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**Hepatocurative Effect of Oral Administration of Aqueous Leaf Extract
 Of *Senna Occidentalis* in CCl₄ Induced Hepatotoxic Rats**

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ABSTRACT

Awareness on the utilization of medicinal plants to curtail diseases is on the increase. This study is to evaluate the phytochemical contents and effect of aqueous leaf extract of *Senna occidentalis* on liver function indices in CCl₄ induced hepatotoxic rats. A total of 32 male albino rats were used for this study. They were divided into six groups of 5 rats each. Group II-VI were induced with CCl₄ at the dose of 150mg/kg for hepatotoxicity. Aqueous extract of *Senna occidentalis* orally administered to animals in groups III, IV and V at doses of 500mg/kg, 250mg/kg and 166mg/kg for four weeks respectively. Animals in group I were used as normal control, while those in group II were CCl₄ induced hepatotoxic control. Group VI were orally administered with livolin at a dose of 100mg/kg for four weeks. At the end of fourth week, the animals were sacrificed and their serum activities of aspartate amino transferase (AST), alkaline phosphatase (ALP) and alanine amino transferase (ALT) as well as serum levels of total bilirubin, direct bilirubin, total protein (TP) and Albumin (Alb) were determined. Results of phytochemical screening revealed the presence of tannins, saponins, cardiac glycosides, resins and flavonoids. The results showed significant ($p < 0.05$) increase in mean serum activities of AST, ALP, and ALT, serum total bilirubin and direct bilirubin levels, and decrease in mean serum TP and albumin levels in the group II when compared with group I. Results of the effect of oral administration of aqueous leaf extract of *Senna occidentalis* at the doses of 500mg/kg, 250mg/kg and 166mg/kg for four weeks showed a significant ($p < 0.05$) decrease in the serum AST, ALP, ALT, total bilirubin, direct bilirubin, and significant increase ($p < 0.05$) in TP and albumin when compared with group II. Thus, it could be suggested that, aqueous leaf extract of *Senna occidentalis* could have a hepatocurative effect against CCl₄ toxicity.

Keywords: Liver function, *Senna occidentalis*, Rats.

1.0 INTRODUCTION

The use of plant extracts in traditional medicine is a worldwide practice. Medicinal plants form the basis of primary healthcare for majority of the people living in the rural and remote areas in Nigeria and other third world countries. According to World Health Organization (WHO, 2001), about 80% inhabitants of the world rely essentially on herbal medicine for their primary healthcare needs (WHO, 2001). In some cases, the plants offer alternative healthcare services where the patients are poor or live far away from the hospitals, for curing ailments and diseases. A number of medicinal plants have been found and put into use in ethnomedicine by traditional healers in the management of many diseases (Sofowora, 1993).

Medicinal plants are plants that possess therapeutic properties or exert beneficial pharmacological

effect on animals. Although there are no apparent morphological characteristics in the medicinal plants, growing them is medicinally important. It is now been established that the plants, which naturally synthesizes and accumulate some secondary metabolites e.g alkaloids, glycosides, tannins, volatile oils and certain minerals and vitamins possess medicinal properties (Sinhg *et al.*, 2012). These medicinal plants, which are often called traditional medicines, need to be evaluated, given due recognition and develop so as to improve their safety, availability and wider application at low cost. According to world health organization (WHO) reports, it is the people's own healthcare system and is well accepted by them (WHO, 2001).

Senna occidentalis belong to the family *leguminosae*, subfamily *caesalpinoidae*. It is botanically classified as both *Senna occidentalis*

and *Cassia occidentalis*. It is small, erect, annual herb that can be up to 2-meter-tall and is found abundantly in the rain forest and tropical areas of the world. *Senna* has compound leaves with narrow linear-to-oval-shaped dark green leaflets. During spring and summer it has yellow pea-like flowers which are followed by brown pods which contain brown seeds. Leaves, fruit and flowers of *Senna* are used for medicinal purpose. *Senna occidentalis* is one of the plants employed in ethnomedicine in many parts of the world. It is commonly called "Rai-Rai" in Hausa. Other common names include Fedegoso in (Brazil), Negro coffee, Brusca and maritnaca (in Peru) (Saraf, 1994).

