RESEARCH COMMUNICATION

Chemopreventive Efficacy of *Moringa oleifera* Pods Against 7, 12-Dimethylbenz[a]anthracene Induced Hepatic Carcinogenesis in Mice

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Abstract

Oxidative stress is a common mechanism contributing to initiation and progression of hepatic damage in a variety of liver disorders. Hence there is a great demand for the development of agents with potent antioxidant effect. The aim of the present investigation is to evaluate the efficacy of *Moringa oleifera* as a hepatoprotective and an antioxidant against 7, 12-dimethylbenz[a]anthracene induced hepatocellular damage. Single oral administration of DMBA (15 mg/kg) to mice resulted in significantly ($p<0.001$) depleted levels of xenobiotic enzymes like, cytochrome P450 and b5. DMBA induced oxidative stress was confirmed by decreased levels of reduced glutathione (GSH) and glutathione-S-transferase (GST) in the liver tissue. The status of hepatic aspartate transaminase (AST), alanine transaminase (ALT), and alkaline phosphatase (ALP) which is indicative of hepatocellular damage were also found to be decreased in DMBA administered mice. Pretreatment with the *Moringa oleifera* (200 and 400 mg/kg) orally for 14 days significantly reversed the DMBA induced alterations in the liver tissue and offered almost complete protection. The results from the present study indicate that *Moringa oleifera* exhibits good hepatoprotective and antioxidant potential against DMBA induced hepatocellular damage in mice that might be due to decreased free radical generation.

Keywords: *M. oleifera* - 7 - 12-dimethylbenz[a]anthracene - xenobiotic - hepato-protective - oxidative stress

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Introduction

Chemoprevention is regarded as one of the most promising and realistic approaches in the prevention of cancer. Chemotherapy is often the first choice for treatment of many cancers, and chemotherapeutic drugs work by disrupting the growth of cancer cells. Several bioactive compounds present in fruits and vegetables have revealed their cancer curative potential on cancer. An alternative approach to cancer avoidance could be to increase the intake of chemopreventive compounds, which might be reasonably expected to interfere with the initiation, promotion or progression of carcinogenesis (Ramakrishnan et al., 2007). A larger number of chemopreventive agents have been elucidated in epidemiological and experimental studies, preclinical and clinical observations (Radhakrishnan et al., 2007). However, the toxic side effects produced by some of these agents have limited their extensive use. Therefore, there is a need to identify natural compounds that have significant chemopreventive potential without undesirable toxic effects. However, use of these synthetic antioxidants has been questioned due to their potential health risks and toxicity (Sharma et al., 2011). In addition, these naturally occurring antioxidants can be formulated to give neutraceuticals that can help to prevent oxidative damage from occurring in the body.

However, the use of natural antioxidants is limited by a lack of knowledge about their molecular composition, amount of active ingredients in the source material and the availability of relevant toxicity data. The competing demands of taste and health pose a dilemma for consumers as well as the food industry. Food based strategies advocate a diet including easily accessible and inexpensive green leafy vegetables to alleviate micronutrient deficiencies.

Therefore, the evaluation of antioxidant activity of various plant extracts is considered as an important step in the identification of their ability to scavenge the free radicals. Medicinal properties of plants have also been investigated in the light of recent scientific developments through out the world, due to their potent pharmacological activities, low toxicity and economic viability, when compared with synthetic drugs (Vadlapudi and Naidu, 2010; Janmeda et al., 2011). In this regard, many compounds have been tested with proved efficacy against experimentally-induced hepatocarcinogenesis.

Moringa oleifera Lam. (Syn *Moringa pterygosperma* Gaertn) 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 (Moringaceae).

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Different parts of this plant are being employed for the treatment of various ailments in the indigenous system of medicine (Paliwal et al., 2011a, b, c). It possesses antitumor, antipyretic, antiepileptic, anti-inflammatory, antiulcer, antispasmodic, diuretic, antihypertensive, cholesterol lowering, antioxidant, antidiabetic, renal (Paliwal et al., 2011b) and hepatoprotective activities (Paliwal et al., 2011c). Early studies documented the presence of Phenolics, flavonoids, saponins, terpenoids, proanthocyanadins, and cardiac glycosides in the Moringa oleifera pods (Paliwal et al., 2011d; Sharma et al., 2011). Although much has been learned about the nutritional value of Moringa oleifera additional knowledge remains to be secured.

