ANTICARCINOGENIC ASSESSMENT OF MORINGA OLEIFERA AND ITS ISOLATED SAPONIN IN ATTENUATION OF 7, 12-DIMETHYLBENZ[A]ANTHRACENE INDUCED HEPATIC CARCINOGENESIS

Veena Sharma*, Ritu Paliwal and Lokendra Singh*

Department of Bioscience and Biotechnology, Banasthali University, Banasthali-304022, Rajasthan, India
*Department of Botany, Meerut College Meerut
E-mail: drvshs@gmail.com, paliwal.ritu18@gmail.com

Abstract: The present investigation was carried out to elucidate anticarcinogenic potential of hydro-ethanolic extract of M. oleifera (MOHE) and its isolated saponin (SM) in attenuation of 7, 12-dimethylbenz[a]anthracene (DMBA) induced hepatocarcinogenesis in male mice. Single oral administration of DMBA (15 mg/kg) to mice resulted in elevated levels of xenobiotic enzymes, hepatic malondialdehyde, with reduction in hepatic glutathione content, superoxide dismutase, catalase and phase-II metabolizing enzymes such as glutathione-S-transferase. The status of hepatic biochemical markers and total protein content were also found to be decreased along with increase in total cholesterol in DMBA administered mice. Pretreatment with the Moringa oleifera and its isolated saponin orally for 21 days offered almost complete protection against DMBA induced tissue toxicity. The current investigation supports Moringa oleifera and its isolated saponin as a potential chemopreventive agent and suppresses DMBA-induced hepatic carcinogenesis in mice that might be due to decreased free radical generation.

Keywords: Moringa oleifera; 7, 12 dimethyl benz[a]anthracene, Hepatocarcinogenesis; Saponin, Xenobiotic, Mice

INTRODUCTION

Hepatocellular carcinoma (HCC) is the sixth most common cancer and the third leading cause of cancer mortality worldwide (Parkin et al., 2002). The majority of HCC cases are attributable to under lying infections caused by hepatitis B and C viruses. However, several other risk factors, including alcohol consumption, obesity, iron overload, environmental pollutants (PAHs), as well as several dietary carcinogens, such as aflatoxins and nitrosamines, have been shown to be involved in its etiology (Paraskevi and Ronald, 2006). Currently, there is no proven effective systemic hemotherapy for HCC. Considering the limited treatment and grave prognosis of liver cancer, chemoprevention has been considered to be the best strategy in lowering the current morbidity and mortality associated with this disease (Yates and Kensler, 2007).

Accordingly, many new classes of chemical compounds are being evaluated in clinical trials as cancer preventive agents for several malignancies. In this regard, many compounds have been tested with proved efficacy against experimentally-induced hepatocarcinogenesis. The polycyclic aromatic hydrocarbons (PAHs) are reasonably anticipated to be human carcinogens based on sufficient evidence of carcinogenicity in experimental animals (IARC 1983, 1987). The PAH 7, 12-dimethyl benz [a] anthracene (DMBA) is well known as cytotoxic, carcinogenic, mutagenic and immunosuppressive agent. DMBA is known to induce damage in many enzymes involved in DNA repair and is normally used to induce liver cancer in experimental animal models (Sharma et al., 2012a, b).

Identification of a successful hepatoprotective agent will provide a useful tool for the treatment of hepatic diseases. Medicinal plants, since time immemorial have been in use for treatment of various diseases all over the world (Satyanarayana et al., 1969). Moringa oleifera Lam (Syn Moringa pterygosperma Gaertn) is well known for its different therapeutic uses. It possesses antitumor, antiinflammatory, antispasmodic, diuretic, antihypertensive, cholesterol lowering, antioxidant and antiulcer, renal, antidiabetic, cardiac glycosides in the Moringa oleifera pods (Paliwal et al., 2011d; Sharma et al., 2011). Saponins exhibited a range of biological activities. Recently, cytotoxicity and antitumor activities of saponins have also been intensively investigated. This is reflected by numerous reports on the highly cytotoxic properties of saponins in vitro against a variety of human tumor cell lines (Sparg et al., 2004). Saponins, by virtue of their multiple apoptotic actions on cancer cells, may provide a new line of anticancer agents. To date, over hundreds of saponins have been described. However, given the diverse distribution of saponins, it can be conceived that a lot of novel anticancer saponins remain unexploited. To the best of knowledge, till date no scientific data available to validate the folkloric anticancer claims of this isolated saponin from Moringa oleifera.

