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Antioxidant, hepatoprotective and lipid lowering activity of Sarcopoterium Spinosum on carbon tetrachloride (CCl4)- induced hepatic damage in rats

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Abstract

Background: Sarcopoterium spinosum, which is commonly named as "shrub thorny barnet", is a well-known medicinal plant in the Mediterranean region. Traditionally, it acts as an antidiabetic agent by improving the tolerance of glucose in vivo. It is also used in inflammation and digestive disorders.

Aims: This study aims to investigate the efficacy of aerial extract Sarcopoterium spinosum in protecting the liver of male Wistar rats against the induced hepatotoxic damage by the effect of carbon tetrachloride

Methods: In this experimental work, 30 male Wistar rats weighing between 160 -180 g were divided in to five tested groups each with 6 rats. Two groups were considered as normal control group and CCl4- intoxicated group. While the other 3 groups were treated with aqueous extract of *S. spinosum* with different concentration (150, 250 and 500 mg/kg body weight) by oral gavage for 21 days just before injecting the rats with hepatotoxic-carbon tetrachloride (CCl4: 3 mL/kg). Serum samples were collected from rats for assessment of certain biochemical parameters as: hepatic antioxidant enzymes (CAT, GPx and SOD), aminotransferase (AST, ALT and ALP), GSH levels, lipid peroxidation (LPO) by measuring MDA levels as well as the lipid profile (TG, LDL, VLDL and HDL).

Results: This study showed that *S. spinosum* extract has a hepatoprotective activity by reduction of lipid peroxidation and oxidative stress as well as the aminotransferase activities in the liver resulted from exposure to CCl4. Likewise, *S. spinosum* seems to have a lipid lowering potential with a beneficial increase in HDL levels of the treated rats.

Conclusion: Regular consumption of oral *S. spinosum* showed improvement in the hepatic biochemical parameters and lipid profile; which indicates that the studied extract is potent natural hepatoprotective agent. However, further studies are recommended to identify its phytochemicals that particularly exhibit these effects.

Keywords: *Sarcopoterium spinosum*, hepatoprotective, antioxidant enzyme activity, aminotransferase, Carbon tetrachloride, serum lipid.

INTRODUCTION:

Liver is a major dynamic organ with an extensive range of biological functions, such as metabolism including protein synthesis and degradation, production of adrenal hormones, glycogen storage and also contributed in regulating the innate immune system [1]. Nowadays, liver damage becomes a very common cause of various metabolic disorders and can lead to even mortality [2]. Hepatotoxicity is defined as an injury or impaired liver function due to chronic exposure to drugs or other chemical agents [3]. There are three common experimental models which influence acute liver injury including surgical interventions, exposure to toxins or chemical agents and viral infection [4]. Xenobiotics are known to be hepatotoxic agents; this can be attributed to the main role of liver in xenobiotic metabolism in addition to its portal circulation and anatomic structure [5]. Among those xenobiotics, carbon tetrachloride (CCl4) which is a halogenated alkane, contributes to liver damage by lipid peroxidation [6]. Limited pharmacotherapeutic options are available for the liver diseases; therefore, more efforts are needed for development of new therapeutic agents [7]. Avicenna's book collected many medicinal plants with a wide range of healing power [8].

Sarcopoterium spinosum species is a well- known Mediterranean medicinal plant that is widely used for diabetes, GI problems, pain and maybe cancer among Moslem Arabs and Bedouins. It is related to Rosaceae family and also known as Thorny or Shruppy barnet (called "Natsh" in Arabic language) [9], [10]. In the late 1960s and 1980s, several studies tested the hypoglycemic effect of Sarcopoterium spinosum in rats. Dafni et al. investigated that the root extract of this plant has an significant antidiabetic activity for treating diabetes in Muslim folk medicine; However, very few studies confirmed this claim [11], [12]. The aqueous root extract of this plant exhibited an insulin- like effect in hyperglycemic mice by increasing the insulin secretion (in vitro) and enhancing the glucose uptake by hepatocytes (in vivo). The optimal hypoglycemic effect can be seen at concentration 0.01 mg/ml of the aqueous root extract. Therefore, these results support the traditional use of Sarcopoterium spinosum as an antidiabetic agent [13].

On the other hand, limited researches discussed the hepatoprotective effect of Sarcopoterium spinosum. On this background, the study investigates the hepatoprotective effect of *Sarcopoterium spinosum* aerial extract against CCl4 induced hepatotoxicity in male Wistar rats in the search for a new natural agent.

