Biokemistri
Vol. 33, No. 4, December 31, 2021
Printed in Nigeria

0795-8080/2021 $10.00 + 0.00

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BKR 33404

Hepatoprotective effect of methanol extract of *Senna* occidentalis seeds in carbon tetrachloride induced hepatotoxic rats

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ABSTRACT: Effect of methanol seed extract of *Senna occidentalis* in carbon tetrachloride (CCl_4) induced hepatic damage in Wistar albino rats was investigated. Biochemical parameters such as serum AST (Aspartate Aminotransferase), ALT (Alanine Amino Transferase), ALP (Alkaline Phosphatase) and Total proteins were determined. The level of Malondialdehyde (MDA) and the liver antioxidant enzymes such as superoxide dismutase (SOD), and Catalase were also determined. Phytochemical screening and subsequent quantification reveal the presence of bioactive compounds such as Flavonoids (0.27±0.01mg/g), Phenols (0.79±0.021 mg/g), Tannins (0.87±0.03mg/g) and Alkaloids (62.57±2.35mg/g). The level of AST, ALT, and ALP significantly increased in CCL_4 induced groups. However, the administration of methanol extract of *S. occidentalis* at 100mg/kg and 200mg/kgbw decreased ($p<0.05$) the activity of ALT, AST and ALP in the treated groups. The activity of SOD and CAT in the CCl_4 induced group was decreased. Also, increase in the level of MDA in CCl_4treated group was observed when compared with the normal group and this was decreased ($p<0.05$) on administration with methanol seed extract of *Senna occidentalis* at 100mg/kg and 200mg/kg (B/W). It can be concluded that methanol seeds extract of *Senna occidentalis* possess important phytoconstituents and hepatoprotective effect. Thus, the extract can be used as a potent therapeutic agent for the management of liver damage.

Introduction

The liver is the main organ involved in the metabolism of several substances in the body which include breakdown and synthesis of fats, proteins and most especially carbohydrates to produce energy, bile production and excretion and enzymes activation. It has a major role in detoxification and excretion of various substances including xenobiotics. This physiological process through several biochemical reactions results in the generation of highly reactive free radicals. These free radicals attack the membrane lipids causing lipid peroxidation which alters the membrane permeability and causes tissue injury [1]. Hepatic

tissue damage causes impairment to liver function which may have deleterious effect on human health. Management of liver diseases is still a challenge to the modern scientific community [2].
Environmental toxins often disturb hepatic metabolic function and increase the expression of liver enzymes, leading to liver fibrosis, cirrhosis, and even cancer [3]. Liver injury can be caused by a combination of oxidative stress, necrosis, inflammation, and apoptosis [4]. Carbon tetrachloride (CCl4) is frequently used to induce liver injury and to study the effects of chemical compounds on the liver [5].

Carbon tetrachloride-induced liver injuries are the most common experimental model for monitoring the hepatoprotective activity of certain drugs. A single exposure to CCl4 as being a strong hepatotoxic xenobiotic directly leads to severe liver necrosis and steatosis [6, 7]. The free radicals induce cell membrane lipid peroxidation via disrupting polyunsaturated fatty acids within these membranes, initiating a sequential free radical chain reaction [6]. Existing hepatoprotective drugs are used sparingly because of their side effects [8]. Therefore, there is an urgent need to develop safer hepatoprotective drugs.

Currently, modern medicine and conventional modern drugs are used to treat liver diseases. However, these drugs such as Ursodiol, silymarin still have many adverse effects which include diarrhea, constipation, dizziness, fullness and pains in the stomach and back [9]. Thus, patients often resort to herbal products as an alternative source of treatment for their ailments. There are several hepatoprotective herbal-based compounds that purportedly possess oxidative stress detoxification properties with little or no side effects [10].