Polycyclic aromatic hydrocarbons (PAH) are a class of organic pollutants that are released into the environment in large quantities, mainly due to human activities. The polycyclic aromatic hydrocarbons (PAHs) are reasonably anticipated to be human carcinogens based on sufficient evidence of carcinogenicity in experimental animals (IARC, 1973, 1983, 1984, 1985, 1987). The PAH 7, 12-dimethyl-benz[a]anthracene (DMBA) acts as a potent carcinogen by generating various reactive metabolic intermediates leading to oxidative stress.

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. Emerging evidences suggest that DMBA induces the production of reactive oxygen species (ROS) that result in lipid peroxidation, DNA damage, and depletion of cell antioxidant defense systems (Bharli et al., 2003). Change in lipid peroxidation production reactions and antioxidant defense systems were associated with changes in a variety of biochemical pathways (Paliwal et al., 2011c)

Although there are many strategies for the treatment of liver cancer, the therapeutic outcome of this cancer remains very poor. In spite of tremendous advances in modern medicine, there are not many effective drugs available that stimulate liver function, offer protection to the liver from damage or help to regenerate hepatic cells (Chattopadhyay, 2003). However, to the best of our knowledge, there is scanty scientific data available to validate the folkloric anticancer claims of this plant. Hence, the aim of this current investigation was to explore more the chemopreventive effects of the hydroethanolic pods extract of *M. oleifera* against environmental toxicant DMBA.

Materials and Methods

Chemicals and reagents

All chemicals used in the study were of analytical reagent grade and of highest quality available and were purchased from reliable firms (SRL (India), MERCK, RANBAXY, HIMEDIA, QUALLIGENS and SUYOG, Mumbai, India). DMBA was purchased from SIGMA Chemical Co. USA.

Experimental plant and preparation of Hydro-ethanolic plant extract (MOHE)

The experimental plant Moringa oleifera was collected from Krishi Vigyan Kendra, Banasthali University, Banasthali, India, in the month of October 2010. 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. Further refluxed for 18 hours at 80ºC for two days. 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 50ºC for 48 hours and stored in a desiccator. The percentage yield of extract (MOHE) was found to be 22% and stored at 4ºC in airtight containers. Suspensions of the extract was prepared in distilled water and used to assess hepatoprotective and antioxidant activity.

Experimental animals

Male Swiss Albino mice (*Mus musculus*) weighing 15-30 g were obtained from Haryana Agricultural University, Hissar (India) for experimental purpose. The animals were acclimatized for a month prior to experiment. The Institutional Animal Ethical Committee approved the animal studies. All experiments were conducted on adult male albino mice when they weighed 25-35 g (3-4 months old). Colony bred adult male albino mice were maintained under standard laboratory conditions at a temperature of 22 ± 3 ºC, relative humidity of 50 ± 5 % and photoperiod of 12 h (12 h-dark and 12 h-light cycle).

Experimental Design

Adult Swiss albino male mice divided into ten groups of 6 mice each were treated by oral gavage. Treatment consisted of pretreatment phase of MO in distilled water followed by the second phase in which the animals were given 15 mg/kg DMBA on day 15. The animals were then euthanized 4 days after DMBA administration. The groups were as follows-

a) Group 1: served as control (normal untreated mice), and received 1ml distilled water daily by oral gavage.

b) Group 2: received pretreatment with distilled water for 14 days prior to a single dose of DMBA (15 mg/kg body weight: p.o) served as DMBA control group.

c) Group 3 and 4 were administered with hydro-ethanolic extract of pods of MO (200 and 400 mg/kg body weight: p.o) daily for 14 days, served as MO treated control group.

d) Group 5 and 6: received BHA (0.5 % and 1%: p.o) daily for 14 days, dissolved in 0.5% acetone and served as standard treated control group.

e) Group 7 and 8: were treated with hydro-ethanolic extract of pods of MO (200 and 400 mg/kg body weight; p.o) daily for 14 days, before being intoxicated with DMBA (15 mg/kg body weight; p.o, once) dissolved in olive oil.

f) Group 9 and 10: received BHA (0.5 % and 1%: p.o) daily for 14 days, before being intoxicated with DMBA (15 mg/kg body weight; p.o, once) dissolved in olive oil.