Senna occidentalis has rich history in natural medicine. The parts of the plant used include roots, leaves and seeds (Saraf, 1994). In Peru, the roots are used as diuretic and decoction for treating fever (Soukup, 1970). In Brazil, the roots are used in the treatment of fever, anaemia, liver complaints and a reconstituent for general body weakness (Raul, 1994). The leaves are also used in Brazil for treating gonorrhoea, fever, urinary tract infection, oedema and menstrual problems (Cruz, 1995). In Panama, tea made from the leaf is used for stomach colic; the crushed leaves are also taken orally to expel intestinal worms and parasites (Gupter, 1979). In many countries around the world, the fresh and/or dried leaves of *Senna occidentalis* are crushed and brewed into tea and applied externally for treating skin disorders, wounds, skin fungi and other parasitic diseases etc. (Caceres, 1991). In Nigeria, the leaves of *Senna occidentalis* are employed in treating various skin infections. Leaf decoction also made for treating typhoid, fever, constipation and also as diuretic. The seeds of *Senna occidentalis* are also ground and injected for treating diseases like hypertension, stomach upset etc (Sule and Abubakar, 2008). It is particularly important to know if the leaf of *Senna occidentalis* has hepatocurative ability.

2.0 MATERIALS AND METHODS

2.1 Collection and Preparation of Plant

Material

Senna occidentalis leaves were collected from Bayero University Kano, Nigeria. It was identified at Department of Plant Science Bayero University Kano and voucher number was deposited as

BUK/HAN/073. It was then dried in shade and pulverized into a powder and 200g of the powder was weighed and soaked in 400cm³ of distilled water for 24 hours. The mixture was filtered using whatman's filter paper no (1), the filtrate was dried. The dried extract was reconstituted with distilled water for further use.

The volume to be administered to animals was calculated according to the method of Alhassan *et al.* (2015).

$$vol. (CM^3) = \frac{Weight\ of\ the\ Rat\ (kg) \times Dose\ (\frac{mg}{kg})}{Concentration\ of\ the\ Extract\ (\frac{mg}{cm^3})}$$

2.2 Experimental Animals

Thirty two (32) male albino rats weighing between 80g to 120g were obtained from Department of Zoology Bayero University Kano. They were housed in cages in a well-ventilated room at room temperature under standard condition and had free access to water and food throughout the period of the experiment. They were fed with standard vital feed diet (growers pelletized manufactured by grand cereals Ltd a subsidiary of UAC of Nigeria). The experiments were performed according to the principles of laboratory animal care.

CCL₄ Induced Hepatotoxicity

Hepatotoxicity was induced in rats by administering 150mg/kg body weight of CCl₄ via intraperitoneal injection.

2.3 Experimental Design

Twenty seven (27) male albino rats were given an intraperitoneal injection of CCl₄ (150mg/kg) to induce liver damage five remaining rat were normal control. After two days, two rats were sacrificed to confirm the induction (Alhassan *et al.*, 2009), while the remaining thirty rats were divided into six equal groups (of five rats each).

Group I was the control group (normal).

Group II was administered with CCl₄ and no extract was given (test control)

Group III was administered orally with 500mg/kg ALESO daily for four weeks after CCl₄

Group IV was administered orally with 250mg/kg ALESO daily for four weeks after CCl₄ induction

Group V was administered orally with 166mg/kg ALESO daily for four weeks after CCl₄ induction

Group VI was administered orally with 100mg/kg livoline daily for four weeks after CCl₄ induction. At fourth week, all the rats were sacrificed (24 hours after last treatment). Blood samples were collected for analysis of AST, ALT, ALP, TB, DB, TP and albumin.

2.4 Qualitative Determination of Some Phytochemicals (Tiwari *et al.*, 2011).

2.4.1 Test for Alkaloids

The Aqueous leaf extract of *Senna occidentalis* was rendered slightly acidic. The acetic acid extract was divided into 4 portions, one served as a blank (control). To one portion, few drops of Mayer's reagent (potassium mercuric iodide solution) were added, to the other few drops of Dragendorff's reagent (potassium bismuth iodide) and to the third few drops of Wagner's reagent. A precipitate/turbidity observed in all the tests as compared with the control indicates the presence of alkaloids.

2.4.2 Test for Flavonoids (NaOH Test)

To 1% hydrochloric acid, extract of *Senna occidentalis* (2cm³), 20% NaOH (few drops) were added. A canary yellow colour indicates the presence of a flavonoid.

