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# Hepatoprotective Effect of Sarcophine Isolated from Soft Coral (*Sarcophyton glaucum*) in Rats

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**Abstract:** The aim of the current study was to evaluate the hepatoprotective effects of sarcophine against carbon tetrachloride-induced hepatotoxicity in rats. Forty male Sprague-Dawley rats were divided into 4 groups including the control group, the group treated orally with sarcophine (20 mg/kg. b.w.), the group treated orally with carbon tetrachloride (1 mg/kg b.w.) and the group treated orally with $CCl_4$ for 2 weeks then treated with sarcophine for another 2 weeks. Although, sarcophine alone-treated group was more or less comparable to the control, the combined treatment resulted in significant improvement in all biochemical parameters and the histopathological picture of the liver tissue. Animals treated with $CCl_4$ for 2 weeks then with sarcophine for other 2 weeks showed a significant decrease in total cholesterol accompanied with a significant improvement in triglycerides. Moreover, the serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and creatinine were decreased significantly compared to $CCl_4$-treated group. It could be concluded that sarcophine has a significant protection against $CCl_4$ induced hepatocellular injury.

**Key words:** Sarcophine · Soft Coral · Hepatoprotective · Liver · Lipid Profile

## INTRODUCTION

As a result of the potential for new drug discovery, marine natural products have attracted scientists from different disciplines, such as organic chemistry, bioorganic chemistry, pharmacology, biology and ecology. This interest has led to the discovery of almost 8,500 marine natural products to date and many of the compounds have shown very promising biological activity [1].

The soft coral of the genus *Sarcophyton* is one of the famous soft corals found to contain a diversity of cembrane diterpenes [2]. Sarcophine is one of the most abundant cembranolide isolated from the *S. glaucum* collected from the Red Sea with yields up to 3% of animal dry weight [3]. It also contains sesquiterpenes [4], tetraterpenoids [5], steroids [6], fatty acids [7] and amino acids [8].

Many of these compounds possess a biological activity beside activities such as cytotoxic [9], antimetastatic [10], antileukemic [11] and their chemopreventive activities [12]. Recently, Cheng *et al.* [13] reported that several sarcophine metabolites have cytotoxic effects against selected cancer and normal cell lines and antiviral activity against human cytomegalovirus and antibacterial activity against *Salmonella enteritidis*.

Chronic liver diseases are common worldwide and are characterized by a progressive evolution from steatosis to chronic hepatitis, fibrosis, cirrhosis and hepatocellular carcinoma [14,15]. Indeed, hepatocellular carcinoma (HCC) is the fifth most common neoplasm and the major cause of death in patients with liver cirrhosis and the third most common cause of cancer-related death in the world [16]. Moreover, autoimmune hepatitis is triggered by different factors. There has been evidence implicating measles virus, hepatitis virus, cytomegalovirus

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and *Epstein-Barr* virus as indicator of the autoimmune hepatitis; the most convincing evidence related hepatitis viruses [17-19].

HCC is the fifth most common malignancy in the world complicating liver cirrhosis in most cases [20]. Its incidence is increasing worldwide ranging between 3 and 9% annually [21]. In Egypt, the annual prevalence of HCC has increased significantly during the past decade [22]. HCC was reported to account for about 4.7% of chronic liver disease (CLD) patients where its epidemiology is characterized by marked demographic and geographic variations [23]. The aims of the current study were to isolate sarcophine from the soft coral (*Sarcophyton glaucum*) collected from the Red sea and to evaluate its hepatoprotective activity against carbon tetrachloride-induced hepatotoxicity in rats.

## MATERIALS AND METHODS

**Chemicals and Kits:** Alanine aminotransferase (ALT), Aspartate aminotransferase, (AST), creatinine, cholesterol, triglycerides, arginase, α-L-fucosidase and total antioxidant capacity kits were purchased from Randex Laboratories (San Francisco, CA, USA). Malondialdehyde (MDA) was obtained from Eagle Diagnostics (Dallas, TX, USA). Other chemicals were of the highest purity commercially available.

**Soft Coral:** The soft coral *Sarcophyton glaucum* was collected from the Red Sea at Hurgada, Egypt during August 2005 at a depth of 10-15 m and was identified by Marine Science Department, Faculty of Science, Suez Canal University, Ismailia, Egypt.

