MODULATORY EFFECT OF THE RED SEA SOFT CORAL EXTRACTS ON HEPATOTOXICITY INDUCED BY CARCINOGENIC AGENTS IN RAT MODEL

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ABSTRACT

Recently, there has been a growing interest in the presence of pharmacologically active components in the aquatic environment. Soft corals are prominent reef organisms in the Red Sea and are prolific sources of terpenoids, especially cembranoid diterpenes. The objective of this study was to investigate the inhibitory effect of the extract from the aqueous MeOH (80%) extract of each of the three Red Sea soft corals: Sinularia polydactyla, Sarcophyton trocheliophorum and Xenia macrospiculata on hepatic toxicity induced by the carcinogenic agents 7,12 dimethyl benz (a) anthracene (DMBA) and 12-O-tetradecanoyl phorbol-13-acetate (TPA) in adult female rats. The results revealed that the liver functions were markedly improved and the levels of tumor markers as well as the inorganic free radical “NO” in serum were significantly decreased as a result of the treatment with three coral extracts. Moreover, treatment of DMBA and TPA-intoxicated groups with the coral extracts resulted in significant reduction in hepatic oxidative stress in concomitant with significant elevation in hepatic SOD activity as compared to the group intoxicated with DMBA and TPA only. Serum estradiol and corticosterone levels were high significantly decreased in the groups intoxicated with DMBA and TPA and treated with the three extracts compared to the group intoxicated with DMBA and TPA only. The soft coral Xenia macrospiculata extract, exerted the highest potential to inhibit hepatotoxicity induced by the tested carcinogenic agents. Each of the soft coral extracts has played a vital role in modulating the severe hepatotoxicity caused by the administration of the two carcinogenic agents DMBA and TPA. The liver functions were significantly promoted beyond normal status, while the hepatotoxic oxidative stress was markedly depressed. These results may provide new concept for development of effective therapies for some diseases involving hepatotoxicity..

Key words: Hepatotoxicity, 7, 12-dimethylbenz (a) anthracene, 12-O-tetradecanoyl phorbol-13 acetate, Soft corals, Sinularia polydactyla, Sarcophyton trocheliophorum, Xenia macrospiculata.

INTRODUCTION

There has been a growing interest in therapeutic applications of pharmacologically active components derived from aquatic organisms. An increasing number of marine natural products, the structures of which are quite different from the terrestrial organisms, have been reported (Faulkner, 1998). Soft corals are prominent reef organisms in the Red Sea and are prolific sources of terpenoids, especially cembranoid diterpenes (Grote et al., 2005). For example, members of the genus Sinularia have been the source of many terpenoids but have also yielded spermidine derivatives (Choi and Schmitz, 1997). Earlier studies showed that physiologically relevant concentrations of diterpenes have a pronounced cytotoxic effect on several human tumor cell lines (Yamauchi et al., 1991). In biological assay systems some cembranoids display significant ichthyotoxic, cytotoxic, anti-inflammatory, and Ca-antagonistic potential (Kobayashi et al., 1983). The active protective diterpenoids, present in soft coral extracts, can reduce the incidence of hepatomas (Yamauchi et al., 1991). The crude extracts of many soft corals seem to contain natural antioxidants that can protect the compounds from oxidation (Groweiss and Kashman, 1983).

A great number of biologically active diterpene derivatives have been isolated from the extracts of several soft corals (Dong et al., 2000; Sheu et al., 2000 and Grote et al., 2005). Sarcophytol A, a cembrane-type diterpene, was isolated from a marine soft coral, Sarcophyton glaucum and proved to have antitumor activity and potent inhibitory activity against the various classes of tumor promoters (Takayanagi et al., 1994). It inhibited the development of spontaneous hepatoma in mice without toxicity. Inhibition of TNF-α release from cells was proposed as a mechanism
of anticarcinogenesis of sarcophytol A (Komori et al., 1993). It also mediated dose-dependent diminution of TPA-induced transformation of JB6 cells and it could also suppress oxidant formation (Wei and Frenkel, 1992). Histological examination showed that sarcophytol A alleviated the TPA-induced inflammatory response and infiltration of phagocytes and neutrophils via its inhibitory effects on tumor promoter-mediated migration and activation of phagocytes, oxidant formation and DNA base oxidation (Wei and Frenkel, 1992).

