

Potential Chemoprevention of 7,12-Dimethylbenz[a]anthracene Induced Renal Carcinogenesis by *Moringa oleifera* Pods and Its Isolated Saponin

Veena Sharma · Ritu Paliwal

Received: 23 January 2013 / Accepted: 27 April 2013 / Published online: 21 May 2013
© Association of Clinical Biochemists of India 2013

Abstract Present investigation shows that hydroethanolic extract of *Moringa oleifera* (MOHE) and its isolated saponin (SM) attenuates DMBA induced renal carcinogenesis in mice. Isolation of SM was achieved by TLC and HPLC and characterization was done using IR and ^1H NMR. Animals were pre-treated with MOHE (200 and 400 mg/kg body weight; p.o), BHA as a standard (0.5 and 1 %) and SM (50 mg/kg body weight) for 21 days prior to the administration of single dose of DMBA (15 mg/kg body weight). Administration of DMBA significantly ($p < 0.001$) enhanced level of xenobiotic enzymes. It enhanced renal malondialdehyde, with reduction in renal glutathione content, antioxidant enzymes and glutathione-S-transferase. The status of renal aspartate transaminase, alanine transaminase, alkaline phosphatase and total protein content were also found to be decreased along with increase in total cholesterol in DMBA administered mice. Pretreatment with MOHE and SM significantly reversed the DMBA induced alterations in the tissue and effectively suppressed renal oxidative stress and toxicity.

Keywords *Moringa oleifera* · Xenobiotic · Saponin · Carcinogenesis · Renal tissue · Mice

Introduction

Cancer is one of the leading cause of death in the world and is notoriously difficult to treat effectively. It is a frightful disease because the patient suffers pain, disfigurement and loss of many physiological processes [1, 2]. It is caused by a complex, poorly understood interplay of genetic and environmental factors. All cancers occur due to activation or mutation of oncogenes, or inactivation of suppressor genes. Most of these genes are involved in activation and detoxification of polycyclic aromatic hydrocarbons (PAHs), suggesting a potential role of these compounds in carcinogenesis [3, 4]. The lack of cell–cell adhesion and increased migration are key characteristics of cancer cells. The development of renocellular carcinoma (RCC) has been linked with innumerable risk factors including environmental exposure to different toxicants.

7,12-Dimethylbenz[a]anthracene (DMBA) a PAH, is a procarcinogen and thus needs metabolic activation to become an ultimate carcinogen [5]. DMBA is known to induce damage in many enzymes involved in DNA repair and is normally used to induce liver and kidney cancer in experimental animal models [2, 4, 6, 7]. Chemoprevention, a novel and appealing strategy, deals with the inhibition, reversal or suppression of carcinogenesis by the use of natural or synthetic agents [8]. The possible mechanism so far reported for the chemopreventive potential of natural products include carcinogen detoxification, suppression of genetic mutation, suppression of cell proliferation, induction of apoptosis and modulation of the immune system [5, 9, 10].

In the recent times natural products have been used to prevent the toxicities induced by chemicals, drugs and carcinogenic xenobiotics. Plant based products are generally considered safe and proved to be effective against various human ailments and their medicinal uses have been

V. Sharma (✉) · R. Paliwal
Department of Bioscience and Biotechnology, Banasthali
University, Banasthali 304022, Rajasthan, India
e-mail: drvshs@gmail.com

R. Paliwal
e-mail: paliwal.ritu18@gmail.com

gradually increasing in developed countries. Epidemiological studies have also proven that consumption of vegetables and fruit have caused lower incidence of cancers [11]. *Moringa oleifera* Lam. (Syn *Moringa pterygosperma* Gaertn; Fam: Moringaceae) have been reported to known by regional names such as drumstick tree, sajiwan and sajna, is a natural as well as cultivated variety of the genus *Moringa* [12, 13]. *M. oleifera* possesses antitumor, antipyretic, antiepileptic, anti-inflammatory, antiulcer, antispasmodic, diuretic, anti-hypertensive, cholesterol lowering, antioxidant, antidiabetic, renal [6] and hepatoprotective activities [7]. Early studies documented the presence of phenolics, flavonoids, saponins, terpenoids, proanthocyanadins, and cardiac glycosides in the *M. oleifera* pods [14, 15]. Medicinal plants represent a rich source of cancer drug leads. Saponins are plant glycosides with a triterpene or steroid aglycone. Saponins, by virtue of their multiple apoptotic actions on cancer cells, may provide a new line of anticancer agents. They are also effective against drug-resistant cancer cells [16]. Saponins have been found in many medicinal plants used in folk medicines. In this study, further analysis was done to know anticarcinogenic potential of these isolated saponins.

However, to the best of our knowledge, there are no scientific data available to validate the folkloric anticancer claims of this isolated saponin from *M. oleifera*. Hence, the aim of this current investigation was to explore the chemopreventive effects of the hydroethanolic pods extract of *M. oleifera* and effect of isolated saponin against environmental carcinogen DMBA.

Materials and Methods

Chemicals

All chemicals used in the study were of analytical reagent grade and of highest quality available and were purchased from reliable firms. DMBA (CAS No. 57-97-6) was purchased from SIGMA chemical Co. (USA).

