

## ***Xeromphis nilotica* Stem-Bark Extract Attenuates CCl<sub>4</sub>-Induced Hepatic Damage and Oxidative Stress in Albino Rats**

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### **ABSTRACT**

*Xeromphis nilotica* is used by traditional practitioners in North East Nigeria to treat or manage various ailments such as inflammations, pains and stomach disorders. This study aimed to evaluate the probable protective effect of *Xeromphis nilotica* stem-bark extract against CCl<sub>4</sub> induced hepatic injury in albino rats. Thirty-six rats were randomly divided into six groups. Group 1 served as the control and normal feed and 3ml/kgbw olive oil intraperitoneally. Groups 2, 3, 4,5&6 were injected with 3ml/kgbw of carbon tetra chloride (CCl<sub>4</sub>) in olive oil intraperitoneally to induce oxidative stress and thereafter group 4,5 &6 were treated with 100, 200, 400 mg/kgbw of the extracts respectively. Group 2 serves as toxic control, while group 3 was treated with 25mg/kgbw silymarin as standard control. Liver marker enzymes ALT, AST, total protein and albumin were assessed and results obtained was extrapolated by performing histological analysis of the liver tissues. The study revealed that treatment of rats with CCl<sub>4</sub> caused marked weight loss, induced liver damage through elevated marker enzymes as well as significant decrease in catalase activity. However, administration of graded doses of *X. nilotica* extract effectively ameliorated the deviation caused by oxidative stress induced liver damage. Pathological examination of the liver tissues also supported the biochemical findings. It was concluded that supplementation of *X. nilotica* extract was beneficial in modulating the alteration induced in liver and serum variables of rats under the effect of CCl<sub>4</sub> induced oxidative stress.

**Keywords:** *Hepatoprotective, Xeromphis Nilotica, Albino rats.*

### **INTRODUCTION**

Carbon tetrachloride (CCl<sub>4</sub>) is a xenobiotic used to induce chemical hepatitis and liver injuries in experimental animals. Carbon tetrachloride-induced liver injuries are the most common test model for monitoring the hepatoprotective activity of certain drugs. A single exposure to CCl<sub>4</sub>, being a strong hepatotoxic xenobiotic could directly leads to severe liver necrosis and steatosis (El-Boshy et al., 2017). The major metabolites of CCl<sub>4</sub> are trichloromethyl (CH<sub>3</sub> CCl<sub>3</sub>·) and trichloromethyl peroxy (CH<sub>3</sub> Cl<sub>3</sub> O<sub>2</sub>·) free radicals. These radicals are extremely reactive and are capable of covalently

binding to cellular macromolecules such as DNA, protein and fatty acids of the membrane phospholipids. The free radicals induce cell membrane lipid peroxidation via disrupting polyunsaturated fatty acids within these membranes, initiating a sequential free radical chain reaction (Knockaert et al.,2012).

*Xeromphis nilotica* is a lowland shrub that grows wild in savannah regions of Africa and Asia (Farooqui et al., 2003). The vernacular names for *Xeromphis nilotica* are *gial-gotel*, *kwanarya* in northern Nigeria.

Ethnobotanical and ethno-pharmacological studies of *Xeromphis nilotica* indicate the

potential use of these plants for the treatment of a large variety of diseases ((Adzu et al.,2014). Hence, *Xeromphis nilotica* is expected to have antioxidant potentials, as it is reputed to be of medicinal value in folkloric treatment and management of various diseases such as inflammation, stomach disorder and pain related ailment. There is dearth of scientific information to substantiate its folkloric uses. Hence there is a need for more precise validation of ethno-pharmacological claims. To the best of our knowledge no studies have been conducted on the hepatoprotective effect of *Xeromphis nilotica*. Therefore, the current study was designed to investigate the probable protective effect of *Xeromphis nilotica* stem-bark extract against CCl<sub>4</sub> induced hepatotoxicity rats.

## MATERIALS AND METHODS

### Chemicals and Reagents

All chemicals and reagents used in this study are of analytical grade and obtained from Sigma Chemicals (St. Louis, MO, USA).

### Plant Material and Extract preparation

Fresh *Xeromphis nilotica* stem-bark was collected from Kumo Gombe State Nigeria in the month of January 2017. It was authenticated by Toxanapist, Professor Halima M Abba and specimen voucher (BUM 326) was deposited in herbarium at the Department of Biochemistry, University of Maiduguri, Nigeria

*Xeromphis nilotica* stem-bark was washed and allowed to dry under shade and pulverize to fine powder using mortar and pestle. The sample(100g) was macerated in 70% ethanol for 72h with intermittent shaking. The mixture was filtered twice through Whatman filter paper (size). The resulting filtrate was subjected to evaporation in a Rotary Evaporator for 10 min at 60°C. Dried extract was packed in air tight container and reconstituted when

required.