Xenobiotics such as 7, 12-dimethylbenz (a) anthracene (DMBA) and 12-O-tetradecanoyl phorbol-13-acetate (TPA) generate the reactive oxygen species (ROS) and superoxide anion radical ($^{•}$O$_2^-$) by neutrophils, macrophages and nonphagocytic cells. These powerful oxidizing free radicals are known to play an important role in mutagenesis and carcinogenesis, particularly in tumor promotion (Breiner, 1990). They also overcome the protection afforded by the antioxidant defence mechanism thereby leading to oxidative damage to tissue macromolecules including DNA, proteins and lipids. Oxidative stress has been proven to involve hepatotoxicity, neurotoxicity and nephrotoxicity, and even to result in disease such as carcinogenesis in mammalian systems (Stohs et al., 1995 and Kasprzak, 2002). Either lipid peroxidation or protein carbonyl (PCO) content showed significant positive free radicals ($^{•}$OH) production (Shi et al., 2005). TPA depressed the specific activities of lactate dehydrogenase and gamma glutamyl transpeptidase of a diploid rat liver epithelial cell line (Tsao et al., 1984). Tumor promotion and ROS generation by stimulated neutrophils are often associated with the activation of protein kinase C and /or ornithine decarboxylase (OCD) induction (Bhimani et al., 1993). Moreover, other potent chemotactic factors such as interleukin-1 and interleukin-8 are also formed by TPA-treated cells (Kupper, 1990).

There is a paucity of information concerning the effect of administration of soft coral extracts on the biochemical markers in toxin-induced hepatotoxicity in living organisms. So the authors think it would be of interest to explore the modulatory effect of the aqueous methanol extracts of the three soft corals: Sarcophyton trocheliophorum, Sinularia polydactyla and Xenia macrospiculata on the various biochemical parameters affecting hepatotoxicity in rat model.

**MATERIALS AND METHODS**

1. Chemicals

7,12-dimethylbenz (a) anthracene (DMBA) and 12-O-tetradecanoyl phorbol-13-acetate (TPA) were purchased from Sigma Chemical Co. (St. Luis, MO, USA). Other chemicals and solvents were reagent grades.

2. Corals

The soft corals of *Sarcophyton trocheliophorum*, *Sinularia polydactyla* and *Xenia macrospiculata* were collected from the Gulf of Suez, Egypt, at a depth of 10-15 meter. The corals were identified by Dr. Mohammad, M. Hagazi, Suez Canal University (Paul, 1993).

3. Preparation of coral extracts

Each one of the fresh soft corals of *Sarcophyton trocheliophorum* (1750 g), *Sinularia polydactyla* (3700 g) and *Xenia macrospiculata* (400 g) was blended and exhaustively extracted with aqueous MeOH (80%) for 24 hrs. Then the extracts were evaporated until dryness under reduced pressure (temperature not exceeding 45°C) (Harbore, 1984). *Sarcophyton trocheliophorum* yielded 8.5 g of crude extract (C1), *Sinularia polydactyla* yielded 28 g of crude extract (C2) and *Xenia macrospiculata* yielded 1.9 g of crude extract (C3). Diterpenes were identified as the main component and sterols as minor components in these extracts. Diterpenes were detected according to Liebermann-Burchard test on the basis of their responses towards specific spray reagents, after separation by hexane/ethyl acetate (6:4) and chloroform/methanol (8:2) on thin layer chromatography, Merk type 60 F254 (Egonstahl, 1969).

4. Experimental animals

This study was conducted on eighty adult female Sprague Dawley rats (ten weeks old) weighing 140-150 g. The animals were provided by the Animal House Colony of the National Research Centre, Dokki, Cairo, Egypt. The animals were kept in wire bottomed cages at room temperature (25 ± 2°C) under 12 hrs dark-light cycle. All animals were maintained on the standard laboratory diet consisting of casein 10%, salt mixture 4%, vitamins mixture 1%, corn oil 10%, cellulose 5% completed to 100g with corn starch (AOA.A.C. 1995) and water ad libitum. After an acclimation period of one week, the animals were divided into eight groups, of ten rats each. This experimental study was performed with the approval of the Animal Care and Experimental Committee, National Research Centre, Cairo, Egypt.

5. Experimental design

An amount of 60 mg of either one of the three extracts C1, C2 or C3 was dissolved in 10 ml 3% Tween 80. The groups of animals were treated as follows: Group (1) untreated control group; Groups (2), (3) and (4) were treated intraperitoneally with 20 mg/kg b.wt/day of either one of the three coral extracts C1, C2 or C3 respectively for 10 successive days; Group (5) was treated intraperitoneally with the carcinogenic substances 10 μg of 7, 12-dimethylbenz (a) anthracene (DMBA) dissolved in 0.2 ml acetone per rat, then after thirty minutes, each rat was treated intraperitoneally with 10μg of 12-O-tetradecanoyl phorbol-13-acetate (TPA) dissolved in 0.2 ml acetone for inducing hepatotoxicity; Groups 6, 7 and 8 were treated...
intraperitoneally with the same doses of the carcinogenic substances then after 72 hrs, the animals were treated intraperitoneally with 20 mg /kg B.wt/ day of either (C1), (C2) or (C3) respectively for 10 successive days. The dose of coral extracts was depicted from the literature (Fuji et al., 1989).

