



Original article

Effects of Aqueous and Methanol Extracts of *Spondias mombin* Fruit on CCl₄-Induced Hepatotoxicity in Albino Rats

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ABSTRACT

The effect of aqueous and methanol extracts of *Spondias mombin* fruit on Carbon tetrachloride (CCl₄) induced liver damage in albino rats were determined. Six groups consisting of five (5) albino rats each were used for this study. Dosages of 250 and 500mg/kg bodyweight of Aqueous and Methanol extracts of *S. mombin* were administered to test groups alongside with control group. Biochemical parameters such as liver and antioxidants enzymes concentration were monitored following standard procedures. The level of serum ALT, AST and ALP significantly ($p < 0.05$) increased in the group that was induced with CCl₄ (85.95 ± 0.94 , 64.06 ± 1.76 and 96.26 ± 1.78 U/l) respectively when compared with the normal group. However, the administration of aqueous extract of *S. mombin* at 500mg/kg body weight significantly ($p < 0.05$) decreased the level of ALT, AST and ALP (46.84 ± 1.56 , 9.99 ± 0.90 and 34.43 ± 2.42 U/l) in the serum respectively. The administration of Aqueous and methanol extracts of *S. mombin* at 250 and 500mg/kg body weight significantly ($p < 0.05$) decreased the level of ALT, AST and ALP in the serum. The activities of liver SOD and Catalase in the extracts treated groups were significantly increased when compared with control mostly in a dose - dependent manner. An increase was observed in the level of MDA in the CCl₄ treated group compared to the normal rats group and this was decreased more significantly on administration with aqueous extract at 500mg/kg. Thus, *S. mombin* fruit extract may serve as promising candidate for the management of hepatic damage.

Keywords: *Spondias mombin*, Liver damage, Biochemical parameters, Carbon tetrachloride

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INTRODUCTION

Liver is the most important organ in our body that is responsible for the detoxification of toxic drugs and chemicals, thus it is the target organ for all toxic chemicals (Behrouz *et al.*, 2012). Liver cancer is the most typical fifth type of cancer in men and seventh most prevalent type in women that is caused by viral and some exogenous environmental toxins (Rasool *et al.*, 2014). Various xenobiotics such as paracetamol, acetaminophen, bromobenzene, ethanol and polycyclic aromatic hydrocarbons have been implicated in the etiology of liver injury (Adesanoye, and Farombi, 2010). Liver damage occurs as a result of over production of free radicals such as Reactive Oxygen Species (ROS) during cell metabolism that induces oxidative state which leads to an alteration in metabolic process (Singh *et al.*, 2008). Intracellular concentration of ROS is a consequence of both their production and removal by various endogenous antioxidants including both enzymatic and non-enzymatic components (Sreelatha *et al.*, 2009). All aerobic organisms including humans have antioxidant defense mechanism that help reduce the production of ROS.

The effectiveness of orthodox medicines is inconsistent in the treatment of liver diseases and are often accompanied by devastating adverse reactions such as renal dysfunction, poor tolerance and anemia. Most often, the treatment is worse than the disease (Rutherford, 2013). According to the World Health Organization, 85% countries including Africa and Asia are using plant-derived natural medicines due to the presence of many bioactive compounds that act as

antioxidant in biological system. The synthetic drugs might have various side effects due to the fact that the focus of scientists is getting shifted to the plants-derived herbal medicines. CAT and GPx are involved in the conversion of O₂ to hydrogen peroxide and catalyze into water to provide protection against reactive oxygen species (ROS) activity (Hafiz *et al.*, 2014). The available drugs are also laden with high relapse rate and expensive. Therefore, the search for natural antioxidants for the prevention and treatment of liver diseases arising from oxidative stress becomes very necessary in drug discovery and formulation of nutraceuticals.

Spondias mombin L which belongs to the family of Anacardiaceae, is a flowering plant that grows in the coastal areas in the rain forest into a big tree up to 15-22m in height. It is native to the tropical Americas, including the West Indies which has been naturalized in parts of Africa, India, Sri Lanka and Indonesia It is readily common in Nigeria and other tropical forest of the world (Ayoka *et al.*, 2008). The leaf, bark and fruit juices of the plant have been widely used for both medicinal and non-medicinal purposes. It is used as remedy for numerous ailments. The fruit is eaten fresh and has potential use to make jelly, juice, jams and ice cream and their leaves are used in folk medicine for the treatment of several topic and systemic diseases like inflammation of the mouth and throat and in cases of prostatitis and herpes labialis (Lorenzi and Matos, 2008). The fruit decoction is drunk as a diuretic, the decoction of the bark and the leaves is said to possess anti-diarrheal property and thus used in the treatment of

dysentery. In addition to the various uses of *S. mombin*, it is useful in the treatment of liver disease locally. In view of the undesirable side effects of synthetic drugs and traditional therapeutic potential of *S. mombin*, there is a need to evaluate the effect of *S. mombin* fruit extract in carbon tetrachloride induced hepatotoxicity.