**Isolation and Identification of Sarcophine:** The fresh soft coral *Sarcophyton glaucum* (3.8 kg) was chopped into small pieces, blended and extracted exhaustively with $CH_2Cl_2$ and filtered. The filtrate was dried over anhydrous $Na_2SO_4$ then the solvent was removed under reduced pressure (temperature not exceeding 40°C) until dryness to give 112 g dark brown residue. The residue was applied to silica gel column chromatography (150×5 cm) and eluted first with n-hexane, followed by EtOAc in n-hexane/ (2-50 %). All fractions were screened by TLC using n-Hexane: ethyl acetate (9:1), (8:2) and (7:3) as solvent systems. A pure compound was isolated and crystallized from the 20% ethyl acetate fraction to give 4.9 g of colorless needles of the compound, sarcophine. It showed a dark spot under short UV light (254 nm), changed to pink color by heating after spraying with 10% sulfuric acid [24]. Rf value was 0.38 in a solvent system of n-hexane/EtOAc (7:3). MS (JEOL JMS-700 Mastation), NMR (Varian Unity-400 machine).

**Experimental Animals:** Male Sprague-Dawley rats (100-120 g, purchased from Animal House Colony, Giza, Egypt) were maintained on standard lab diet (protein: 160.4; fat: 36.3; fibre: 41 g/kg and metabolizable energy 12.08 MJ) purchased from Meladco Feed Co. (Aubor City, Cairo, Egypt). Animals were housed in a room free from any source of chemical contamination, artificially illuminated and thermally controlled at the Animal House Lab., National Research Centre, Dokki, Cairo, Egypt. After an acclimatization period of 1 week, the animals were divided into four groups (10 rats/group) and housed in filter-top polycarbonate cages. All animals were received humane care in compliance with the guidelines of the Animal Care and Use Committee of the National Research Center, Dokki, Cairo, Egypt.

**Experimental Design:** Animals within treatment groups were maintained on their respective diets for 4 weeks as follows: group 1, untreated control; group 2, treated orally with sarcophine (20 mg/kg. b.w.); group 3, treated orally with carbon tetrachloride (1 mg/kg b.w.) and group 4, treated orally with $CCl_4$ for 2 weeks then treated with sarcophine for another 2 weeks. At the end of experimentation period (i.e. day 28), blood samples were collected from all animals from retro-orbital venous plexus for biochemical analysis. The following biochemical methods were performed: ALT, AST, triglycerides, cholesterol, creatinine, arginase, α-L-Fucosidase and total antioxidant capacity. All biochemical analyses were carried out according to the manufacturer's instructions

After collecting the blood samples, all animals were killed and liver samples were excised and fixed in formalin 10% and were hydrated in ascending grades of ethanol, cleared in xylene and e mbedded in paraffin. Sections (5 mm thick) were cut and stained with hematoxylin and eosin (H and E) for the histological examination [25].

**Statistical Analysis:** All data were statistically analyzed using the General Linear Models Procedure of the Statistical Analysis System [26]. The significance of the differences among treatment groups was determined by Waller-Duncan k-ratio [27]. All statements of significance were based on probability of $P \leq 0.05$.

Table 1: $^{13}$C-and $^{1}$H-NMR Data of sarcophine ($CDCL_3$)

| No. | $\delta_H$ | $\delta_C$ |
|---|---|---|
| 1 | 5.54 d (10.3) | 78.73, d |
| 2 | 5.01 m | 120.58, d |
| 3 | | 144.01, s |
| 4 | 2.34 m | 37.34, t |
| 5 | 1.64 m, 1.67 m | 25.17, t |
| 6 | 2.64 m | 61.38, d |
| 7 | | 59.90, s |
| 8 | 1.98 m, 2.14 m | 36.33, t |
| 9 | 2.72 m | 27.51, t |
| 10 | 5.11 m | 124.87, d |
| 11 | | 135.50, s |
| 12 | 2.07 m, 1.06 m | 38.98, t |
| 13 | 2.23 m, 1.90 m | 23.27, t |
| 14 | | 162.20, s |
| 15 | | 122.90, s |
| 16 | | 174.65, s |
| 17 | 1.82 s | 8.96, q |
| 18 | 1.86 s | 15.38, q |
| 19 | 1.25 s | 16.08, q |
| 20 | 1.59 s | 17.10, q |