At the end of the experimental period, all animals were fasted for 12 hrs, then the blood samples were collected from the retroorbital plexus under light diethyl ether anaesthesia (Schermer, 1967). Blood samples were left to clot and then centrifuged at 3000 r.p.m for 10 min at 4°C where the clear sera were obtained and stored at −20°C until further analysis. After blood collection all animals were rapidly sacrificed and the liver of each animal was dissected, weighed and immediately homogenized in phosphate buffer (pH =7.4) to give 20% w/v homogenate (Lin et al., 1998). Then the homogenate was centrifuged at 1700 r.p.m and 4°C for 10 min. and the supernatant was stored at −70°C until analysis. A portion of this supernatant (20%) was used for the determination of hepatic lipid peroxidation. Another portion of the 20% supernatant was further diluted with phosphate buffer solution to give 0.5% dilution for the determination of hepatic superoxide dismutase activity and protein content of the liver (Manna et al., 2005).

Serum alanine aminotransferase (ALT) activity was determined by kinetic spectroscopy using Randox Laboratories kit, UK, according to the method recommended by The Committee on Enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology (1974). Colorimetric determination of serum bilirubin was carried out by the method described by Sherlock (1951) using kit purchased from Randox Laboratories Co. Serum alpha fetoprotein (AFP) was estimated by immuno enzymatic assay procedure using Dia, Metra s.r.i. kit (Italy) according to the method described by Uotila et al. (1981). Serum carcinoembryonic antigen (CEA) was determined by enzyme linked immunosorbent assay (ELISA) using International Immuno-Diagnostics kit (USA) according to the method of Gold and Freedman (1965). Quantitative determination of tumor necrosis factor-alpha (TNF-α) was done by ELISA procedure according to the method of Corti et al. (1992) using kit produced by Diaclone Research Co. (France). Serum nitrate concentration as a stable end product of nitric oxide was determined photometrically on microtitre plate according to the method of Moshage et al. (1995) using kit produced by R & D system GmbH (Germany). Serum interleukin-1 alpha (IL-1α) was measured by ELISA procedure of Krakauer and Krakauer (1991) using Cytimmune Sciences Co.kit, (USA). Hepatic lipid peroxidation was estimated by a spectrophotometric method according to Ruiz- Larrea et al. (1994). The level of lipid peroxides was expressed as nmol/mg protein. The protein content in liver tissue was measured by applying the method of Lowry et al. (1951). Hepatic superoxide dismutase activity was assayed spectrophotometrically by the red formazan dye reduction procedure (Suttle, 1986) with 50 μl diluted liver homogenate using a Ransod kit from Randox Laboratories Co., UK. The specific activity of hepatic superoxide dismutase was expressed as units/mg protein. Serum estradiol was determined by enzyme linked immunosorbent assay according to the method of Ratcliffe et al. (1988) using kit produced by Biochem ImmunoSystem Co. (Italy). Serum corticosterone was estimated by enzyme linked immunosorbent assay technique according to the method described by Blake-Tyrrell and Forsham (1985) using kit produced by Adaltis Italia S.P.A.

**Statistical analysis**

The results were expressed as mean ± SE. Statistical differences between means were carried out using Student “t” test (Snedecor and Cochran, 1967).

**RESULTS**

Treatment of the non-toxicated rats (groups 2, 3 and 4) with either one of the three extracts of the soft corals *Sarcophyton trocheliophorum* C1, *Simularia polydactyla* C2 and *Xenia macrospiculata* C3 resulted in significant decrease in serum ALT and bilirubin levels in groups (2) (P<0.05) and (4) (P<0.01), and nonsignificant decrease (P>0.05) in both parameters in group (3) as compared to the control group (1) (Table 1). In general, the intoxicated group (5) displayed highly significant (P<0.01) decrease in each of serum tumor markers AFP and CEA as compared to the control group (1) (Table 5). In addition, the serum estradiol and corticosterone levels were nonsignificantly decreased (P≥0.05) in groups (2), (3) and (4) as compared to the control group (1) (Table 4). The rate of lipid peroxidation (LPO) in groups (2), (3) and (4) were nonsignificantly decreased (P>0.05), while the hepatic SOD activity was nonsignificantly increased in groups (2) and (3) but significantly increased (P<0.05) in group (4) as compared to the control group (1) (Table 4).