Hence, the aim of this current investigation was to explore the chemopreventive effects of the hydroethanolic pods extract of Moringa oleifera and effect of isolated saponin against DMBA induced intoxication in liver of male mice.
MATERIAL AND METHOD

Chemicals: All chemicals used in the study were of analytical reagent grade and of highest quality available and were purchased from reliable firms. DMBA was purchased from SIGMA chemical Co. (USA).

Procurement of experimental plant: The experimental plant *Moringa oleifera* was collected from Krishi Vigyan Kendra, Banasthali University, Banasthali, India, in the month of October 2009. The plant material was taxonomically identified by Botanist of Krishi Vigyan Kendra, Banasthali, Tonk district.

Preparation of hydro-ethanolic plant extract (MOHE): For preparation of hydro-ethanolic extract, dried powdered pods were placed in the Soxhlet thimble with 80% ethanol in 250 ml flat bottom flask. Collected solvent was cooled at room temperature and poured in a glass plate. The extract was concentrated under vacuum at 40 °C to yield a semisolid mass, dried in hot air oven below 50 °C for 48 hours and stored in a desiccator. The percentage yield of extract (MOHE) was found to be 22% and stored at 4 °C in airtight containers.

Isolation and characterization of saponin: Sequential extraction of plant material was performed using solvents (polar to non-polar) that is pet ether, benzene, chloroform, ethyl acetate than ethanol for 16 h in soxhelet apparatus. The extracts were than concentrated on a rotary evaporator below 50 °C and were stored in air-tight containers in cold room or at 4 °C temperature for further studies. Thin layer chromatography (TLC) study was carried out for different extracts. The solvent system for isolation of saponin used was: chloroform: methanol: H₂O (7:3:1). Characterization of isolated saponin having R₅ of 0.90 was done using HPLC, IR and •H NMR. The isolated compound was nomenclatures as SM (isolated saponin from *Moringa oleifera* pods) then assessed for its anticarcinogenic potential.

Animal care and monitoring: Male Swiss Albino mice (*Mus musculus*) weighing approximately 15 to 30 g were procured from Haryana Agricultural University, Hisar (Haryana, India). Animals were maintained under standard laboratory conditions at a temperature of (22 ± 3) °C, relative humidity of 50 ± 5% and photoperiod of 12 h (12 h dark and 12 h light cycle). They were housed in polypropylene cages throughout the experiment and were provided standard food pellet (Hindustan Lever Ltd.) and drinking water *ad libitum*. Experiments described in the present study were approved by the Institutional Animal Ethical Committee (IAEC) of Banasthali University, Rajasthan (CPCSEA Reg. No: IAEC/814 dated. 23/01/2010).

Procedure and design of experiments: Adult male Swiss albino mice (*Mus musculus* L.) were used for various parameters (72 mice; six in each group). Treatment consisted of pretreatment phase of MOHE, BHA and SM followed by the second phase in which the animals were given 15 mg/kg DMBA on day 22. The animals were sacrificed 10th day after DMBA administration. The groups were as follows:

- **Group 1** served as control (normal untreated mice), and received 1ml distilled water daily by oral gavage.
- **Group 2** received distilled water for 21 days prior to a single dose of DMBA (15 mg/kg body weight: p.o) served as DMBA control group.
- **Group 3 and 4** were administered with hydro-ethanolic extract of MOHE pods (200 and 400 mg/kg body weight: p.o) daily for 21 days, served as MOHE treated control group.
- **Group 5 and 6** received BHA (0.5 % and 1%: p.o) daily for 21 days, dissolved in 0.5% acetone and served as standard treated control group.
- **Group 7** was administered with isolated saponin component of *Moringa oleifera* pods (SM; 50 mg/kg body weight: p.o) daily for 21 days, served as SM control group.
- **Group 8 and 9** were treated with hydro-ethanolic extract of MOHE pods (200 and 400 mg/kg body weight: p.o) daily for 21 days, before being intoxicated with DMBA (15 mg/kg body weight; p.o, once) dissolved in olive oil for 10 days.
- **Group 10 and 11** received BHA (0.5 % and 1%: p.o) daily for 21 days, before being intoxicated with DMBA (15 mg/kg body weight; p.o, once) dissolved in olive oil for 10 days.
- **Group 12** received isolated saponin component of *Moringa oleifera* pods (SM; 50 mg/kg body weight: p.o), before being intoxicated with DMBA (15 mg/kg body weight; p.o, once) dissolved in olive oil for 10 days.

