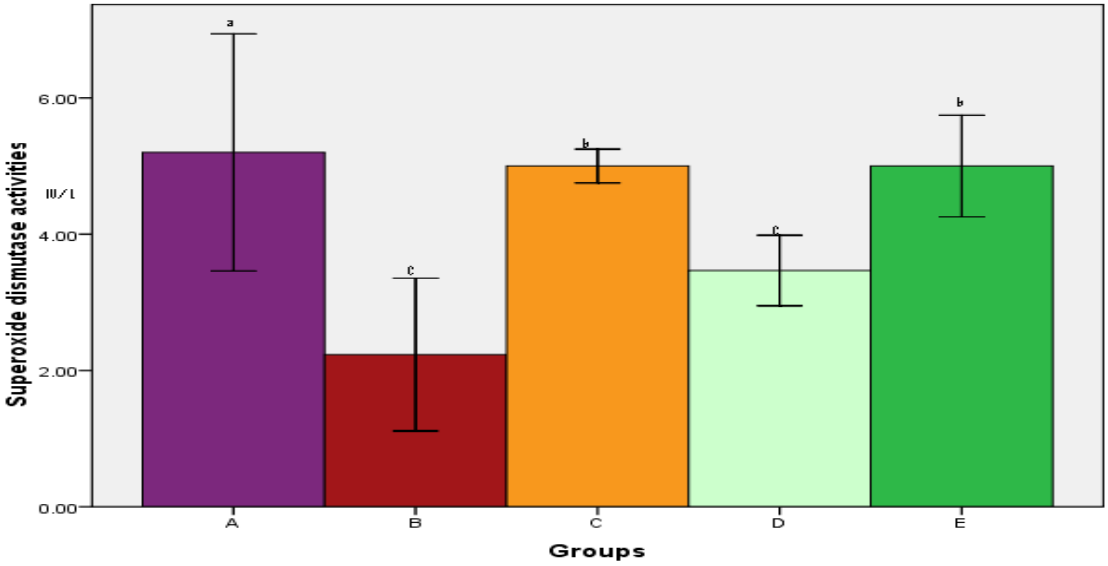

Fig. 4. Superoxide dismutase activity of hepatotoxic rat treated with methanolic extract of *Solanum melongena*

Key; A: Normal, B: Normal +CCl_4, C:CCl_4 + Vitamin E, D: CCl_4 +500 mg/kgbw S. melogena, D: CCl_4 +1500 mg/kgbw S. Melogena.
Values are expressed as Mean ±SEM

plant have different ingredient profile and different extraction procedure and solvent used may yield different active component [38].

The activities of medicinal ingredient in this plant were further confirmed by hepatoprotective study in CCl_4 induced hepatotoxic rats. The liver is one of the most important organs in our body that is responsible for detoxification of toxic drugs and chemicals. Thus, it is the target organs for all toxic chemicals [39].

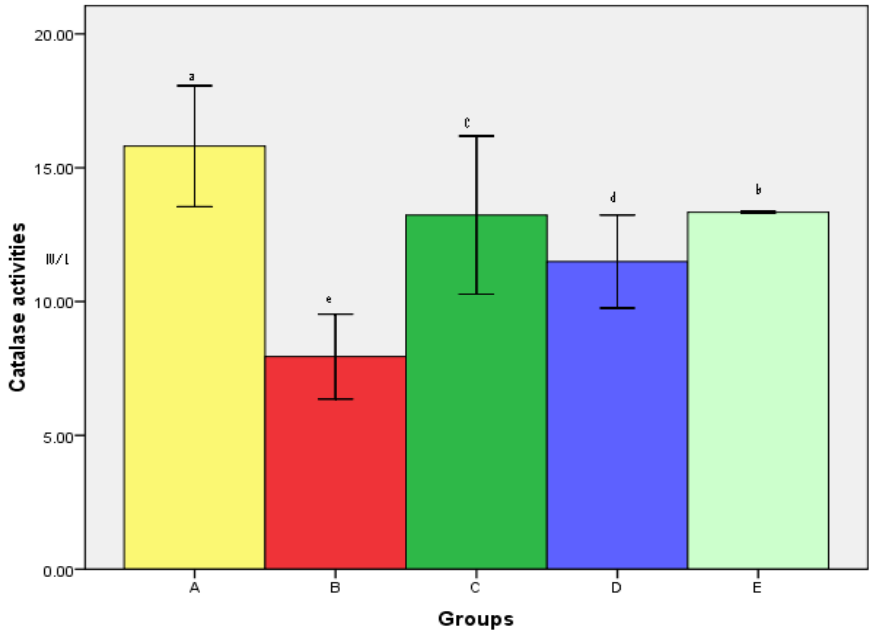

Fig. 5. Liver catalase activity in hepatotoxic rat treated with methanolic extract of *Solanum melongena*

Key; A: Normal, B: Normal +CCl_4, C:CCl_4 + Vitamin E, D: CCl_4 +500 mg/kgbw S. melogena, D: CCl_4 +1500 mg/kgbw S. Melogena.
Values are expressed as Mean ±SEM