**On the other hand, treatment of rats in groups (6), (7) and (8) which were intoxicated with the carcinogenic agents DMBA and TPA, with either one of the three soft coral extracts C1, C2 or C3 resulted in highly significant decrease (P<0.01) in the serum ALT and bilirubin levels as compared to the intoxicated group (5) (Table 1). In general, high significant decrease (P<0.01) was observed in each of serum tumor markers AFP and CEA levels as compared to the intoxicated group (5) (Table 2). High significant decrease (P<0.01) in serum TNF-α level was observed in groups (6) and (8) while significant decrease (P<0.05) was observed in group (7). Serum IL-1α and NO levels
Table (1): Modulatory action of coral extracts on liver function of DMBA and TPA-intoxicated female rats.

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>ALT (U/L) Mean ± SE</th>
<th>Bilirubin (mg/dl) Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group (1) control</td>
<td>32.1 ± 1.7</td>
<td>0.74 ± 0.05</td>
</tr>
<tr>
<td>Group (2) C1</td>
<td>26.7 ± 1.2</td>
<td>0.60 ± 0.04</td>
</tr>
<tr>
<td>Group (3) C2</td>
<td>27.1 ± 2.3&lt;sup&gt;(00)&lt;/sup&gt;</td>
<td>0.69 ± 0.07&lt;sup&gt;(00)&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group (4) C3</td>
<td>22.1 ± 1.5&lt;sup&gt;**&lt;/sup&gt;</td>
<td>0.57 ± 0.03&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>Intoxicated group (5)</td>
<td>65.8 ± 4.2&lt;sup&gt;**&lt;/sup&gt;</td>
<td>1.87 ± 0.10&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>Intoxicated group (6) + C1</td>
<td>44.5 ± 2.3&lt;sup&gt;**&lt;/sup&gt;</td>
<td>1.25 ± 0.13&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>Intoxicated group (7) + C2</td>
<td>48.2 ± 3.5&lt;sup&gt;**&lt;/sup&gt;</td>
<td>1.43 ± 0.18&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>Intoxicated group (8) + C3</td>
<td>39.7 ± 2.1&lt;sup&gt;**&lt;/sup&gt;</td>
<td>1.10 ± 0.14&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

- Sample size (n) = 10 rats
- ALT: Alanine aminotransferase.
- ± Compared with the control group (1).
- ‡ Compared with the intoxicated group (5).
- (ns) Differences are insignificant at P < 0.05.
- * P <0.05  * * P<0.01.

Table (2): Modulatory action of coral extracts on tumor markers of DMBA and TPA-intoxicated female rats.

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>AFP (ng/ml) Mean ± SE</th>
<th>CEA (ng/ml) Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group (1) control</td>
<td>1.56 ± 0.48</td>
<td>3.65 ± 0.27</td>
</tr>
<tr>
<td>Group (2) C1</td>
<td>1.51 ± 0.12&lt;sup&gt;(00)&lt;/sup&gt;</td>
<td>3.48 ± 0.30&lt;sup&gt;(00)&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group (3) C2</td>
<td>1.56 ± 0.16&lt;sup&gt;(00)&lt;/sup&gt;</td>
<td>3.55 ± 0.27&lt;sup&gt;(00)&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group (4) C3</td>
<td>1.48 ± 0.22&lt;sup&gt;(00)&lt;/sup&gt;</td>
<td>3.32 ± 0.23&lt;sup&gt;(00)&lt;/sup&gt;</td>
</tr>
<tr>
<td>Intoxicated group (5)</td>
<td>4.88 ± 0.60&lt;sup&gt;**&lt;/sup&gt;</td>
<td>8.15 ± 0.61&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>Intoxicated group (6) + C1</td>
<td>2.72 ± 0.32&lt;sup&gt;**&lt;/sup&gt;</td>
<td>4.99 ± 0.26&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>Intoxicated group (7) + C2</td>
<td>3.12 ± 0.35&lt;sup&gt;**&lt;/sup&gt;</td>
<td>6.12 ± 0.42&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>Intoxicated group (8) + C3</td>
<td>2.60 ± 0.20&lt;sup&gt;**&lt;/sup&gt;</td>
<td>4.76 ± 0.15&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

- Sample size (n) = 10 rats
- AFP: Alpha fetoprotein.
- CEA: Carcinoembryonic antigen.
- ± Compared with the control group (1).
- ‡ Compared with the intoxicated group (5).
- (ns) Differences are insignificant at P ≥ 0.05.
- * P <0.05  * * P<0.01.

Table (3): Modulatory action of coral extracts on inflammatory promoters of DMBA and TPA-intoxicated female rats.