Experimental Plant and Preparation of Hydro-ethanolic Plant Extract (MOHE)

The experimental plant *M. 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.

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 h and stored in a desiccator. The percentage yield of extract (MOHE) was found to be 22 % and stored at 4 °C in airtight containers. Suspensions of the extract was prepared in distilled water and used to assess renoprotective activity.

Isolation and Characterization of Saponin

Successive extraction of plant material was performed using solvents that were pet ether, benzene, chloroform, ethyl acetate than ethanol for 16 h in soxhlet apparatus. The extracts were then concentrated on a rotary evaporator below 50 °C and were stored in air-tight containers in cold room for further studies. Isolation of SM was achieved by TLC and HPLC and characterization was done using IR and ¹H NMR. The isolated compound was nomenclatures as SM then assessed for its anticarcinogenic potential.

Experimental Animals

Male Swiss albino mice (*Mus musculus*) weighing approximately 15–30 g were procured from Haryana Agricultural University, Hissar (Haryana, India). The animals were acclimatized for a month prior to experiment. 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 (CPC-SEA Reg. No. IAEC/814 dated. 23/01/2010).

Procedure and Design of Experiments

Adult male Swiss albino mice (*M. musculus* L.) weighing 25–30 g (72 mice; six in each group) were used for various parameters. 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 1 ml 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 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 *M. 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 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 *M. 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. Kidneys were dissected out, washed immediately with ice-cold saline to remove blood, and the wet weight noted and then stored at -80°C for various oxidative stress and biochemical assays.

Preparation of Kidney Homogenate

Kidney homogenate was prepared in cold 50 mM potassium phosphate buffer (pH 7.4), using Remi homogenizer. The unbroken cells and debris were removed by centrifugation at 10,000 rpm for 15 min at 4°C using a Remi cooling centrifuge and the supernatant was used for the estimation of renoprotective property [7, 17].

Determination of Xenobiotic Enzymes

Cytochrome P450 and cytochrome b5 content were assayed in the homogenate by the method of Omura and Sato [18], using an absorption coefficient of 91 and $185\text{ cm}^2\text{ M}^{-1}\text{ m}^{-1}$, respectively.

Oxidative Stress Parameters and Enzymatic Variables

The various oxidative stress variables viz., lipid peroxidation (LPO) by the method of Ohkawa et al. [19], superoxide dismutase by Marklund and Marklund [20] and catalase by the method of Abei [21] were performed.

The enzymatic variables viz., reduced glutathione (GSH) content was assayed by the method of Jollow et al. [22] and glutathione-S-transferase (GST) was determined by method of Habig et al. [23].

Determination of Biochemical Assays

The activities of various biochemical parameters like aspartate and alanine transaminase (AST and ALT) were assayed by the method of Reitman and Frankal [24]. Activities of alkaline phosphatase (ALP) were determined according to the protocol described in laboratory manual [25]. Total protein content was estimated by the method of Lowry et al. [26] using bovine serum albumin as standard. Total cholesterol level was determined by using cholesterol as standard by the method of Zak's [27].

Statistical Analysis

The experimental results obtained were expressed as mean \pm standard error of mean. The data was subjected to one way analysis of variance 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 $p < 0.05$.

Results

Effect on Xenobiotic Enzymes in Kidney of DMBA Treated Mice

Cytochrome P450 and b5 showed a significant increase ($p < 0.001$) above their basal level in their contents in DMBA treated group as compared to control group. In comparison to DMBA treated group MOHE at low dose (200 mg/kg body weight; $p < 0.01$), high dose (400 mg/kg body weight; $p < 0.001$), BHA low dose (0.5 %; $p < 0.01$), BHA high dose (1 %; $p < 0.001$) and SM (50 mg/kg body weight; $p < 0.001$) significantly increased both the enzyme level. The renoprotective effect of the extract was comparable to the effect seen with BHA treatment (Table 1).

Intake of MOHE at dose of 200 and 400 mg/kg body weight ($p < 0.001$), BHA (0.5 and 1 %) and SM (50 mg/kg body weight; $p < 0.001$) before DMBA challenge significantly ($p < 0.01$) improved the enzyme level as compared to DMBA treated group. The increase in the activity of enzymes due to DMBA challenge was significantly ($p < 0.001$) restored by pre-administration of MOHE at both the doses (200 and 400 mg/kg) and SM (50 mg/kg body weight; $p < 0.001$) for 21 days. From the results it is clear that pods exhibit dose dependent activity.

Renal Lipid Peroxidation (TBARS) Level

The data displays a considerable and significant ($p < 0.001$) boost of in the level of TBARS in kidney (120.12 %) after DMBA administration (15 mg/kg b.wt).