## RESULTS

**Sarcophine:** The results of Mass spectrum of the isolated compound indicated a formula of $C_{20}H_{28}O_3$ and Mw. 316 with a melting point 136°-136.5°C. The $^{13}$C-NMR spectrum showed the presence of 20 carbon signals. $^{1}$H and $^{13}$C NMR spectra (Table 1) as well as the DEPT experiment indicated the presence of four tertiary methyls (C-17, C-18, C-19, C-20), two oxymethines (C-1, C-6), six methylenes (C-4, C-5, C-8, C-9, C-12, C-13) carbons, one Q-substituted quaternary carbon (C-7), two methane olefins (C-2, C-10) and five quaternary olefinic carbons (C-3, C-11, C-14, C-15, C-16, carbonyl). The correlations of $^{1}$H-$^{1}$H COSY revealed four proton-proton networks, $H_3$-18/H-1/H-2/$H_2$-4/$H_2$-5/H-6, $H_2$-8/$H_2$-9, H-10/$H_3$-20 and $H_2$-12/$H_2$-13. These data together with HMBC cross peaks between H-2/C-1, H-1/C-2, $H_3$-18/C-2, H-1/C-3, $H_2$-4/C-3, $H_2$-5/C-3, $H_3$-18/C-3, $H_3$-18/C-4, $H_2$-5/C-4, H-6/C-4, H-6/C-5, $H_2$-4/C-5, $H_3$-19/C-6, H-6/C-7, $H_3$-19/C-7, $H_2$-9/C-8, $H_2$-8/C-9, $H_3$-20/C-10, $H_2$-12/C-10,

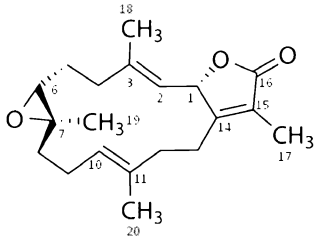

Fig. 1: Chemical structure of isolated sarcophine.

$H_3$-20/C-11, $H_2$-9/C-11, $H_2$-13/C-12, $H_2$-12/C-13 and H-1/C-14 confirmed the connections from C-1 to C-14 of the 14-membered ring of the isolated compound. The most downfield carbon signals at δ162.20 and δ174.65 (Table 1) corresponding to C-14 and C-16 confirmed the presence of a α-β-unsaturared lactone moiety. Taken together, HMBC couplings $H_3$-17/C-14, $H_3$-17/C-15 and $H_3$-17/C-16 confirmed the structure of sarcophine (Fig. 1) [28].

In the *in vivo* study, animals treated with $CCl_4$ showed significant changes in serum biochemical parameters and histological picture typical to those reported in the literature. The effects of sarcophine alone or after $Ccl_4$ administration on serum biochemical parameters are depicted in Table (2). These results showed that sarcophine alone did not affect ALT, AST activity or creatinine level. However, administration of $CCl_4$ alone resulted in a significant increase in both enzymes activity and creatinine level. Animals treated with $CCl_4$ for 2 weeks then treated with sarcophine for other 2 weeks showed a significant improvement in these enzymes activity and creatinine level. The levels of both enzyme activity and creatinine level decreased significantly compared to $CCl_4$-treated group although it still higher than the control group.

The effect of different treatments on serum total cholesterol and triglycerides (Table 2) revealed that the group treated with $CCl_4$ alone showed a significant increase in both parameters. Animals treated with sarcophine alone were comparable to the controls regarding total cholesterol and showed a significant decrease in triglycerides compared to the control group.

Table 2: Effect of sarcophine on serum biochemical parameters in rats treated with $CCl_4$ (mean ± SE)

| Parameter | Control | Sarcophine | $CCL_4$ | $CCL_4$ then Sarcophine |
|---|---|---|---|---|
| ALT(U/L) | 12.4± 0.51$^{a}$ | 13.8± 1.16$^{a}$ | 30.6± 0.87$^{b}$ | 15.4± 1.29$^{c}$ |
| AST(U/L) | 65.32± 2.9$^{a}$ | 66.32± 3.86$^{a}$ | 122.66± 3.6$^{b}$ | 80.86± 2.19$^{c}$ |
| Creatinine (mg/dl) | 6.33± 0.74$^{a}$ | 6.74± 0.64$^{a}$ | 17.05± 0.61$^{b}$ | 9.8± 0.47$^{c}$ |
| Total Cholesterol (mg/dl) | 94.9±1.97$^{a}$ | 97.8± 3.98$^{a}$ | 140.18± 2.85$^{b}$ | 87.99± 5.19$^{c}$ |
| Triglyceride (mg/dl) | 130.42± 1.31$^{a}$ | 106.47± 8.14$^{b}$ | 231.47 ± 13.05$^{c}$ | 150.93± 2.15$^{d}$ |