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>TNF-α (Pg/ml) Mean ± SE</th>
<th>NO (μmol/L) Mean ± SE</th>
<th>IL-1α (ng/ml) Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group (1) control</td>
<td>55.88 ± 1.7</td>
<td>35.5 ± 3.9</td>
<td>0.60 ± 0.05</td>
</tr>
<tr>
<td>Group (2) C1</td>
<td>53.78 ± 1.4&lt;sup&gt;(00)&lt;/sup&gt;</td>
<td>33.9 ± 3.6&lt;sup&gt;(00)&lt;/sup&gt;</td>
<td>0.58 ± 0.06&lt;sup&gt;(00)&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group (3) C2</td>
<td>54.82 ± 1.2&lt;sup&gt;(00)&lt;/sup&gt;</td>
<td>35.2 ± 3.4&lt;sup&gt;(00)&lt;/sup&gt;</td>
<td>0.60 ± 0.07&lt;sup&gt;(00)&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group (4) C3</td>
<td>52.12 ± 1.1&lt;sup&gt;(00)&lt;/sup&gt;</td>
<td>33.2 ± 3.1&lt;sup&gt;(00)&lt;/sup&gt;</td>
<td>0.55 ± 0.05&lt;sup&gt;(00)&lt;/sup&gt;</td>
</tr>
<tr>
<td>Intoxicated group (5)</td>
<td>86.36 ± 4.2&lt;sup&gt;**&lt;/sup&gt;</td>
<td>63.9 ± 4.8&lt;sup&gt;**&lt;/sup&gt;</td>
<td>1.66± 0.16&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>Intoxicated group (6) + C1</td>
<td>65.62 ± 3.9&lt;sup&gt;**&lt;/sup&gt;</td>
<td>48.8 ± 3.8&lt;sup&gt;**&lt;/sup&gt;</td>
<td>1.25 ± 0.11&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>Intoxicated group (7) + C2</td>
<td>71.70 ± 4.2&lt;sup&gt;**&lt;/sup&gt;</td>
<td>53.5 ± 4.1&lt;sup&gt;**&lt;/sup&gt;</td>
<td>1.32± 0.13&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>Intoxicated group (8) + C3</td>
<td>61.51 ± 3.3&lt;sup&gt;**&lt;/sup&gt;</td>
<td>43.3 ± 3.4&lt;sup&gt;**&lt;/sup&gt;</td>
<td>1.15 ± 0.07&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

- Sample size (n) = 10 rats
- TNF-α: Tumor necrosis factor alpha
- NO: Nitric oxide
- IL-1α: Interleukin-1 alpha.
- ± Compared with the control group (1).
- ‡ Compared with the intoxicated group (5).
- (ns) Differences are insignificant at P ≥ 0.05.
- * P <0.05  * * P<0.01.
Table (4) : Modulatory action of coral extracts on hepatic oxidative stress for DMBA and TPA-intoxicated female rats.

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Lipid peroxidation (nmol/mg protein) Mean ± SE</th>
<th>SOD (U/mg protein) Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group (1) control</td>
<td>18.64 ± 0.83</td>
<td>104.6 ± 5.2</td>
</tr>
<tr>
<td>Group (2) C1</td>
<td>18.24 ± 0.77†(ns)</td>
<td>116.2 ± 4.8†(ns)</td>
</tr>
<tr>
<td>Group (3) C2</td>
<td>18.48 ± 0.82†(ns)</td>
<td>110.6 ± 3.9†(ns)</td>
</tr>
<tr>
<td>Group (4) C3</td>
<td>17.68 ± 0.52†(ns)</td>
<td>120.8 ± 5.4†</td>
</tr>
<tr>
<td>Intoxicated group (5)</td>
<td>23.55 ± 1.12**</td>
<td>65.3 ± 3.4**</td>
</tr>
<tr>
<td>Intoxicated group (6) + C1</td>
<td>20.42 ± 0.69‡</td>
<td>84.9 ± 5.5‡</td>
</tr>
<tr>
<td>Intoxicated group (7) + C2</td>
<td>20.93± 0.78‡(ns)</td>
<td>80.3 ± 4.2‡</td>
</tr>
<tr>
<td>Intoxicated group (8) + C3</td>
<td>19.68 ± 0.73‡**</td>
<td>92.5 ± 7.2‡**</td>
</tr>
</tbody>
</table>

- Sample size (n) = 10 rats
- † Compared with the control group (1).
- ‡ Compared with the intoxicated group (5).
- (ns) Differences are insignificant at P ≥ 0.05.
- * P <0.05
- ** P<0.01.

Table (5) : Modulatory action of coral extracts on serum estradiol and corticosterone levels for DMBA and TPA-intoxicated female rats.