### Experimental Animals

Healthy albino rats were procured from NITR Vom, Plateau state Nigeria. The animals were housed under standard laboratory condition of 12h light- dark cycle, temperature 25±2°C, humidity 55±5% and fed with standard rat pellets (vital feeds Jos. Nigeria) and clean tap water ad libitum in the animal house of University of Maiduguri, Department of Biochemistry. Prior to the commencement of the experiment, an approval was obtained from the animal ethics committee for the study protocol

### Determination of Acute Toxicity (LD<sub>50</sub>)

Median lethal dose (LD<sub>50</sub>) was determined for evaluating the safety of *Xeromphis nilotica* using slightly modified procedure reported by (Chineodu et al.,2013) and ten albino rats were used for the study.

At the first stage four animals and were divided into 4 groups of one rat each. Then different doses (1000-2000mg/kg b.wt) of the extract were administered to the rats and were observed for 1 hours post-administration and then 10 minutes every 2 hrs interval for 24 hrs. The behavioural signs of toxicity and also mortality were recorded. No mortality was recorded at this stage, the testing was continued to the next stage

Three animals were divided into three groups of one rat each. Different doses of the extract (2000-3000mg/kg b.wt) were given orally to the animals and then observed for 1 hour after administration and periodically for 24 hours. Behavioural signs of toxicity were noted and mortality as well. No sign of toxicity or death were observed.

This stage three animals which were distributed into three groups of one rat each. Doses of extract (3500- 4000 mg/kg as the highest) were administered to the different rats and was observed for 1 hour after administration and then 10 minutes every 2

hours for 24 hours. Behavioural toxicity signs and also mortality was recorded. This was the final stage of testing and no death was observed, then the LD50 of the extract is greater than 4000 mg/kg. Therefore, *X. nilotica* stem bark extract was considered safe up to 4000 mg/kg b.wt.

### Animal Treatment

Adult albino rats weighing 165-200g were randomly divided into six groups of 6 animals each

Group 1a. Normal control: no treatment only feed and water.

Group 2. Toxic control: 3ml/kg body weight carbon tetrachloride in olive oil.

Group 3. Standard control: 140mg/kg body weight silymarin and 3ml/kg body weight CCl<sub>4</sub>.

Group 4. Test I: 100mg/kg bodyweight *X. nilotica* extract and 3ml/kg bodyweight CCl<sub>4</sub>

Group 5. Test II: 200mg/kg bodyweight *X. nilotica* extract and 3ml/kg bodyweight CCl<sub>4</sub>.

Group 6. Test III : 200mg/kg bodyweight *X. nilotica* extract and 3ml/kg bodyweight CCl<sub>4</sub>

Three (3)ml per kg body weight single dose of carbon tetrachloride, in olive oil at ratio 1:1 was given to the animals in groups 2, 3,4,5, using intraperitoneal route of administration according to protocol previously described by Dutta et al (2018) and the plant extract dose of 200, 400mg per kg body weight were administered orally for a period of 28 days to the animals in groups 4,5 & group 3 was treated with standard drug silymarin. Group 1b was given 400mg/kg bodyweight of the extract

### Sample Collection

Twenty-four hours after the last treatment, blood was obtained under anaesthesia from each rat through direct cardiac puncture.

Blood was allowed to clot for 30 minutes and then centrifuged at 4000 rpm for 10 minute, serum was harvested and immediately analysed. The liver was removed from each animal and kept in 10% formalin for histopathological analysis.

### Determination of Biochemical Variables

Activities of aspartate amino transferase and alanine amino transferase were assayed by using the methods previously described by Uphadyay et al (2010). Serum Albumin and total protein were determined according to the methods described by Daumass et al (1975)

#### *Catalase activity* (Jeong et al 2011)

Catalase activity was determined according using method previously described by . Catalase content of the samples react with hydrogen peroxide to produce water and oxygen. 12  $\mu$ L 1 mM fresh hydrogen peroxide solution was added to 40  $\mu$ L serum and samples were incubated at 25°C for 30 min and then 10  $\mu$ L stop solution was added. To standard samples already containing the stop solution, OxiRed™ probe solution was added and incubated at 25°C for 10 min. Then absorbance was measured at 570nm. A standard curve was prepared by adding 10  $\mu$ L stop solution to 0, 2, 4, 6, 8 and 10  $\mu$ L 1 mM hydrogen peroxide solution.

