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Hepatoprotective potentials of *Sida corymbosa* (broom weed) ethanolic leaf extract against carbon tetrachloride (ccl₄)-induced hepatotoxicity on male albino wistar rats

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Abstract

This study aimed at evaluating the hepatoprotective potentials of *Sida corymbosa* ethanolic leaf extract against carbon tetrachloride (CCL₄)-induced hepatotoxicity in male Albino Wistar rats. Determination of serum aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) were done spectrophotometrically. The acute toxicity study carried out suggests that the extract may have an LD₅₀ above 5000 mg/kgbw. serum aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase levels decreased significantly ($P < 0.05$) in rats given the extract at 5000, 3000, and 1000mg/kgbw and injected carbon tetrachloride when compared to those injected carbon tetrachloride only. This suggests protection of the liver of rats by the extract against carbon tetrachloride-induced toxicity. Histological examination confirmed the results of the biochemical findings. *Sida corymbosa* ethanolic leaf extract may therefore protect the liver of male Albino Wistar rats against carbon tetrachloride and may be dependent on dosage and time.

Keywords: Carbon tetrachloride; induced-hepatotoxicity; *Sida corymbosa*, ethanolic extract

Introduction

The liver is among the largest organs of a human being. It is the main location for metabolism and excretion of unwanted materials. Liver disease is among the most common diseases globally and one of the largest health challenges of the world presently [1]. It has been reported that xenobiotics and microbial infiltration from ingestion or infection can destroy the liver [2, 3, 4, 5]. Carbon tetrachloride (CCL₄) has been used in many occasions as a model for inducing minor and severe liver damages. It has been reported as well established xenobiotics [6, 7]. A lot of the findings had shown that kidney and liver are prone to CCL₄ toxicity [8, 9, 10]. Organic chemistry had given credence to the manufacture of synthetic products which are rich in chemicals as normal constituents of feeding products or as preservatives. These arrays of chemical pollutants resulting from increased industrial activities to which human is exposed to have increased the incidence of hepatic disease.

Traditional medicine existed before the introduction of synthetic drugs and it is older than civilization. From the onset, human being has been searching for traditional ways to be used to take care of the diseases that have been affecting many people in the society [11, 12]. The use of parts of plants in curing diseases is among the traditions of Africa. According to [13], man has been using products from plants to survive from either hunger or illness. The reason is because plants are obtained easily and cheaper. Man acquired the knowledge of the usefulness of plants by trial and error and passed on the information from one generation to another in the environment mostly without documentation. Nature has been a source of medicinal agents for years. A number of synthetic drugs had been isolated from natural sources [14, 15]. Medicinal plants play a key role in human health care system [16, 17, 18, 19, 20].

Sida is one of the ethnomedicinally important genus of plants [14] which belongs to the family called Malvaceae [16, 21]. *Sida* plants have more than 200 species which are used in the treatment of disease such as dysentery, ulcer, gonorrhea, bleeding, diarrhea, and hepatic diseases. *Sida corymbosa* or *Sida garckeana* popularly known as Udoike or Acharaiki or Udonwatakaike by the people of South Eastern Nigeria, is a common weed found in most South Eastern, South Western and Northern parts of Nigeria growing along the roadside and streets. It survives all weathers and it is propagated through all modes of propagations. It belongs to the class magnoliopsida.

There have been claims by some traditional medical practitioners on the usefulness of *Sida corymbosa* in treating and managing liver diseases. There were some publications on the haemostasis potentials, anti-bleeding potentials, anti-ulcer potentials and anti-rust potentials of *Sida corymbosa* ethanolic leaf extract, but there is still scanty information on the hepatoprotective effects of the plants' extract on liver diseases. This work sought to close this gap by investigating the hepatoprotective potentials of *Sida corymbosa* ethanolic leaf extract on CCL4-induced liver damages in male Albino Wistar rats and dose dependent effect of the extract at various durations.



Fig 1: Photograph of *Sida corymbosa* plant

Materials and methods

All the chemicals used were of analytical grade and were obtained from the British Drug House (BDH) Ltd, Poole, England through their sales representatives in Lagos State, Nigeria. Reagent kits used were obtained from Randox Laboratory Ltd, United Kingdom via their sales representative in Ikeja, Lagos State, Nigeria. The distilled water used was obtained from the Human Biochemistry Research Laboratory, College of Health Sciences, Nnamdi Azikiwe University, Nnewi campus, Anambra State, Nigeria.