After 31 days of duration the mice were fasted overnight and then sacrificed under light ether anesthesia. Liver lobules were dissected out, washed immediately with ice-cold saline to remove blood, and the wet weight was noted and then stored at -80 °C for various oxidative stress and biochemical assays. Post-mitochondrial supernatant (PMS) was prepared using method of Mohandas et al (1984) with some modifications. The percentage change was calculated for all the parameters by using formula:

\[
\text{% Change} = \frac{\text{Test} - \text{Control}}{\text{Control}} \times 100
\]

**Determination of xenobiotic enzymes:** Cytochrome P450 and cytochrome b5 content were assayed in the PMS suspension by the method of Omura and Sato, 1964, using an absorption coefficient of 91 and 185 cm⁻¹ M⁻¹ l⁻¹, respectively.

**Oxidative stress, enzymatic and non-enzymatic variables:** The various oxidative stress variables viz., lipid peroxidation (LPO) by the method of Ohkawa et al. (1979), superoxide dismutase (SOD) by Marklund and Marklund (1974) and Catalase by the method of Abei (1983) were performed. The non enzymatic and enzymatic variables viz., reduced glutathione (GSH) content was assayed by the method of Jollow et al. (1974) and glutathione-S-
transferase (GST) was determined by method of Habig et al. (1974).

**Determination of hepatic damage marker enzymes and biochemical assays:** The activities of various biochemical parameters like aspartate and alanine transaminase (AST and ALT) were assayed by the method of Reitman and Frankel (1979). Activities of alkaline phosphatase (ALT) were determined according to the protocol described in laboratory manual (Sadashivam and Manickam, 2004). Total protein content was estimated by the method of Lowry et al. (1951) using bovine serum albumin as standard. Total cholesterol level was determined by using cholesterol as standard by the method of Zak’s (1977).

**Statistical analysis:** The experimental results obtained were expressed as mean ± standard error of mean (S.E.M). The data was subjected to one-way analysis of variance (ANOVA) and differences between samples were determined by Tukey multiple comparison test using the SPSS 16.0 (Statistical program for Social Sciences) program. The level of significance was set at p<0.05.

**RESULT**

**Xenobiotic drug metabolizing (phase I) enzyme activities:** The components of cytochrome P450 and b5 system were determined in tissue fraction of liver. Table 1 shows significant (p<0.001) elevation in specific activity of cytochrome P450 by 360.95 % in their contents in DMBA treated group. Cytochrome b5 also showed a significant (p<0.001) increase (225.43 %) in their contents in DMBA treated group as compared to control group (group 1).

Oral administrations of plant extract, BHA (at both the dose) and isolated saponin (SM) during 21 days treatment significantly increased (p<0.001) the activity of cytochrome P450 and b5 in hepatic tissue as compared to control group. The specific activity of both the cytochrome was recovered significantly (p<0.001) by the oral intake of MOHE, SM and BHA at both the dose before DMBA challenge as compared to DMBA treated group. The extract showed a dose dependent study, in which the activity of high dose was found to be more effective in comparison to lower dose.

**Hepatic lipid peroxidation (TBARS) levels**

Table 2 shows the activity of TBARS (Thio barbituric acid reactive substrate) in liver of mice. Tabulated data reveals a tremendous increase in the concentration of TBARS in hepatic tissue (78.71 %) of DMBA treated group (p<0.001) as compared to control group.

Pre-administration of MOHE, BHA (at both dose) and SM before DMBA challenge resulted in significant reduction (p<0.001) in the TBA-reactive product (-31.49 %, -39.06 %, -19.72 %, -23.74 % & -35.18 %), in groups 8 to 12 respectively as compared to DMBA treated group. The overall antilipid peroxidative effect of isolated compound was considered in comparison to the effect shown by MO 400.