Histopathological evaluation of liver and kidney were done according to the method of Janquera and Carneiro (2005).

### Statistical Analysis

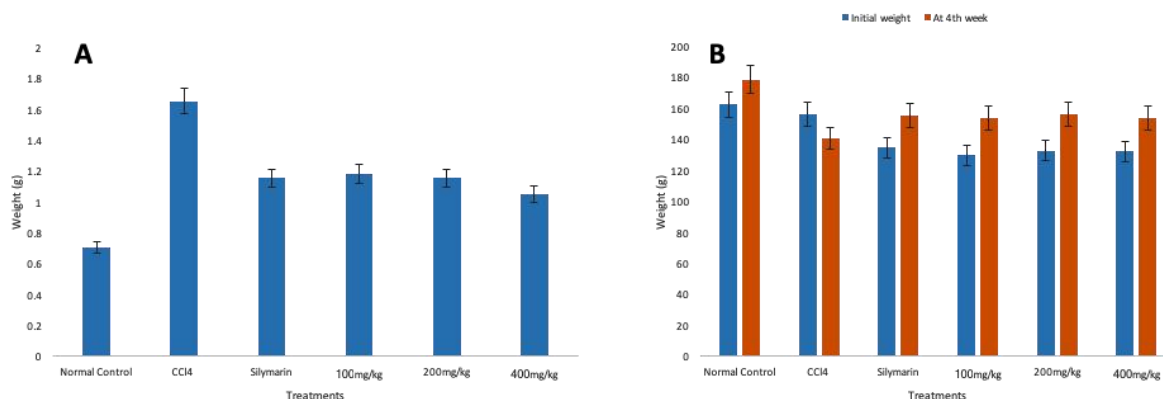
Statistical analyses were carried out using GraphPad Prism. Data were expressed as mean  $\pm$  SEM. Hypothesis testing methods included one-way analysis of variance (ANOVA) followed by *Tukey's post hoc* test to determine the differences among the mean values of different groups.  $P < 0.05$  was considered to indicate statistical significance.

## RESULTS

### Effect of *Xeromphis nilotica* Extract on Body and Liver Weight of Rats

The effect of *X. nilotica* stem-bark extracts on bodyweight of CCl<sub>4</sub> intoxicated albino rats is presented in table 1. The variation in both body weight was examined to assess the impact of the extract treatment on the overall changes following liver injury induction with CCl<sub>4</sub>. The body weights and

the liver weight were measured at the end of the experiment. The *Xeromphis nilotica* treatment markedly improved the body weight in contrast to the negative control. Figure 1A. Similarly, following injection of the CCl<sub>4</sub>, a marked increase in liver weight was observed, however, the extract treatment decreased the liver inflammation and hence, subsequently the liver weight was strikingly reduced in the extract and silymarin treated groups Figure 1B.



**Figure 1A.** Effect of *Xeromphis nilotica* extract treatment on liver weight in CCl<sub>4</sub> treated albino rats. Values are represented as mean  $\pm$  standard error of mean (SEM) of six different replicates. The extract treatment markedly attenuated the liver weight variation ratio compared to the CCl<sub>4</sub> treated group. The data was reported as mean  $\pm$  standard deviation  $p < 0.05$ , shows significant difference comparison with CCl<sub>4</sub> treated group. **Figure 1B** Effect of *X. nilotica* stem-bark extract on bodyweight in CCl<sub>4</sub> treated albino rats. The body weight was slightly decreased in CCl<sub>4</sub> group compared to the normal control group. But an increase in body weight compared to the initial was observed in silymarin treated group and the treatment group at different doses (100mg, 200mg and 400mg)

a) CCl<sub>4</sub> induced marked increase in serum level of hepatic biomarkers; ALT and AST were reversed by *Xeromphis nilotica* stem-bark extract.

Administration of 0.5ml of CCl<sub>4</sub> in olive oil (1:1v/v) twice weekly for 28 days to albino mice caused an increase ( $P < 0.05$ ) in serum level of ALT and AST activities compared to the control. However, the group that received 100mg/kg bodywt silymarin, 100mg/kgbw, 200 mg/kgbw, and 400 mg/kgbw of the extract, a significant reduction ( $P < 0.05$ ) in serum level of ALT and AST was observed compared to CCl<sub>4</sub>

treated (Table 1)

b) *D. mesopiliformis* stem-bark extract ameliorates the CCl<sub>4</sub> induced decrease in serum level of Albumin and Total protein in albino mice.