Senna occidentalis is one of the most widely used herbal plants among people of tropical and sub-tropical regions of the world [11].It has been documented in literatures that extract of *Senna occidentalis* has antimicrobial activity [12,13], larvicidal and pupicidal activity [14], antioxidant and hepatoprotective activity (methanol leaf extract) [15], anti- inflammatory actions [16], antimalarial activity [17], antianxiety and antidepressant activity [18], analgesic activity [19] and antidiabetic activity [20, 21, 22]. The present study was, therefore, undertaken to assess the effect(s) of methanol extract of *Senna occidentalis* seeds against CCl4induced hepatotoxicity in rats liver.

Materials and Method

Plant collection

Seeds of Fresh matured *Senna occidentalis* plant were collected from Bosso Local Government area of Niger state. The plant material was identified by Mal. Mohammed Dagana of the Biological Science Department of Federal University of Technology Minna.

Reagents and chemicals

The reagent used in this study include; Carbon tetrachloride, Mayer's reagent, Wagner's reagent, phosphate salt, HCL, NaOH, 3,5-dinitrosalic acid, $FeCl_3$, concentrated ammonium hydroxide, concentrated H_2SO_4, distilled water, Randox kits (ALT, AST, ALP). All chemicals and reagents used for this study are of analytical grade.

Experimental animal

Twenty (20) albino rats weighing 100-180 g were procured from National Veterinary Research Instituted (VOM), Jos plateau state. They were housed in plastic cages and were allowed to acclimatize for 2 weeks. During this period, the rats were fed on standard rats-chaw diets *ad libitum.*

Sample preparation

Seeds of matured *Senna occidentalis* plant were dried under room temperature and made into powder using mortar and pestle.

Extraction procedure

The extract was obtained from the powder (150g) by maceration in 50% methanol for 2 hours at 64°C (23Abdul *et al*., 2009). The extract was filtered and the excess methanol was evaporated under a reduced pressure in a water bath at 40^0C. The dried extract was put in a clean sterile container and stored in a refrigerator for further use.

Phytochemical analysis

Quantitative phytochemical screening of the methanol leaf extracts of *S. occidentalis*leaves was conducted according to standard procedures [24].

Quantitative Phytochemical Components Determination

Estimation of alkaloids

Methanol extract (0.5g) was dissolved in 96% ethanol and 20% H_2SO_4 (1:1) mixture. 1ml of the filtrate was added to 5ml of 60% H_2SO_4 and allowed to stand for 5 minutes. Then, 5ml of 0.5% formaldehyde was added and allowed to stand for 3 hours. The reading was taken at absorbance of 565nm [25].

Total flavonoid determination

Aluminium chloride colometric method was used to determine flavonoid [26]. The seeds extract was separately mixed with 1.5 ml of methanol, 0.1ml of 10% aluminium chloride, 0.1ml of 1M sodium acetate and 2.8ml of distilled water. It remained at room temperature for 30 minutes; the absorbance of the reaction mixture was measured at 415 nm with a double beam spectrophotometer.

Estimation of Saponin

Methanol extract (0.5g) was added to 20ml of 1N HCL and was boiled for 4 hours. After cooling it was filtered and 50ml of petroleum ether was added to the filtrate for ether layer and evaporated to dryness. 5ml of acetone ethanol was added to the residue 0.4ml of each was taken into 3 different test tube.6ml of ferrous sulphate reagent was added into them followed by 2ml of concentrated H_2SO_4. It was thoroughly mixed after 10 minutes and the absorbance was taken at 490nm [25].

Tannin determination

Methanol extract (0.5g) were weighed into a 50ml of distilled water and shake for 1 hour using mechanical shaker, it was then now filtered into a 50ml volumetric flask and make up to the mark. Then 5ml of the filtrate was pipette into another test tube and mixed with 2ml of 0.1M Fecl3 in 0.1 M HCL solution and 0.008 potassium ferrocyanide. The absorbance was taken at 395nm [27].