Within each row, means superscript with different letters are significantly different (P≤ 0.05)

Table 3: Effect of sarcophine on serum lipid peroxidation, total antioxidant capacity and tumor markers in rats (mean ± SE)

| Parameters | Control | Sarcophine | $CCl_4$ | $CCl_4$ then Sarcophine |
|---|---|---|---|---|
| MDA (nmol/ml serum) | 148.20± 14.91[a] | 130.26± 12.16[b] | 185.54± 2.42[c] | 152.17± 7.59[d] |
| TAC (µmol/ml serum) | 41.02± 1.73[a] | 54.44± 2.65[b] | 21.7± 1.08[c] | 36.5± 2.93[d] |
| α-L-Fucosidase (U/L) | 7.33± 0.36[a] | 4.87± 0.09[b] | 12.38±0.49 [c] | 8.09±0. 69[a] |
| Arginase (U/L) | 116.35± 2.01[a] | 94.64± 5.94[b] | 145.63± 3.57[c] | 104.84± 7.73[d] |

Within each row, means superscript with different letters are significantly different (P≤ 0.05), (TAC) total antioxidant capacity, (MDA) an end product of lipid peroxidation

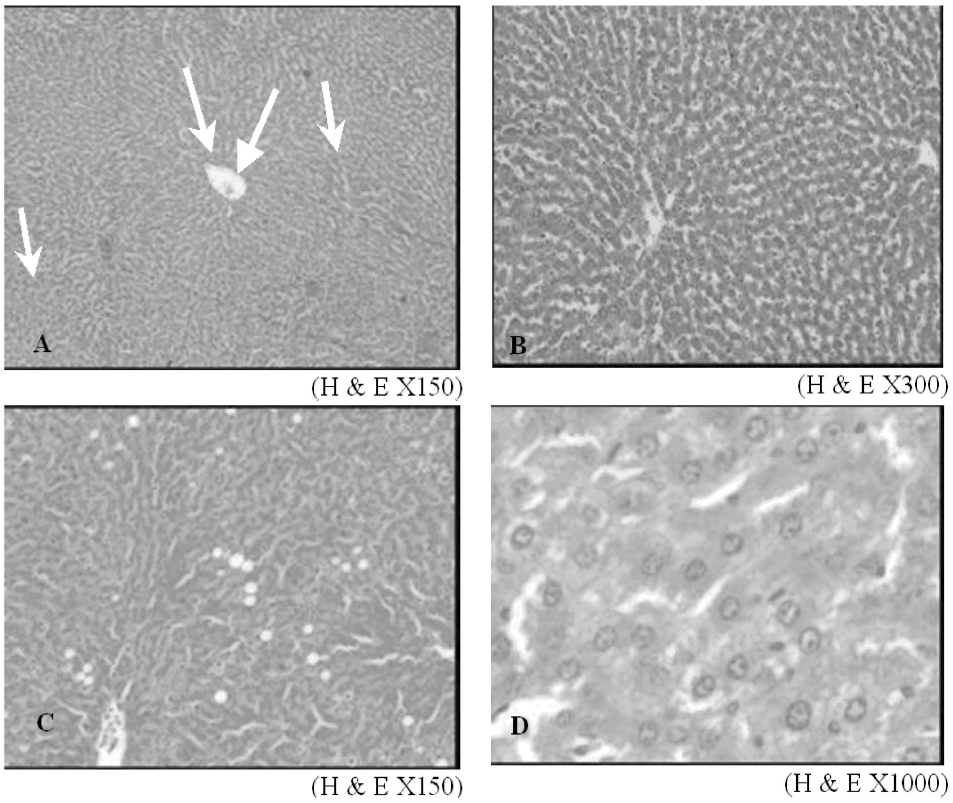


Fig. 2: A photomicrograph of a section in liver of (A) control rat showing central veins and hepatic cords separated with blood sinusoids, (B) control rat showing central veins and normal hepatocytes architecture, (C, D) sarcophine-treated rat showing more or less normal hepatocytes architecture. Few fatty degenerative changes are noticed.

On the other hand, animals treated with $CCl_4$ for 2 weeks then sarcophine for other 2 weeks showed a significant decrease in total cholesterol accompanied with a significant improvement in triglycerides.