Sida corymbosa plants were gotten from Otolu Nnewi, Anambra State, Nigeria. They were identified and authenticated by Prof. J.C. Oka for of Botany Department, Enugu State University, Nigeria. They were reauthenticated by Dr. Ogbuzobe Okwudili Gabriel of Botany Department, Nnamdi Azikiwe University, Awka and issued with the voucher number- NAU Herbarium No 75G.

The leaves of the plant were prepared according to the method described by [19]. The leaves were washed with distilled water, air-dried at room temperature and powdered using blender (Philips, England, 350W). The ethanol extract was obtained by soaking 25g of the leaves in round bottom flasks containing 200 ml of absolute ethanol (98%) for forty-eight hours with shaking at room temperature using orbital shaker. The ethanolic extract was filtered with muslin cloth and filtered through 40mm Whatman filter paper and evaporated using a rotary evaporator (Model: TT22, USA) at 65°C. The crude extract was dried using the oven at 45°C.

Animal studies

All methods in the animal research complied with standards for the use and care of experimental animals as recommended

by WHO and were approved by the Animal Ethical Committee of Faculty of Basic Medical Sciences, College of Health Sciences, Nnamdi Azikiwe University- Awka, Nnewi Campus, Anambra State, Nigeria. The letter of the Ethical approval is attached to this work.

The LD₅₀ of *Sida corymbosa* ethanolic leaf extract was determined according to the method described by [22]. The procedure was carried out in three stages with the outcome of each stage determining whether to terminate or move on to the next stage. The initial stage, which is stage one requires four rats. The extract was administered to the rats orally. The rats were grouped into four groups of one rat each. Rat in group one was administered 10 mg/kgbw of the extracts while those in groups two, three and four were each administered 100, 300 and 600 mg/kgbw of the extract respectively. Since no mortality and signs of toxicity were observed, another three groups of one rat in each group were administered 1000, 1,500 and 2000 mg/kgbw (stage 2). Again, no mortality and signs of toxicity were observed. Based on this, another three groups of rats with one rat in each group were administered 3,000, 4000 and 5,000 mg/kg bw of the extract respectively (stage 3). There was no mortality and signs of toxicity observed at 5,000 mg/kg bw. A confirmatory test was carried out by administering 5,000 mg/kgbw of the extract to each of two groups of one rat each. Observation was done on the rats for 1 h after administration and 10 min for every 2 h interval. Calculation:

$$LD_{50} = \frac{[M0 + M1]}{2} \text{ Eq. 1}$$

Where M0= highest dose of the test substance that gave no mortality

M1 = Lowest dose of the test substance that gave mortality

$$\text{Mortality} = \frac{\text{No of dead rats}}{N} \text{ Eq. (2)}$$

Where n= number of rats used

A total of 100 male- adult Albino Wistar rats of about three months weighing between 180 to 200g and maintained at the Animal Facility Unit of College of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, were used in this study. They were acclimatized for two weeks, housed under the same condition of temperature, humidity and a 12 h of light and dark cycle. The rats were grouped into 20 groups of five rats each. Twelve groups were administered *Sida corymbosa* ethanolic leaf extract orally using oral canular at 5,000, 3,000 and 1,000 mg/kgbw for seven days, 14 days, 21 days and 28 days respectively. The rats were then injected 0.4ml/kgbw of CCL4 at the end of each duration intraperitoneally (ip) using olive oil as a vehicle in the ratio of 50:50 and left to stay for another seven days before taking blood samples. Four groups served as positive controls or normal rats (rats, which were neither given extract nor injected CCL4 but given feed and water only) from seven days to twenty eight days while another four groups served as negative control groups (groups injected CCL without given any extract and left to stay from seven days to twenty eight days). Blood samples were collected via ocular puncture using heparin specimen bottles and the serum separated by centrifugation at 3000 RPM for 15 min at room temperature. The animals were anaesthetized, sacrificed and the liver tissues were weighed and stored in 10 % formalin at room temperature for histopathological

examination. Relative weights of the rat liver were calculated using the formula:

$$\text{Relative weight} = \frac{\text{Weight of liver}}{\text{Final body weight of the animals}} \times 100 \text{g Eq. (3) }^{[23]}$$

The animals were fed on a commercial pellet diet (Vital growers) obtained from Gland Cereals Ltd., a subsidiary of UAC Nigeria PLC, Zawana, Jos, Plateau State, Nigeria, through her sales representative at Nnewi, Anambra State. Animal care and handling were done according to guidelines given by ^[24, 25]. The rats were kept in metal cages, given tap water and feed ad libitum. The body weights of the rats were measured every week throughout the experiment using weighing balance (Cuinry J1106337616, China) while water consumption was measured every day using calibrated water bottles.

$$\text{Volume of water consumed by each rat per day} = V_1 - V_2 \text{ Eq. (4)}$$

Where V_1 = Initial volume of water in the water bottle
 V_2 = Final volume of water

Hepatotoxicity studies

Hepatotoxicity assessment was conducted using routine diagnostic indicators for liver function. These include; serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase spectrophotometrically by using WHO ^[24] recommended method.