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Estradiol (Pg/ml) Mean ± SE</th>
<th>Corticosterone (ng/ml) Mean ± SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group (1) control</td>
<td>21.8± 0.68</td>
<td>12.5 ± 1.1</td>
</tr>
<tr>
<td>Group (2) C1</td>
<td>21.0 ± 1.10†(ns)</td>
<td>12.1 ± 1.2†(ns)</td>
</tr>
<tr>
<td>Group (3) C2</td>
<td>21.5 ± 0.87†(ns)</td>
<td>12.4 ± 0.94†(ns)</td>
</tr>
<tr>
<td>Group (4) C3</td>
<td>20.1 ± 0.20†(ns)</td>
<td>11.9 ± 1.5†(ns)</td>
</tr>
<tr>
<td>Intoxicated group (5)</td>
<td>32.1 ± 0.63**</td>
<td>19.8 ± 1.5**</td>
</tr>
<tr>
<td>Intoxicated group (6) + C1</td>
<td>26.3 ± 1.31‡**</td>
<td>14.2 ± 0.97‡**</td>
</tr>
<tr>
<td>Intoxicated group (7) + C2</td>
<td>27.0 ± 1.15‡**</td>
<td>15.5 ± 1.2‡</td>
</tr>
<tr>
<td>Intoxicated group (8) + C3</td>
<td>24.4 ± 1.16‡**</td>
<td>13.7 ± 0.82‡**</td>
</tr>
</tbody>
</table>

- Sample size (n) = 10 rats
- † Compared with the control group (1).
- ‡ Compared with the intoxicated group (5).
- (ns) Differences are insignificant at P ≥ 0.05.
- * P <0.05
- ** P<0.01.

DISCUSSION

Recently, interest of natural product research has actively moved to marine organisms. As a result, almost 50% of reported natural cytotoxic compounds were isolated from marine organisms such as soft corals and sponges (Kim & Park, 2002).

Terpenoids, including sesquiterpenes, cembrane, norcembrane, flexibilene, cladillane and lobane diterpenoids and steroids compose the main secondary metabolites isolated from the genus Sinularia (Kobayashi, 1994 and Coll et al., 1998). Specimens belonging to the Xenia genus have proved to be a rich source of a class of diterpenes known as xenicans (Anta et al., 2002). Two cytotoxic cembrane diterpenes, isosarcophytoxide and isosarcophene were isolated from the soft coral Sarcophyton trocheliophorum (Wu et al., 1992), whereas, two cembranoids : sarcophytol A and B were isolated from the Red Sea soft coral Sarcophyton glaucum (El-Sayed et al., 1998). Many biological activities were reported for these metabolites including: cytotoxicity, enhancement of glucose transport in rat adipocytes, histamine- release inhibition and also inhibited the development of spontaneous hepatomas (Shindo et al., 1992; Yokomatsu et al., 1994 and Shoji et al., 1994).
In this study, we report on the use of selected serum markers for assessment of acute liver cell damage and regeneration in adult female rats with exposure to the carcinogenic agents (DMBA) and (TPA). Several authors have expressed the adverse effects of these toxins on the liver (Goerttler et al., 1981 and Bagchi et al., 1998). Tsao et al. (1984) reported that TPA depressed the specific activity of lactate dehydrogenase and gamma glutamyl transpeptidase of a diploid rat liver epithelial cell line, but rather caused remarkable proliferation of rat AH 66 hepatoma cells as a result of alterations in the functions of cell membrane (Kaneko et al., 1980). Wei and Frenkel (1992) found that TPA induced inflammation and infiltration of phagocytes and transformation of JB6 cells. Recently, Shi et al. (2005) suggested that TPA may induce free radicals generation by inhibiting the electron transfer chain or by removing the iron from cell membrane, which overcome the protection afforded by the antioxidant defense mechanism, thereby leading to oxidative damage to tissue macromolecules including DNA, proteins and lipids. Bhimani et al. (1993) reported that tumor promotion and ROS generation are often associated with the activation of protein tyrosine kinase (PTK) and induction of ornithine decarboxylase (OCD) (Randi et al., 2003). Koppel (1993) suggested that toxins exert their effect on the hepatocyte through binding to and inhibition of RNA polymerase. This action markedly decreases DNA transcription, which results in loss of cellular integrity, function and ultimately cell death.

Actually, the authors hypothesize that both carcinogenic agents DMBA and TPA are metabolized by the liver cytochrome P-450 system to produce toxin intermediates associated with the generation of reactive oxygen species (ROS). ROS can be converted in mammalian cells, via the Fenton reaction using iron or copper, into free hydroxyl radical (•OH), the most highly oxidative species among the ROS. This reactive •OH forms adducts with DNA leading to mutagenesis and carcinogenesis. One of the most prevalent adducts formed by the adherence of •OH to DNA bases is 8- hydroxy-2- deoxyguanosine (8-OHdG) (Floyd, 1990). Bhimani et al. (1993) reported that TPA treatment caused oxidative stress in Hela cells, and caused 7-fold increase in H2O2 levels, 5-10 fold increase in 8-OHdG and 5- hydroxymethyl-2`- deoxyuridine (HMdU). As metabolism proceeds and the toxic intermediates accumulate in the hepatic parenchyma, hepatocytic damage occurs with the release of bilirubin and the aminotransphrases ALT and AST. Moreover, accumulation of polymorph nuclear leukocytes (PMN) in the liver after exposure to endotoxins stimulated superoxide anion (•O2) generation (Mayer and Spitzer, 1993).