MATERIALS AND METHODS:

Plant material

The aerial S. spinosum were collected from Ramallah rural area during both flowering and fruiting periods. The plant root was dried and powdered, then extracted by a mixture of water/methanol in a ratio 70:30 v/v for 6 hrs. After repeating the previous steps three times, the extraction was filtered and the concentrated filtrate was collected under vacuum at a temperature not exceeding 50 ℃. This filtrate was evaporated to dryness using a freeze dryer until 9.0% yield. Later, the extract was dissolved (400 mg/kg in 1 ml normal saline) and administered orally to the experimental rats using the animal feeding intubation's needles. [14]

Experimental animals

The experimental Male Wistar albino rats weighing between 140 and 180 g were maintained in the laboratory animal house at College of Pharmacy and Health Sciences in Ajman University. The rats were kept inside plastic cages and received a standard rodent diet with water ad libitum.

Rats were allowed to adapt the laboratory environment by exposing them to alternative cycle of 12 hrs of dark and light for at least one week before investigation. Principles of laboratory animal care as described in the European Community guidelines were followed [15]. All experiments were approved by the Animal Welfare Committee of the University (approval ref no. 6ZAWC/2016).

Acute toxicity experiment
Thirty male wistar rats were randomly assigned into five different groups (6 animals each). The first group served as control which received only the vehicle (normal saline) while the second group was injected with CCl4 only to induce toxicity. The other three test groups were received graded doses of *Sarcopoterium spinosum* aqueous extract orally (1500 – 4000 mg/kg), assessed for mortality up to 48 hrs and the LD50 was calculated for each.

Doses Selection
The selected doses of the aqueous extract of *Sarcopoterium spinosum* was considered based on the acute toxicity study and no adverse effect was detected after oral administration up to 5000 mg/kg. According to modified method of [16], the experimental oral doses of 150, 250 and 500 mg/kg were selected after determining the maximum possible dose of the extract based on the acute toxicity study.

Induction of hepatic damage experiment
Carbon tetra chloride was dissolved in groundnut oil in the ratio of 1:1 v/v. A single subcutaneous (SC) injection of CCl4 (3 ml/kg) was administered in the lower abdomen of the rats once daily for 7 consecutive days to induce liver toxicity [17].

Hepatoprotective activity trial
Experimental rats were randomly assigned in to five groups (each with six rats) and treated as the following manner:
- 1st (normal control) and 2nd (CCl4 only) groups: Both received 5 ml/kg PO of the vehicle (normal saline).
- 3rd group: Received 150 mg/kg PO of the aqueous extract of *Sarcopoterium spinosum* for 21 days.
- 4th group: Received 250 mg/kg PO of the aqueous extract of *Sarcopoterium spinosum* for 21 days.
- 5th group: Received 500 mg/kg PO of the aqueous extract of *Sarcopoterium spinosum* for 21 days.

All drugs were managed by gastric intubation for 21 consecutive days. The normal control group was administered a single subcutaneous dose of groundnut oil at dose of 3 ml/kg two hours after the last dose. While rats of 2nd to 5th group were received a single dose of CCl4 (3 ml/kg) subcutaneously. One day later (after 24 hrs), blood samples were collected from all rats and allowed to clot for 30 min at room temperature in separately labeled centrifuging tubes. The collected samples were centrifuged at 10,000 rpm for 5 min until serum was separated for analysis of certain biochemical parameters. Livers of rats were collected and sliced up then reserved in liquid nitrogen for assessment of antioxidant status.

Measurement of liver function markers
Evaluation of liver functions was done by measuring the serum activity of ALT and AST as per Reitman and Frankel method [18] while the serum activities of ALP were determined according to Arthur et al. method [19].

Evaluation of CCl4 mediated oxidative stress
This method was done by homogenizing the liver tissue in 10 ml of 100 mM KH2PO4 buffer containing mM EDTA at pH of 7.4, then it was centrifuged at 12,000 rpm at low temperature (~4℃) for 30 min. The antioxidant activities of hepatic enzymes (SOD, GPx and CAT) were evaluated in normal control and all tested rats as per previously described methods [20-22]. In addition to that, the lipid peroxidation level (LPO) was estimated by mixing the liver homogenate of all rats with aqueous thiobarbituric acid product (malondialdhyde – MDA) in acetate buffer at pH of 3.5 according to Ohkawa et al. method [23]. The content of the reduced glutathione (GSH) was determined by its reaction with DTNB to produce a compound that was measured by standard spectrophotometric method at 412 nm [24].