Determination of serum aspartate aminotransferase (AST) activities

Principles: Aspartate aminotransferase (AST) catalyzes the transfer of amino group between L-aspartate and oxoglutarate. The oxoglutarate formed reacts with NADH to form NAD^+ . The activities of this enzyme were then determined by measuring the rate of oxidation of NADH^+ .

Procedure: Hundred microliters of various serum samples were added into the respective test tubes for tests. This was followed by the addition of 0.5 ml of AST reagent 1 to all the test tubes for test and that of blank. Hundred microliters of distilled water was added into the test tube for blank. These were mixed and incubated for 30 min at 37°C using incubator (DNP-P052A, China). After this, 0.5ml, of AST reagent 2 was added into all the test tubes. These were mixed and incubated at room temperature for 20 min. This was followed by the addition of 5 ml of sodium hydroxide (0.4M) into all the test tubes and the absorbance read at 546 nm against the reagent blank after five minutes using UV-VIS spectrophotometer (Model 752G, China). The activity levels of AST in the serum were obtained from the AST chart by reading off the values which correspond with the absorbance obtained.

Determination of serum alanine aminotransferase (ALT)

Principle: The amino group is transferred from alanine to oxoglutarate to form L-glutamate and pyruvate in the presence of alanine aminotransferase. The pyruvate formed is reduced to lactate dehydrogenase and NADH. The activities of ALT are determined by measuring the rate of oxidation of NADH.

Procedure: The procedure is the same as that of serum AST mentioned above.

Determination of serum alkaline phosphatase

Principles: Alkaline phosphate (ALP) acts on

p- nitrophenylphosphate, thereby converting it to phosphate and p-nitrophenol. The absorbance of the colour developed is measured spectrophotometrically which is equivalent to the activities of the enzymes.

Procedure: Alkaline phosphate reagent 1a (R1a) was constituted with 10 ml of ALP reagent 1b (R1b) and called ALP reagent. Twenty microlitres of various samples were added to the respective test tubes. This was followed by the addition of 0.50 ml of ALP reagent to all the test tubes and properly mixed. The absorbance was read at one minute interval for 3 times using UV-VIS spectrophotometer at 405 nm and average found in each sample.

The activities of the enzyme were calculated using the formula:

$$\text{Enzyme activities (U/L)} = 2760 \times \Delta \text{ AEq. (5)}$$

Where Δ = average absorbance

Histopathological examination

This was done according to the method described by Paola ^[25].

Procedure: Ten percent formalin-fixed paraffin-embedded sections of liver tissues of various groups of rats were cut into 4μ thick and mounted onto slides with cover slips. Sections for histological examinations were stained with haematoxylin and eosin and Malloy trichrome stain using standard procedure.

Statistical analysis

Results were expressed as mean \pm STD of triplicate determinations. Graphs, students' "T"-test and one way ANOVA were used to analyze the results using SPSS statistical software version 21.

Results

The results of liver function test (AST, ALT and ALP assays) carried out on rats given *Sida corymbosa* ethanolic leaf extract before injecting carbon tetrachloride (CCL4) are hereby presented in figures two to four. In all instances of significant difference, the rats injected CCL4 without giving any extract (untreated liver damage) had the highest level of AST, ALT, and ALP activities from seven days to twenty eight days when compared to those rats given the extract before injecting CCL4 at 5000 mg/kgbw, 3000 mg/kgbw and 1000 mg/ kgbw from seven days to twenty eight days and those neither given the extract nor injected CCL4 (Normal rats). Those given 5000 mg/kgbw of the extract before injecting CCL4 had higher levels of AST, and ALT from seven to twenty one days and ALP significantly at 28 days when compared to those given 3000 and 1000 mg/kgbw ($P < 0.05$).

Figures five to six shows the results of the body and liver weights of rats given *Sida corymbosa* ethanolic leaf extract before injecting CCL4 while figures seven shows the results of mean water consumption of the rats. The body weights of rats given 5000 mg/kgbw, 3000 mg/kgbw and 1000 mg/kgbw were observed to increase progressively from seven days to twenty eight days of treatment. The reverse was the case for live weights. The body weights of those injected CCL4 without giving any extract decreased significantly ($P < 0.05$) from seven days to twenty eight days. The reversed was the case for the liver weights. The water consumption of the treatment groups increased from 14 days to 28 days while that of the untreated liver damage groups witnessed a decrease in water consumption throughout the period from seven days to twenty eight days.

