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## **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<sub>4</sub>) 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<sub>4</sub> 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<sub>4</sub> induced group was decreased. Also, increase in the level of MDA in CCl<sub>4</sub>treated 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 (CCl<sub>4</sub>) 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 CCl<sub>4</sub> 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 subtropical 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 CCl<sub>4</sub>induced 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<sub>3</sub>, concentrated ammonium hydroxide, concentrated H<sub>2</sub>SO<sub>4</sub>, 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°C. 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<sub>2</sub>SO<sub>4</sub> (1:1) mixture. 1ml of the filtrate was added to 5ml of 60% H<sub>2</sub>SO<sub>4</sub> 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 colorimetric 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<sub>2</sub>SO<sub>4</sub>. 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 FeCl<sub>3</sub> 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) CCl<sub>4</sub> 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 + CCl<sub>4</sub>(Toxic group)

Group 3: received silymarin 50 mg/kg p.o + CCl<sub>4</sub> (standard group)

Group 4: methanol extract of *Senna occidentalis* seeds (100mg/kg/day) + CCl<sub>4</sub> group 5: methanol extract of *Senna occidentalis* seeds (200mg/kg/day) + CCL<sub>4</sub>

### **Blood collection and preparation of tissue homogenate**

All animals were anaesthetized with diethyl ether vapour at the end of the experiment on the 10<sup>th</sup> 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 for 10 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:**

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

Where  $A_0$  = 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<sub>2</sub>O 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<sub>2</sub>O<sub>2</sub> 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<sub>2</sub>O<sub>2</sub> 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 =  $1.56 \times 10^5 \text{ cm}^{-1}\text{m}^{-1}$  MDA concentration = Absorbance/  $1.56 \times 10^5 \text{ cm}^{-1}\text{m}^{-1}$  MDA activity ( $\mu\text{mol}/\text{mg}$  of protein) = MDA concentration/ mg of protein.

### Statistical analysis

All data were expressed as mean  $\pm$  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 ( $62.57 \pm 2.35$ ) mg compared to Tannins ( $0.87 \pm 0.03$ ) mg, Flavonoids ( $0.27 \pm 0.01$ ) mg and Total phenol ( $0.79 \pm 0.021$ ) 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**

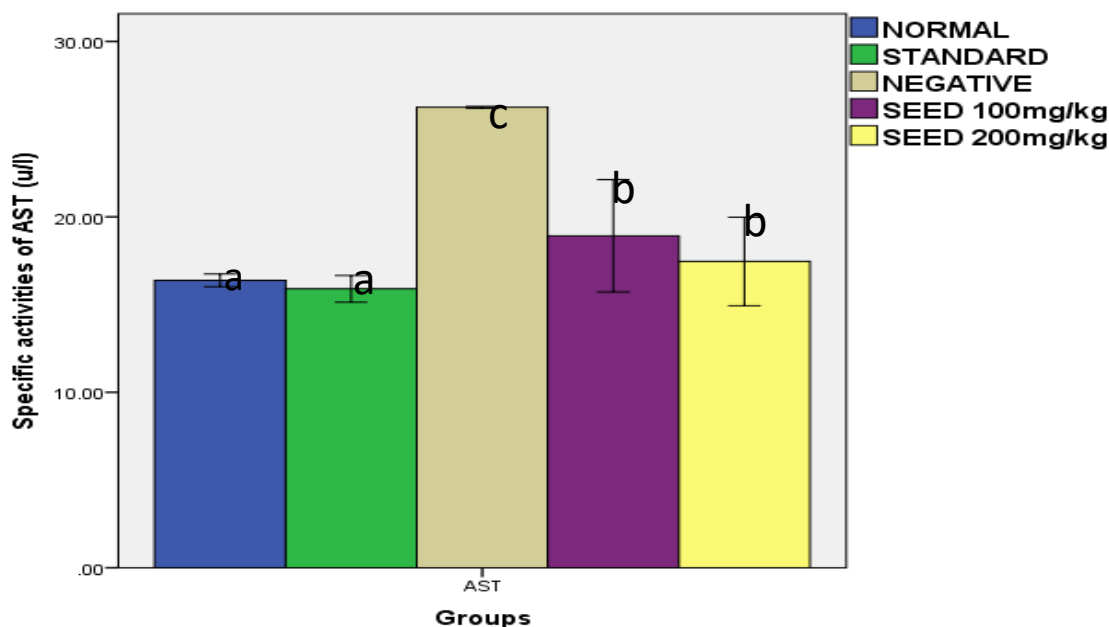
Table 2: Quantitative phytochemical composition of methanol extract of *S. occidentalis* seeds