MATERIALS AND METHODS

Samples Collection and Extraction

S. mombin fruits were collected from a farm in Odenku, Okene Local Government Area, Kogi State Nigeria in the month of September, 2012 and were authenticated at the Department of Biological Science, Federal University of Technology, Minna, Nigeria.

S. mombin fruits were washed with distilled water and the fruits pulp were separated from the seeds. They were pulverized with an electric blender into a paste. The pasty *S. mombin* (100g) was weighed into a round bottom flask to which 400 ml of methanol or distilled water were added separately and kept for 72 hours with continuous stirring. The samples were filtered using a clean muslin cloth and the solvents were evaporated using rotary evaporator. The samples were then concentrated further in a water bath. The methanol and aqueous extracts were collected in sterile sample containers and stored in the refrigerator until required for use.

Animals

Wistar albino rats weighing between 100-180g were used in the study. The rats were procured from animal house of the Department of Biochemistry, Federal University of Technology, Minna, Niger State, Nigeria. They were housed in well

ventilated cages at room temperature ($24\pm 2^{\circ}\text{C}$) under hygienic condition and were fed on standard laboratory diet. Food and water were given ad libitum.

Acute Toxicity Test

Doses were selected and determined according to the acute toxicity test reported earlier by Lorke's (1983). Doses of 10,100 and 1000 mg/kgbw were used in phase one of the test while 1600, 2900 and 5000mg/kgbw were used in phase two of the toxicity study.

Animal Groupings and Treatments

Thirty Wistar rats weighing between 100-180g were divided randomly into six groups of five rats each. Carbon tetrachloride (CCl_4) in olive oil (1:1) was used to induce hepatotoxicity in rats intraperitoneally at a concentration of 2ml/kgbw of rats on the ninth day post oral administration of the extracts. The animals were grouped as follows: Group 1 (Positive Control; distilled water), Group 2 (Negative Control; Not Treated), Group 3 (Aqueous extract 250mg/kgbw), Group 4 (Aqueous extract 500mg/kgbw), Group 5 (Methanol extract 250mg/kgbw), and Group 6 (Methanol extract 500mg/kgbw).

The feeds were withdrawn 12 hours before CCl_4 administration after the treatment with various extracts. Twenty four hours after the last day administration of CCl_4 , the animals were euthanized, the blood was collected via cardiac puncture of the animals into serum bottles as reported by (Adebayo *et al.*, 2003). The blood samples were allowed to clot and the serum was obtained by centrifuging at 3000 rpm for 5 minutes (Ogbu, & Okechukwu, 2001). The clear serum was used for the assay of

Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline phosphatase (ALP) activities using randox kits.

The liver from each rat in each group was homogenized in ice-cold 0.25 M sucrose solution (1:5 w/v) and centrifuged at 4000 rpm for 10 minutes. The supernatant was used for the estimation of liver antioxidant enzymes. Superoxide dismutase (SOD), Catalase (CAT) and lipid peroxidation were determined

following the method of Stroev & Makarova (1989).

RESULTS

Acute Toxicity Profile of *S. Mombin* Fruit Extracts

Aqueous and Methanol extract of *S. mombin* fruit did not produce any toxic symptoms or mortality up to the dose level of 5000mg/kgbw in rats, hence the extracts were considered to be safe and non-toxic (Table 1)

Table 1: LD₅₀ determination of both aqueous and methanol extract of *S. mombin* in Albino rats.