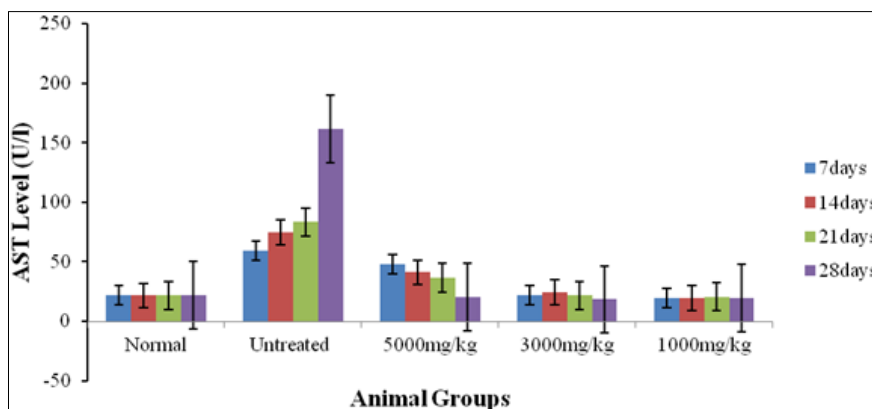


Fig 2: Graph of mean serum AST levels of rats given *Sida corymbosa* ethanolic leaf extract before injecting CCL4 vs extract dosage. Ms Excel version 2007 was used to plot the graph.

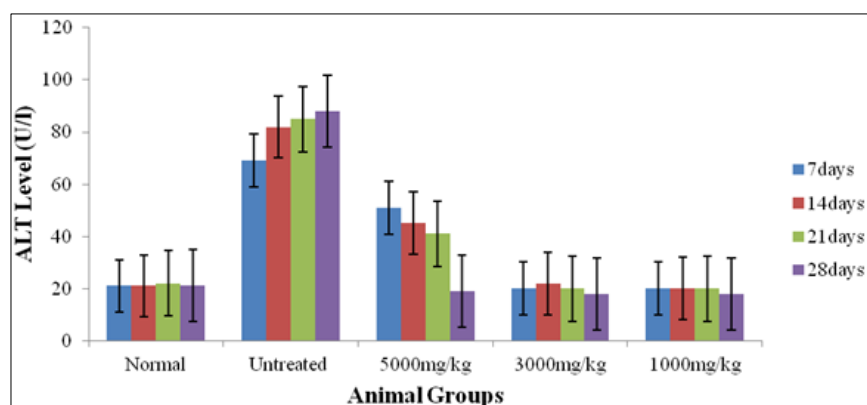


Fig 3: Graph of mean serum ALT levels of rats given *Sida corymbosa* ethanolic leaf extract before injecting CCL4 vs extract dosage. The graph was plotted using Ms Excel version 2007

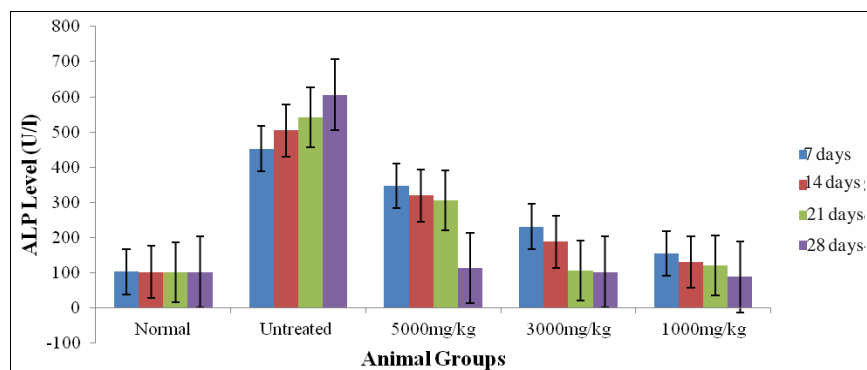


Fig 4: Graph of mean serum ALP levels of rats given *Sida corymbosa* ethanolic leaf extract before injecting CCL4 vs extract dosage. The graph was plotted using Ms excel version 2007.