| PHYTOCHEMICALS | CONCENTRATION (mg) |
|----------------|--------------------|
| Alkaloids      | $62.57 \pm 2.35$   |
| Tannins        | $0.87 \pm 0.03$    |
| Flavonoids     | $0.27 \pm 0.01$    |
| Total phenol   | $0.79 \pm 0.021$   |

**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<sub>4</sub> 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<sub>4</sub> 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*.

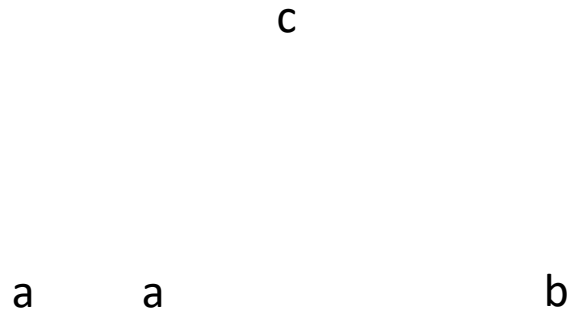


Figure 2: Effect of methanol extract of *Senna occidentalis* seed on serum ALT activity in CCL<sub>4</sub> hepatotoxic rats and other treated rats.

#### **Alkaline Phosphatase**

The result of serum alkaline phosphatase activity of CCL<sub>4</sub> induced hepatotoxic rats treated with methanol extract of *Senna occidentalis* shown in Figure 3. Induction with CCl<sub>4</sub> 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.

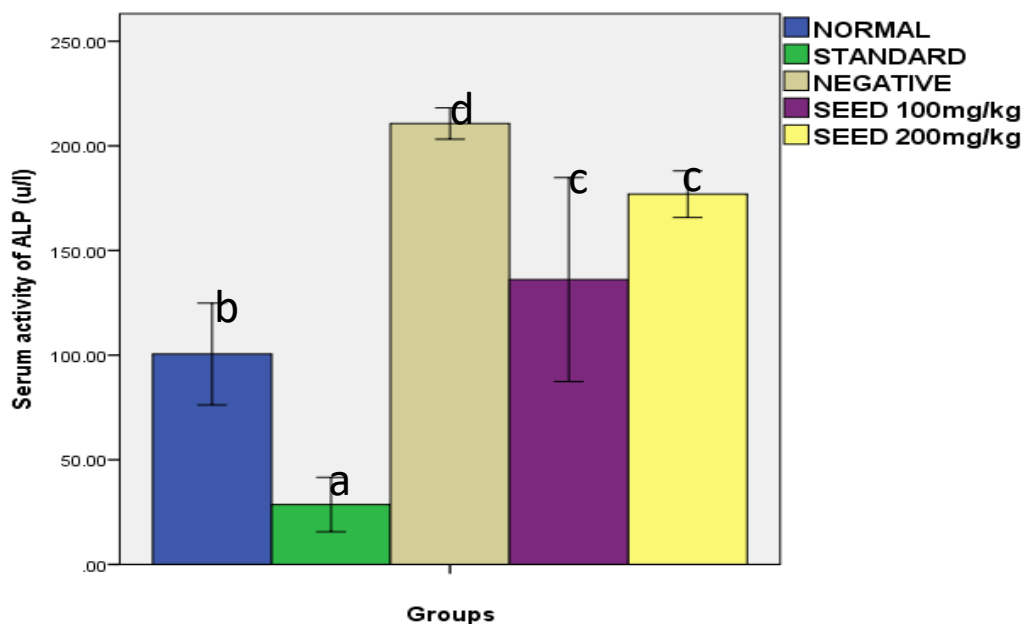


Figure 3: Effect of methanol extract of *Senna occidentalis* seeds on serum ALP activity in CCL<sub>4</sub> hepatotoxic rats and other treated rats.