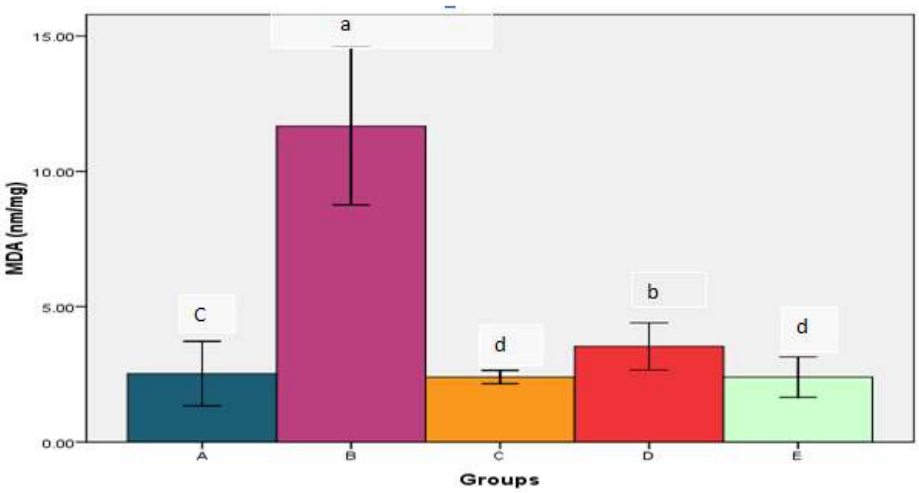

Fig. 6. Level of MDA in hepatotoxic rats treated with methanolic extract of *Solanum melongena*

Key; A: Normal, B: Normal +CCl_4, C:CCl_4 + Vitamin E, D: CCl_4 +500 mg/kgbw S. melogena, D: CCl_4 +1500 mg/kgbw S. Melogena.
Values are expressed as Mean ±SEM

In the evaluation of hepatic damage, determination of level of AST, ALT, ALP, is largely applied [40]. In this study, oral administration of CCl_4 in rat caused liver damage as indicated by significant increase in serum ALT, AST, ALP as compared to the control group.

AST and ALT are sensitive indicators of necrotic lesion within the liver [41]. Hence, the marked

release of transaminase into circulation is indicative of severe damage to hepatic tissue membrane during CCl_4 intoxication [42]. However, administration of methanol extract of *Solanum melongena* significantly lowered the elevated transaminase level towards their normal value thereby reflecting the hepatoprotective effect of the extract.

Alkaline phosphatase is biomarker enzymes for assessing the integrity of plasma membrane [43]. Increase in the activities of alkaline phosphates is an indication that there was a leakage of this enzyme from the liver into the serum. Such increase in alkaline phosphates level can constitute threat to the life of cells that are dependent on a variety of phosphates esters for their vital process since there may be indiscriminate hydrolysis of phosphate esters of the tissue [44]. The methanol extract of *Solanum melongena* exhibited the ability to counter the CCl_4 induced hepatotoxicity by decreasing the enzyme levels in the blood significantly. The serum activities of ALT, AST and ALP were almost brought to normal indicating that the methanol extract of *Solanum melongena may have* hepatoprotective effect. The hepatoprotective effect demonstrated by this extract increased with concentration and comparable at 1500 mg/kg with the standard drug (vitamin E). This finding is an indication that *Solanum melongena* is a good source of agent that could be as promising as vitamin E in the maintenance of hepatic integrity.

Free radicals have been implicated in the aetiology of many degenerative disorder including cancer, diabetes, arthrosclerosis neurodegenerative diseases malaria and aids [45]. In this study administration of CCl_4 induced oxidative stress as manifested by significant increase in the level of MDA and significant decrease in the level of antioxidant enzyme (SOD and CAT) compared to the control group. Similar alteration in the level of antioxidant enzyme and MDA following CCl_4 administration to experimental rat has been reported by [46,47].

The antioxidant activities and inhibition of free radicals generation are important in terms of protecting the liver from CCl_4 induced damage. SOD reduces the concentration of highly reactive superoxide radical by converting it to H_2O_2 whereas Catalase converts harmful hydrogen peroxide into water and oxygen and protects the tissues from highly reactive hydroxyl radicals [48]. The reduction in the activity of this enzyme may result in number of deleterious effect due to accumulation of highly toxic metabolites and hydrogen peroxide on CCl_4 administration which can induce oxidative stress in the cells [49]. Administration of *Solanum melongena* increases the activity of catalase in animals to prevent the accumulation of excessive free radicals and protects the liver from CCl_4 intoxication.

Lipid peroxidation has been implicated in the pathogenesis of hepatic injury by compounds like CCl_4 and is responsible for cell membrane alterations [50]. In the present study, significantly elevated level of MDA (P<0.05) observed in CCl_4 administered rats indicated excessive formation of free radicals and activation of lipid peroxidation system resulting in hepatic damage. The significant decline in the MDA content in *S. melongena* treated groups (1500 and 500 mg/kg) indicated antilipid peroxidative effect of *S. melongena.*

4. CONCLUSION

In conclusion, the results obtained from this study showed that methanol extract of *Solanum melongena* possess important phytoconstituents and hepatoprotective effect. Thus, the extract possess therapeutic agent for the management of liver damage.

CONSENT

It is not applicable.

ETHICAL CLEARANCE

Ethical clearance was given by Federal University of Technology, Minna/Nigerian Ethical Review Board (CUERB) in accordance with internationally accepted principles for laboratory animal use and care as contained in the Canadian Council on Animal Care Guidelines and Protocol Review.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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