Experimental Design

Twenty albino rats weighing (100g-180g) were divided into five groups of 4 rats each. Hepatic injury was induced in the four groups through injecting an intraperitoneal dose of 2.0 ml/kg body weight (50% v/v) CCl4 in olive oil at every 72 hours for nine days. The animals were treated with the extracts orally and standard (silymarin) for nine days. The groups include;

Group 1: serve as the normal control and was given normal saline (2 ml/kg p.o)
Group 2: receive normal saline + CCl4(Toxic group)
Group 3: received silymarin 50 mg/kg p.o + CCl4 (standard group)
Group 4: methanol extract of *Senna occidentalis* seeds (100mg/kg/day) + CCl4 group 5: methanol extract of *Senna occidentalis* seeds (200mg/kg/day) + CCL4

Blood collection and preparation of tissue homogenate

All animals were anaesthetized with diethyl ether vapour at the end of the experiment on the 10th day. Animals were euthanized by cervical dislocation and their blood was collected into a plain tube. The blood samples were allowed to clot. The clotted blood samples were centrifuged in a bench top centrifuge (3000 rpm for10 min) to obtain serum. This separated serum was used for the following biochemical analyses: Alanine Aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline Phosphatase (ALP) and total protein. These tests were assayed using commercially available kits (RANDOX) following the manufacturers instruction.

The liver was excised, weighed and 0.5 g of the liver was homogenized in 5 ml of ice- cold potassium chloride solution using ceramic mortar and pestle placed in a bowl of ice cubes, then transferred into a clean centrifuge tube and centrifuged at 4000 rpm for 10 minutes and the supernatant was stored in the refrigerator.

Determination of liver Enzymes Activity

Liver enzymes including aspartate transferase (AST), alanine transferase (ALT) and alkaline phosphatase (ALP) were determined enzymatically using commercially available kits (Randox kits).

Determination of Antioxidant Markers

Estimation of Superoxide Dismutase (SOD) Activity

Superoxide dismutase (SOD) was determined by the method of [28].A 10 dilution of the supernatant was prepared. An aliquot of 0.1ml of the diluted tissue supernatant was added to 1.25ml of 0.05M phosphate buffer (pH 7.8) to equilibrate in the spectrophotometer and the reaction started by the addition of 0.15ml of freshly prepared 0.3M adrenaline to the mixture which was quickly mixed by inversion. The reference cuvette contained 1.25ml of the phosphate buffer, 0.15ml of substrate (adrenaline) and 0.1ml of distilled water. The increase in absorbance at 480 nm was monitored every 30 seconds for 150 seconds.

Calculation:

SOD activities (U/L)=Increase in absorbance per minute = $\frac{A_3 - A_0}{2.5}\underline{A}$

Where A_o = absorbance after 30 seconds

A_S = absorbance after 150 seconds

Catalase Activity Determination

This was carried out by the method described by [29].A 50 dilution of the liver homogenate was prepared. 1ml of the diluted homogenate was dispensed into a test tube, 4ml of H_20 solution and 5ml of phosphate buffer was added. The reaction mixture was thoroughly mixed again by a gently swirling motion. 1ml portion of the reaction mixture was withdrawn and blown into 2ml of dichromate acetic acid reagent at 60 seconds intervals. The absorbance of the reaction mixture was determined at 570 nm and the H_20_2 content was extrapolated from the standard curve. A standard curve of hydrogen peroxide was first prepared by dispensing various volumes (0.00, 0.05, 0.10, 0.15, 0.30 etc.) of H_20_2 into test tubes and 2ml of dichromate/acetic acid was added to each. It was heated for ten minutes in a boiling water bath after cooling the volume of the reaction mixture was made up to 3ml with distilled water and absorbance was read at 570 nm on a spectrophotometer.