**Enzymic and non-enzymic parameters of hepatic tissue:** DMBA at the dose of 15mg/kg body wt. caused significant (p<0.001) decrease in SOD activity (-44.48 %), CAT activity (-57.84 %), GSH content (-37.07 %) and GST activity (-42.28 %), in comparison to control group (table 2).

The subsequent 21 days treatment of mice with plant extract (at both dose; p<0.001) and standard antioxidant BHA 0.5%; (p<0.01) and 1%; (p<0.001) before DMBA administration for 10 days significantly increased the SOD activity amounting to 66.33 %, 95.07 %, 42.91 % and 51.96 % respectively as compared to that of DMBA treated mice (group 2). CAT activity (111.37 %; p<0.01, 122.75 %; p<0.001, 74.83 %; p<0.01 and 82.70 %; p<0.001), GSH content (44.04 %, 55.95 %, 38.69 % and 47.61 %) and GST activity (57.71 %, 70.47 %, 50.72 % and 62.89 %) enhanced significantly (p<0.001) in group 8 to group 11 respectively, when compared to DMBA control group. It was also observed that pretreatment with isolated saponin (SM) before DMBA challenge (group 12) potentially restored the depleted activity of SOD (p<0.01) by 77.24 %, and CAT (p<0.01) by 116.63 %. The GSH content (51.11 %) and GST activity (63.83 %) were significantly increased (p<0.001) in the hepatic tissue of DMBA intoxicated mice, the order of efficacy being MO 400 followed by SM and MO 200.

**Effect on hepatic damage markers and biochemical assays:** In the present study, exposure of DMBA (15 mg/kg b. wt) alone induced a significant (p<0.001) drop off in AST (-41.55 %), ALT (-37.79 %) and ALP (-40.80 %) in comparison to control animals (group 1). However, DMBA administration significantly elevated (p<0.001) total cholesterol content (64.83 %) in group 2, when compared with normal mice (table 3). Pretreatment with MOHE, BHA (at both dose) and SM before DMBA challenge (group 8-12) significantly (p<0.001) enhanced the level of marker enzymes i.e. AST (40.56 %, 66.71 %, 12.80 %, 27.22 % & 57.23 %), ALT (35.16 %, 56.20 %, 12.69 %, 25.99 %, & 47.88 %), and ALP (49.54 %, 106.13 %, 12.89 %, 26.73 %, & 58.36 %), whereas a significant (p<0.001) decline was observed in total cholesterol content (-24.88 %, -35.10 %, -8.16 % -17.51 %, -30.05%)in all the groups except group 10 as compared to DMBA treated group (group 2). When the values observed in DMBA (group 2) were compared with normal control group 1, it was explored that total protein lowered significantly (p<0.001) by -5.65 % in the former group. However, the reduced protein content was significantly (p<0.001) increased and hence normalized in the plant extract, BHA and SM treated groups by 101.56
DISCUSSION

In recent times, there is an increased risk of malignancy because of environmental pollution such as exposure to genotoxic and carcinogenic chemicals. This has created awareness to prevent the harmful effect of these chemical agents. This has lead to the development of several preventive agents. These agents significantly reduce tumor incidence, delay tumor onset and also have minimal long-term toxicity.

Medicinal plants represent a rich source of cancer drug leads. Saponins are plant glycosides with a triterpene or steroid aglycone. Saponins have been found in many medicinal plants used in folk medicines. Saponins also have beneficial pharmacological effects. The activities include anti-inflammation, anti-parasite and anti-virus (Traore et al., 2000). Recently, cytotoxicity and antitumor activities of saponins have also been extensively investigated (Sparg, 2004). In this study, analysis was done to know anticarcinogenic potential of isolated saponin (SM) and crude hydro-ethanolic extract of M. oleifera pods (MOHE).