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

Groups	Dose (mg/kg)	Cyt P450 (nmol/mg)	Cyt b5 (nmol/mg)
Control (1)	–	2.97 ± 0.13	1.87 ± 0.18
DMBA (2)	15	8.13 ± 0.10 ^a	6.73 ± 1.15 ^a
MOHE (3)	200	3.11 ± 0.11 ^{**a}	2.08 ± 0.14 ^{**a}
MOHE (4)	400	3.82 ± 0.16 ^{*a}	2.78 ± 1.04 ^{*a}
BHA (5)	0.5 %	2.95 ± 0.12 ^{**}	1.98 ± 0.15 ^{**}
BHA (6)	1 %	3.34 ± 1.06 [*]	2.05 ± 1.09 [*]
SM (7)	50	3.58 ± 0.89 ^{*a}	2.19 ± 0.10 ^{*a}
MOHE + DMBA (8)	200 + 15	4.81 ± 1.02 ^{*a}	3.27 ± 1.04 ^{*a}
MOHE + DMBA (9)	400 + 15	3.06 ± 1.08 ^{*a}	1.92 ± 1.06 ^{*a}
BHA + DMBA (10)	0.5 % + 15	5.15 ± 1.07 ^{*a}	5.12 ± 1.08 ^{*a}
BHA + DMBA (11)	1 % + 15	4.38 ± 3.02 ^{*a}	4.87 ± 1.11 ^{*a}
SM + DMBA (12)	50 + 15	3.19 ± 1.21 ^{*a}	2.25 ± 1.13 ^{*a}

Values are expressed as mean ± SEM ($n = 6$)

^a $p < 0.001$ vs. control group; * $p < 0.001$; ** $p < 0.01$ vs. treated (DMBA) group

Cyt P450 cytochrome P450, Cyt b5 cytochrome b5, DMBA 7,12-dimethylbenz[a]anthracene, MOHE hydro-ethanolic extract of *M. oleifera*, BHA butylated hydroxyanisole, SM isolated saponin

Intake of MOHE at the dose of 200 and 400 mg/kg body wt. before DMBA challenge showed a significant ($p < 0.001$) decrease in TBA-reactive product (–48.50, –50.87 %) compared to DMBA treated group (group 2). BHA (0.5 and 1 %) administration before DMBA challenge showed a significant ($p < 0.01$) decrease in TBA-reactive product (–38.54, –43.42 %) compared to DMBA treated group. Intake of SM at the dose of 50 mg/kg b. wt. before DMBA challenge (group 12) showed a significant ($p < 0.001$) protection against LPO and restoration of the LPO level towards normal values (–48.78 %) as compared to DMBA treated group (Table 2).

Antioxidant Related Parameters (SOD, CAT, GSH and GST) of Renal Tissue

Data informs a significant ($p < 0.001$) declination in the content of SOD (–52.82 %), CAT (–59.76 %), GSH (–22.87 %) and GST (–29.06 %) activities, respectively, in DMBA treated group (group 2) when compared to untreated group (Table 2).

Compared with DMBA control (group 2), the oral administration of MOHE at 200 and 400 mg/kg b. wt and BHA at 0.5 and 1 %, respectively before DMBA challenge (group 8–11) showed a significant increase ($p < 0.001$) in the values of SOD (94.83, 105.68, 70.28 and 83.72 %) and GSH content (20.68, 28.27, 11.03 and 16.05 %). The oral intake of MOHE at 200 and 400 mg/kg b. wt ($p < 0.001$) and BHA at 0.5 and 1 % ($p < 0.01$), respectively before DMBA challenge (group 8–11) showed a increase in the value of CAT (104.96, 118.05, 33.94 and 70.40 %) and

GST (31.35, 36.73, 15.54 and 24.99 %) activities, respectively. When SM was given before DMBA challenge (group 12); it showed a significant augmentation ($p < 0.001$) in the values of the antioxidant variables i.e. SOD (100 %), CAT (112.01 %), GSH content (25.51 %) and GST activity (34.51 %) when compared with DMBA control (group 2).

Effect on Renal Biochemical Variables (AST, ALT, ALP, Total Cholesterol and Total Protein Level)

DMBA toxicity produced a significant ($p < 0.001$) decrease in AST (–47.68 %), ALT (–37.66 %) and ALP (–38.62 %) level in kidney homogenate as compared to untreated mice (group 1). The total cholesterol content in kidney of DMBA administered mice was significantly ($p < 0.001$) increased by 63.34 % as compared to control animals (Table 3).

Compared with DMBA (group 2) oral administration of MOHE, BHA and SM before DMBA administration (group 8–12) results significantly ($p < 0.001$) increased the renal AST (67.83, 85.55, 20.76, 47.07, and 75.40 %), ALT (30.60, 47.78, 9.48, 16.44, and 41.10 %), and ALP (47.68, 74.57, 14.76, 33.74, and 54.03 %) levels respectively. The renal total cholesterol content in groups 8 to 12 decreased significantly (group 8–9, $p < 0.001$; group 10–12, $p < 0.01$) to about –26.00, –35.31, –4.40, –17.72, and –31.06 % respectively, as compared to DMBA control values (group 2).