Data analysis
Data collected from the experiment were expressed as mean ± standard error for all experimental rats. A one- way analysis of variance (ANOVA) was used for calculating the significant difference in means between the control and treated rats' groups (CCl4 only, 150 mg/kg + CCl4, 250 mg/kg + CCl4 and 500 m/kg + CCl4). The p- value of <0.05 was considered statistically significant and all data analysis was carried out using Microsoft SPSS (23.0).

RESULTS:

Activity of liver marker enzymes:
After 24 hours of intoxication by subcutaneous injection of CCl4 in rats, liver marker enzymes (ALT, AST and ALP) were significantly elevated. Also, the total bilirubin level which is normally processed in the liver was accumulated while albumin levels were reduced in the serum of CCl4-intoxicated control compared to the normal control group. However, the treated groups with aqueous extract of *Sarcopoterium spinosum* at different doses (150, 250 and 500 mg/kg) for 21 days prior to the CCl4 exhibited a remarkable hepatoprotective effect. As a result of that, the liver marker enzymes and the total bilirubin level were significantly reduced, [Figure 1] whereas the albumin levels were significantly elevated [Figure 2] compared to the CCl4-intoxicated group.

Activity of liver antioxidant enzymes:
Table 3 & 4 presented the antioxidant activity of hepatic enzymes (SOD, GPx and CAT), level of GSH and malondalyde (MDA) after the induced oxidative stress in rats' liver by CCl4. Subcutaneous injection of CCl4 caused a significant reduction in the antioxidant activities of superoxide dismutase (SOD), peroxidase (GPx), catalase (CAT) and glutathione reduced (GSH) level [Figure 3], while MDA levels was increased in the liver tissue as compared to the normal control group [Figure 4]. On the other hand, pre-treated rats with the aqueous extract *S. spinosum* (150, 250 and 500 mg/kg) showed minimal CCl4 toxicity, as non- significant differences were observed in the antioxidant liver enzymes and the levels of oxidative stress indicators.

Lipid profile measurement:
The lipid profile of CCl4-intoxicated rats showed a significant elevation ($p < 0.05$) in the serum TG, LDL and VLDL while the HDL level was significantly declined when compared to normal control group [Figure 5]. However, pre- administration of aqueous extract of *S. spinosum* significantly lowered these parameters into normal in concentration dependent manner as compared to CCl4 control group ($p < 0.05$). Moreover, the lipid lowering effect in the treated rats with the aqueous extract was even lower than that of normal control group; which indicates that aqueous extract of *S. spinosum* itself has a lipid lowering potential regardless the CCl4 treatment.

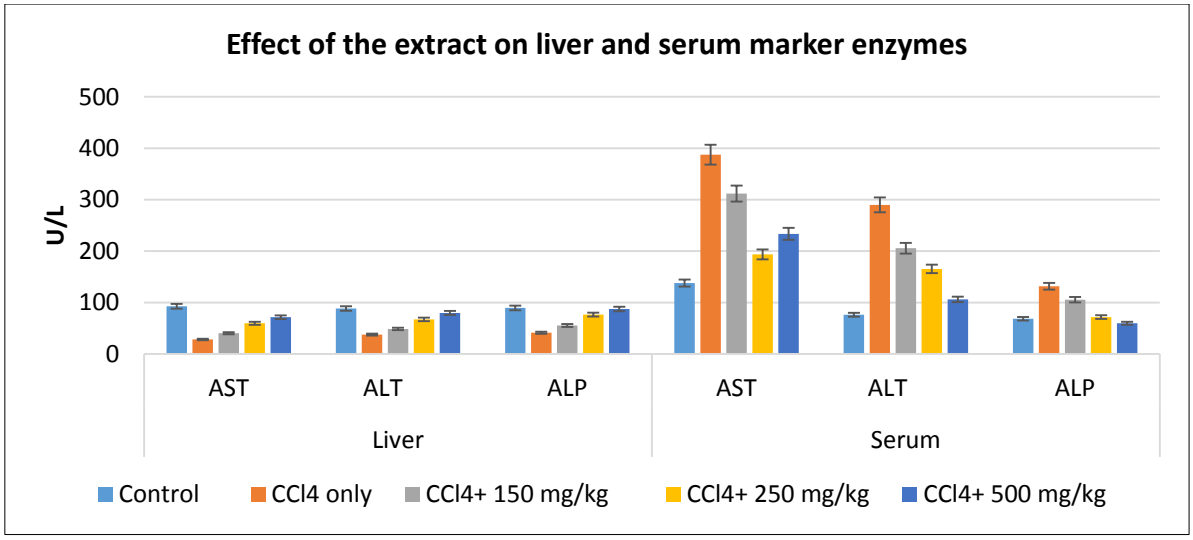

Figure 1: Changes in the activities of serum and liver marker enzymes in CCl4-induced rats treated with aqueous extract of Sarcopoterium spinosum