Extracts	Doses (mg/kgbw)		Mortality /No of Animals		
Phase 1:			Phase 2:		
Aqueous & Methanol	10	0/3	Aqueous & Methanol	1600	0/3
	100	0/3		2900	0/3
	1000	0/3		5000	0/3

Hepatoprotective Activity of *S. Mombin* Fruit Extracts

Aspartate Transaminase (AST), Alanine Transaminase (ALT) and Alkaline Phosphatase (ALP) Activity

A significant ($p < 0.05$) increase in the activities of AST, ALT and ALP were observed in the group treated with CCl₄ without treatment compared with the

normal group as shown in Figures 1-3. However, treatment with aqueous and methanol extract of *S. mombin* resulted in an appreciable reduction in serum AST, ALT, and ALP activities compared with the normal group. The aqueous extract at a dose of 500mg/kgbw showed the highest reduction

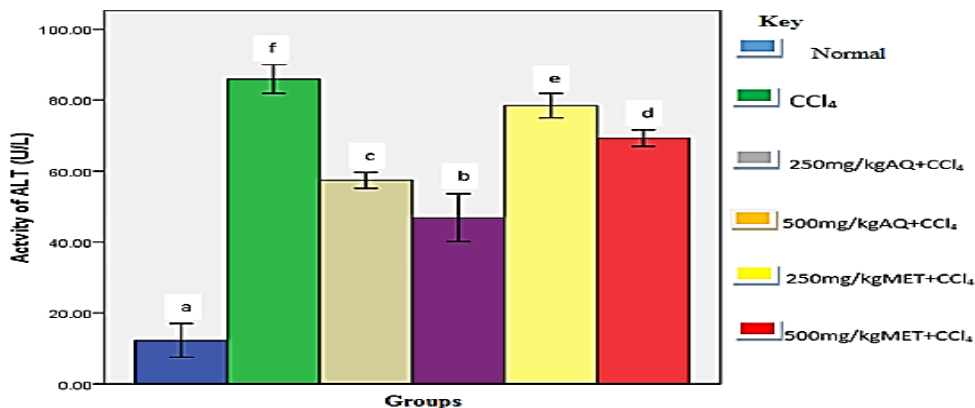


Figure 2: Effect of *S. mombin* extract (Aqueous and methanolic) on ALT in rats induced with CCl₄. Values are expressed as mean \pm SEM (n=5).