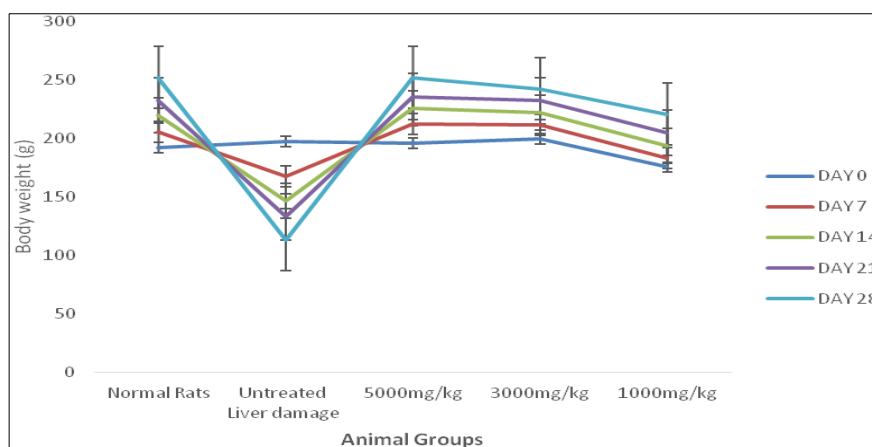


Fig 5: Graph of mean body weights of rats given *Sida corymbosa* ethanolic leaf extract before injecting CCL4. The graph was plotted using Ms Excel version 2007.

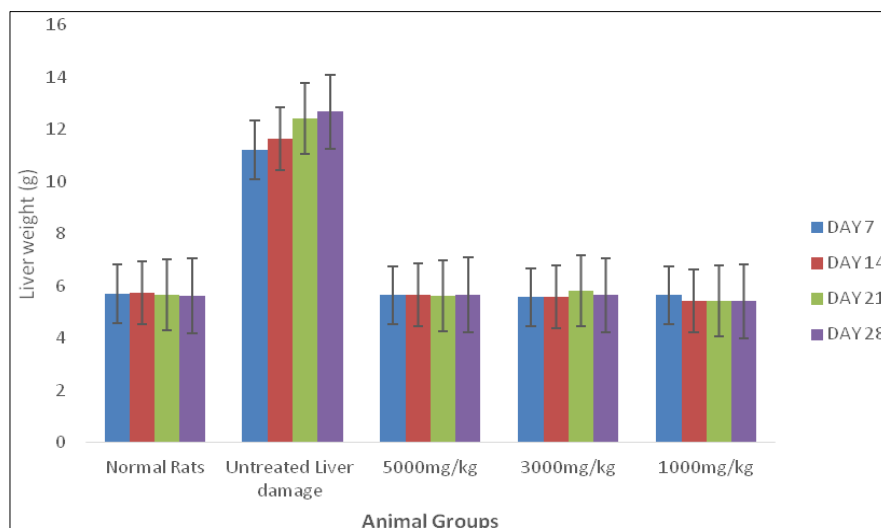


Fig 6: Graph of mean liver weights of rats given *Sida corymbosa* ethanolic leaf extract before injecting CCL4. The graph was plotted using Ms Excel version 2007.

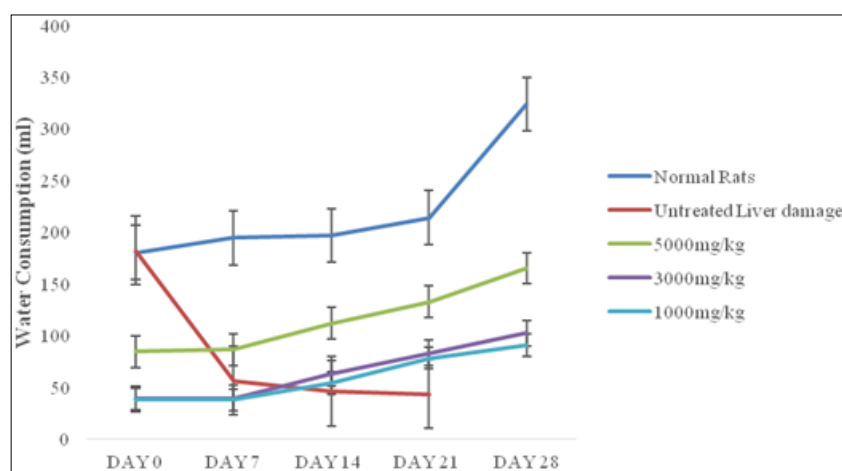


Fig 7: Graph of mean water consumption of rats given *Sida corymbosa* ethanolic leaf extract before injecting CCL4. The graph was plotted using Ms Excel version 2007.