Estimation of Lipid Peroxidation

Lipid peroxidation as evidenced by the formation of TBARS was described by [30] and measured by the modified method of [31].To 0.15ml of tissue homogenate, 0.25M sucrose solution was treated with 2ml of (1:1:1 ratio) TBA-TCA-HCl reagent (0.37%TBA, 0.25N HCl and 15% TCA) and was placed in water bath for 1h at 90°C. The mixture was cooled and centrifuged at 3000rpm for 5mins. The absorbance of the

pink supernatant was measured against a reference blank using spectrophotometer at 535nm. The MDA activity was calculated as follows: Molar extinction of MDA = I.56 x 10^5 $cm^{-1}m^{-1}$ MDA concentration = Absorbance/ I.56 x 105 cm-1m-1 MDA activity (μmol/mg of protein) = MDA concentration/ mg of protein.

Statistical analysis

All data were expressed as mean ± S.E.M (standard error mean). Statistical differences between groups were assessed using the SPSS software, version 20 and Results was statistically analyzed by ANOVA.

Results

Phytochemical Composition of Methanol Extract of *Senna occidentalis* Seeds

The qualitative phytochemical screening of methanol extract of *Senna occidentalis* seeds was found to contain phenols, flavonoids, tannins, saponin, steroid, phlobatannins, cardioglycoside, reducing sugars. However, alkaloids and anthraquinone were absent (Table 1).

In Table 2, the quantitative phytochemical composition of methanol extract of *Senna occidentalis* indicated that alkaloids had the highest value (62.57±2.35) mg compared to Tannins (0.87±0.03) mg, Flavonoids (0.27±0.01) mg and Total phenol (0.79±0.021) mg respectively.

Table 1: Qualitative phytochemical composition of methanol extract of *S.occidentalis* seeds.

PHYTOCHEMICALS	INFERENCE
Phenols	+
Tannins	+
Flavonoids	+
Saponins	+
Alkaloids	+
Steroids	+
Phlobatannins	_
Cardioglycoside	+
Anthraquinone	_
Reducing sugar	_

Key: +=Present, -=Absent

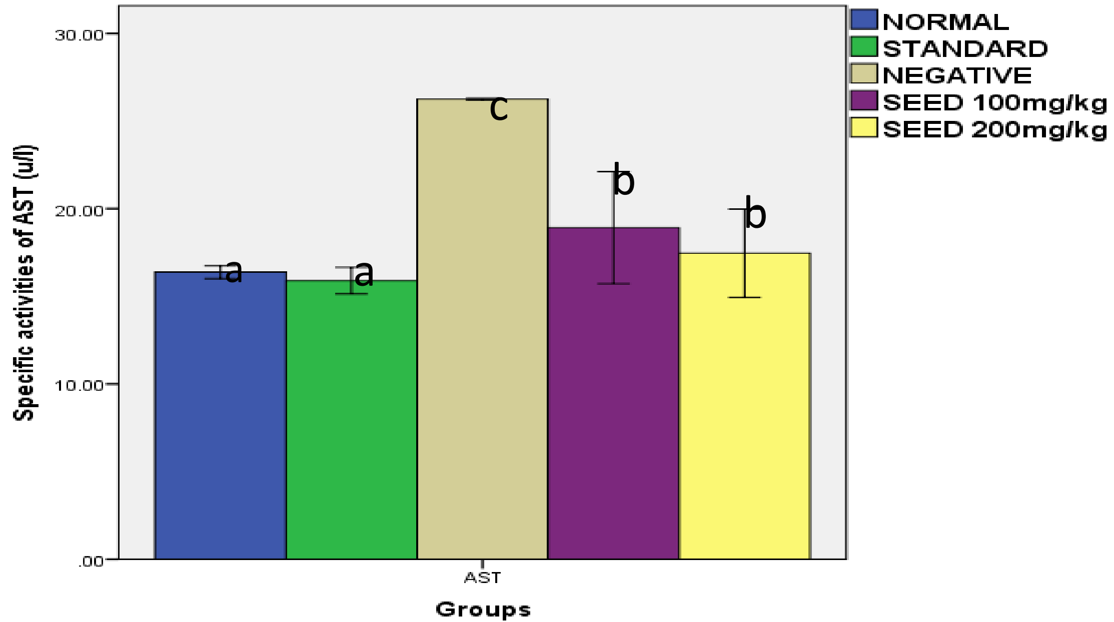
Table 2: Quantitative phytochemical composition of methanol extract of *S. occidentalis* seeds