2.4.3 Test for cardiac glycosides (Baljet's Test)

Baljet's reagent was prepared by freshly mixing equal volumes of 1% picric acid in 95% alcohol and 10% NaOH in water. Few drops of this reagent were added to *Senna occidentalis* (2cm³), which gave a light orange to red colour indicating the presence of cardinolides.

2.4.4 Test for saponins (Froth Test)

Senna occidentalis (2cm³) was shaken vigorously; a persistent froth lasting for 3 minutes indicate presence of saponins.

2.4.5 Test for tannins (Ferric Chloride Test)

Few drops of 5% ferric chloride (FeCl₃) were added to *Senna occidentalis* (2cm³). A blue green colour observed indicates the presence of tannins.

2.4.6 Test for Resins

Extract of *Senna occidentalis* (2cm³) was dissolved in acetic anhydride (10cm³). Concentrated sulphuric acid (1 drop) was added. Presence of

purple colour (which rapidly changes to violet) indicates absence of resins

2.5 Analysis of Liver Function

Activities of serum transferases (ALT and AST) were estimated according to the method of Reitman and Frankel (1957).

Serum alkaline phosphatase activity was measured using the method of Rec (1972). The serum bilirubin was measured using the method of Jendrassik and Grof (1938). Total protein was determined using biuret method (Tietz, 1995). Albumin was determined using the method of Grant (1987).

2.5.1 Histopathology

Histological examination was done using a binocular microscope at x100 magnification.

3.0 Statistical Analysis

Results were expressed as mean \pm standard deviation and analysed using one way analysis of variance (ANOVA) in which $p < 0.05$ differences in mean were considered significant.

4.0 RESULTS

4.1 Phytochemical Screening

Results of qualitative phytochemical screening of aqueous leaf extract of *Senna occidentalis* are presented in Table 1. The results of the analysis indicated the presence of tannins, flavonoids, saponins, cardiac glycosides and resins

4.2 Enzymatic Markers for Liver Function

The results for the hepatocurative effect of aqueous leaf extract of *Senna occidentalis* on liver enzymes (AST, ALT and ALP) were depicted in figure 1. There was a significant increase in the serum AST, ALT and ALP level ($p < 0.05$) in CCl₄ induced control group (group II) compared to the normal control (group I). A significant decrease ($p < 0.05$) in ALT, and ALP was observed in group treated with 500mg/Kg, 250mg/kg, 166mg/kg of ALESO and group treated with standard drug (livolin) and there was also significance decrease ($p < 0.05$) in AST in group treated with 500mg/kg, 250mg/kg of ALESO in dose defendant pattern and the group treated with standard drug (livolin) compared CCl₄ induced control group (group II), after induction of liver damage.

4.3 Non-Enzymatic Markers

Results for the hepatocurative effect of aqueous leaf extract of *S. occidentalis* on liver Total bilirubin (TB), direct bilirubin (DB) and Indirect bilirubin (IB) and that of Total protein (TP), and Albumin (ALB), were shown in figure 2 and Figure 3 respectively. There was a significant increase in the serum TB and DB level ($p < 0.05$) in CCl_4 induced control group (group II) compared to the normal control (group I). A significant decrease ($p < 0.05$) in TB and DB was observed in rats orally administered with 500mg/Kg, 250mg/kg, 166mg/kg of ALESO in dose dependant pattern and

also a significant increase ($p < 0.05$) in total protein and albumin was observed in a dose dependant pattern.

4.4 Histopathology

The results obtained for histopathological study is presented in Figure 4 shows a photomicrograph of cross section of liver of a rat in Group I (normal control), figure 5 the liver for a rat induced with damage at a dose of 150mg/kg of CCl_4 (group II test control), figure 6,7 and liver of a group of rats treated with the varying doses of 500,250 and 166mg/kg of ALESO and figure 9 the liver of a rats treated with livolin, for four weeks.