The present study thus investigates the induction of the activities of hepatic detoxification system enzymes and antioxidant enzyme profiles in mice by Moringa oleifera Lam. extract. The findings of the present study reveals that administration of MOHE at both dose levels (200mg/kg body weight and 400mg/kg body weight) and its isolated saponin (SM; 50mg/kg body weight) for 21 days have enhanced the levels of hepatic superoxide dismutase and catalase, elucidating that Moringa oleifera acts as bifunctional inducer as it modulates both Phase-I and Phase-II system enzymes that furnish the balance of xenobiotic metabolism towards detoxification (Singh et al., 2000). The antioxidant defense enzymes have been suggestive of playing an important role in maintaining physiological levels of oxygen and hydrogen peroxide and eliminate peroxides generated from inadvertent exposure to xenobiotics and drugs (Bharali et al., 2003).

One of the effective ways to screen the compounds or plant extracts that might be of use in inhibiting the chemically induced carcinogenesis is to look for its ability in inducing the phase I and phase II drug metabolizing enzymes (Singh et al., 2000). Cytochrome P450 system, which is a product of the CYP super family of genes, constitutes a major electron transport chain in the membrane of endoplasmic reticulum. The production of phase I enzymes is measured as a cancer risk factor because of starting carcinogens to ultimate carcinogens (Parimalakrishnan et al., 2009). In the present study, MOHE and SM pretreatment significantly improved the levels of the measured components of cytochrome P450 system (cytochrome P450 and cytochrome b5).

MOHE and SM also protects against oxidative stress by reducing the level of lipid peroxidation (Singh et al., 2000; Arabshahi et al., 2007; Paliwal et al., 2011b, c). The depletion in the hepatic GSH level may be due to oxidative stress caused by DMBA treatment. GSH has been endowed with an important function in maintaining the reducing milieu of cells, in addition to its conjugating ability owing to nucleophilic center and is involved in detoxification of xenobiotics that cause toxicity and carcinogenicity. GST catalyses the conjugation of a variety of endogenous and exogenous compounds with the non protein thiol, glutathione.

During carcinogenesis, some enzymes can be used as a biochemical indicator of cancer response to therapy (Ramakrishnan et al., 2007). In this study, DMBA administration to mice lead to a marked depletion in levels of tissue AST, ALT and ALP levels, which is indicative of hepatocellular damage. The decrease in AST, ALT and ALP levels might be due to the possible release from cytoplasm into the blood circulation rapidly after rupture of plasma membrane and cellular damage. Low levels of AST in tissue indicate liver damage, such as that caused by viral hepatitis as well as cardiac infarction and muscle injury. ALT catalyses the conversion of alanine to pyruvate and glutamate and is released in similar manner. Therefore ALT is more specific to the liver and is thus a better parameter for detecting liver injury (Eesha et al., 2011). These enzymes are the most sensitive markers employed in the diagnosis of hepatic damage because they are in cytoplasmic location and hence released into the circulation after cellular damage (El Mesallamy et al., 2011).

Treatment with Moringa oleifera pods extract and its saponin significantly enhanced the activities of the enzymes in DMBA intoxicated mice. In the present study the data indicate the protein damage in liver may be due to oxidative stress generated by DMBA. Free radicals that are generated by DMBA decreased the total protein content in liver that was elevated by the administration of M. oleifera pods extract in experimental groups. Saponins also form complexes with proteins and could decrease protein degradability (Parmar et al., 2011). In the present study DMBA intake increased the mean values of cholesterol in tissues. DMBA mediated development of hypercholesterolemia entails the activation of cholesterol biosynthetic enzymes and the simultaneous suppression of cholesterol catabolic enzymes.

In DMBA induced hepatocarcinogenesis in mice the free radical scavenging mechanism pathway may be operating, hence the mechanism of cancer chemoprevention may be postulated as:

- By blocking the carcinogen (DMBA) so that it cannot pass through the plasma membrane.
- By induction of enzymes that detoxify carcinogens.
- By inhibiting competitively DNA adduct formation.
- MOHE and SM may be used as a cancer chemopreventive agent by virtue of its antioxidant property. The antioxidant property of Moringa may be due to the presence of various phytochemicals that was confirmed in this study by phytochemical screening of the extract (Paliwal et al., 2011d; Sharma et al., 2011)

Table 1: Modulatory influence of MOHE, SM and BHA on status of xenobiotic (phase I) drug metabolizing enzymes in the liver of control and experimental mice in each group.