The dose for DMBA, standard antioxidant, and plant

were decided and selected on the basis of LD_{50} calculated in the laboratory and on the basis of published reports (Song et al., 2000; Bharali et al., 2003; Paliwal et al., 2011b; 2011c).

Hepatoprotective Activity

After 19 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 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.

Determination of xenobiotic enzyme and oxidative stress activities

Cytochrome P_{450} and cytochrome b_5 content were assayed in the microsomal suspention by the method of Omura and Sato (1964), using an absorption coefficient of 91 and 185 cm^2 M^{-1} m^{-1}, respectively. The 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 Biochemical assays

The activities of various biochemical parameters like asparatate and alanine transaminase (AST and ALT) were assayed by the method of Reitman and Frankal (1957). Activities of alkaline phosphatase (ALT) were determined according to the protocol described in laboratory manual (Sadashivam and Manickam, 1996). Total protein content was estimated by the method of Lowry et al. (1951) using bovine serum albumin as standard. The cholesterol level was determined by using cholesterol as standard by the method of Zak's (1977).

Statistical Analysis

The experimental results obtained are expressed as mean ± standard deviation (SD) of three replicates. The data was subjected to one way analysis of variance (ANOVA) and differences between samples were determined by Tukey multiple comparison test and Bonferroni's test using the SPSS 16.0 (Statistical program for Social Sciences) program. The level of significance was set at $p<0.001$.

Results

The results of the study clearly indicate that *Moringa oleifera* pods possess chemo protective activity. Table 1, 2 and 3 illustrates effect of hydroethanolic extract of *Moringa oleifera* and BHA on Xenobiotic enzymes, Oxidative stress and biochemical parameters in control and treated groups against DMBA-induced hepatic toxicity in male mice.

Effect on xenobiotic enzymes in Liver of DMBA treated mice

For studying the effect of MOHE pods on drug metabolizing phase I enzymes, specific activities of cytochrome P_{450} and cytochrome b_5 were measured (Table 1). Cytochrome P_{450} and b_5 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, intake of hydro-ethanolic extract of Moringa oleifera pods at low dose (200 mg/kg body weight), high dose (400 mg/kg body weight) and BHA (0.5% and 1%) significantly ($p<0.01$) decreased both the enzyme level, protecting liver from hepatocellular damage due to DMBA. The hepato-protective effect of the extract was comparable to the effect seen with BHA treatment.

Pre-treatment with hydro-ethanolic extract of *Moringa oleifera* at dose of 200 and 400mg/kg body weight and BHA (0.5% and 1%) before DMBA challange significantly ($p<0.001$) improved the enzyme level as compared to DMBA treated group. The increase in the activity of enzymes is due to DMBA challenge which was significantly ($P<0.001$) restored by pre-administration of MOHE at both the doses (200 and 400mg/kg) for 14 days. Thus the results clearly indicate that pods exhibit dose dependent activity. BHA showed less significant effect as compared to MOHE.

Table 1. Modulatory Influence of Hydro-Ethanolic Extract of *Moringa oleifera* pods on Hepatic Xenobiotic (phase I) drug Metabolizing Enzymes in DMBA-Exposed Male Mice

Groups	Treatments (mg/kg)	Cyt P_{450} (nmole/mg)	Cyt b_5 (nmole/mg)
Control	-	2.82±0.03[a]	1.72±0.06[a]
DMBA	15	8.17±0.07[*]	5.71±0.16[*]
MO	200	3.57±0.16[**/a]	1.96±0.12[**/a]
	400	4.59±0.01[*/a]	2.31±0.05[*/a]
BHA	0.50%	2.93±0.11[**/a]	1.87±0.14[**/a]
	1%	3.92±0.13[**/a]	2.09±0.09[**/a]
MO+DMBA	200 + 15	5.67±0.16[*/a]	4.73±0.12[*/a]
MO + DMBA	400 + 15	3.92±0.09[*/a]	2.81±0.05[*/a]
BHA+ DMBA	0.5% + 15	6.21±0.16*	5.11±0.17[*]
BHA+DMBA	1% + 15	5.11±0.07[**/a]	3.53±0.08[**/a]