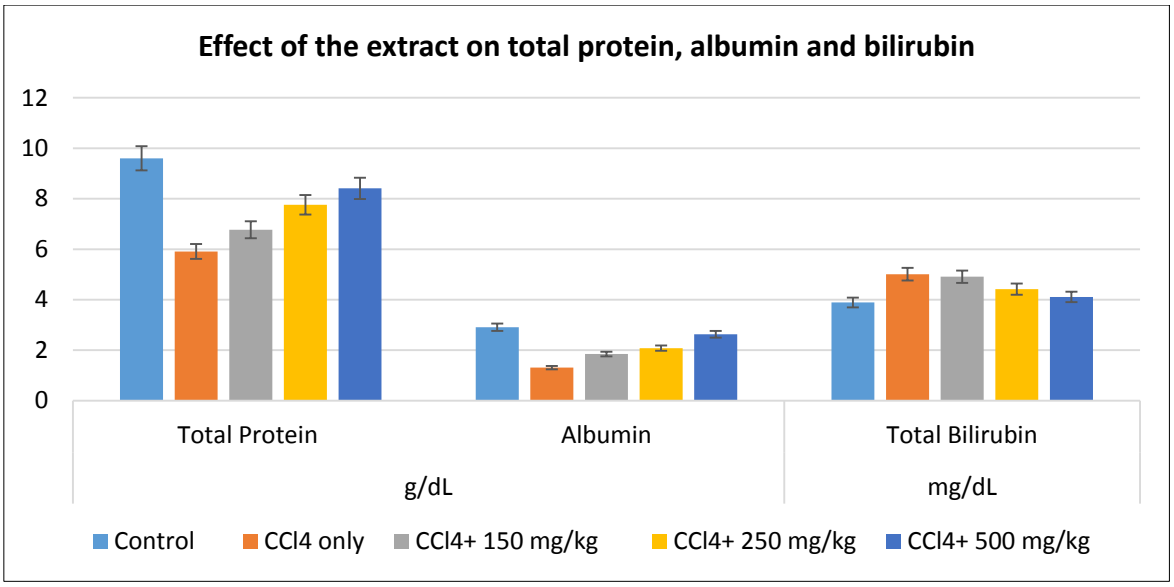

Figure 2: Levels of total protein and bilirubin and albumin in the serum of rat's with CCL4 induced-hepatotoxicity

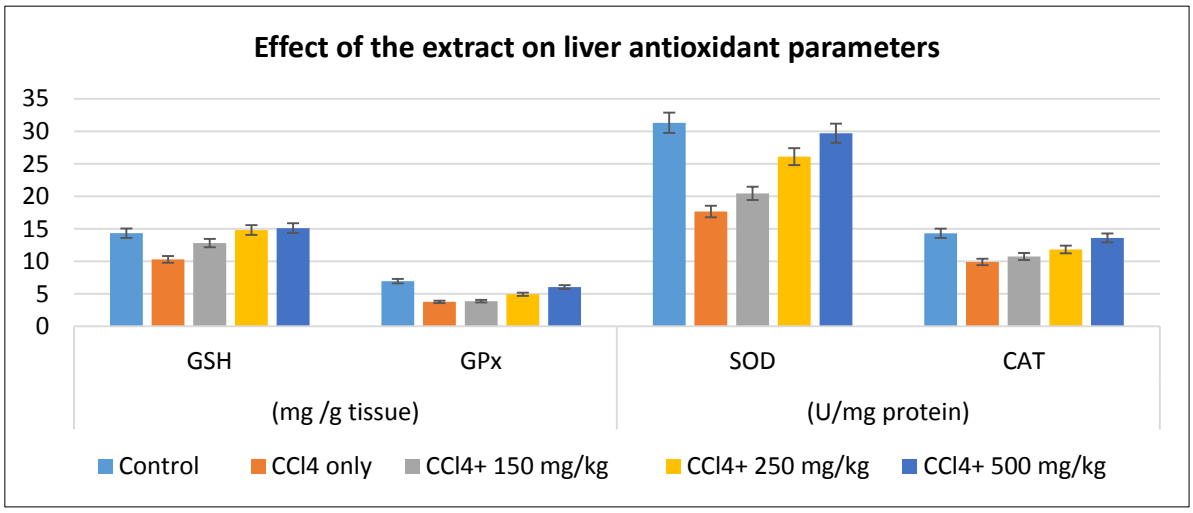

Figure 3: Changes in the levels of liver antioxidant parameters in CCl4-induced rats treated with aqueous extract of Sarcopoterium spinosum