Plates 1 to 20 show photomicrographs of liver sections of groups of rats given *Sida corymbosa* ethanolic leaf extract from seven days to twenty eight days at 5000, 3000, and 1000 mg/kgbw before injecting CCL4. The physical

examination carried out showed that all the groups excepts those injected CCL4 only had deep brown colors with smooth surfaces. Those injected CCL4 only had light brown colors with white spots all over the surfaces.

Plates 1-20: Microscopy of Liver Tissues of Rats

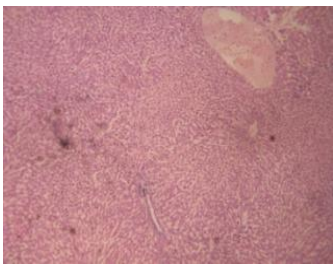
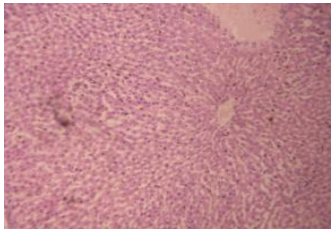
Description	Photomicrograph (x40)	Histological Observations
Plate 1: Normal rat liver for 7 days		well preserved liver architecture
Plate 2: Normal rat liver for 14days		well preserved liver architecture

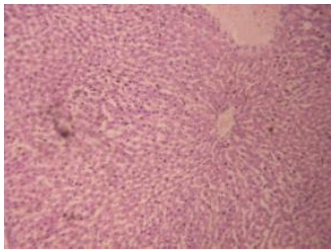
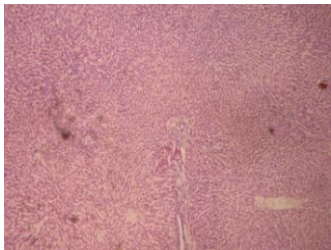
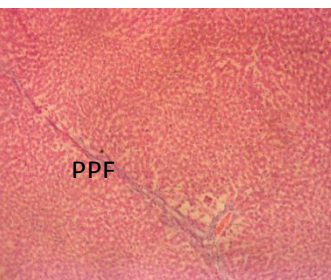
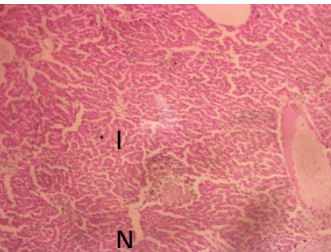
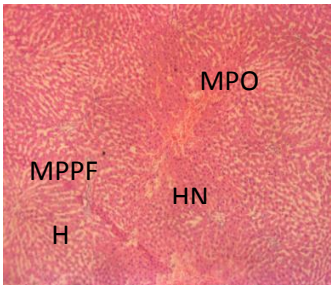
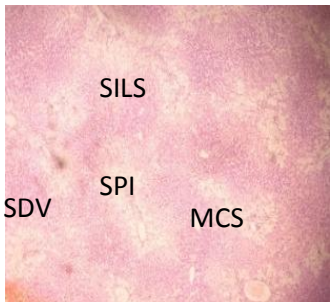
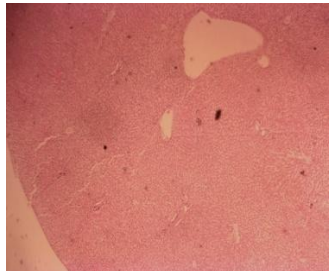
Plate 3: Normal rat liver of for 21days		well preserved liver architecture
Plate 4: Normal rat liver for 28days		Well preserved liver architecture
Plate 5: Untreated liver damage for 7days		Classic micrograph of Liver damage showing portal to portal fibrosis (PPF)
Plate 6: Untreated liver damage for 14days		Classic micrograph of liver damage showing necrosis (N) and inflammation(I)
Plate 7: Untreated liver damage for 21 days		Classic micrograph of liver damage showing: mild parenchymal oedema(MPO), mild portal to portal fibrosis(MPPF), haemorrhage (H) and cells with hyperchromatic nuclei(HN)
Plate 8: Untreated liver damage for 28 days		Classical micrograph of liver damage showing severe vacuolar degeneration of hepatocytes (SVD), Severe portal inflammation (SPI), mild congestion of sinusoidal spaces (MCS) and severe infiltration of liver sinusoids (SILS)
Plate 9: Rat liver given leaf extract at 5000mg/kgbw for 7days before injecting CCL4.		The liver architecture was intact. The erythrocyte cells were intact