A significant reduction ( $P < 0.05$ ) in serum level of albumin and total protein were observed in CCl<sub>4</sub> treated group compared to the control. But in group that received 100mg/kgbw silymarin, 200 mg/kgbw, and 400 mg/kgbw of the extract, a significant increase ( $P < 0.05$ ) in serum level of albumin and total protein were observed compared to CCl<sub>4</sub> treated group (Table 1).

**Table 1:** Effect of *X. nilotica* stem-bark extract on Liver marker enzymes in CCl<sub>4</sub> treated Rats

Group	ALT	AST	Albumin	Total Protein
Control	60.7±5.3 <sup>#</sup>	138.7±0.5 <sup>#</sup>	38.8±0.8	6.8±0.8 <sup>#</sup>
CCL <sub>4</sub>	124.2±8.1	231.7±0.5	28.0±0.9	3.9±0.5
Silymarin	75.2±2.0*	147.5±0.5*	44.7±1.0*	7.8±1.0*
100mg	75.8±2.9*	211.7±2.0*	41.0±1.4	6.3±1.0
200mg	66.5±3.1*	201.7±2.0	47.2±0.4	7.8±0.4
400mg	58.0±3.0*	189.0±1.0	49.8±0.9	7.7±0.4

All values are expressed as mean ± SEM (n = 6). <sup>#</sup>P < 0.05 compared with normal control group with the negative control (CCl<sub>4</sub> treated). \*P < 0.05 compared with negative control group and the treatment groups. There is significant difference (p<0.05) between the normal group and negative control group, also there is significant different (p<0.005) the extract treatment groups when compared to negative control group

### Effect of *X. nilotica* Stem-Bark Extract on Catalase Activity

The result of *X. nilotica* stem-bark extract on catalase activity was presented on table 2. A marked lipid peroxidation associated with

significant (p<0.05) decrease in the catalase activity was observed in the CCl<sub>4</sub> intoxicated group compared to the normal control. This decrement was about 76.2%, which was statistically significant (p<0.05). However, oral administration of the plant extract at graded doses of 100, 200, 400 mg/kgbw, attenuated the lipid peroxidation with percentage increase of catalase activity 65.2, 68.3, 71.7 respectively. The increase in catalase activity in response to extract treatment is significantly (p<0.05) higher compared to the CCl<sub>4</sub> intoxicated group. Also, in the positive control group silymarin induced the antioxidant mechanism to lessen the oxidative stress posed by the CCl<sub>4</sub> Table 2.

**Table 2:** Effect *X. nilotica* stem-bark extract on hepatic catalase activity in CCl<sub>4</sub> treated rats.

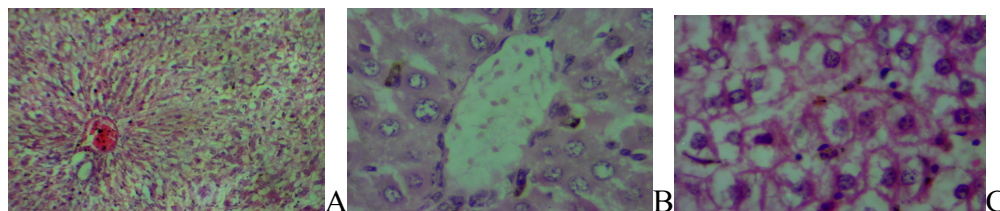
Group	Catalase(units/mg of protein)
Normal Control	117.7± 2.
Toxic control (CCl <sub>4</sub> )	28.0 ± 0.9
Standard control (Silymarin)	74.5 ± 2.5
Treatment group(100mg/kg)	80.5 ± 1.5
Treatment group(200mg/kg)	88.5 ± 1.6
Treatment group(400mg/kg)	95.8 ± 1.9