Values are expressed as mean ± SD (n=6), *p<0.001, **p<0.01 vs. control group, ap<0.001 vs. Treated (DMBA) group, Cyt P450: Cytochrome P_{450} expressed as (nmole/mg); Cty b5: Ctochrome b_5 expressed as (nmole/mg)

Table 2. Chemo-Protective Effect of Hydro-Ethanolic Extract of *Moringa oleifera* Pods on Oxidative Stress Parameters in DMBA-Exposed Male Mice

Groups	Treatments (mg/kg)	GSH(nmole/g) (nmole	GST CDNB/min/mg)
Control	-	2.07±0.13[a]	220.55±0.04[a]
DMBA	15	1.52±0.04[***]	147.31±0.08[**]
MO	200	2.24±0.09[***/a]	234.16±0.03[**/a]
	400	2.43±0.02[***/a]	263.60±0.06[**/a]
BHA	0.50%	2.12±0.09[***/a]	224.73±0.06[**/a]
	1%	2.19±0.11[***/a]	229.31±0.15[**/a]
MO+DMBA	200 + 15	1.71±0.06[*/a]	182.17±0.05[*/a]
MO + DMBA	400 + 15	2.06±0.12[*/a]	215.26±0.07[*/a]
BHA+ DMBA	0.5% + 15	1.53±0.08[*/a]	157.50±0.01[*/a]
BHA+DMBA	1% + 15	1.85±0.24[*/a]	184.61±0.08[*/a]

Table 3. Hepatoprotective Effect of Hydro-Ethanolic Extract of *Moringa oleifera* Pods on Hepatic Biochemical Parameters in DMBA-Exposed Male Mice

Groups	Treatments (mg/kg)	AST (IU/ml)	ALT (IU/ml)	ALP (μM PNP/min/g)	Total Protein (g/ml)	Cholesterol (mg/g)
Control	-	98.32±0.18[a]	54.24±0.16[a]	104.42±0.09[a]	5.65±0.02[a]	71.65±0.04[a]
DMBA	15	54.21±0.02[**]	31.34±0.28[**]	67.55±0.01[**]	1.96±0.05[*]	123.54±0.05[*]
MO	200	112.13±0.07[a]	67.32±0.07[**/a]	118.43±0.05[**/a]	7.21±0.13[*/a]	61.54±0.02[*/a]
	400	132.11±0.08[*/a]	73.11±0.23[**/a]	129.21±0.08[**/a]	8.54±0.11[*/a]	52.14±0.11[*/a]
BHA	0.50%	101.21±0.28[**/a]	58.12±0.14[*]	106.23±0.11[*]	6.57±0.09[**]	70.47±0.08[**]
	1%	105.12±0.18[**/a]	62.32±0.18[**/a]	112.34±0.07[**/a]	6.93±0.07[**/a]	66.23±0.13[*/a]
MO+DMBA	200 + 15	84.23±0.02[*/a]	44.34±0.08[*/a]	84.22±0.08[*/a]	5.12±0.15[*/a]	89.56±0.14[*/a]
MO + DMBA	400 + 15	93.21±0.12[*/a]	51.76±0.07[*/a]	98.65±0.12[*/a]	5.82±0.11[*/a]	76.44±0.08[*/a]
BHA+ DMBA	0.5% + 15	65.11±0.09[*/a]	39.32±0.09[*/a]	71.76±0.08[*/a]	4.21±0.16	107.32±0.12
BHA+DMBA	1% + 15	76.27±0.03[*/a]	41.65±0.13[*/a]	75.39±0.03[*/a]	4.86±0.09[*/a]	98.23±0.07[*/a]