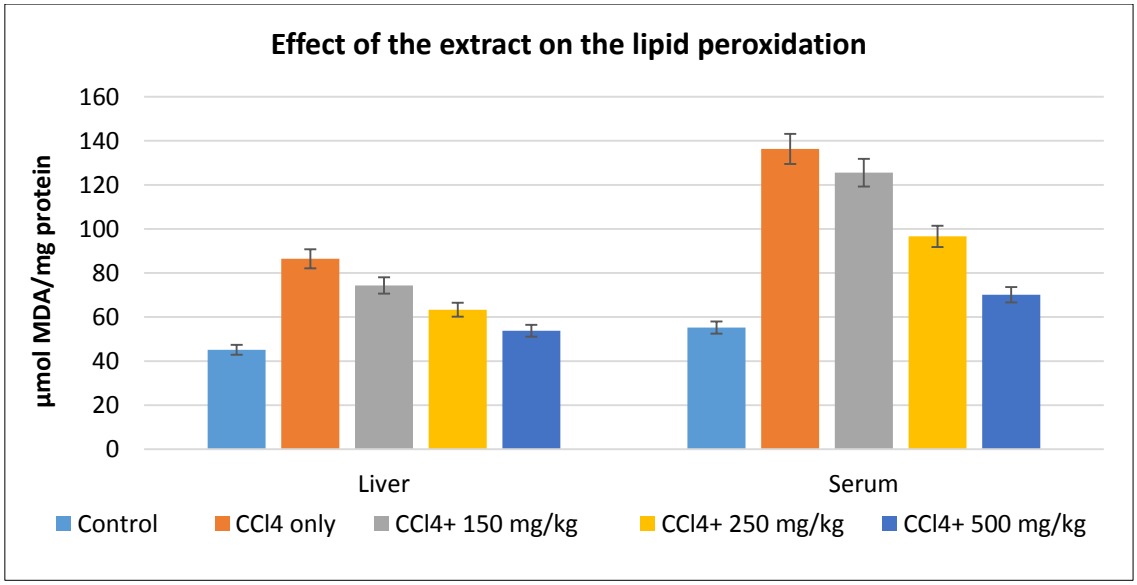

Figure 4: Changes in the levels of lipid peroxidation in CCl4-induced rats treated with aqueous extract of S. spinosum

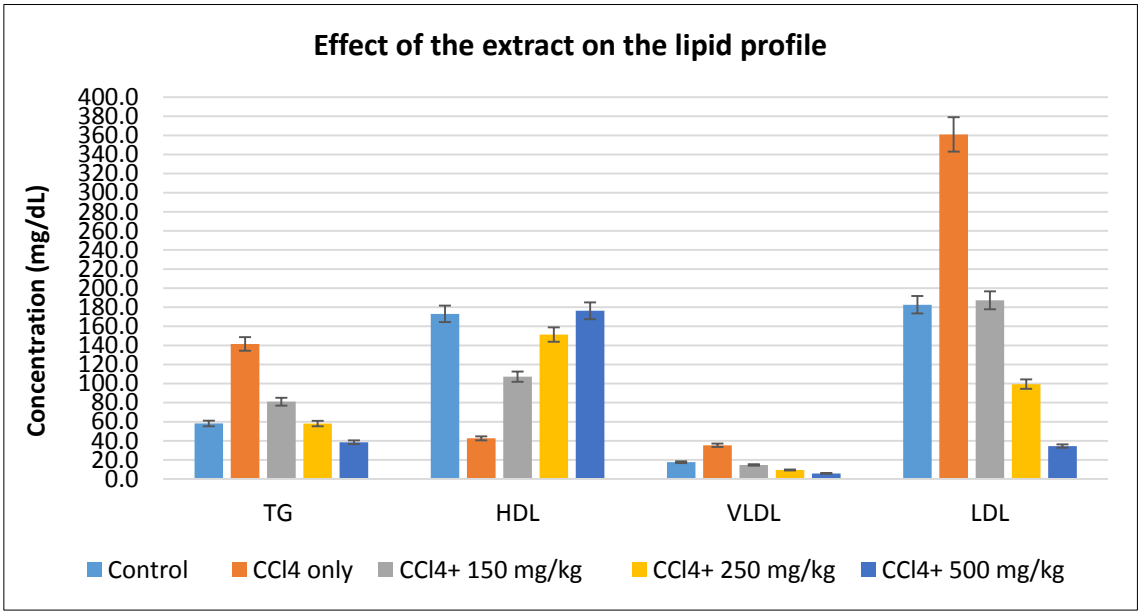

Figure 5: Effects of aqueous extract of Sarcopoterium spinosum on lipid profile of CCl4-induced rat