### Histopathological Examination

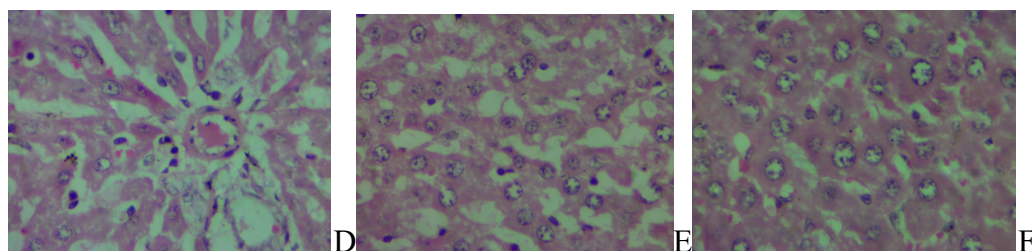
Histopathological examination of the liver tissue (Plate. 1A) showed no pathological abnormalities in the normal control group. The hepatocytes are radiating and arranged in cord from the central vein and sinusoids. But the rats intoxicated with CCl<sub>4</sub> showed expansion of the portal tract by inflammatory cell infiltration and the portal veins are thrombosed with an amorphous eosinophilic material within, compared to the control section Also, binucleated hepatocytes some undergoing karyolysis

and karyorrhexic also massive vacuolation seen within the nucleus of vacuolated hepatocytes shifted to the periphery. (Plate B). There were also haemorrhages (arrow) following the intraperitoneal administration of single dose of 3ml/kgbdwt of CCl<sub>4</sub> and treatment with silymarin (Plate C). However, *X. nilotica* extract treatment markedly reduced vacuolation, karyolysis and karyorrhexic caused by CCl<sub>4</sub> toxicity. Also, in the hepatocytes some of the nuclei were shifted to the periphery with mild inflammatory cell infiltration showing gradual hepatoprotective efficacy of the

extract.



**Plate1A.** section of a liver (Normal control) showing a central vein (arrow) and hepatocytes arranged in cords, H and E, X100. **Plate1B**  $\text{CCl}_4$ -intoxicated group showing dense bridging fibrosis with pseudo-lobules formation, hepatocytes undergoing karyorrhexis and karyolysis. Haemosiderin and the central vein are also seen following intraperitoneal administration of single dose of 3ml/kgbdwt  $\text{CCl}_4$ , H and E, X400. **Plate1C: (silymarin treated group)** section of the liver showing hepatocytes, where few of them are apoptotic (arrow heads) and a few of them are vacuolated (stars) with the nucleus of some of the cells shifted to the periphery. There were also haemorrhages (arrow) following the intraperitoneal administration of single dose of 3ml/kgbdwt of  $\text{CCl}_4$  and treatment with 140mg/kg bwt Silymarin administered orally H and E, X400



**Plate1D** section of liver showing markedly reduced vacuolation with some of the nuclei shifted to the periphery and diminished karyorrhexic hepatocytes following administration of  $\text{CCl}_4$  and treatment with 100 mg of ethanolic extract of *Xeromphis nilotica*, H and E, X400. **Plate 1E** section of liver showing hepatocytes, some of them binucleate is markedly reduced karyolysis indicative of tissue repair process. Massive vacuolation are also seen with nucleus of vacuolated hepatocytes shifted to the periphery following the intraperitoneal administration of  $\text{CCl}_4$  and treatment with 200 mg of ethanolic extract of *Xeromphis nilotica*, H and E, X400. **Plate1F** section of liver showing hepatocytes with very minimal undergoing karyorrhexis (arrows) and a very mild vacuolation (arrow heads) with few areas of haemorrhages following intraperitoneal administration of  $\text{CCl}_4$  and treated with 400 mg of ethanolic extract of *Xeromphis nilotica* orally, H and E, X400

## DISCUSSION

The liver is the central organ of the body and part of digestive tract regulating numerous functions including metabolism, synthesis and detoxification (Al-Seeni et al., 2016). The  $\text{CCl}_4$  is well known noxious chemical associated with tissue damage, both renal and hepatic through generation of volatile free radicals. However, they have propensity to become stable through electron pairing with cellular

macromolecules such as DNA, lipids and protein in healthy human cells thus causing tissue damage (Ying et al., 2018).

Ethno-pharmacology of plants have been employed in traditional herbal medicines for eras to cure variety of liver disease-related symptoms, but the underlying mechanisms are still unknown. Natural antioxidants from plant sources reduce the risk of adverse side effect caused by conventional treatments. *Xeromphis nilotica* is used in ethno-

medicinal practice for treatment and management of various ailment, and the therapeutic benefits might be ascribing to its antioxidant potentials (Musa et al., 2021).

Adult albino rat were used to assess the protective effect of *Xeromphis nilotica* stem-bark extract on CCl<sub>4</sub> induced hepatotoxicity. CCl<sub>4</sub> was given via intraperitoneal injection twice weekly for 28 days. This procedure, is a typical model for reproducible fibrosis, which can also be reversible after discontinuation of the treatment (Liedtke et al., 2013). Thus, this ideal is often used in study of fibrosis and analysis of liver repair mechanisms, emphasizing the systematic importance of this model.