Table 1: Phytochemical analysis of aqueous leaf extract of *S. occidentalis*

Phytochemicals	inference
Tannins	+
Saponins	+
Flavonoids	+
Cardiac glycoside	+
Resins	+
Alkaloids	-

Key ; (+) means present,(-) means not detected

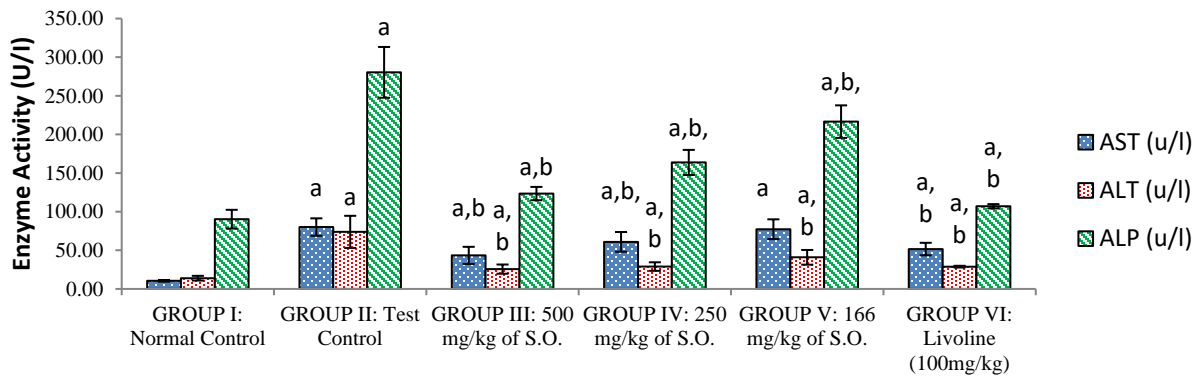


Figure: 1 Effect of aqueous leaf extract of *senna occidentalis* on Rats AST, ALT, and ALP

Results are expressed as mean±SD, n=5

Key. AST: Aspartate amino transferase, ALT: Alanine amino transferase, ALP: Alkaline phosphatase

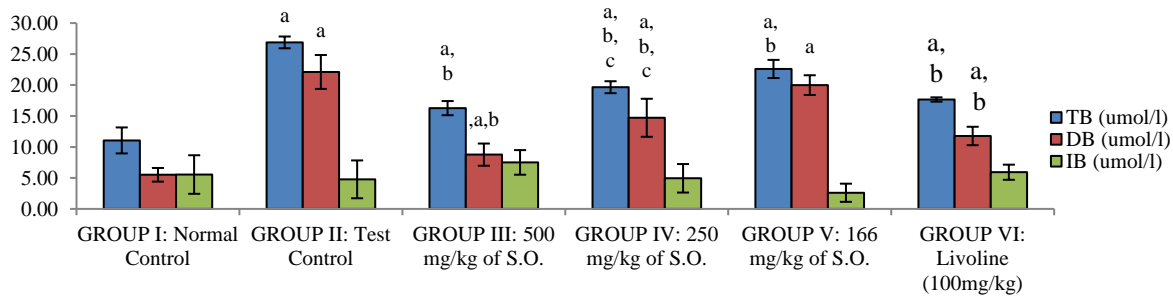


Figure 2: Effect of aqueous leaf extract of senna occidentalis on TB, DB and IB
 Results are expressed as mean±SD, n=5
 Key. TB: Total bilirubin DB: Direct bilirubin, IB: Indirect bilirubin Alkaline phosphatase

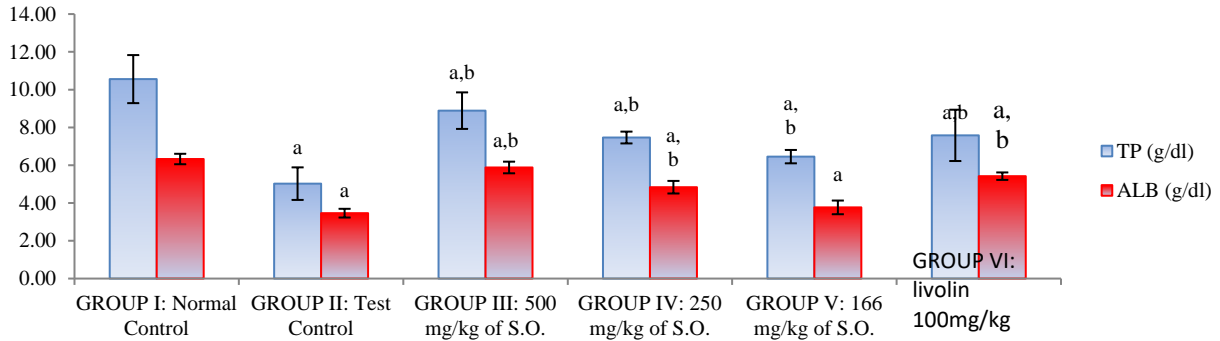


Figure 3: Effect of aqueous leaf extract of senna occidentalis on TP and Albumin
 Results are expressed as mean±SD, n=5
 Key.TB: Total bilirubin DB:Direct bilirubin, IB:Indirect bilirubinAlkaline phosphatase