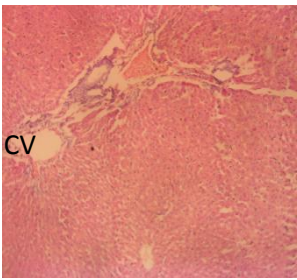
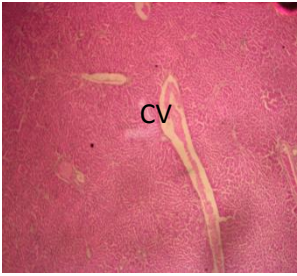
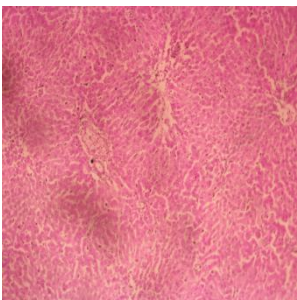
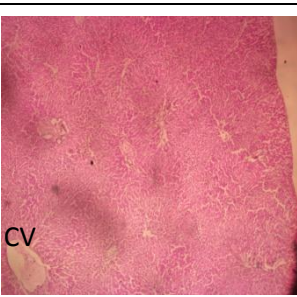
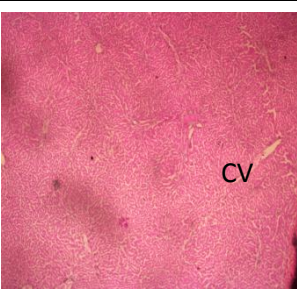

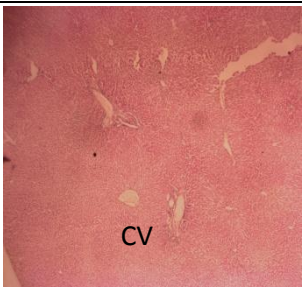
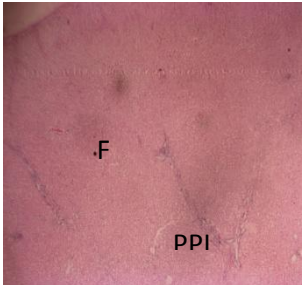
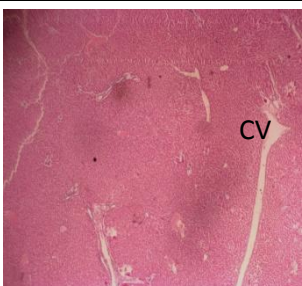
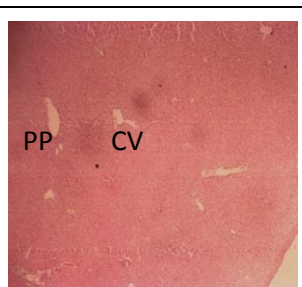

Plate 10: Rat liver given leaf extract at 5000mg/kgbw for 14 days before injecting CCL4		The liver architecture was intact, the erythrocyte cells were intact. The central vein (CV) was clear.
Plate 11: Rat liver given leaf extract at 5000 mg/kgbw for 21 days before injecting CCL4.		The liver architecture was intact, the erythrocyte cells were intact, the central vein (CV) showed mild congestion of blood cell.
Plate 12: Rat liver given leaf extract at 5000 mg/kgbw for 28 days before injecting CCL4.		Normal liver tissue. The liver architecture was intact, the erythrocyte cells were intact.
Plate 13: Rat liver given leaf extract at 3000mg/kgbw for 7days before injecting CCL4.		The liver architecture was intact, the erythrocyte cells were intact. The central vein (CV) showed mild congestion of blood cells.
Plate 14: Rat liver given leaf extract at 3000mg/kgbw for 14days before injecting CCL4.		The liver architecture was intact, the erythrocyte cells were intact and the central vein (CV) showed mild congestion of blood cells.
Plate 15: Rat liver given leaf extract at 3000mg/kgbw for 21days before injecting CCL4.		The liver architecture was intact, the erythrocyte cells were intact and the central vein (CV) was clear.

Plate 16: Rat liver given leaf extract at 3000mg/kgbw for 28days before injecting CCL4.		The liver architecture was intact, the erythrocyte cells were intact. The central vein (CV) was clear.
Plate 17: Rat liver given leaf extract at 1000mg/kgbw for 7days before injecting CCL4.		The liver architecture was not intact. There was periportal inflammation (PPI), and fibrosis (F).
Plate 18: Rat liver given leaf extract at 1000 mg/kgbw for 14 days before injecting CC		The liver architecture was intact, the erythrocyte cells were intact and the central vein (CV) was clear.
Plate 19: Rat liver given leaf extract at 1000 mg/kgbw for 21days before injecting CCL4.		The liver architecture was intact, the erythrocyte cells were intact, the central vein (CV) and periportal triad (PPT) were clear.
Plate 20: Rat liver given leaf extract at 1000 mg/kgbw for 28days before injecting CCL4.		The liver architecture was intact, the erythrocyte cells were intact and there were no inflammation and fibrosis.