### Total protein

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



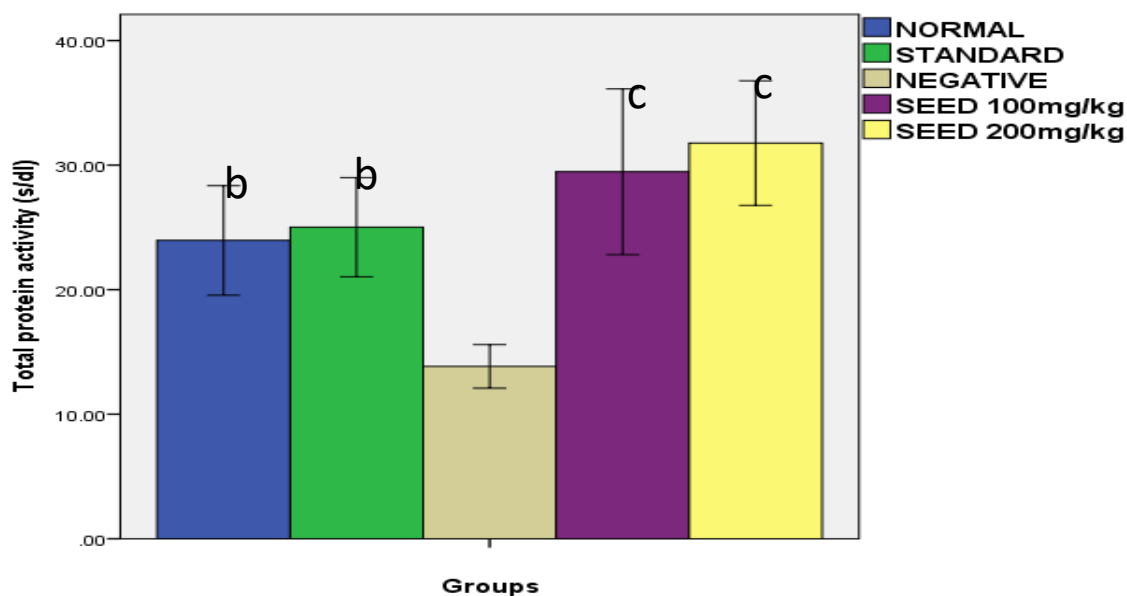


Figure 4: Effect of methanol extract of *Senna occidentalis* seeds on total protein level in CCl<sub>4</sub> hepatotoxic rats and other treated rats.

### 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<sub>4</sub> induced hepatotoxic rats (negative group). Significant reduction (0.58u/L) in superoxide dismutase activity was observed in negative group administered with CCl<sub>4</sub>. 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<sub>4</sub> 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 weight increased catalase activity (76.0u/L) significantly when compared with the CCl<sub>4</sub> induced group.