<table>
<thead>
<tr>
<th>Groups</th>
<th>DOSE (mg/kg)</th>
<th>Cyto P450 (nmole/mg)</th>
<th>Cyto b5 (nmole/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (1)</td>
<td>-</td>
<td>3.15 ± 0.81</td>
<td>2.87 ± 0.82</td>
</tr>
<tr>
<td>DMBA (2)</td>
<td>15</td>
<td>14.52 ± 1.15</td>
<td>9.34 ± 0.96</td>
</tr>
<tr>
<td>MO (3)</td>
<td>200</td>
<td>4.32 ± 0.97*</td>
<td>3.08 ± 0.93*</td>
</tr>
<tr>
<td>MO (4)</td>
<td>400</td>
<td>5.62 ± 0.90*</td>
<td>3.61 ± 0.71*</td>
</tr>
<tr>
<td>BHA (5)</td>
<td>0.5%</td>
<td>3.75 ± 1.21*</td>
<td>2.96 ± 0.94**</td>
</tr>
<tr>
<td>BHA (6)</td>
<td>1%</td>
<td>4.97 ± 1.09*</td>
<td>3.18 ± 1.18*</td>
</tr>
<tr>
<td>SM (7)</td>
<td>50</td>
<td>5.21 ± 1.11*</td>
<td>3.38 ± 1.16*</td>
</tr>
<tr>
<td>MO+DMBA (8)</td>
<td>200+15</td>
<td>8.17 ± 1.21*</td>
<td>7.35 ± 1.03*</td>
</tr>
<tr>
<td>MO + DMBA (9)</td>
<td>400+15</td>
<td>5.12 ± 1.13*</td>
<td>4.73 ± 0.77*</td>
</tr>
<tr>
<td>BHA+DMBA (10)</td>
<td>0.5%+15</td>
<td>9.72 ± 2.10*</td>
<td>8.83 ± 1.09*</td>
</tr>
<tr>
<td>BHA+DMBA (11)</td>
<td>1%+15</td>
<td>7.93 ± 3.16*</td>
<td>6.87 ± 1.12*</td>
</tr>
<tr>
<td>SM+DMBA (12)</td>
<td>50+15</td>
<td>6.65 ± 1.13*</td>
<td>5.15 ± 1.18*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM (n=6); ap<0.001 vs. control group; *p<0.001; **p<0.01 vs. Treated (DMBA) group

Cyto P450: Cytochrome P450; Cyto b5: Cytochrome b5; DMBA: 7, 12-dimethylbenz[a]anthracene; MOHE: hydro-ethanolic extract of Moringa oleifera; BHA: butylated hydroxyanisole