The total protein content in renal tissue of DMBA administrated mice (group 2) significantly ($p < 0.001$)

Table 2 Renal protective effect of MOHE and SM on antioxidative stress parameters in DMBA-exposed male mice

Groups	Dose (mg/kg)	LPO (nmole MDA/g protein)	SOD (U/ml)	CAT ($\mu\text{mol H}_2\text{O}_2$ degraded/min/mg protein)	GSH (nmol/g)	GST (nmol CDNB/min/mg)
Control (1)	–	54.75 \pm 1.02	8.20 \pm 0.88	9.52 \pm 0.92	1.88 \pm 0.74	120.80 \pm 0.66
DMBA (2)	15	120.52 ^a \pm 1.04	3.87 ^a \pm 1.03	3.83 ^a \pm 1.08	1.45 ^a \pm 1.05	85.69 ^a \pm 1.09
MOHE (3)	200	41.76 ^{*,a} \pm 1.12	9.84 ^{*,a} \pm 1.09	11.16 ^{*,a} \pm 1.13	2.17 ^{*,a} \pm 1.09	139.57 ^{*,a} \pm 1.12
MOHE (4)	400	31.41 ^{*,a} \pm 1.06	10.20 ^{*,a} \pm 2.18	11.85 ^{*,a} \pm 1.18	2.28 ^{*,a} \pm 1.02	145.59 ^{*,a} \pm 1.07
BHA (5)	0.5 %	49.02 ^{**} \pm 2.09	8.77 ^{***,a} \pm 2.13	9.93 ^{***,a} \pm 2.09	2.07 ^{***,a} \pm 1.11	124.02 ^{***,a} \pm 2.09
BHA (6)	1 %	45.84 [*] \pm 3.07	9.34 ^{***,a} \pm 4.17	10.52 ^{***,a} \pm 2.18	2.15 ^{***,a} \pm 0.98	130.46 ^{***,a} \pm 2.08
SM (7)	50	34.63 ^{*,a} \pm 1.16	10.02 ^{***,a} \pm 2.04	11.59 ^{*,a} \pm 1.12	2.25 ^{***,a} \pm 0.14	142.53 ^{***,a} \pm 1.14
MOHE + DMBA (8)	200 + 15	62.06 ^{*,a} \pm 1.11	7.54 ^{*,a} \pm 1.23	7.85 ^{*,a} \pm 1.04	1.75 ^{*,a} \pm 0.71	112.56 ^{*,a} \pm 1.13
MOHE + DMBA (9)	400 + 15	59.21 ^{*,a} \pm 1.10	7.96 ^{*,a} \pm 1.10	8.35 ^{*,a} \pm 1.06	1.86 ^{*,a} \pm 0.83	117.17 ^{*,a} \pm 1.07
BHA + DMBA (10)	0.5 % + 15	74.07 \pm 2.04 ^{***,a}	6.59 ^{*,a} \pm 1.21	5.13 ^{*,a} \pm 1.07	1.61 ^{*,a} \pm 1.01	99.01 ^{***,a} \pm 3.08
BHA + DMBA (11)	1 % + 15	68.18 ^{***,a} \pm 2.09	7.11 ^{*,a} \pm 2.13	6.53 ^{*,a} \pm 2.09	1.69 ^{*,a} \pm 0.81	107.11 ^{***,a} \pm 4.18
SM + DMBA (12)	50 + 15	61.73 ^{*,a} \pm 1.01	7.74 ^{*,a} \pm 2.11	8.12 ^{*,a} \pm 2.08	1.82 ^{*,a} \pm 0.88	115.27 ^{*,a} \pm 2.08

Values are expressed as mean \pm SEM ($n = 6$)

LPO lipid per oxidation, SOD superoxide dismutase, CAT catalase, GSH reduced glutathione, GST glutathione-S-transferase, DMBA 7,12-dimethylbenz[a]anthracene, MOHE hydro-ethanolic extract of *Moringa oleifera*, BHA butylated hydroxyanisole, SM isolated saponin

^a $p < 0.001$ vs. control group; * $p < 0.001$; ** $p < 0.01$; *** $p < 0.05$ vs. treated (DMBA) group

Table 3 Protective effect of MOHE and SM on renal biochemical parameters in DMBA-exposed male mice

Groups	Dose (mg/kg)	AST (IU/l)	ALT (IU/l)	ALP ($\mu\text{M PNP/min/g}$)	TC (mg/g)	TP (g/ml)
Control (1)	–	98.31 \pm 1.02	58.35 \pm 1.03	118.64 \pm 1.08	90.38 \pm 1.05	6.19 \pm 1.06
DMBA (2)	15	51.43 ^a \pm 1.03	36.33 ^a \pm 2.09	72.81 ^a \pm 3.03	147.63 ^a \pm 1.09	4.20 ^a \pm 1.08
MOHE (3)	200	112.43 ^{**} \pm 2.06	65.44 ^{***} \pm 1.07	98.09 ^{***,a} \pm 1.11	81.30 ^{*,a} \pm 1.07	7.92 ^{*,a} \pm 2.03
MOHE (4)	400	125.32 ^{*,a} \pm 1.14	74.23 ^{***,a} \pm 1.02	82.90 ^{***,a} \pm 1.06	70.25 ^{*,a} \pm 1.02	8.29 ^{*,a} \pm 1.01
BHA (5)	0.5 %	99.32 ^{***,a} \pm 2.18	59.22 ^a \pm 2.13	112.74 \pm 2.08	130.01 ^{**} \pm 1.11	6.88 ^{**} \pm 2.19
BHA (6)	1 %	106.76 ^{***,a} \pm 2.15	61.74 ^{***} \pm 4.09	105.85 ^a \pm 2.09	85.24 ^{***,a} \pm 3.18	7.14 ^a \pm 1.13
SM (7)	50	118.43 ^{*,a} \pm 1.09	69.65 ^{***,a} \pm 2.07	88.54 ^{***,a} \pm 1.04	76.12 ^{***,a} \pm 1.09	8.11 ^{*,a} \pm 0.06
MOHE + DMBA (8)	200 + 15	86.32 ^{*,a} \pm 1.07	47.33 ^{*,a} \pm 3.14	129.28 ^{*,a} \pm 1.07	109.24 ^{*,a} \pm 2.08	5.77 ^{*,a} \pm 1.11
MOHE + DMBA (9)	400 + 15	95.43 ^{*,a} \pm 1.08	53.71 ^{*,a} \pm 1.10	121.40 ^{*,a} \pm 1.01	95.50 ^{*,a} \pm 1.03	6.02 ^{*,a} \pm 1.02
BHA + DMBA (10)	0.5 % + 15	62.11 ^{*,a} \pm 3.09	39.21 ^{*,a} \pm 2.19	142.15 ^{*,a} \pm 2.11	141.12 ^{***,a} \pm 5.11	4.88 ^{*,a} \pm 2.09
BHA + DMBA (11)	1 % + 15	75.64 ^{*,a} \pm 3.06	42.44 ^{*,a} \pm 2.14	135.12 ^{*,a} \pm 3.18	121.46 ^{***,a} \pm 7.07	5.27 ^{*,a} \pm 1.05
SM + DMBA (12)	50 + 15	90.21 ^{*,a} \pm 2.08	51.65 ^{*,a} \pm 1.05	125.17 ^{*,a} \pm 2.11	101.77 ^{***,a} \pm 2.03	5.88 ^{*,a} \pm 1.01