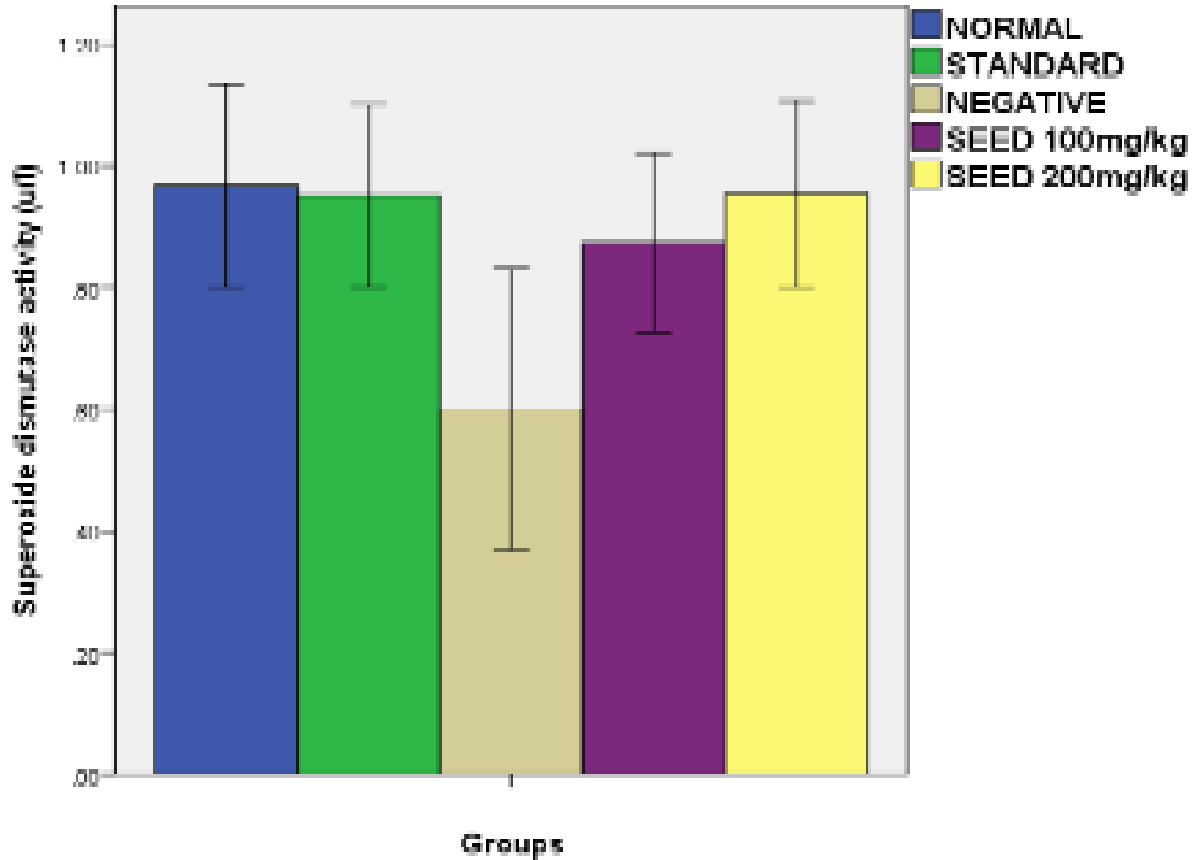


Figure 5: Effect of methanol extract of *Senna occidentalis* seed on superoxide dismutase activity in CCL4hepatotoxic rats and other treated rats.

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<sub>4</sub> induced group and the other treated group. There was observed significant ( $p < 0.05$ ) increase ( $13.1 \times 10^{-5} \text{u/mol}$ ) 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 ( $4.1 \times 10^{-5} \text{u/mol}$ ) and 200mg ( $4.3 \times 10^{-5} \text{u/mol}$ ) in the treated groups.

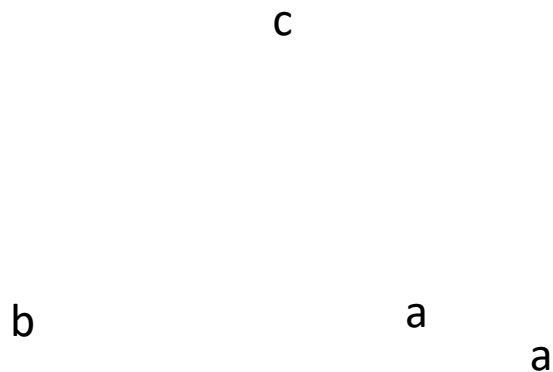


Figure 7: Effect of MDA in hepatotoxic rat treated with methanol extract of *Senna occidentalis* seeds in CCL<sub>4</sub> hepatotoxic rats and other treated rats.

### 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<sub>4</sub> treatment is known to invigorate lipid peroxidation, reactive oxygen species generation, and centrilobular necrosis and steatosis; it has been widely used to induce acute 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<sub>4</sub> 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<sub>3</sub>· 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<sub>4</sub> 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<sub>4</sub> 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<sub>4</sub>, 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<sub>4</sub> induced hepatotoxicity 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<sub>4</sub> 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<sub>4</sub> had significant effects on the activities of liver SOD by reducing its activity. This decrease in SOD activities may be due to the overwhelming 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 SOD usually catalyzes superoxide 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<sub>4</sub> only compared to rats in the treated groups. This can be attributed to free radicals and oxidative stress formation in CCl<sub>4</sub> 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 CCl<sub>4</sub> was able to significantly 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<sub>4</sub> 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<sub>4</sub> by enhancing activities of SOD and CAT, decreasing lipid peroxidation and protecting the integrity of the liver cells.