Effect Oxidative stress parameters in Liver of DMBA treated mice

The toxic effect of DMBA was justified by the significant decrease in the activity of GSH ($p<0.05$) and GST ($p<0.01$) in liver when compared with healthy controls (Table 2). The antioxidant effect of MOHE (200 and 400 mg/kg) and BHA (0.5% and 1%) was observed by significant increase in the activity of GSH ($p<0.05$) and GST ($p<0.01$) when compared to DMBA positive control, thus protecting liver from oxidative damage due to carcinogen. Pre-administration of MOHE (200 and 400 mg/kg) and BHA (0.5% and 1%) showed a significant elevation ($p<0.001$) in GSH and GST level inspite of post DMBA challenge i.e. on 15th day. The hepatoprotective effect of MOHE was comparable to the effect seen with BHA treatment.

Effect on Biochemical parameters in Liver of DMBA treated mice

DMBA toxicity produced a significant ($p<0.01$) depletion of AST, ALT, ALP and total protein content in liver tissue, as compared to control mice (Table 3), which indicated severe hepatic damage. The total cholesterol level in liver of DMBA exposed mice showed a significant ($p<0.001$) elevation, in comparison to control animals.

The levels of hepatic marker enzymes (AST, ALT and ALP) and total protein content were significantly ($p<0.001$) elevated by pre- administration of hydro-ethanolic extract of Moringa oleifera (200 and 400 mg/Kg body weight) and BHA (0.5% and 1%). The cholesterol level was significantly ($p<0.001$) reduced as compared to DMBA treated group. Administration of MOHE showed significant protection in almost all the above biochemical variables.

Discussion

Cancer prevention research involves the use of either natural or synthetic components to delay, inhibit or reverse the development of cancer in normal or preneoplastic conditions. Numerous experimental and epidemiological studies show that chemoprevention has the potential of providing an important means for cancer prevention, for both the general population and even more importantly for individuals at high risks (Sporn, 2000).

The cellular systems may be the target for the toxicological effects of wide variety of chemical compounds such as PAH, if these compounds are not biotransformed to easily excretable hydrophilic metabolites. DMBA treatments generate ROS in affected area of organism and ultimately lead to carcinogenesis (Das et al., 2010). Antioxidant enzymes as well as antioxidant molecules protect the cells against oxidative damage (Burton et al., 1985).

One of the most 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). Microsomal cytochrome P_{450} 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). Cytochrome P_{450} catalyzes the oxidation of lipophilic chemicals through the insertion of one atom of molecular oxygen into substrate thereby rendering the latter to either less harmful or totally harmless hydrophilic metabolites for their ultimate removal from living system.

In the present study, Moringa pretreatment significantly increased the levels of the measured components of cytochrome P_{450} system (cytochrome P_{450} and cytochrome b_5). During oxidative metabolism, in the microsomal microenvironment involving the cytochrome P_{450} system, the electron flows from NADPH or NADH through a flavoprotein cytochrome P_{450} reductase or cytochrome b_5 reductase to different isomorphic forms of cytochrome P_{450} and cytochrome b_5 (Gibson & Skett, 1994). Cytochrome P_{450} is the terminal oxidase component of an electron transport system present in the endoplasmic reticulum responsible for many drug oxidation reactions. Thus, the investigated plant extract by virtue of its action as inducer of cytochrome P_{450} may be speculative of acting as blocking agent.

The result of this study clearly demonstrates that carcinogen DMBA decreased the activities of antioxidant enzymes (GSH and GST) and the levels of hepatic marker enzymes (AST, ALT and ALP) in liver of mice. The total protein content in liver of mice was also declined when treated with DMBA. *Moringa oleifera* pods extract restored the loss of enzyme activities caused by DMBA

treatment. GSH and GST play a critical role in protection of tissues from deleterious effects of carcinogen.

The pathophysiological consequences owing to depletion of GSH have been well studied. Glutathione is highly abundant in cytosol, nuclei and mitochondria, and is the major soluble antioxidant in these cell compartments (Sharma et al., 2010). The depletion of GSH promotes generation of reactive oxygen species and oxidative stress with cascade of effects thereby affecting functional as well as structural integrity of cell and organelle membranes (De Leve et al., 1996; Singh et al., 2000). The elevated level of GSH protects cellular proteins against oxidation through glutathione redox cycle and also directly detoxifies reactive oxygen species and/or neutralizes reactive intermediate species generated from exposure to xenobiotics including chemical carcinogens (Kettrer, 1998). 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.