PHYTOCHEMICALS	CONCENTRATION (mg)
Alkaloids	62.57±2.35
Tannins	0.87±0.03
Flavonoids	0.27±0.01
Total phenol	0.79±0.021

Effect of methanol extract of *Senna occidentalis* seeds on liver marker enzymes

Aspartate transaminase

The aspartate transaminase (AST) activity of methanol extract of *Senna occidentalis* seeds in normal and CCL_4 induced hepatotoxic rats is shown in Figure 1. There was a significant ($p<0.05$) increase (26.5u/L) in AST activity in the negative group compared to the other groups. Treatment with the seeds extract at 100mg/kg and 200mg/kg significantly reduced AST activity (18.1u/L and 17.5u/L respectively) with the higher dose of 200mg/kg body weight having a better effect



rats.

Figure 1:

Alanine transaminase

Figure 2 shows the effect of serum alanine transaminase (ALT) activity in Normal and CCL_4 induced hepatotoxic rats treated with methanol extract of *Senna occidentalis*. The activity of ALT was significantly ($p>0.05$) increased in negative group (8.5u/L) compared to the other groups. Administration of seed extracts at (100mg and 200mg/kg) significantly decreased the level of ALT activity. The extract at the dose of 100mg/kg body weight showed a more significant reduction (1.1u/L) than in the group that were administered 200mg/kg (3.6u/L) of seed extract of *Senna occidentalis*.

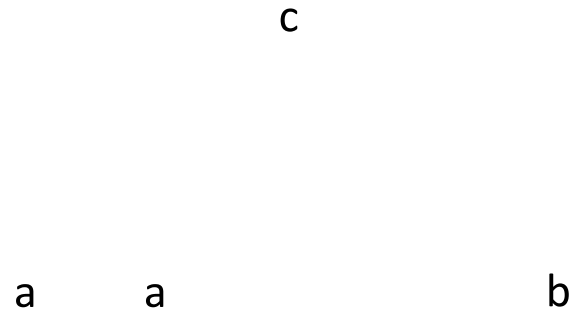


Figure 2: Effect of methanol extract of *Senna occidentalis* seed on serum ALT activity in CCL4 hepatotoxic rats and other treated rats.

Alkaline Phosphatase

The result of serum alkaline phosphatase activity of CCL_4 induced hepatotoxic rats treated with methanol extract of *Senna occidentalis*is shown in Figure 3. Induction with CCl_4 significantly increased the concentration of ALP in the serum to a value of 220.5u/L. However, treatment with seed extracts at 100mg and 200mg/kg significantly reduced ($p>0.05$) the level of ALP activity to 140.2u/L and 175.0u/L respectively.