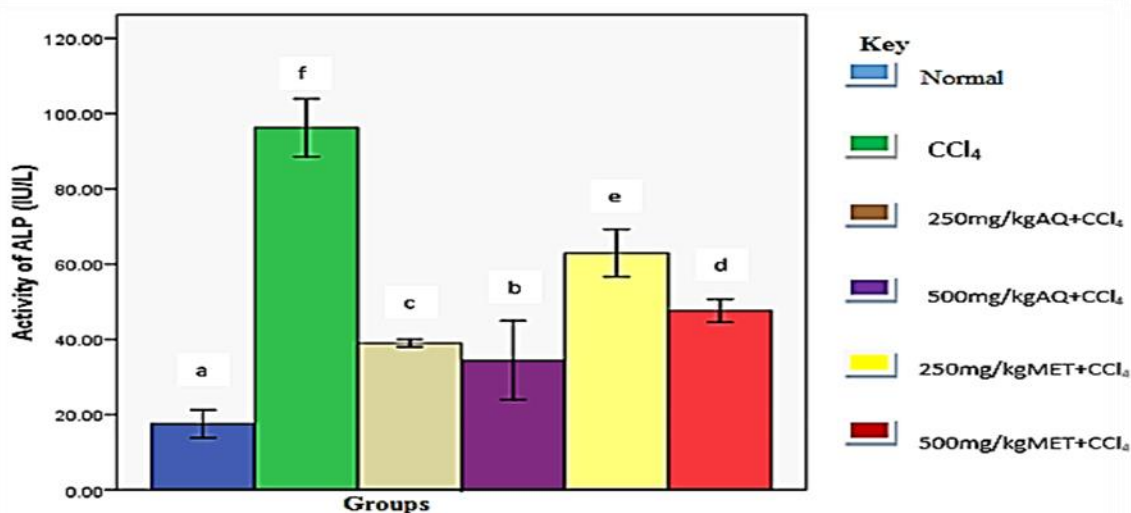


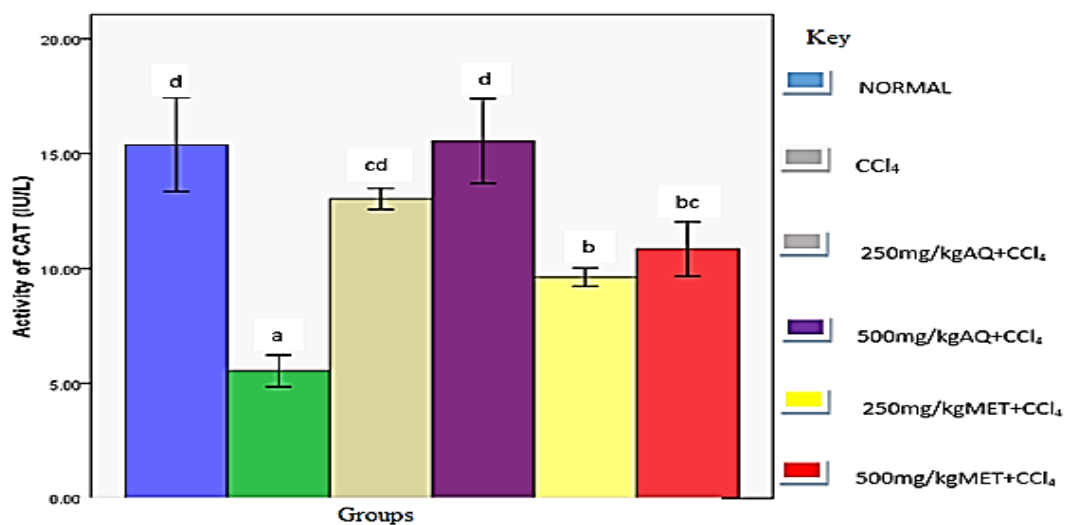
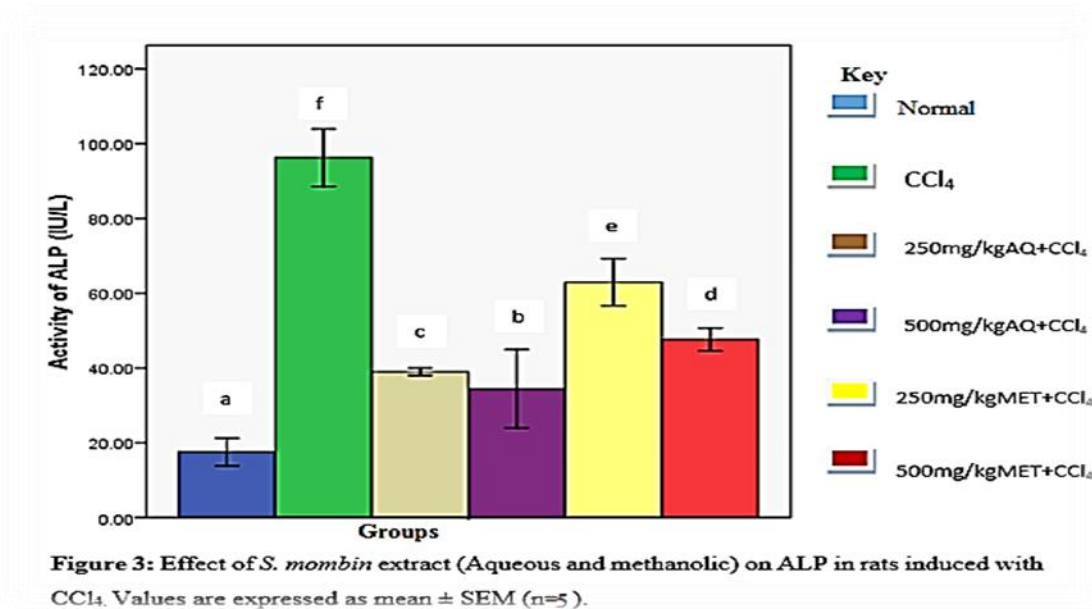
Figure 3: Effect of *S. mombin* extract (Aqueous and methanolic) on ALP in rats induced with CCl₄. Values are expressed as mean \pm SEM (n=5).

Antioxidant Enzymes

Catalase, Superoxide Dismutase (SOD) and Lipid Peroxidation

A decrease in the level of Catalase activity in the liver was observed in CCl₄ induced group compared with the normal group. However, administration of Aqueous and Methanol extract of *S. mombin* significantly ($p < 0.05$) increase Catalase activity when compared with CCl₄ induced group. Aqueous extract of *S. mombin* at a dose of 500mg/kgbw was

found to be more effective in increasing the enzymes activity compared with other treated groups as shown in Figure 4-6. A significant ($p < 0.05$) increase in the activity of SOD and MDA (Malondialdehyde) as an index of lipid peroxidation was seen in the normal group compared with other experimental groups. Induction of CCl₄ brought about a reduction in the activities of both. However, a significant increase in these antioxidant enzymes was elicited after 9 days of treatment.