Table 2: Hepatoprotective effect of MOHE, SM and BHA on antioxidative stress related parameters in DMBA-exposed male mice.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (mg/kg  )</th>
<th>LPO (µM/mg protein)</th>
<th>SOD (U/ml)</th>
<th>CAT (nmol/min/ ml)</th>
<th>GSH (nmoll/g)</th>
<th>GST (nmoleCDNB min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (1)</td>
<td>-</td>
<td>92.10 ± 1.08</td>
<td>9.15 ± 0.85</td>
<td>10.84 ± 0.80</td>
<td>2.67 ± 0.81</td>
<td>267.43 ± 1.09</td>
</tr>
<tr>
<td>DMBA (2)</td>
<td>15</td>
<td>164.60 ± 1.09</td>
<td>5.08 ± 0.99</td>
<td>4.57 ± 0.98</td>
<td>1.68 ± 0.74</td>
<td>154.35 ± 1.07</td>
</tr>
<tr>
<td>MO (3)</td>
<td>200</td>
<td>61.29 ± 1.17</td>
<td>10.86*±0.92</td>
<td>12.20*±0.84</td>
<td>2.85*±0.92</td>
<td>286.67*±0.82</td>
</tr>
<tr>
<td>MO (4)</td>
<td>400</td>
<td>48.05 ± 1.12*</td>
<td>11.18*±0.96</td>
<td>12.90*±0.98</td>
<td>2.96*±0.97</td>
<td>263.60*±0.82</td>
</tr>
<tr>
<td>BHA (5)</td>
<td>0.5%</td>
<td>82.39 ± 2.14**</td>
<td>9.61*±1.11</td>
<td>11.05*±2.13</td>
<td>2.69*±1.11</td>
<td>310.21*±3.13</td>
</tr>
<tr>
<td>BHA (6)</td>
<td>1%</td>
<td>74.34 ± 3.09*</td>
<td>10.13*±1.08</td>
<td>11.71*±1.21</td>
<td>2.75*±1.14</td>
<td>274.45*±4.15</td>
</tr>
<tr>
<td>SM (7)</td>
<td>50</td>
<td>53.80 ± 1.12*</td>
<td>11.09*±1.05</td>
<td>12.71*±1.07</td>
<td>2.89*±0.67</td>
<td>281.65*±1.14</td>
</tr>
<tr>
<td>MO+DMBA (8)</td>
<td>200+15</td>
<td>112.76 ± 1.04</td>
<td>8.45*±1.06</td>
<td>9.66*±1.16</td>
<td>2.42*±0.89</td>
<td>243.43*±1.07</td>
</tr>
<tr>
<td>MO + DMBA (9)</td>
<td>400+15</td>
<td>100.30 ± 1.09*</td>
<td>9.91*±0.97</td>
<td>10.18*±1.04</td>
<td>2.62*±0.95</td>
<td>263.13*±1.05</td>
</tr>
<tr>
<td>BHA+DMBA (10)</td>
<td>0.5%+15</td>
<td>132.13 ± 3.09</td>
<td>7.26*±1.14</td>
<td>7.99*±2.09</td>
<td>2.33*±0.71</td>
<td>232.65*±2.06</td>
</tr>
<tr>
<td>BHA+DMBA (11)</td>
<td>1%+15</td>
<td>125.54 ± 4.07*</td>
<td>7.72*±1.15</td>
<td>8.35*±2.13</td>
<td>2.48*±0.94</td>
<td>251.43*±3.11</td>
</tr>
<tr>
<td>SM+DMBA (12)</td>
<td>50+15</td>
<td>106.69 ± 1.17*</td>
<td>8.75*±1.16</td>
<td>9.90*±1.11</td>
<td>2.54*±0.97</td>
<td>257.51*±1.10</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± S.E.M (n=6); ap<0.001 vs. control group; *p<0.001; **p<0.01; ***p<0.05 vs Treated (DMBA) group, DMBA: 7, 12-dimethylbenz[a]anthracene; MOHE: hydro-ethanolic extract of Moringa oleifera.
Moringa oleifera; BHA: butylated hydroxyanisole; LPO: Lipid Per oxidation; SOD: Superoxide dismutase; CAT: Catalase; GSH: Reduced glutathione; GST: glutathione-S-transfserase, CDNB: 1-chloro-2, 4-dinitrobenzene.

Table 3: Hepatoprotective effect MOHE, SM and BHA on hepatic biochemical parameters in DMBA-exposed male mice.