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## References

- [1] Osadebe PO, Okoye FB, Uzor PF et al. Phytochemical analysis, hepatoprotective and antioxidant activity of *Alchornea cordifolia* methanol leaf extract on carbon tetrachloride-induced hepatic damage in rats. *Asian Pac J Trop Med.* 2012; 5:289-293.

- [2] Jha M, Nema N, Shakya K, Ganesh N, Sharma V. In vitro hepatoprotective activity of *Bauhinia variegata*. *Pharmacologyonline*. 2009;3:114-118.
- [3] Jaeschke H, McGill MR, Ramachandran A. Oxidant stress, mitochondria, and cell death mechanisms in drug-induced liver injury: Lessons learned from acetaminophen hepatotoxicity. *Drug Metab Rev.* 2012;44(1): 88-106.
- [4] Cong M, Zhao WH, Liu TH et al. Protective effect of human serum amyloid P on CCl<sub>4</sub>-induced acute liver injury in mice. *Int J Mol Med*. 2017;40(2): 454-464.
- [5] Tian XT, Liu H, Qiao SD et al. Exploration of the hepatoprotective chemical base of an orally administered herbal formulation (YCHT) in normal and CCl<sub>4</sub>-intoxicated liver injury rats. Part 2: Hepatic disposition in vivo and hepatoprotective activity in vitro. *J Ethnopharmacol*. 2019; 236:161-172.
- [6] Manibusan MK, Odin M, and Eastmond DA. "Postulated carbon tetrachloride mode of action: a review," *Journal of Environmental Science and Health, Part C: Environmental Carcinogenesis and Ecotoxicology Reviews*. 2007. vol.25, no3, pp.185– 209.
- [7] Brautbar N and Williams J. "Industrial solvents and liver toxicity: risk assessment, risk factors and mechanisms," *International Journal of Hygiene and Environmental Health*. 2002. vol. 205, no.6, pp.479–491.
- [8] Yousefi-Manesh H, Shirooie S, Partoazar A, Nikoui V, Estakhri MRA, Bakhtiarian A. Hepatoprotective effects of phosphatidylserine liposomes on carbon tetrachloride-induced hepatotoxicity in rats. *J Cell Biochem*. 2019;120(7):11853-11858.
- [9] Tukappa NKA, Londonkar RL, Nayaka HB, Kumar C. Cytotoxicity and hepatoprotective attributes of methanolic extract of *Rumex vesicarius* L. *Biol Res*. 2015;48:19–27.
- [10] Bezenjani SN, Pouraboli I, Afshar RM, Mohammadi G. Hepatoprotective effect of *Otostegia persica* Boiss. shoot extract on carbon tetrachloride induced acute liver damage in rats. *Iran J Pharm Res*. 2012;11:1235–1241.
- [11] Veronique JE. and Gabriel NM. Sub-chronic Toxicity of the Beverage made from *Cassia occidentalis* seeds in mice. *International Journal of Nutrition and Food Sciences*. 2013; 2(5):237-242.
- [12] Mariano-Souza DP, Paulino CA, Maiorka PC, Gorniak, SL. Administration *Senna occidentalis* seeds to adult and juvenile rats: Effects on Thymus, Spleen and in Haematological parameters. *Journal of Pharmacology and Toxicology*. 2010; 5:46-54.