Glutathione S-transferase is a critical detoxification enzyme that primarily functions in conjugating 'functionalized P_{450} metabolites' with endogenous ligands (reduced glutathione) favouring their elimination from the body of the organisms. There are persuasive evidences to support the induction of glutathione S-transferase and protection against a wide spectrum of cytotoxic, mutagenic and carcinogenic chemicals (Singh et al., 2000).

Aminotransferases, are group of enzymes that catalyse reversible transfer of the amino acid group from an α-amino acid to an oxo acid. These enzymes are not normal components of plasma and its function outside organ of origin is unknown (James et al., 2003; Fakurazi et al., 2008). The largest pool of ALT is found in cytosol of hepatic parenchymal cells (Okuda, 1997). AST is found in cytosol and mitochondria of hepatocytes and also found in cardiac muscle, skeletal muscle, pancreas and kidney (Shyamal et al., 2006). Therefore, measurement of ALT is more liver specific to determine hepatocellular damage (Shyamal et al., 2006). Nevertheless, AST is still being commonly used as a laboratory test to assess liver function since it is considered to be a sensitive indicator of mitochondria damage particularly in the centrilobular regions of liver (Panteghini, 1990). Meanwhile, ALP comprises of a family of enzymes that hydrolyse phosphate esters at alkaline pH and is often used as a marker for cholestatic liver dysfunction.

Free radicals that are generated by DMBA decrease the total protein content in liver mice that were elevated by the administration of *M. oleifera* pods extract in experimental groups. Saponins also form complexes with proteins and could decrease protein degradability (Sen et al., 1998; Parmar et al., 2011). Inhibitory role of DMBA in protein synthesis may be due to reduced DNA and RNA content. Moreover the carcinogen perturbs intracellular Ca^{2+} homeostasis and damages the endoplasmic reticulum, which in turn results in protein depletion (Sharma et al., 2010). 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 this study, hepatoprotective effect of *Moringa oleifera* is evident by the restoration of ALT, AST and ALP along with total protein and cholesterol content. Significant preservation of liver was observed in the groups that were pretreated with MOHE.

Recovery towards normalization of the enzymes following MO pre-treatment suggested that the plant extract have some roles in preserving structural integrity of hepatocellular membrane, thus prevented enzymes leakage into the blood circulation.

The present investigation has demonstrated that MO 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 phenolic compounds that was confirmed in this study by phytochemical screening of the extract (Paliwal et al., 2011d; Sharma et al., 2011). In this respect, Moringa pods contain important bioactive compounds including glucosinolates, isothiocyanates, thiocarbamates, and flavonoids (Guevara et al., 1999; Lalas & Tsaknis, 2002; Bennett et al., 2003; Bharli et al., 2003). These compounds quench of ROS, chelate metal ions and regenerate membrane-bound antioxidants. This finding is consistent with previous studies, which demonstrated the antioxidant activity of MO extract (Kumar & Pari, 2003; Paliwal et al., 2011d; Sharma et al., 2011). MO also protects against oxidative stress via the elevation of antioxidative defense enzyme, while significantly reducing the level of lipid peroxidation (Singh et al., 2000; Arabshahi et al., 2007; Paliwal et al., 2011b, c)

The results obtained from this study prompt further investigation of the mechanism of hepatoprotective activities of MO and the role of bioactive components of the plant extract responsible for this action. Each part of the plant has been reported useful with various medicinal properties and nutritional values. Therefore, scientifically proven hepatoprotective activities of MO pods may certainly provide benefits following human consumption.

In conclusion, the present study demonstrated that *Moringa oleifera* mediates its chemopreventive effects by enhancing antioxidant status and quenching of ROS. Traditional use of natural plant part as food ingredients may confer some protection from cancer. The results of present study indicate that *Moringa oleifera* may emerge as putative chemopreventive agents against carcinogen toxicity.

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