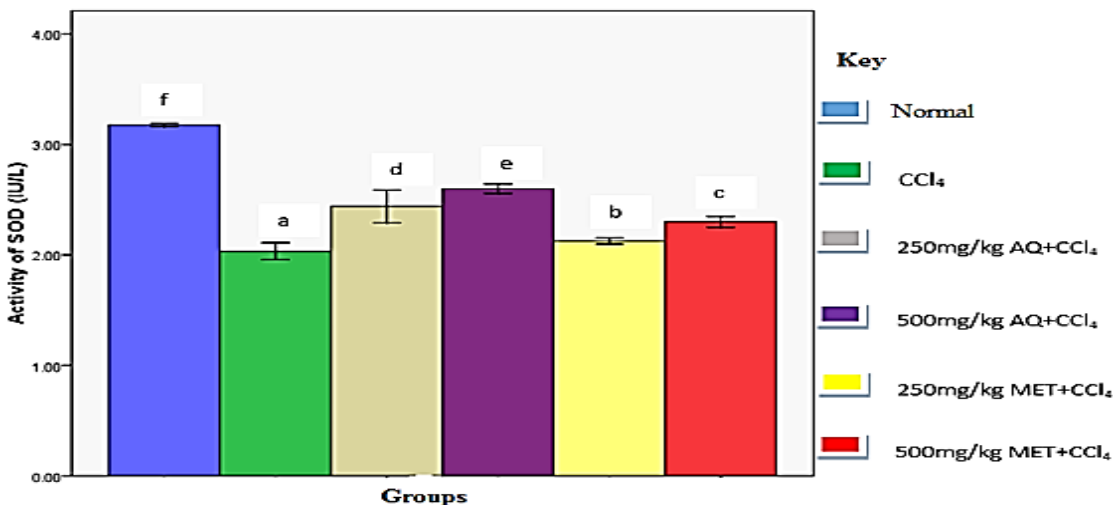


Figure 5: Effect of *S. mombin* extract (Aqueous and methanolic) on SOD in rats induced with CCl₄. Values are expressed as mean \pm SEM (n=5).

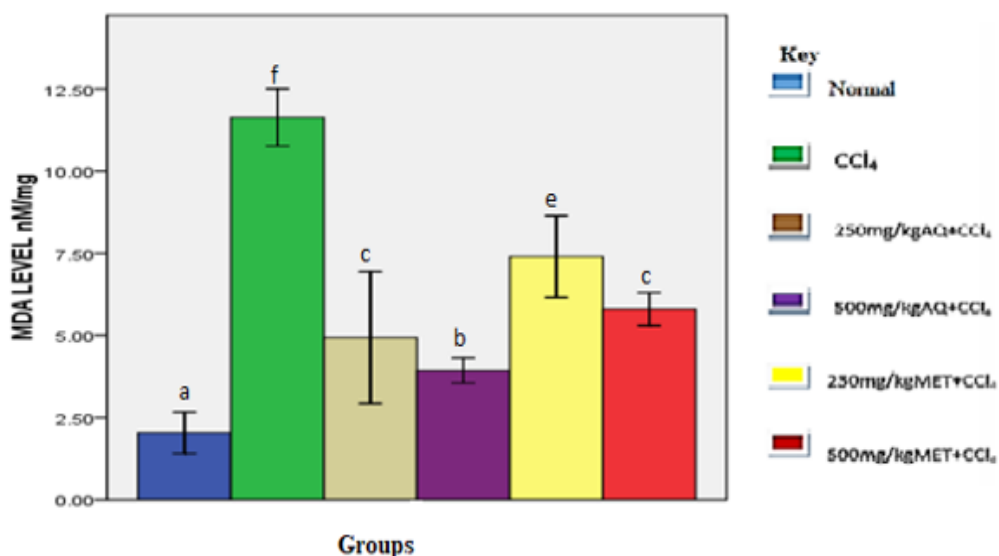


Figure 6: Effect of *S. mombin* extract (Aqueous and methanolic) on MDA in rats induced with CCl₄. Values are expressed as mean \pm SEM (n=5).