The results of lipid peroxidation estimated as MDA and the total antioxidant capacity (TAC) are presented in Table (3). These results indicated that $CCl_4$ administration resulted in a severe oxidative stress as indicated by the significant increase in MDA accompanied with the significant decrease in TAC. Sarcophine alone decreased MDA and increased TAC significantly compared to the control group. Animals treated with sarcophine after $CCl_4$ showed a significant improvement in the levels of both parameters although it did not normalize them.

The effect of different treatments on tumour markers (α-L-Fucosidase and Arginase) are depicted in Table (3). These data indicated that animals treated with $CCl_4$ alone showed a significant increase in both tumor markers. Whereas, animals treated with sarcophine alone showed a significant decrease in these parameters. On the other hand, treatment with sarcophine for 2 weeks after $CCl_4$ treatment could normalize α-L-fucosidase and decreased arginase significantly compared to the control value.

The biochemical results were confirmed by the histopathological examinations of the liver which revealed that rats in the control group showed normal hepatocytes architecture and central vein (Fig. 2 A, B). The microscopic examination of the liver tissues of animals

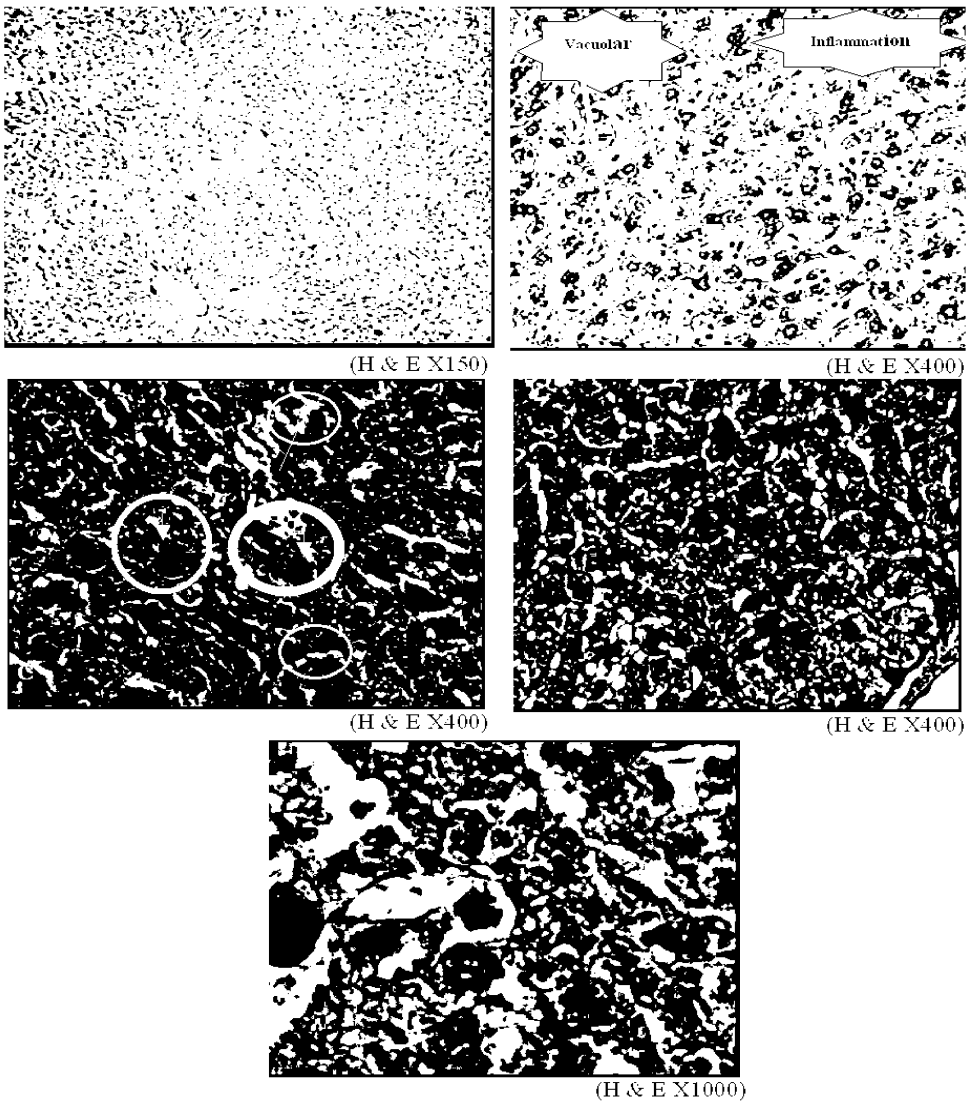


Fig. 3: A photomicrograph of a section in liver of (A) $CCl_4$-treated rat showing scattered of fibrous tissues around blood vessels all over section, (B) $CCl_4$-treated rat showing dilated blood sinusoids with aggregation of inflammatory cells arrow. Hepatocytes showing vacuolar degeneration and necrosis, (C) $CCl_4$-treated rat showing different degrees of damage some are apoptotic and necrotic, (D) $CCl_4$ then Sarcophine-treated rat showing prominent regeneration in hepatocytes. Blood sinusoids revealed small droplets of fatty degeneration and (E) $CCl_4$ then sarcophine-treated rat showing same picture of degenerative changes and fatty degeneration.

treated with sarcophine alone showed more or less normal hepatocytes architecture and few fatty degenerative changes (Fig.2 C, D). The liver of animals treated with $CCl_4$ alone showed a scattered fibrous tissue around blood vessels (Fig. 3 A). The same animals showed dilated blood sinusoids with aggregation of inflammatory cells (Fig. 3 B). The hepatocytes were damaged in the form of vacuolar degeneration and necrotic nuclei with some apoptotic nuclei (Fig. 3 C). On the other hand, the microscopic examination of liver section of the animals treated with $CCl_4$ then sarcophine showed a prominent regeneration in the hepatocytes and the blood sinusoids revealed small droplets of fatty degeneration (Fig 3. D, E).