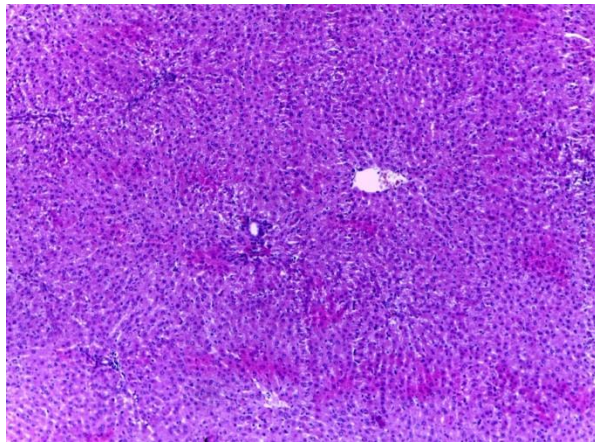


Figure 4: Stained cross section of the liver showing unremarkable liver biopsy with hepatocytes arranged in cord radiating from central venule (H and E Stain (×100))

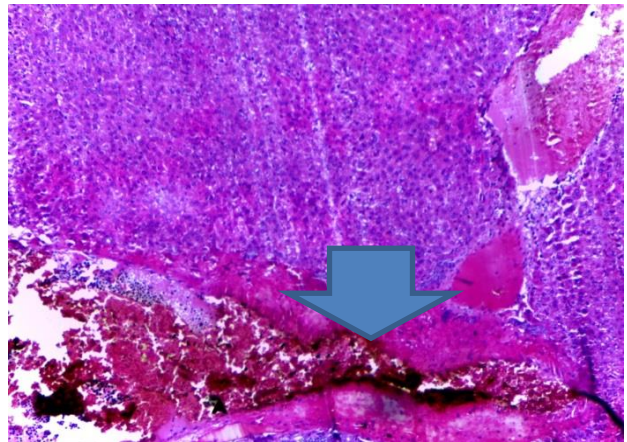


Figure 5: Stained cross section of the liver showing damages and hemorrhage with the loss of normal architecture after CCl4 administration (H and E Stain (×100))

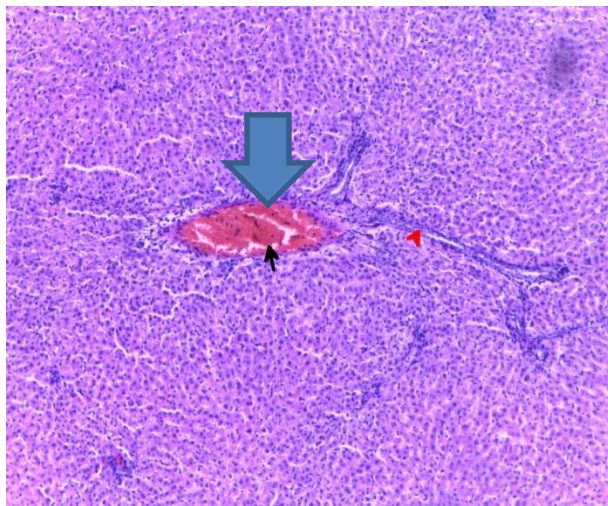


Figure 6: Stained cross section of liver of CCl4 hepatotoxicity rats administered with daily dose of 500mg/Kg of ALESO for four weeks showing areas of fibrosis and vascular congestion. (H and E Stain (×100))