Discussion

This work investigated the protective potentials of *Sida corymbosa* ethanolic leaf extract against CCL4 toxicity study (LD50) carried out showed that *Sida corymbosa* ethanolic leaf extract has an LD50 above 5000 mg/kgbw. This suggests that the extract may be safe for consumption at a concentration above 5000 mg/kgbw. This means that the extract has a low toxicity profile. This agrees with the earlier reports of [12, 18, 26]. The results of findings on male Albino Wistar rats given *Sida corymbosa* ethanolic leaf extract before injecting CCL4 revealed that the level of liver enzymes (AST, ALT and ALP) of groups of rats injected only CCL4 without any treatment increased significantly ($P < 0.05$) from seven days to twenty eight days against those injected CCL4 after given the extract at 5000 mg/kgbw, 3000 mg/kgbw and 1000 mg/kgbw from seven days to twenty eight days and the normal rats (Figures 2

to 4). This suggests that the liver tissues of those rats given the extract before injecting CCL4 may be better than those injected CCL4 only. This may be a sign of protection of the liver against CCL4 toxicity to some extent. *Sida* species had been reported to have hepatoprotective activities against CCL4 toxicities [20, 14, 27, 28]. Carbon tetrachloride is a known model for inducing toxicity in the liver of experimental animals [29]. The levels of the above mentioned enzyme markers were observed to be higher in those rats given 5000mg/kgbw of the extract from seven days to twenty days than those given the same extract at 3,000 mg/kgbw and 1000 mg/kgbw before injecting CCL4. This again suggests that the treatment is dependent on dosage and duration between seven days to twenty eight days of treatment. There are several reports on the dose dependent effects of plant extracts on CCL4 induced toxicities [27, 28]. At 28 days of treatment, it was

revealed that there were no significant changes in the levels of AST, ALT and ALP among the treatment groups and normal rats (Positive control groups). This may also be suggesting that at this time, the treatment may not be dependent on dosage for AST, ALT and ALP.

The results of the body weights taken revealed that all the groups accept the groups without extract and CCL4 (normal rats) had decreased body weights significant at seven days of treatment (Fig. 5). Those of the treated groups regained weights again from 14 days to 28 days. The normal rats maintained progressive weight gain while those injected CCL4 without giving any extract maintained progressive weight loss from seven days to twenty eight days. This may be suggesting that the extract may have the potentials to protect the animals from water imbalance as a result of CCL4 toxicity. This is in line with similar findings of [30]. The reverse was the case for the liver weights. The liver weights of the groups injected CCL4 without giving *Sida corymbosa* ethanolic extract were observed to have increased significantly ($P < 0.05$) from seven days to twenty eight days against other groups (Fig. 6) suggesting possible liver enlargement in these groups (Groups + CCL4 without extract) as a result of liver damage. Similar findings had been reported by [31]. The decrease in the liver weights as witnessed in groups given 5000 mg/kgbw, 3000mg/kgbw and 1000 mg/kgbw from seven days to twenty eight days against groups injected CCL4 without giving any extract may be an indication that the extract may have protected the rats against liver enlargement caused by CCL4 toxicity.

The histological investigation carried out so far supported the biochemical findings on the liver functions of the rats. Microscopic examination done revealed that the architectures and erythrocyte cells of both the normal rats groups and those given the extract from seven days to twenty eight days before injecting CCL4 were intact (Plates 1 to 4 and 9 to 20). Severe liver damage were observed in the liver sections of rats injected CCL4 without giving any treatment with the extract which progressed to fibrosis and necrosis (Plates 5 to 8). There were severe inflammation and degeneration of erythrocyte cells in these groups of rats injected CCL4 without administering the extract and left for 28 days (Plate 8). This suggests that the damages caused in the liver of rats by CCL4 toxicity without treatment became worse as the time progressed. This agrees with the similar findings of [28, 30, 31].

Sida corymbosa ethanolic leaf extract therefore has some degree of protection against hepatotoxicity of CCL4 on male Albino Wistar rats since the liver functions of rats given the extract and injected CCL4 were observed to be better than those injected CCL4 without giving any extract. The treatment may be dependent on dosage with time from seven days to twenty one days. Protection of rats against CCL4 hepatotoxicity with *Sida corymbosa* ethanolic leaf extract may be better at 3000 mg/kgbw and 1000 mg/kgbw for 21 days. This extract may equally have positive effects on the body weights and water consumption of the rats.

Declaration of Interest: None

Consent to Participate: Not applicable

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