Values are expressed as mean \pm SEM ($n = 6$)

AST aspartate transaminase, ALT alanine transaminase, ALP alkaline phosphatase, TC total cholesterol, TP total protein, DMBA 7,12-dimethylbenz[a]anthracene, MOHE hydro-ethanolic extract of *Moringa oleifera*, BHA butylated hydroxyanisole, SM isolated saponin

^a $p < 0.001$ vs. control group; * $p < 0.001$; ** $p < 0.01$; *** $p < 0.05$ vs. treated (DMBA) group

decreased by -32.14% in comparison to control animals (group 1). Administration of MOHE and BHA at both dose before DMBA (group 8–11) significantly ($p < 0.001$) augmented the protein level to about 37.38, 43.33, 16.19, 25.47 and 40.00 % as compared to DMBA treated group (group 2). Similarly intake of SM before DMBA also significantly ($p < 0.001$) enhanced the protein level (40.00 %) as compared to DMBA treated group.

Discussion

Cancer is a group of diseases that can occur in all living cells in the body. Different cancer types have different natural history. Epidemiological studies have shown that 70–90 % of all cancers are environmental. Life style related factors are the most important and preventable among the environmental exposures [1]. There are several

treatment modalities to control cancer. Unfortunately, surgery and radiation are only effective against local neoplasms. Moreover, chemotherapy indiscriminately destroys both normal and tumor cells, as a consequence of their remarkably low therapeutic windows. The latter profiles trigger an endless series of serious adverse reactions that can be life-threatening. Further, the frequent eruption of resistance to chemotherapeutic agents poses a serious challenge to their efficacy in cancer management [28].

Environmental factors, may act as initiators, promoters, or both of carcinogenesis. Chemical carcinogens are commonly employed to initiate and promote neoplastic transformation in experimental animals. However, the most commonly employed environmental and chemical carcinogen for inducing experimental carcinogenesis is DMBA. The PAHs are reasonably anticipated to be human carcinogens based on sufficient evidence of carcinogenicity in experimental animals [29–31]. The PAH DMBA is well known as cytotoxic, carcinogenic, mutagenic and immunosuppressive agent [5, 32, 33]. Medicinal plants represent a rich source of cancer drug leads. Saponins have been found in many medicinal plants used in folk medicines. Saponins also have beneficial pharmacological effects. They are anticholesterolemic due to the formation of a complex with cholesterol in gastrointestinal tract thus preventing absorption [34]. Other activities include anti-inflammation, anti-parasite and anti-virus [35, 36]. Recently, cytotoxicity and antitumor activities of saponins have also been intensively investigated [37]. On the other hand, *M. oleifera* is used as phytomedicine such as antioxidant, anticancer and anti-inflammatory. Hence, the present study was undertaken to investigate the toxicity induced by DMBA and anticancer potential of *M. oleifera* and its isolated saponin (SM).

The cancer chemopreventive efficacy is assessed by its ability to modulate the activities of enzymes associated with drug metabolism and bifunctional modulators reduced the availability of ultimate carcinogen metabolites in the epithelial stage [38]. Cytochrome P450 (CYP) isoenzymes, are necessary to begin the conversion of metabolize lipophilic carcinogens compounds to more water-soluble metabolites, which are then acted upon by phase II enzymes to promote their polarity and assisting in their excretion [38, 39].