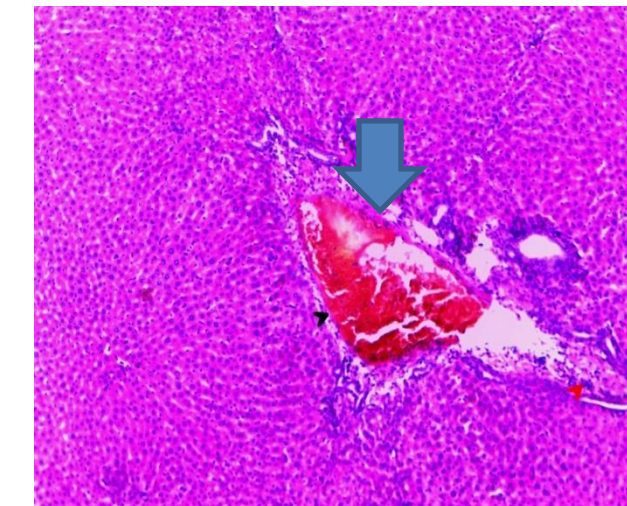


Figure 7: Stained cross section of liver of CCl4 hepatotoxicity rats administered with daily dose of 250mg/Kg of ALESO for four weeks showing areas of fibrosis and vascular congestion. (H and E Stain (×100))

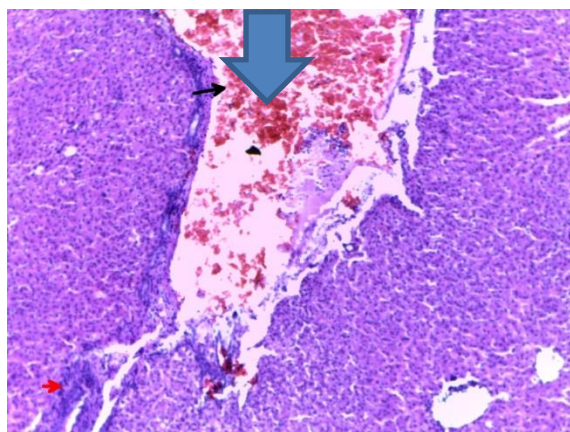


Figure 8: Stained cross section of the liver of liver of CCl₄ hepatotoxicity rats administered with daily dose of 166mg/kg showing damages, some areas of vascular congestion and fibrosis (H and E Stain (×100))

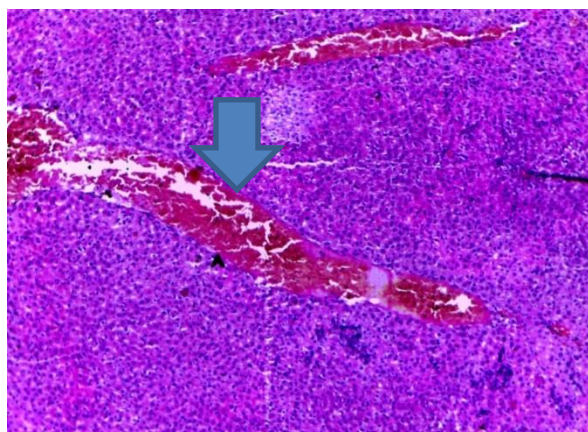


Figure 9: Stained cross section of liver of CCl₄ hepatotoxicity rats administered with 100mg/kg daily dose of livolin for four weeks showing areas of fibrosis and vascular congestion. (H and E Stain (×100))

Figure 4: Histopathological studies of Liver hepatocytes of control and test rats

5.0 DISCUSSION

5.1 Phytochemical Screening

Result of phytochemical screening of this study shows that *Senna occidentalis* contain tannins, flavonoids, cardiac Glycosides, saponins and resins (Table 1). This is in accordance with the findings of Nuhu and Aliyu (2008) which also reported the presence of the above phytochemicals in *Senna occidentalis*. Phytochemicals have potential physiological and pharmacological effect. They have disease preventive properties. The presence of the phytochemicals compounds in the plant provide empirical basis for its traditional medicinal uses (Tella and Ojo, 2005).

5.2 Hepatocurative Effect

Liver is an important organ in the body. It plays a key role in metabolic processes. Carbon tetrachloride is one of the most widely used chemical for induction of hepatotoxicity. In the present study, the result showed a significant increase ($p < 0.05$) in mean serum levels of AST, ALT, and ALP (figure 1), total bilirubin and direct bilirubin (figure 2), with a significant decrease ($p < 0.05$) in mean serum levels of total protein and albumin (figure 3) in test control (Group II) after 150 mg/kg of CCl₄ administration when compared with the normal control (Group I). Extensive evidence demonstrated that CCl₄ is activated in the liver to a highly reactive trichloromethyl radical

which initiate free radical mediated lipid peroxidation of the cytoplasmic membrane phospholipids causing functional and morphological changes in the cell membrane which leads to accumulation of lipid derived oxidants causing liver injury. Elevated levels of (AST, ALP, ALT, total bilirubin, direct bilirubin) and subsequent reduction of total protein and albumin is an indication of the liver dysfunction as a result of the induction with the CCl₄.