DISCUSSION

Carbon tetrachloride (CCl₄) has been extensively used in animal model in investigating hepatotoxicity and screening of hepatoprotective agents and alteration of antioxidant profile of the liver (Vilwanathan *et al.*, 2005). The increase in liver enzymes activity such as

AST, ALT and ALP are usually associated to the damaged structural integrity of the liver releasing them into circulation from the cytoplasm as it occurred in this study. This may be due to leakage from the cells through peroxidative damage of the membrane and release the enzymes into the bloodstreams. ALT is specifically produced in the hepatocytes, making it relatively a better determinant of hepatic

injury than AST, which is observed in higher levels in a variety of other tissues like cardiac muscle, skeletal muscle, red blood cell, kidney and testes. Consequently, muscle injury/trauma (intra muscular injections, severe restraint during handling) and haemolysis can lead to significant AST elevations (Ramaiah, 2007). Again, AST has a shorter half-life of about 12 h while ALT has a half-life of about 60 h (Meyer, &Harvey, 2004). Alkaline phosphatase (ALP) is another biomarker for assessing the integrity of plasma membrane (Akanji & Ngaha, 1993). From this study, increase in the activity of serum alkaline phosphatase in CCl₄ induced group when compared with controls may be as a result of leakage of this enzyme from the cells through peroxidative damage of the liver. Such increase in alkaline phosphatase activities can constitute threat to the life of cells that are dependent on a variety of phosphate esters for their vital process, since there may be indiscriminate hydrolysis of phosphate esters of the tissue (Yakubu *et al.*, 2006).

The Aqueous extract of the *S. mombin* fruit extract at 500mg/kgbw significantly decreased the elevated ALT, AST and ALP than other extracts. The reversal of liver enzymes activities by these extracts agrees with the generally accepted view that serum levels of transaminases return to normal with the healing of hepatic parenchyma and regeneration of hepatocytes (Thabrew *et al.*, 1987). Thus the aqueous extract at 500mg/kgbw offered a more significant protection than the other extracts.

The body has an effective defense mechanism to prevent and neutralize free radicals-induced damage. Lipid peroxidation has been implicated in the

pathogenesis of hepatic injury by compounds like CCl₄ and is responsible for cell membrane alterations (Liu *et al.*, 2009). The increased MDA level in liver damage induced by CCl₄ is an indication of enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanism to prevent formation of excessive free radicals (Khan *et al.*, 2012). Treatment with the *S. mombin* extracts significantly reversed these changes. Again the aqueous extract was observed to be the best in this reduction than the methanol extract. Hence it is likely that the mechanism by which this extract exerted its protective property may be via its ability to scavenge the excessive reactive free radicals, trichloromethyl radical generated from carbon tetrachloride induction.

Decreased activity of superoxide dismutase (SOD) is usually a sensitive index in hepatocellular damage. SOD has been reported as one of the most important enzymes in the enzymatic antioxidant defense system. It scavenges the superoxide anion to form hydrogen peroxide and thus lessening the toxic effect caused by this radical (Tamilarasi *et al.*, 2012). The aqueous extract was much more effective and substantially enhanced the antioxidant activities since it was able to reduce reactive free radical induced oxidative damage to liver better than the methanol extract.

Catalase is an antioxidant enzyme ubiquitous to most aerobic cells in animals and is especially concentrated in the liver and erythrocytes. It is known to decompose hydrogen peroxide into water and oxygen. The result obtained from this study showed that there was a significant reduction in CAT activity in CCl₄ administered rats compared to normal control while all treated groups showed a

significant increase in activity, with the aqueous extract of *S. mombin* at 500mg/kgbw showing greater increment compared to the other group. This decrease in the activity of SOD and CAT observed in CCl₄ induced but not treated group is consistent with various reports already documented (Tamiliarasi *et al.*, 2012; Okpala *et al.*, 2014). Treatment with aqueous extract at 500mg/kg body weight showed a more significant increase in catalase activity and this may be due to presence of some phytochemicals capable of converting hydrogen peroxide to non – harmful substances, oxygen and water

The effectiveness of these extracts generally may be due to the presence of some active compounds that may be responsible for the potency of the extract in combating toxicity and consequent peroxidation caused by CCl₄ on the albino rats.

CONCLUSION

Conclusively, aqueous and methanol extracts of *S. mombin* exhibited a hepatoprotective effect at dose levels of 250mg/kgbw and 500mg/kgbw in CCl₄ treated rats. Aqueous extract of *S. mombin* at the dose level of 500mg/kg showed the highest protective effect. Hence, the extracts may be a potential candidate towards development of therapeutic agent for the management of liver damage.

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