- [13] Mohammed M, Aboki MA, Saidu HM, Victor O, Tawakalitu A and Maikano SA. Phytochemical and Some Antimicrobial Activity of *Cassia Occidentalis* L. (Caesalpinaceae). *International Journal of Science and Technology*. 2012; 2:4.
- [14] Ibrahim MA, Aliyu AB, Sallau AB, Bashir M, Yunusa I, Umar TS. *Senna occidentalis* leaf extract possesses antitrypanosomal activity and ameliorates the trypanosome-induced anaemia and organ damage. *Pharmacognosy Research*. 2010; 2(3):175-180.
- [15] Gowrisri M, Sarita K., Vrushabendra SBM, Archana SP, Vishwanath KM. Anti-oxidant and Nephroprotective Activities of *Cassia occidentalis* Leaf Extract against Gentamicin Induced Nephrotoxicity in Rats. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*. 2012;3(3):684-694.
- [16] Yadav JP, Vedpriya A, Sanjay Y, Manju P, Sandeep K., Seema D. *Cassia occidentalis* L.: A review on its ethnobotany, phytochemical and pharmacological profile. *Fitoterapia*. 2010; 81(4):223-230.
- [17] Gwarzo U.S, Gimba CE, Adeyemo DJ, Paul ED. Neuron Activation Analysis (NAA) of *Senna occidentalis* Linn. *Journal of Natural Sciences Research*. 2014;4(11):22-28.
- [18] Shafeen S, Srinath RT, Arafath S, Nagarjuna S, Padmanabha RY. Evaluation of antianxiety and antidepressant activity of *Cassia occidentalis* leaves. *Asian Journal of Pharmaceutical and Clinical Research*. 2012;5(3):47-50.
- [19] Silva MG, Aragao TP, Vasconcelos CF et al. Acute and subacute toxicity of *Cassia occidentalis* L. stem and leaf in Wistar rats. *Journal of Ethnopharmacology*. 2011;136(2): 341-346.
- [20] Emmanuel S, Rani MS, Sreekanth MR. Antidiabetic activity of *Cassia occidentalis* Linn in streptozocin-induced diabetic rats: A dose dependent study. *International Journal of Pharmacology and Bioscience*. 2010;1(4): 14-25.
- [21] Laxmi V, Singour PK, Chaurasiya PK, Rajak H, Pawar RS, Patil UK. Effect of ethanolic extract of *Cassia occidentalis* Linn. For the management of alloxan induced diabetic rats. *Pharmacognosy Research*. 2010;2(3):132-137.
- [22] Onakpa MM, and Ajagbonna OP. Antidiabetic Potentials of *Cassia occidentalis* leaf extract on Alloxan induced Diabetic albino mice. *International Journal of Pharm Tech Research*. 2012;4(4): 1766-1769.
- [24] Trease GE, Evans WC *Pharmacognosy*. 15<sup>th</sup> Ed. London: Saunders Publishers; 2002. Pp. 42-44. 221- 229, 246-249, 304- 306, 331- 332, 391- 393.
- [25] Oloyede OI. Chemical profile of unripe pulp of *Carica papaya*. *Pak. J. Nutr.* 2005;4:379-381.
- [26] Chang C, Yang M, Wen H and Chern J, "Estimation of total flavonoid content in propolis by two complementary colorimetric methods," *Journal of Food and Drug Analysis*. 2002; vol 10, no 3, pp. 178- 182.