Daily oral administration of *Senna occidentalis* aqueous leaf extract for four weeks at the doses of 500, 250 and 166mg/kg showed a significant decrease ($P < 0.05$) in mean serum levels of AST, ALP, ALT, Total bilirubin and Direct bilirubin (figure 1 and 2) with a significant increase ($P < 0.05$) in the mean serum levels of total protein and albumin in dose dependent pattern (figure 3) when compared with the test controls (group II). Administration of the aqueous extract at a dose of 500mg/kg (group III) showed a significant decrease ($P < 0.05$) in mean serum AST, ALT and ALP activities, mean serum level of total and direct bilirubin (figure 1 and 2) and also with a significant increase ($P < 0.05$) in mean serum level of total protein and albumin (figure 3) when compared with 166mg/kg. The result in group III also showed no significant different ($P > 0.05$) in ALT but significantly differ in AST, ALP, TB, DB, TP and

albumin ($P>0.05$) when compared with 250mg/kg (group IV) ALESO treated group (figure 1, 2 and 3). Oral administration of the aqueous extract at a dose of 250mg/kg showed a significant decrease ($P<0.05$) in the mean serum activities of AST, ALT and ALP, mean serum level of total and direct bilirubin (figure 1 and 2) and significant increase ($P<0.05$) in mean serum total protein and albumin levels (figure 3) when compared with oral administration of 166mg/kg ALESO dose. The decrease in ALT, AST, ALP, TB, DB and subsequent increase in TP and albumin goes in dose dependent manner (i.e 500mg/kg>250mg/kg>166mg/kg). The daily oral administration of 100mg/kg of livolin shows significant decrease ($P<0.05$) in ALT, AST, ALP, TB and significant increase ($P<0.05$) in total protein and albumin when compared with 166mg/kg ALESO treated groups and no significant decrease ($P>0.05$) in ALT, AST, DB and total protein when compared to 250mg/kg and 500mg/kg ALESO treated rats (figure 1,2 and 3).

Decrease in the levels of (AST, ALP, ALT) in the groups orally administered with the aqueous leaf extract of *Senna occidentalis* when compared with the test control shows that the extract has hepatocurative effect. This agrees with the Usha *et al*, (2007), Uzzi and Grillo, (2013) and contrast with Nuhu and Aliyu (2008) whose study reported an elevation of ALT, AST and ALP activities after treatment with *Senna occidentalis* on CCl₄ induced liver damage, thereby indicating that, the crude extract of *Senna occidentalis* leaves may be slightly toxic as concoction for liver ailments.

The induction of the liver damage was confirmed by histopathological analysis of the liver

of the intoxicated rats which showed a severely damaged liver. The liver of rats treated with 500mg/kg, 250mg/kg and livoline for four weeks (Figure 6 and 9) shows areas of fibrosis and vascular congestion, while for rats orally administered with 166mg/kg aqueous leaf extract of *Senna occidentalis* for four weeks shows areas of fibrosis (Figure 8) with a vascular congestion and damaged tissue. The severely damaged and hemorrhagic liver in the test controls is as a result of the CCl₄ exposure (figure 5). Exposure to CCl₄ had severe adverse health effect such as causing fulminant, hepatic necrosis and hemorrhage. In this study, CCl₄ induced a severe liver damage as represented by significantly elevated serum marker enzymes. This is with the agreement of the previous research that CCl₄ induces liver damage and is also able to alter the antioxidant status of the tissue, which is manifested by the abnormal histopathological changes (Raj, 2004).

In this study, the liver at the doses of 500mg/kg and 250mg/kg showed a mild damage after four weeks of the extract administration indicating some ameliorative effects of the extract on the organ.

6.0 CONCLUSION

The observed decrease in some serum liver function indices, with concomitant increase in total protein and albumin in hepatotoxic rats orally administered with aqueous leaf extract of *Senna occidentalis* could suggest hepatocurative potentials of the plant. This finding could also provide some biochemical basis for the use of leaf extract of *Senna occidentalis* in treatment of liver toxicity which could be attributed to some of the phytochemical content of the plant.

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