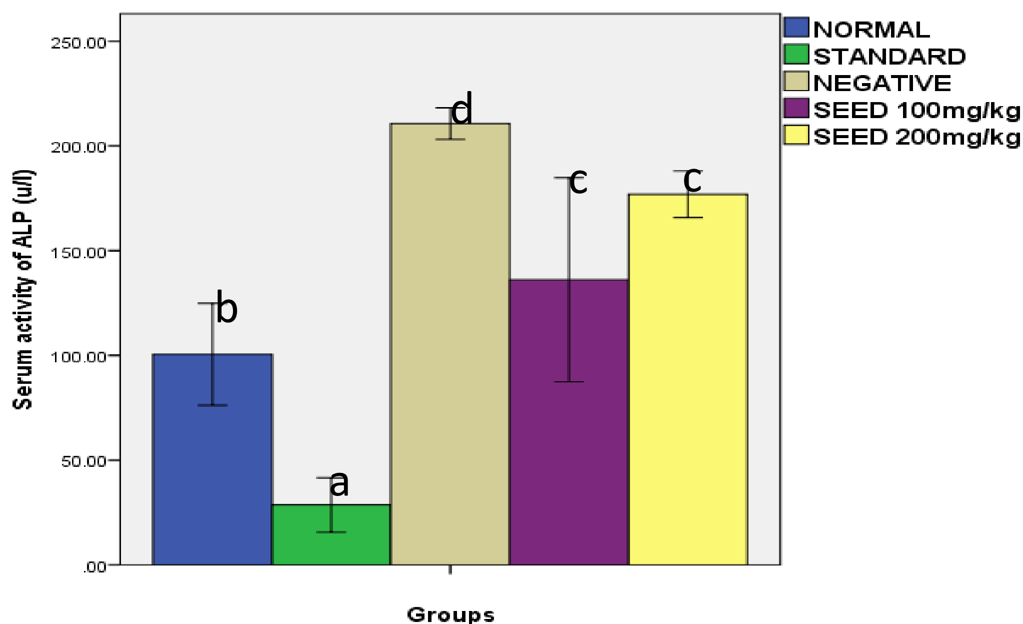


Figure 3: Effect of methanol extract of *Senna occidentalis* seeds on serum ALP activity in CCL4 hepatotoxic rats and other treated rats.

Total protein

The effect of methanol extract of *senna occidentalis* on the total protein concentrations in normal and CCl_4 induced hepatoxic rats is showed in Figure 4 There was significant($p>0.05$) reductionin total protein concentration upon induction with CCL_4.However, administration of the seeds extract at 100 mg/kg and 200 mg/kg significantly increased total protein concentration.

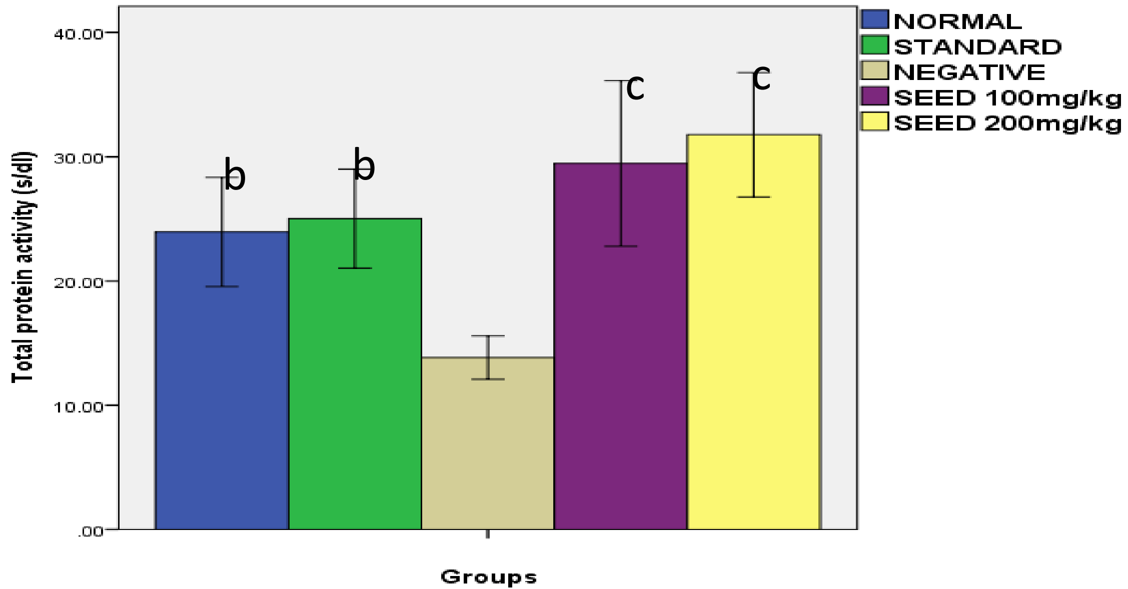


Figure 4: Effect of methanol extract of *Senna occidentalis* seeds on total protein level in CCl4 hepatotoxic rats and other treated rats.