## DISCUSSION

Sarcophine has been isolated before from many *Sarcophyton* species. Melting point, mass spectra as well as $^{1}H$ and $^{13}C$ NMR data were superimposed with those previously reported [28]. The chemopreventive effects of

sarcophine against $CCl_4$-induce hepatic toxicity in rats were also evaluated. The selective dose of sarcophine and $CCl_4$ were literature based [29, 30]. The significant increase in ALT, AST activities and createnine level in animals treated with $CCl_4$ may indicate degenerative changes and hypofunction of liver [31,32] as well as hepatic cell necrosis [30] which increased the release of these enzymes in blood stream [35]. Similar to the current results, Bhattachrjee and Sil [36] reported that $CCl_4$ administration cause hepatotoxicity, which is accompanied with elevation of ALT and AST enzymes in rats and mice.

The elevation in cholesterol and triglyceride (TG) levels reported herein in the $CCl_4$-treated group indicated necrosis or hepatocellular injury as suggested by El-Nekeety *et al.* [37]. Taken together, the increased activity of ALT, AST with the elevated serum cholesterol and triglycerides are probably associated with biliary obstruction and acute hepatic injury [32-34, 38].

$CCl_4$ is one of the most extensively studied hepatotoxicant. The mechanism by which $CCl_4$ causes hepatotoxicity is well documented in a series of reports. The hepatotoxicity of $CCl_4$ undergoes 2 phases. The first results from its metabolic conversion to free radical product $CCl^{\cdot}_3$ by cytochrome-P450 [39]. Once $CCl^{\cdot}_3$ has been formed, it reacts very rapidly with $O_2$ to produce $CCl_3OO^{\cdot}$, a much more reactive radical than $CCl^{\cdot}_3$ [40]. These free radicals attack microsomal lipids leading to its peroxidation and also they covalently bind to microsomal lipids and proteins resulted in the generation of reactive oxygen species (ROS), which includes the super-oxide anion $O^{\cdot}_2$, $H_2O_2$ and the hydroxyl radical ($OH^{\cdot}$). Although various enzymatic and non-enzymatic systems have been developed by cells to cope up with the ROS and other free radicals, the defence capability against ROS becomes insufficient when a condition of oxidative stress establishes [41]. ROS also affects the antioxidant defence mechanism and decrease the activity of superoxide dismutase. Increasing evidence indicates that oxidative stress cause liver injury then the development of cirrhosis and carcinogenesis [42-44]. The second phase in $CCl_4$ toxicity occurs in kupffer cells and mainly contributed to inflammatory response. Kupffer cells are activated by free radicals and secrete cytokines that attract and activate neutrophils. Neutrophils themselves release ROI, thereby enhancing the liver injury [45, 46]. Excess ROI, a condition referred to as oxidative stress, is considered to be a major contributor to cell injury [47, 48].