DISCUSSION:

The current study elucidated that exposing the studied rats to carbon tetra chloride (CCl4) is attributed to toxic metabolites which interferes with the normal liver functions. In the liver, CCl4 is transformed to CCl3 free radicals; which interact with the unsaturated lipids in the cell membrane causing its damage. The hepatic damage was characterized by elevated the transaminase and serum bilirubin, increased lipid peroxidation disrupted antioxidant activity of liver enzymes (SOD, GPx and CAT) and GSH levels, and elevated serum TG, VLDL, LDL and reduced HDL. Therefore, this alteration of liver biomarkers by CCl4-intoxication destroys the balance between necrosis and regeneration of the hepatocytes. This was clearly demonstrated by previous studies [6].

Many herbal preparations are known to inhibit oxidative stress and liver injuries through modulating the levels of cytochrome P-450 [25]. These herbs exhibit their action according to the phytochemicals composition [26]. The present study investigated that pretreatment of Sarcopoterium spinosum extract at different doses (150, 250, 500 mg/kg) possessed a hepatoprotective activity against the CCl4 through reduction of elevated liver biomarker enzymes and lipid profile. These findings are attributed to the existence of certain phytochemicals in leaves and thorns such as palmitic acid as reported by a recent study [27].

According to our findings, the elevated transaminase (ALT, AST and ALP) were used as indicators of liver dysfunction and CCl4 intoxication [25]. However, *S. spinosum*- treated rats returned the transaminase concentration into normal levels and counteract the CCl4 intoxication.

The accumulation of bilirubin in the circulation was attributed to the excessive conversion of heme into bilirubin by heme oxygenase. The bilirubin normally is conjugated in the liver with glucuronide which excreted in bile and exhibits a protective effect against the oxidative stress induced by CCl4 or other toxicants. However, in case of liver damage or impaired hepatic clearance, the conjugated bilirubin is inactivated and hence increase the oxidative stress [28], [29]. The aqueous extract of *S. spinosum* was able to maintain the integrity of liver tissues and reduced the

oxidative stress caused by CCl4 intoxication. This antioxidant activity could be related to the presence of certain phytochemicals as saponins , phenols and alkaloids [30].

Furthermore, untreated rats (CCl4- treated only) showed a significant elevation of MDA level ($p < 0.05$); as an indicative of oxidative damage and lipid peroxidation (LPO) which may end up with liver necrosis [31]. In addition to that, a significant decline in the antioxidant enzymes activities (CAT, SOD and GPx) and GSH levels ($p < 0.05$) were observed in the CCl4- treated rats when compared to normal control group. The depletion of the antioxidant enzymes and GSH also confirm lipid peroxidation of the tissues [5]. Pre- treated rats with the aqueous extracts *of S. spinosum* showed a significant amelioration of the hepatic enzymes activities ($p < 0.05$) and decreased the MDA levels ($p < 0.05$) as shown in table 3 and 4. These findings confirmed that *S. spinosum* has an antioxidant activity as revealed by [12].

The abnormalities in the lipid parameters are considered as a major risk factor for atherosclerosis and cardiovascular morbidities [32]. According to our findings, there was a significant accumulation ($p < 0.05$) of serum LDL-C, VLDL and TG as well as a significant decline ($p < 0.05$) in HDL-C in the CCl4 control group when compared to normal control group as shown in Table 5. While these parameters were reserved after administration of the aqueous extract of *S. spinosum* even lower than that of the normal control group. This could be attributed to certain bioconstituents present in this extract [30].

CONCLUSION:

The current study revealed that *Sarcopoterium spinosum* has a hepatoprotective effect against the CCl4 toxicity through its antioxidant and lipid parameters amelioration properties. This could be attributed to certain phytophenolic components available in the extract. Further studies are recommended to verify the active phytochemicals and their role underlying the interesting properties of this plant.

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