Administration of DMBA and TPA to female rats in group (5) resulted in highly significant increase in both serum ALT and bilirubin levels as compared to the control group (1) (Table 1) and are released in the blood when plasma membranes of hepatic cells are damaged (Chairello et al., 1998). Elevation in serum levels of these two parameters correlates with hepatocyte damage and necrosis (Nakao et al., 1996).

Highly significant increase in the serum tumor markers alpha fetoprotein (AFP) and carcinoembryonic antigen (CEA) levels was recorded for group (5) as compared to the control group (1) (Table 2). AFP is a typical-onco-developmental protein, mainly synthesized in fetal life. Nevertheless, AFP synthesis may commence again when some adult cells start carcinogenesis (Gitlin and Boseman, 1976). AFP has been utilized as prognostic marker and has been correlated with clinical outcomes following fulminant liver failure (Nakao et al. 1996). Kaneko et al. (1980) reported that TPA as well as epidermal growth factor (EGF) caused remarkable proliferation of rat AH66 hepatoma cells and also caused 2-fold increase in the production of AFP and other acid perceptible materials. However, elevated serum levels of AFP and the intracellular protein CEA predicted an increased risk of hepatoma. In addition, highly significant increase (P<0.01) in serum inflammatory promoters TNF-α, IL-1α and NO levels was observed in group (5) as compared to the control group (1) (Table 3). TNF-α, a proinflammatory cytokine, is normally released from activated macrophages, monocytes, mast cells and many other cells in immunological inflammatory responses (Takaki et al., 2003). Unregulated TNF-α release plays pivotal roles in chronic inflammatory diseases including: cystic fibrosis, asthma and multiple sclerosis (Bjorsdottir and Cypcar, 1999). A significant positive correlation between serum TNF-α and NO levels was observed. Increased production of TNF-α results in coagulation, thrombosis and local infarcts compromising local tissue perfusion and massive liver damage (Leist et al., 1995). The inorganic free radical NO has been produced in LPS- activated macrophages (Takaki et al., 2003) and it is liable to react with superoxide anion radical (•O2) to form peroxynitrite (Radi et al., 1991) which caused protein nitration with further tissue injury when decomposed to the more powerful oxidant •OH (Billiar, 1995). Increased oxidative stress on the hepatocytes depletes antioxidant activity and consequently induces overproduction of NO that promotes lipid peroxidation.

Table (4) group (5) shows highly significant increase in lipid peroxidation level with concomitant highly significant decrease in the antioxidant activity of superoxide dismutase (SOD) as compared to control group (1). TPA is known to cause a marked decrease in the activities of antioxidant enzymes such as SOD and catalase (Solenki et al., 1981 and Reiners et al., 1990). The authors postulate that the metabolites of both DMBA and TPA may be inactivated by binding to and depleting hepatic stores of the antioxidant defense system. Enhanced generation of reactive oxygen species (ROS) and impairment of cellular and extracellular antioxidant system potential are correlated with incidence of lipid peroxidation.

Highly significant rise in both serum estradiol and corticosterone levels was also observed in group (5) as
compared to the control (group 1) (Table 5). Increased serum levels of these two hormones raise the risk of developing breast cancer, endometrial cancer, stroke, heart disease and blood clots. Murphy and Malley (1969) reported that endogenous corticosterone secretion occurs during acute toxic liver injury in rats. Inflammatory processes stimulate endogenous corticosterone secretion by activating the hypothalamic-pituitary-adrenal axis (Swain et al., 1999).

However, in order to explore the modulatory effect of soft coral extracts on toxin-induced hepatic injury in adult female rats, we firstly assessed the direct effect of each of these extracts on normal rat's liver functions (groups 2, 3 and 4). No marked adverse action of these extracts on the rat's liver could be detected. Only high significant decrease in serum ALT and bilirubin levels, and significant increase in serum SOD level were observed in group (4) which was treated with the extract from the soft coral Xenia macrospiculata C3 as compared to the control group (1) (Tables 1 and 4). Nonetheless, nonsignificant changes in the biochemical markers under test could be detected in groups (2, 3 and 4) as compared to the control group (1) (Tables 1-5).

Fortunately, treatment of the intoxicated female rats (groups 6, 7 and 8) with either one of the three soft coral extracts C6, C7 or C8, which are highly enriched in the bioactive terpenoids (Grote et al., 2005) engendered striking positive outcomes.