Oxidative damage in a cell or tissue occurs when the concentration of reactive oxygen species (O_2 , H_2O_2 and OH) generated exceeds the antioxidant capability of the cells [40]. The status of LPO as well as altered levels of certain endogenous radical scavengers is taken as direct evidence for oxidative stress [41]. Free radical scavenging enzymes like SOD and catalase protect the biological system from oxidative stress. Endogenous antioxidant system may counteract the ROS and reduce the oxidative

stress with the enzymic antioxidants SOD, CAT and GST. SOD accelerates the conversion of superoxide radical ($O_2^{\cdot-}$) to hydrogen peroxide while CAT converts H_2O_2 to H_2O . Depletion in the activity of these three antioxidant enzymes can be owed to an enhanced radical production during DMBA metabolism. In this present observation an increase in MDA was presumably associated with increased free radicals, confirming the fact that these free radicals inhibited the activities of SOD, CAT and GST. This is supported by earlier studies that showed during the DMBA induced renal carcinogenesis [2, 7]. The observed reduction in enzyme activities may be attributed to ROS; here the ROS themselves can reduce the activities of enzymes [42]. Activities of the enzymic antioxidants are reverted to near normal in MOHE (200 and 400 mg/kg) and SM (50 mg/kg) treated animals. This indicates the antioxidant potency of the drug and so preventing the inactivity of these enzymes from ROS.

One of the most important antioxidant systems is the glutathione redox cycle. GSH is one of the essential compounds for maintaining cell integrity because of its reducing properties and participation in the cell metabolism. The depletion in the renal GSH level has been observed in mice in response to oxidative stress caused by DMBA treatment. The depleted levels were restored to normal by supplementation of MOHE and SM. This might be due to release of antioxidants and chemoprotective role of saponins.

In the present investigation, the renoprotective effect of MOHE and SM was evident by restoration of various biochemical variables. Various biocellular enzymes were activated when renocellular damage gave rise to abnormalities of kidney function and these enzymes are remarkably increased in RCC. AST and ALT activities in damaged tissue and blood serum are generally accepted as an index of tissue damage and this tendency is also known to be distinct in rodents [43]. In the present study, a decline in activity of ALP was seen in damaged tissue of mice with RCC, this may be due to disturbance in secretory activity or due to altered gene expression in these conditions. Development of tumor results in tissue damage that lead to the release of ALP into circulation [44, 45] and this enzyme level have been depleted in damaged tissue of mice and this depletion is significantly enhanced by the supplementation of MOHE and SM. In this study, renoprotective effect of *M. oleifera* is evident by the restoration of ALT, AST and ALP. Significant preservation of kidney was observed in the groups that were pretreated with MOHE and SM. Recovery towards normalization of the enzymes suggested that the plant extract and saponins have some potent role in preserving structural integrity of renocellular membrane, thus preventing enzymes leakage into the blood circulation.

In the present study the data indicated the protein damage in kidney may be due to oxidative stress generated by DMBA. Free radicals that are generated by DMBA decreased the total protein content in kidney that was elevated by the administration of MOHE and SM in experimental groups. Reactive species can react directly with protein or they can react with sugars and lipids, generating products that in turn react with the protein [46, 47]. Within the protein, either the peptide bond or the side chain may be targeted. It has been demonstrated that the attack by hydroxyl free radical leads to an abstraction of a hydrogen atom from the protein polypeptide back bone and form a carbon centred radical [40, 48]. 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 [49, 50]. Supplementation of MOHE and SM decreased the level of cholesterol as compared to treated group.

The present investigation has demonstrated that *M. oleifera* may be used as a cancer chemopreventive agent by virtue of its antioxidant property. The antioxidant property of *M. oleifera* may be due to the presence of phytochemicals, especially saponins that was confirmed in the previous study [14, 15]. These compounds quench of ROS, chelate metal ions and regenerate membrane-bound antioxidants [6, 7, 51–53]. Therefore, the result of the present study suggests that *M. oleifera* and its isolated saponin has potential anticarcinogenic and anti-oxidative properties and may be act as a potent chemopreventive agent.