- [28] Winterbourn CC, Hawkins RE, Brian M, Carrell RW. The estimation of red cell superoxide dismutase activity. *J Lab Clin Med.* 1975; 85(2):337-41
- [29] Bock PP, Kramer R and Pavelka M. Peroxisomes and related particles. In: *Cell Biology Monographs 7*. Springer, Berlin. 1980. pp 44-74.
- [30] Niehaus WG, Samuelsson B. formation of malonaldehyde from phospholipid arachidonate during microsomal lipid peroxidation. *Eur J Biochem.* 1968 Oct 17;6(1): 126-130.
- [31] Akanji M. A, Adeyemi O. S, Oguntoye S. O and Sulaimon F. A. Psidium Guajava extract reduces trypanosomiasis associated lipid peroxidation and raises glutathione concentrations in infected animals. *EXCLI Journal* (2009):8:148-154.
- [32] Xiong Rui, Shan Shuzhong, Wang Xiaoming, Zhang Xiaowen, Yu Haixia, Shi Haomin, Wang Xuejiao. Aloperine attenuates carbon tetrachloride-induced mouse hepatic injury via Nrf2/HO-1 pathway. *Tropical Journal of Pharmaceutical Research* May 2020; 19 (5): 983-988.
- [33] Jaeschke H, McGill MR, Ramachandran A. Oxidant stress, mitochondria, and cell death mechanisms in drug-induced liver injury: Lessons learned from acetaminophen hepatotoxicity. *Drug Metab Rev.*2012; 44(1): 88-106.
- [34] Kodai S, Takemura S, Minamiyama Y, Hai S, Yamamoto S, Kubo S, Yoshida Y, Niki E, Okada S, Hirohashi K et al. S-allyl cysteine prevents CCl4-induced acute liver injury in rats. *Free Radical Res.*2007; 41(4): 489-497.
- [35] Ohwokevw OA, Ogunka- Nnoka CU. Effect of Ethanol Leaf Extract of *Spilanthes filicaulis* on Liver induced and selected Biochemicals on Carbon Tetrachloride Induced Hepatic Damage in Wistar Rats. *Med Aromat Plants (Los Angeles)*.2019;8: 342
- [36] Wadhwa S, Panwar M. Agrawal S, A. et al., "A review on pharmacognostical study of *Lepidium sativum*," vol. 2, 2012, 316-323.
- [37] Gupta R, Sharma KA, Sharma MC, Dobhal MP and Gupta RS. Evaluation of antidiabetic and antioxidant potential of lupeol in experimental hyperglycemia, *Natural Product Research.* 2012;26: 12, 1125- 1129.
- [38]. Al-Seeni MN, El Rabey HA, Zamzami MA, Alnefayee AM. The hepatoprotective activity of olive oil and *Nigella sativa* oil against CCl4 induced hepatotoxicity in male rats. *BMC Complement Altern Med.*2016; 16(1):1-14.
- [39]. Elbakry MA, El Rabey HA, Elremaly W, Sakran MI, Almutairi FM. The methanolic extract of *Moringa oleifera* attenuates CCl4 induced hepatonephro toxicity in the male rat. *Biomed Res.*2019;30(1):23-31.
- [40]. Abdelaal S, Mousa HSE, Ahmed SM. Effect of Silymarin versus Silymarin and green coffee extract on Thioacetamide induced liver injury in adult male albino rats (histological and Immunohistochemical study). *Egy J Histol.*2019;42(1):133-46.
- [41]Wahid A, Ashraf NH, Heba ME and Mekky MA. Hepatoprotective activity of ethanoic extract of *Salix subserrata* against CCl4- Induced chronic hepatotoxicity in rats. *BMC Complementary and Alternative Medicine.*2016;16(1): 263.
- [42] Tsado N, Lawal B, Yahaya MA. Antioxidants and Antimicrobial- Activities of Methanol Leaf Extract of *Senna occidentalis*. *Journal of Advances in Medical and Pharmaceutical Sciences.* 2016;8(2):1-7.
- [43] He J, Chi C, Liu H. Identification and analysis of an intracellular Cu/Zn superoxide dismutase from *Sepiella maindroni* under stress of *Vibrio harveyi* and Cd<sup>2+</sup>. *Dev Comp Immunol.*2014;47:1-5
- [44] Ibrahim AM, Lawal B, Tsado NA, Yusuf AA, Jimoh AM. Phytochemical screening and GC-MS determination of bioactive constituents from methanol leaf extract of *Senna occidentalis*. *Journal of Coastal Life Medicine.* 2015;3(12):992-995.
- [45] Rice- Evans CA, Miller NJ, Bolwell PG, Bramley PM, Pridth JB. The relative antioxidant activities of plants derived polyphenolics flavonoids. *Fre Rad. Res.* 1995;22:375-383.
- [46] Lawal B, Ossai PC, Shittu OK, Abubakar AN. Evaluation of phytochemicals, proximate, minerals and anti-nutritional compositions of yam peel, maize chaff and bean coat. *Inter J. Appl. Biol. Res.* 2014;6(2):10-17.
- [47] Usunobun U, Okolie NP and Eze IG. Inhibitory effect of *Vernonia amygdalina* on dimethylnitrosamine (DMN)-induced liver fibrosis in rats. *International Journal of Clinical Pharmacology and Toxicology.* 2015;4(4): 179-184.
- [48] Usunobun U, Ugbeni CO and Agu KC. Protective effect of *Celosia argentea* aqueous leaf extract on liver function in Carbon- tetrachloride (CCl4)- induced hepatotoxicity. *Malaysian Journal of Biochemistry and Molecular Biology.*2019;22(1): 93- 96.