Highly significant drop in both serum ALT and bilirubin levels was observed for the three groups (6, 7 and 8) as compared to the intoxicated group (5) (Table 1). Handa and Sharma (1990) reported that treatment of rats with an active diterpenoid before galactosamine administration led to complete normalization of toxin-induced increase in serum ALT, AST, ALP and bilirubin levels and also hepatic triglycerides, and significantly ameliorated toxin-induced histopathological change in the livers of experimental rats.

Highly significant decrease in serum AFP and CEA levels was obtained for the two groups (6 and 8) and significant decrease in group (7) as compared to the intoxicated group (5) (Table 2). Serum CEA levels may be elevated in some malignant and benign conditions of the gastrointestinal tract and predicts an increased risk of carcinogenesis.

Marked decrease in each of serum TNF-α, IL-1α and NO level was recorded for the three groups (6, 7 and 8) as compared to the intoxicated group (5) (Table 3). However, treatment with the soft coral Xenia macrospiculata extracts C, in particular, has resulted in highly significant drop in the serum levels of these three inflammatory promoter markers in group (8) (Table 3). There have been several reports that TNF-α stimulates growth of human cancer cells and metastasis of sarcoma cells (Orosz et al., 1993). Therefore, the active terpenoids in soft corals which can inhibit TNF-α mRNA expression and TNF-α release may be effective not only in cancer prevention but also in the treatment of diseases related to TNF-α (Suganuma et al., 1996). Takaki et al. (2003) reported that an ethanol extract of Sinul aria sp exhibited an inhibitory effect on NO production not influenced by cytotoxicity. They also added that two norditerpenes isolated from Sinul aria sp inhibited LPS – activated NO production, and also inhibited TNF-α production in a dose-dependent manner.

Marked drop in lipid peroxidation with concomitant marked increase in the antioxidant SOD activity was achieved for the three intoxicated groups (6, 7 and 8) after treatment with either one of the three extracts C6, C7 or C8 as compared to the intoxicated group (5) (Table 4). Actually, group 8, treated with the extract C8, particularly exhibited highly significant drop in LPO and highly significant rise in SOD activity (Table 4). SOD and catalase are important antioxidant enzymes and usually used as biomarkers to indicate ROS production (Oast et al., 2003 and Zhang et al., 2004).

The three extracts C6, C7 and C8 have played a pivotal role in attenuating the severe oxidative stress induced by the carcinogenic agents DMBA and TPA. Kapil et al. (1993) reported that the greater protective activity of diterpenes against lipid peroxidation, glutathione (GSH) depletion and enzymatic release of ALT could be due to their glucoside groups which may act as strong antioxidants, thus inhibiting infiltration of phagocytic cells and ROS generation (Wei and Frenkel, 1992).

Highly significant decrease in both serum hormone estradiol and corticosterone levels was observed for each one of the three intoxicated groups (6, 7 and 8) as compared to the intoxicated group (5) (Table 5). In the current study we have outlined that a striking increase in serum corticosterone levels that accompanies the hepatic inflammatory response due to DMBA and TPA administration.

High levels of estradiol may be linked with greater risks for some diseases including breast cancer, endometrial hyperplasia, coronary vascular disease and thromboembolism and may also stimulate liver proteins such as sex hormone binding proteins (SHBP) and liver protein synthesis such as corticosteroid binding-globulin (Régine Sitruk-Ware, 1990).

We postulate that the mechanisms of action of the terpenoids in the soft coral extracts are closely related to the inhibition of ROS generation and oxidative DNA damage. This inhibition of ROS accumulation might be correlated with three pathways, mainly: 1- Counteracting the TPA-induced decline in the levels of antioxidant defence enzymes, 2-Interfering with the ROS-generating pathways in target cells (Robertson et al., 1990), 3-Subsequent blocking of infiltration and activation of polymorphonuclear leukocytes (PMS’s) (Frenkel, 1992).

Bhimani et al. (1993) tested sarcophytol A, a cembrane-type diterpene isolated from the soft coral Sarcophyton glaucum, as potential chemoprotective agent which dose-
dependently inhibited TPA- induced H$_2$O$_2$, 8-OHdG, 5-HMdU and also inhibited the development of spontaneous hepatomas in mice without toxicity (Yamauchi et al., 1991), and that it should be considered as a possible cancer chemoprotective agent for hepatomas in humans.

Coral extracts hold the promise of being useful chemoprotective agents once their toxicity to human is proved to be negligible, especially since they can be so effective at low doses (Takayanagi et al., 1994). On the whole, this study highlights the need to include the many overlooked marine organisms such as soft corals and sponges in ongoing and planned research on development of health-promoting diets. It seems plausible to establish whether we can incorporate certain coral extracts into our regular diet to prevent the occurrence of hepatoma and cancers. The authors appeal to find ways of breeding coral reefs and of changing the way that they are currently used to verify and maintain their viability.

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