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 P, Parmar J, Verma P, Sharma P, Goyal PK. Chemopreventive effect of *Phyllanthus niruri* on DMBA induced skin papillomagenesis in Swiss albino mice. *Int J Biol Med Res.* 2010;1(4):158–64.
- Sharma V, Paliwal R, Janmeda P, Sharma SH. The reno-protective efficacy of *Moringa oleifera* pods on xenobiotic enzymes and antioxidant status against 7,12-dimethylbenz[a]anthracene exposed mice. *J Chin Integr Med.* 2012;10(10):1171–8.
- Das BN, Biswas BK, Moghal MMR, Basher MA, Ahmed M. Study of analgesic activity of bark of *Xeromphis spinosa*. *Int J Compr Pharm Pharm Glob.* 2010;1(2):1–3.
- Sharma V, Paliwal R, Janmeda P, Sharma SH. Chemopreventive efficacy of *Moringa oleifera* pods against 7,12-dimethylbenz[a]anthracene induced hepatic carcinogenesis in mice. *Asian Pac J Cancer Prev.* 2012;13:2563–9.
- Manoharan S, Vasantha Selvan M. Chemopreventive potential of geraniol in 7,12-dimethylbenz(a)anthracene (DMBA) induced skin carcinogenesis in Swiss albino mice. *J Environ Biol.* 2012;33:255–60.
- Paliwal R, Sharma V, Pracheta, Sharma S, Yadav S, Sharma SH. Antinephrotoxic effect of administration of *Moringa oleifera* Lam. in amelioration of DMBA-induced renal carcinogenesis in Swiss albino mice. *Biol Med.* 2011;3(2):27–35.
- Paliwal R, Sharma V, Pracheta, Sharma SH. Hepatoprotective and antioxidant potential of *Moringa oleifera* pods against DMBA-induced hepatocarcinogenesis in male mice. *Int J Drug Dev Res.* 2011;3(2):128–38.
- Cazzaniga M, Bonanni B. Breast cancer chemoprevention: old and new approaches. *J Biomed Biotechnol.* 2012;2012:1–15.
- Ebeed NM, Abdou HS, Booles HF, Salah SH, Ahmed ES, Fahmy KH. Antimutagenic and chemoprevention potentialities of sweet fennel (*Foeniculum vulgare* Mill.) hot water crude extract. *J Am Sci.* 2010;6:831–42.
- Feng L, Jia X, Zhu M, Chen Y, Shi F. Chemoprevention by *Prunella vulgaris* L. extract of non-small cell lung cancer via promoting apoptosis and regulating the cell cycle. *Asian Pac J Cancer Prev.* 2010;11:1355–58.
- Bharali R, Tabassum J, Azad MRH. Chemomodulatory effect of *Moringa oleifera* Lam. on hepatic carcinogen metabolizing enzymes, antioxidant parameters and skin papillomagenesis in mice. *Asian Pac J Cancer Prev.* 2003;4:131–9.
- Kasolo JN, Bimenya GS, Ojok L, Ochieng J, Okeng JWO. Phytochemicals and uses of *Moringa oleifera* leaves in Ugandan rural communities. *J Med Plants Res.* 2010;4:753–7.
- Paliwal R, Sharma V, Pracheta. A review on horse radish tree (*Moringa oleifera*): a multipurpose tree with high economic and commercial importance. *Asian J Biotechnol.* 2011;3(4):317–28.
- Paliwal R, Sharma V, Pracheta, Sharma S. Elucidation of free radical scavenging and antioxidant activity of aqueous and hydro-ethanolic extracts of *Moringa oleifera* pods. *Res J Pharm Technol.* 2011;4(4):566–71.
- Sharma V, Paliwal R, Pracheta, Sharma S. Phytochemical analysis and evaluation of antioxidant activities of hydro-ethanolic extracts of *Moringa oleifera* Lam. pods. *J Pharm Res.* 2011;4(2):554–7.
- Man S, Gao W, Zhang Y, Huang L, Liu C. Chemical study and medical application of saponins as anti-cancer agents. *Fitoterapia.* 2010;81:703–14.
- Mohandas J, Marshall JJ, Duggin GG, Horvath JS, Tiller DJ. Differential distribution of glutathione and glutathione related enzymes in rabbit kidney. *Biochem Pharmacol.* 1984;33:1801–7.
- Omura T, Sato R. The carbon monoxide binding pigment of liver. *J Biol Chem.* 1964;239:2370–8.
- Ohkawa H, Ohishi N, Yagi K. Assay for LPO in animals tissue by thiobarbituric acid reaction. *Anal Biochem.* 1979;95:351–8.
- Marklund S, Marklund G. Involvement of super oxide anion radical in the auto oxidation of pyrogallol and convenient assay for SOD. *Eur J Biochem.* 1974;47:469–74.
- Abel H. Catalase. In: Bergmeyer H, editor. *Methods in enzymatic analysis*, vol. 2. New York: Academic press; 1983. pp. 876–80.
- Jollow DJ, Mitchell JR, Zampagilone N, Gillette JR. Bromobenzene induced liver necrosis. Protective role of glutathione and evidence for 3,4-bromobenzene oxide as the hepatotoxic intermediate. *Pharmacology.* 1974;11:151–69.
- Habig WH, Pabst MJ, Jakoby WB. Glutathione-S-transferases. The first enzymatic step in mercapturic acid formation. *J Biol Chem.* 1974;249:7130–9.
- Reitman S, Franckal S. A colorimetric method for the determination of serum glutathione oxaloacetate and glutamic-pyruvic transaminase. *J Clin Pathol.* 1979;28:481–90.