Effect of Methanol Extract of *Senna occidentalis* seeds on Antioxidant Enzyme

Superoxide dismutase

Figure 5 shows the Superoxide dismutase activity of methanol extract of *Senna occidentalis* seeds in treated and CCL_4 induced hepatotoxic rats (negative group). Significant reduction (0.58u/L) in superoxide dismutase activity was observed in negative group administered with CCl_4. Treatment with 100mg and 200mg of the seeds extract increases the superoxide dismutase activity with the 200mg/kg body weight exhibiting a higher increment (0.85u/L) in activity than the 100mg/kg groups (0.82u/L).

Catalase

The effect of seed extract of *Senna occidentalis* on catalase activity of CCl_4 induced rats is shown in Figure 6. The catalase activity was significantly ($p<0.05$) decreased in hepatotoxic rats (3.3u/L) compared to other treated groups. However, treatment at 100mg increased the activity (5.7u/L) and at 200 mg/kg body increased catalase activity (76.0u/L) significantly when compared with the CCl_4 induced group.

NORMAL
STANDARD
NEGATIVE
SEED 100mg/kg
SEED 200mg/kg
Superoxide dismutase activity (u/l)
Groups

Figure 5: Effect of methanol extract of *Senna occidentalis* seed on superoxide dismutase activity in CCL4hepatotoxic rats and other treated rats.

b b b b

a

Figure 6: Effect of methanol extract of *Senna occidentalis* seed on Catalase activity in CCL4hepatotoxic rats and other treated rats.

Lipid peroxidation

Figure 7 shows the level of production of Malondialdehyde (MDA) as an index of lipid peroxidation in the CCl_4 induced group and the other treated group. There was observed significant ($p<0.05$) increase (13.1×10^{-5}u/mol) in the level of MDA in CCL4group when compared with other experimental treated groups. Treatment with methanol extracts of *Senna occidentalis* seeds significantly decreased the level generation of MDA at 100mg (4.1×10^{-5}u/mol) and 200mg (4.3×10^{-5}u/mol) in the treated groups.

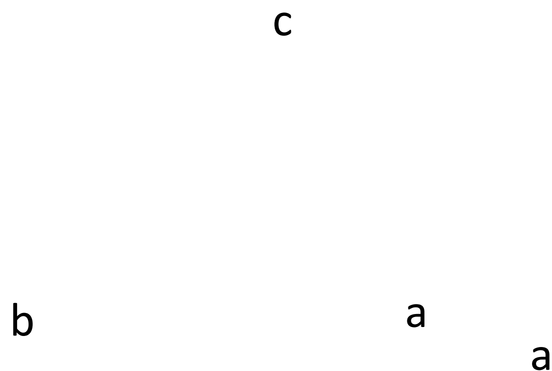


Figure 7: Effect of MDA in hepatotoxic rat treated with methanol extract of *Senna occidentalis* seeds in CCL4 hepatotoxic rats and other treated rats.

Discussion

The liver has various significant functions that are fundamental to life, including protein synthesis or production, glucose homeostasis, and detoxification. Albeit the liver has a solid regenerative capacity, it can still get damaged and harmed because of its exposure to natural poisons and toxins, resulting in organ dysfunctions and metabolic abnormalities [32].

The underlying molecular mechanism of acute liver has been discovered to be related with oxidative stress, apoptosis, and inflammation [33]. CCl_4 treatment is known to invigorate lipid peroxidation, reactive oxygen species generation, and centrilobular necrosis and steatosis; it has been widely used to induceacute hepatic damage in a mouse model of hepatotoxicity [34].