The marginal reduction in body weight was only observed in rats treated with CCl<sub>4</sub> only and this could be linked to CCl<sub>4</sub> toxicity that affect internal organs resulting in less feed intake and malabsorption of the ingested food (Ullah et al., 2020). However, the control and extract treated group exhibited no such decrease in the body weights compared to the control. Thus, the extract might have alleviated the induced anorexia and enhanced feed intake with subsequent weight gain. Also the liver weight was assessed in all the groups, the CCl<sub>4</sub>-treated rats suffered from hepatomegaly with striking increase in the liver weight compared to the normal control group. This scenario could be justified in basis of the progression of liver fibrosis (El-Baset et al.,2022). Also swelling is very common as organs become opaque and boggy during inflammatory conditions (Ullah et al.,2020). However, the groups treated with the silymarin and extract showed marginal change in the liver weight as compared to the control. Thus, interestingly, the relative liver weight was preserved in those groups. The liver is an important organ of the body that detoxifies harmful metabolic products. In the assessment of liver damage by drugs or any

other hepatotoxin, liver biomarkers; ALT, AST and ALP, evaluated. However, serum level of total protein and albumin are widely used to assess the metabolically functional activity of the liver (Yoshiwaka et al., 2002).

Elevation of liver biomarkers and increased lipid peroxidation are further evidences of CCl<sub>4</sub> induced liver injury. ALT and AST that exist in the hepatocytes can certainly leak into the peripheral blood as soon as the hepatocytes are injured. Chemically induced liver injury depends mostly on the oxidative stress in hepatic tissue and underlies the pathology of numerous ailment including cancer, diabetes and arteriosclerosis. In the present study, the hepatoprotective efficacy of *X. nilotica* stem-bark in CCl<sub>4</sub> treated rats was evaluated. Serum level of AST, ALT levels were significantly elevated in response to CCl<sub>4</sub> with subsequent reduction of serum albumin and total protein compared to the control. *Xeromphis nilotica* stem-bark extracts lessened these alterations by restoring serum levels ALT, AST, as well as albumin and total protein to almost near normal. This ameliorative effect of the plant extract mimics silymarin, herbal drug with several bioactivities such as antioxidant, anti-inflammatory, immunomodulatory as well as liver regenerating mechanism (Karimi et al.,2011). In this study we used 100mg per kg b wt of silymarin as standard drug. Administration of 100, 200, 400mg per kg body weight of the *X. nilotica* stem-bark extract ameliorated the oxidative stress induced by CCl<sub>4</sub> in the rats. This observation might in part due to presence of metabolites such as polyphenols, triterpenoid saponin and flavonoids which may be attributed to its free radical scavenging effect which was earlier reported by our group (Musa et al., 2021) and others (AbdurRahman et al., 2018).

Catalase is an enzyme known to be responsible for degradation of H<sub>2</sub>O<sub>2</sub> to H<sub>2</sub>O and oxygen, hence the key component

of antioxidant defence system (Kacem et al., 2014). Therefore, under oxidative stress conditions, the catalase enzyme activity is expected to reduce. Decrease in serum catalase activity reveals declined protection capacity of the body against hydrogen peroxides and the increase of ROS and this is what exactly happened in this experiment. Reduction in catalase activity observed in the CCl<sub>4</sub> group may possibly be attributable to excessive generation of free radicals, followed by depletion of the endogenous antioxidant enzyme catalase. The CCl<sub>4</sub>-intoxicated animals exhibited a significant ( $p < 0.05$ ) decrease in catalase activity, the reduction is about 76% compared to the control group. However, a dose dependent percentage increase in catalase activity was observed in response to *Xeromphis nilotica* stem-bark extract.

Routine treatment of CCL4 intoxicated rats with *X. nilotica* extract for 4 weeks significantly ameliorated the liver injuries and restored serum level of liver marker enzymes as well as total protein, and albumin to normal values. Thus, *X. nilotica* stem-bark extracts have exhibited hepatoprotective activity against CCl<sub>4</sub> induced liver damage. The extract significantly improved the biochemical and histological parameters, while enhanced the activity of the endogenous antioxidant enzyme catalase. The antioxidant and hepatoprotective effects of the extract may in part attributable to the phytochemical components present in the plant.

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