25. Sadashivam S, Manickam A. Phenolics biochemical methods. New Delhi: New Age publishers; 2004. p. 193–4.
26. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. *J Biol Chem.* 1951;193: 265–75.
27. Zak B. Cholesterol methodologies: a review. *Clin Chem.* 1977;23:1201–14.
28. El-Mesery ME, Al-Gayyar MM, Salem HA, Darweish MM, El-Mowafy AM. Chemopreventive and renal protective effects for docosahexaenoic acid (DHA): implications of CRP and lipid peroxides. *Cell Div.* 2009;4:6–12.
29. IARC. Some polycyclic aromatic hydrocarbons and heterocyclic compounds. IARC monographs on the evaluation of carcinogenic risk of chemicals to humans, vol. 3. Lyon: France International Agency for Research on Cancer; 1973. p. 271.
30. IARC. Polynuclear aromatic compounds, part 1. Chemical, environmental and experimental data. IARC monographs on the evaluation of carcinogenic risk of chemicals to humans. Lyon, France: International Agency for Research on Cancer. 1983; p. 32.
31. IARC. Overall evaluations of carcinogenicity. IARC monographs on the evaluation of carcinogenic risk of chemicals to humans, supplement 7, vol. 7. Lyon, France: International Agency for Research on Cancer. 1987; p. 440.
32. Agrawal A, Verma P, Goyal PK. Chemomodulatory effects of *Aegle marmelos* against DMBA-induced skin tumorigenesis in swiss albino mice. *Asian Pac J Cancer Prev.* 2010;11:131–9.
33. Mirunalini S, Karthiashwaran K, Dhamodharan G, Mohan S. Melatonin attenuates lipid peroxidation and enhances circulatory antioxidants during mammary carcinogenesis in rats. *J Biochem Technol.* 2010;2:171–4.
34. Bachran C, Bachran S, Sutherland M, Bachran D, Fuchs H. Saponins in tumor therapy. *Mini Rev Med Chem.* 2008;8:575–84.
35. Bachran C, Heisler I, Bachran D, et al. Chimeric toxins inhibit growth of primary oral squamous cell carcinoma cells. *Cancer Biol Ther.* 2008;7:237–42.
36. Podolak I, Galanty A, Sobolewska D. Saponins as cytotoxic agents: a review. *Phytochem Rev.* 2010;9:425–74.
37. Lee ST, Wong PF, Cheah SC, Mustafa MR. Alpha-tomatine induces apoptosis and inhibits nuclear factor-kappa B activation on human prostatic adenocarcinoma PC-3 cells. *PLoS One.* 2011;6:e18915.
38. Budda S, Butryee C, Tuntipopipat S, Rungsipipat A, Wangnait-hum S, Lee JS, Kupradinun P. Suppressive effects of *Moringa oleifera* Lam pod against mouse colon carcinogenesis induced by azoxymethane and dextran sodium sulfate. *Asian Pac J Cancer Prev.* 2010;12:3221–8.
39. Parimalakrishnan S, Dey A, Manavalan R. Effect of methanolic extract of *Cleome chelidonii* on DRUG metabolising enzymes, antioxidant status and chemomodulatory efficacy of mice. *J Basic Appl Sci.* 2009;5:37–46.
40. Kesba HH, El-Belagi HS. Biochemical changes grape rootstocks resulted from humic acid treatments in relation to nematode infection. *Asian Pac J Trop Biomed* 2012; 2(4):287–93.
41. Khan SM. Protective effect of black tea extract on the levels of lipid peroxidation and antioxidant enzymes in liver of mice with pesticide induced liver injury. *Cell Biochem Funct.* 2006;24:327–32.
42. Vijayabaskaran M, Yuvaraja KR, Babu G, Sivakumar P, Perumal P, Jayakar B. Hepatoprotective and antioxidant activity of *Symplocos racemosa* bark extract on DMBA induced hepatocellular carcinoma in rats. *Int J Curr Trends Sci Technol.* 2010;1:147–58.
43. Bedi PS, Saxena KD, Singh B, Ghosh D, Pal SK. Effect of *Cichorium intybus* Linn. against aflatoxicosis induced in albino rats. *World Cancer Congress, Geneva: Proceedings Publication.* 2008, p. 340.
44. Singh H, Bedi PS, Singh B. Hepatoprotective Activity of turmeric and garlic against 7,12-dimethylbenzanthracene induced liver damage in Wistar albino rats. *Eur J Med Plants.* 2011;1:162–70.
45. Ramakrishnan G, Augustine TA, Jagan S. Effect of silymarin on *N*-nitrosodiethylamine induced hepatocarcinogenesis in rats. *Exp Oncol.* 2007;29:39–44.
46. Klaunig JE, Xu Y, Bachowski S, Jiang J. Free-radical oxygen induced changes in chemical carcinogenesis. In: Wallace K.B., editor. *Free radical toxicology.* Taylor & Francis: London; 1997. pp. 375–400.
47. Freeman TA, Parvizi J, Della Valle CJ, Steinbeck MJ. Reactive oxygen and nitrogen species induce protein and DNA modifications driving arthrofibrosis following total knee arthroplasty. *Fibrogen Tissue Repair.* 2009;2:5.
48. Stadtman ER. Role of oxidant species in aging. *Curr Med Chem.* 2004;11:1105–12.
49. Klaunig JE, Wang Z, Pu X, Zhou S. Oxidative stress and oxidative damage in chemical carcinogenesis. *Toxicol Appl Pharmacol.* 2011;254:86–99.
50. Parmar J, Sharma P, Verma P, Sharma P, Goyal PK. Modulation of DMBA induced biochemical and histopathological changes by *Syzygium cumini* seed extract during skin carcinogenesis. *Int J Cur Biomed Phar Res.* 2011;1(2):24–30.
51. Fakurazi S, Sharifudin SA, Arulselvan P. *Moringa oleifera* hydroethanolic extracts effectively alleviate acetaminophen-induced hepatotoxicity in experimental rats through their antioxidant nature. *Molecules.* 2012;17:8595–603.
52. Sharma V, Paliwal R. Chemo protective role of *Moringa oleifera* and its isolated Saponin against DMBA induced tissue damage in male mice: a histopathological analysis. *Int J Drug Dev Res.* 2012;4(4):215–28.
53. Sharma V, Paliwal R. Isolation and characterization of saponins from *Moringa oleifera* (moringaceae) pods. *Int J Pharm Pharm Sci.* 2013;5(1):179–83.