<table>
<thead>
<tr>
<th>Groups</th>
<th>DOSE (mg/kg)</th>
<th>AST (IU/ml)</th>
<th>ALT (IU/ml)</th>
<th>ALP (µM PNP/min/g)</th>
<th>TC (mg/g)</th>
<th>TP (g/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (1)</td>
<td>-</td>
<td>124.31 ± 1.06</td>
<td>73.42 ± 1.09</td>
<td>138.31 ± 1.10</td>
<td>92.31 ± 1.11</td>
<td>8.81 ± 0.93</td>
</tr>
<tr>
<td>DMBA (2)</td>
<td>15</td>
<td>72.65 ± 2.11</td>
<td>45.67 ± 1.01</td>
<td>81.87 ± 1.09</td>
<td>152.19 ± 2.10</td>
<td>3.83 ± 0.82</td>
</tr>
<tr>
<td>MO (3)</td>
<td>200</td>
<td>136.34 ± 2.21</td>
<td>83.12 ± 1.04</td>
<td>147.38 ± 1.07</td>
<td>81.55 ± 1.12</td>
<td>10.12 ± 0.98</td>
</tr>
<tr>
<td>MO (4)</td>
<td>400</td>
<td>149.65 ± 3.13</td>
<td>92.42 ± 1.02</td>
<td>163.21 ± 1.04</td>
<td>70.32 ± 1.09</td>
<td>10.83 ± 0.63</td>
</tr>
<tr>
<td>BHA (5)</td>
<td>0.5%</td>
<td>127.23 ± 3.21</td>
<td>74.32 ± 2.09</td>
<td>139.67 ± 2.07</td>
<td>90.76 ± 4.17</td>
<td>9.07 ± 1.13</td>
</tr>
<tr>
<td>BHA (6)</td>
<td>1%</td>
<td>129.45 ± 4.20</td>
<td>79.41 ± 3.12</td>
<td>143.21 ± 3.09</td>
<td>86.54 ± 2.09</td>
<td>9.86 ± 1.15</td>
</tr>
<tr>
<td>SM (7)</td>
<td>50</td>
<td>139.27 ± 2.18</td>
<td>85.28 ± 1.12</td>
<td>152.22 ± 3.12</td>
<td>75.43 ± 1.05</td>
<td>10.69 ± 1.10</td>
</tr>
<tr>
<td>MO+DMBA (8)</td>
<td>200+15</td>
<td>102.12 ± 2.13</td>
<td>61.73 ± 2.09</td>
<td>122.43 ± 1.19</td>
<td>114.32 ± 1.08</td>
<td>7.72 ± 1.09</td>
</tr>
<tr>
<td>MO + DMBA (9)</td>
<td>400+15</td>
<td>121.12 ± 1.08</td>
<td>71.34 ± 1.02</td>
<td>168.76 ± 1.13</td>
<td>98.76 ± 1.06</td>
<td>8.30 ± 0.93</td>
</tr>
<tr>
<td>BHA+DMBA (10)</td>
<td>0.5%+15</td>
<td>81.65 ± 4.18</td>
<td>51.47 ± 3.07</td>
<td>92.43 ± 4.19</td>
<td>139.76 ± 3.07</td>
<td>6.60 ± 0.88</td>
</tr>
<tr>
<td>BHA+DMBA (11)</td>
<td>1%+15</td>
<td>92.43 ± 4.19</td>
<td>57.54 ± 3.18</td>
<td>103.76 ± 5.21</td>
<td>127.54 ± 2.15</td>
<td>7.14 ± 1.09</td>
</tr>
<tr>
<td>SM+DMBA (12)</td>
<td>50+15</td>
<td>114.23 ± 2.06</td>
<td>67.54 ± 2.09</td>
<td>129.65 ± 2.09</td>
<td>106.45 ± 2.05</td>
<td>8.11 ± 1.05</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± S.E.M (n=6); ap<0.001 vs. control group; *p<0.001; **p<0.01; ***p<0.05 vs. Treated (DMBA) group. DMBA: 7, 12-dimethylbenz[a]anthracene; MOHE: hydro-ethanolic extract of Moringa oleifera; BHA: butylated hydroxyanisole; AST: aspartate transaminase; ALT: alanine transaminase; ALP: alkaline phosphatase; TC: Total cholesterol; TP: Total protein; PNP: p- nitrophenol.

CONCLUSION

The present investigation has demonstrated that since the plants have shown no toxic effect at the tested doses, it could well be applied in cancer chemoprevention, to reduce the risk of cancer. The antitumor properties of Moringa oleifera may be due to the presence of saponins and other phytochemicals. Their constituents scavenge free radicals and exert a protective effect against free radicals and exert a protective effect against the oxidative damage to cellular macromolecules. Previous in vitro studies performed by us in our laboratories have proved that M. oleifera acts as a free radical scavenger and has wide variety of antioxidants and phytoconstituents. All these observations clearly indicate a significant chemo protective activity of Moringa oleifera and its isolated saponin.

ACKNOWLEDGMENTS

The authors are grateful to University Grants Commission (UGC) for providing financial assistance (Grant No. F. No. 37-68/2009; SR). The authors are thankful to the authorities of Banasthali University for providing support to the study.

Conflict of interest

The authors declare that there are no conflicts of interest.

REFERENCES


Sharma, V.; Paliwal, R.; Janneda, P. and Sharma, S.H. (2012b). Chemopreventive efficacy of *Moringa oleifera* pods against 7, 12-