In this present study, it was observed that there was statistically significant increase ($p<0.05$) in the serum AST, ALT and ALP in the group that was administered CCl_4 alone when compared to control. AST, ALT and AST that exist in the hepatocytes can certainly leak into the peripheral blood as soon as the hepatocytes are injured. Increase in these enzymes may be due to generation of free radical, $CCl_3^{\bullet}$ that alkylates cellular proteins and lipids in the presence of oxygen causing lipid peroxidation, leading to liver damage which result in leakage of hepatic enzymes [35]. This is an indication that CCl_4 could damage the plasma membrane of liver cells (hepatocytes). However, subsequent administration of the methanol extract of *Senna* occidentalis seeds in this study was able to significantly reduce ($p<0.05$) the effect of CCl_4 towards normalization comparable to the control group in rats as observed by reduced concentration of these hepatic

enzymes found in the bloodstream. The hepatoprotective effect of methanol extract of *Senna occidentalis* seeds could be due to the presence of alkaloids, phenol, flavonoids, tannin, and saponins which enhance antioxidant activity and reduce free radical generation from CCl_4, which is the basic triggering factor for hepatotoxicity [36]. The findings in this study with respect to the significant ($p<0.05$) changes recorded in the extract treated group corroborate with finding of the reports of [37] which showed CCl_4 induced hepatoxicity administered with *Solanum xanthocarpum* leaf extract and ethanol leaf extract of *Spilanthes filicaulis* respectively.

Also, the total protein (TP) level was reduced in the CCl_4 rats showing the pulverization in the quantity of hepatic cells, which may result in a diminished hepatic ability to synthesize proteins [38, 39, 40]. Decreased concentration of liver protein may indicate impaired or damaged liver. Whereas the administration of methanol extract of *Senna* occidentalis seeds significantly and advantageously improved serum levels of protein approaching them to the normal group levels. This may be due to the fact that phytochemicals present in*Senna* occidentalis seeds extract act as agonist for the genes that are responsible for protein synthesis.

Antioxidants such as SOD, CAT, and GPx are known to neutralize excess free radicals and hence protect against their toxic effects [41]. It was observed that CCl_4 had significant effects on the activities of liver SOD by reducing its activity. This decrease in SOD activities may be due to theoverwhelming reaction between the antioxidant molecule and the generated trichloromethyl radicals[42]. This effect was ameliorated by the oral administration of methanol extract of *Senna occidentalis* at the dose of 100mg/kg and 200mg/kg. The SODsusually catalyzesuperoxide radical into oxygen and hydrogen peroxide [43]. SOD is the first line of defense against oxygen-derived free radicals and can be rapidly induced in some conditions when exposed to the oxidative stress. Also, in this study, the level of antioxidant enzyme, CAT activity was significantly decreased in rats administered CCl_4 only compared to rats in the treated groups. This can be attributed to free radicals and oxidative stress formation in CCl_4 intoxicated rats. The overall antioxidant effect exhibited by *Senna occidentalis* could be attributed to the presence of phenolic compounds in it [44]. This high scavenging property or activity can be attributed mainly to hydroxyl groups existing in the chemical structure of phenolic compounds that can provide the necessary components as a radical scavenger [45]. This finding support earlier reports that metabolites and phytoconstituents of *Senna occidentalis* like phenol, tannins and flavonoids possess antioxidant properties [46].

The decreased MDA level following *Senna occidentalis* administration can be attributed to the in vivo elevated levels of antioxidants and decreased formation of free radicals. The findings in this study are similar to previous results which elucidated similar liver toxicants and medicinal leaf protection [47,48]. The study showed that CC_{l4} was able to significantly able to induce lipid peroxidation in the liver of the intoxicated test animals, however the role of medicinal plants in ameliorating this effect was observed. There was reduction in lipid peroxidation vis a vis CCl_4 induced hepatotoxicity as a result of the ability of the medicinal plants to raise the activities of antioxidant enzymes that helps to mop up the MDA already generated.

It can be concluded from this study that *Senna occidentalis*seed extracts significantly reverse the effect of oxidative stress induced by $CCl_4$by enhancing activities of SOD and CAT, decreasing lipid peroxidation and protecting the integrity of the liver cells.

ACKNOWLEDGEMENT: We sincerely acknowledgeDepartment of Biochemistry, Federal University of Technology, Minna for giving us the liberty to use its laboratory for this research.

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