The current results revealed that $CCl_4$ is also nephrotoxic as indicated by the significant increase in creatinine level. $CCl_4$ induced its toxic effects on the kidney besides well-known liver [48, 49]. However, the pathogenesis of $CCl_4$-induced renal injury has not been clearly clarified. While Rincon *et al.* [50] suggested that the effects of $CCl_4$ on kidney structure and function depend on the functional state of the liver.

MDA, an end product of lipid peroxidation (LP), is widely used as a marker of LP which is considered a one of the main manifestation of oxidative damage and plays an important role in toxicity and carcinogenicity. The antioxidant enzymes represent the major defence system against liver injury and carcinogenesis. In the present study, the elevation of MDA in the animals treated with $CCl_4$ alone supported the earlier findings of Nwozo *et al.* [51] and Bhattachrjee and Sil [36]. Alteration in the hepatic antioxidant status may therefore be considered a manifestation of oxidative stress caused by $CCl_4$ and its metabolites. In the present study, total antioxidant capacity (TAC) was found to decline significantly in rats treated with $CCl_4$. It is well known that TAC plays an important role in the elimination of ROS derived from the peroxidative process in liver tissues [52, 53]. Moreover, some of the antioxidant enzymes such as SOD remove superoxide by converting it to $H_2O_2$, which can be rapidly converted to water by CAT [54]. Taken together, the increased level of MDA and the decreased TAC may be attributed to free radical formation which initiated chain reactions of direct and indirect bond formation with cellular molecules (nucleic acids, proteins, lipids and carbohydrates) and impairing crucial cellular processes that may ultimately culminate in extensive cell damage and death [55].

Animals treated with $CCl_4$, showed a significant increase in both tumour markers tested. These results were supported by alteration of the tumour response by $CCl_4$, which is indicative for the crucial role of $CCl_4$ in induction of hepatocellular carcinoma in the presence of other material such as aminopyrine and sodium nitrite [55]. Moreover, the increased level of α-L-Fucosidase and Arginase reported herein revealed that $CCl_4$ is not only hepatotoxic agent but also cancer promoter. Similar to these observations, Frezza *et al.* [56] reported that the intragastric administration of $CCl_4$ for 30 weeks induced liver cirrhosis and hepatocellular carcinoma. The biochemical results reported in the present study were confirmed by the histological findings in the liver tissue which indicated that $CCl_4$ induced severe degenerative changes and necrosis in the hepatocytes with some apoptotic nuclei. These results are in agreement with those reported previously [36, 57-59].

Zhang *et al.* [60] reported that sarcophine-diol (SD), a structural modifications of sarcophine, has shown chemopreventive effects on 7, 12-dimethylbenz (a) anthracene-initiated and 12-O-tetradecanoylphorbol-13-acetate-promoted skin tumour developments in mice. The same authors stated that sarcophine-diol treatment also inhibited A431 cell proliferation as measured by testing the amount of BrdU incorporated to DNA during DNA synthesis. Both inhibition of cell viability and cell proliferation contributed to the overall inhibition of cell growth by sarcophine-diol treatment in A431 cells. The current results agree with that previously reported which indicated that sarcophine has antitumor properties due to its cytotoxic activity on tumour cells [60]. Moreover, it had been reported that terpenes could stimulate apoptosis of cancer cells in culture [61]. In this concern, Zhang *et al.* [60] proved that the treatment with sarcophine-diol resulted in a loss of cell viability by testing cell's mitochondrial metabolic activity using MTT assay.

In the present study, animals treated with sarcophine showed a significant decrease in tumour marker against liver toxicity induced by $CCl_4$ and support the earlier findings which stated that sarcophine and its related compounds inhibited cell proliferation. According to Kasibhatla and Tseng [62], apoptosis or programmed cell death is the physiological process by which unwanted or undesirable cells are eliminated during development and other normal biologic process without causing damage to surrounding tissues. An increasing number of chemopreventive agents have been shown to stimulate apoptosis in premalignant and malignant cells *in vitro* or *in vivo* [63, 64].

## CONCLUSION

Sarcophine treatment showed a significant chemopreventive effect against $CCl_4$ toxicity as indicated by the reduction of biochemical parameters, tumour marker and histological picture of the liver, towards the control levels. This protection may be due to the induction of apoptosis of cancer cells.

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