RESEARCH COMMUNICATION

Chemomodulatory Effect of *Moringa Oleifera*, Lam, on Hepatic Carcinogen Metabolising Enzymes, Antioxidant Parameters and Skin Papillomagenesis in Mice

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Abstract

The modulatory effects of a hydro-alchoholic extract of drumsticks of *Moringa oliefera* Lam at doses of 125 mg/ kg bodyweight and 250 mg/ kg body weight for 7 and 14 days, respectively, were investigated with reference to drug metabolising Phase I (Cytochrome b_5 and Cytochrome P_{450}) and Phase II (Glutathione-S- transferase) enzymes, anti-oxidant enzymes, glutathione content and lipid peroxidation in the liver of 6-8 week old female Swiss albino mice. Further, the chemopreventive efficacy of the extract was evaluated in a two stage model of 7,12 – dimethylbenz(a)anthracene induced skin papillomagenesis. Significant increase ($p<0.05$ to $p<0.01$) in the activities of hepatic cytochrome b_5, cytochrome P_{450}, catalase, glutathione peroxidase (GPx), glutathione reductase (GR), acid soluble sulfhydryl content (-SH) and a significant decrease ($p<0.01$) in the hepatic MDA level were observed at both dose levels of treatment when compared with the control values. Glutathione-S- transferase (GST)activity was found to be significantly increased ($p<0.01$) only at the higher dose level. Butylated hydroxyanisol (BHA) fed at a dose of 0.75% in the diet for 7 and 14 days (positive control) caused a significant increase ($p<0.05$ to $p<0.01$) in the levels of hepatic phase I and phase II enzymes, anti- oxidant enzymes, glutathione content and a decrease in lipid peroxidation. The skin papillomagenesis studies demonstrated a significant decrease ($p<0.05$) in the percentage of mice with papillomas, average number of papillomas per mouse and papillomas per papilloma bearing mouse when the animals received a topical application of the extract at a dose of 5mg/ kg body weight in the peri-initiation phase 7 days before and 7 days after DMBA application, Group II), promotional phase (from the day of croton oil application and continued till the end of the experiment, Group III) and both peri and post initiation stages (from 7 days prior to DMBA application and continued till the end of the experiment, Group IV) compared to the control group (Group I). The percentage inhibition of tumor multiplicity has been recorded to be 27, 72, and 81 in Groups II, III, and IV, respectively. These findings are suggestive of a possible chemopreventive potential of *Moringa oliefera* drumstick extract against chemical carcinogenesis.

Key Words: Chemoprevention - hepatic biotransformation enzymes - anti-oxidant defense - DMBA - skin papillomagenesis - *Moringa oliefera* Lam

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Introduction

A promising approach to reduce the occurance of cancer is its prevention, specifically by chemical intervention through minor dietary constituents. Epidermiological studies suggests that specific pharmacologically active agents present in the diet might reduce the relative risk of cancer development (Tanaka et al,2001). A remarkable surge of interest in chemoprevention research has, thus, led to the identification of many phytochemicals of dietary origin as effective potential chemopreventive agents (Cordell et al, 1992). *Moringa oliefera*, Lam (Fam: Morigaceae) a perennial plant, is native to tropical Africa and is also widely distributed in India, Ceylon and Madagascar (Eilert et al, 1981). The medicinal value of different parts of the plant has long been recognised in folk medicine and is extensively used in the treatment of ascites, rheumatism, venomous bites and as a cardiac and circulatory stimulant (Guenera et al,

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1996). The seed oil is a strong anti-inflammatory agent and used for skin diseases (Villasenor, 1994). Preliminary analysis suggested that LD 50 value of the crude extract is 2.8g/ kg, intraperitoneal in mice indicating a less toxic action of the crude extract of *Moringa oliefera* (Majumdar et al, 1999). From the stem of *Moringa oliefera* vanillin, b-sitosterol, b-sitostenone, 4-hydroxymellin, octacosanoic acid have been isolated while alkaloids, waxes, qurecetic acid and kaemferol are reported from its flowers (Faizi et al ,1994). The plant is a rich source of vitamin A and C (Dhar and Gupta, 1982).The drumsticts are specifically rich in carotene, which is efficiently converted into vitamin A in the body and it has a significant hepatoprotective effect (Geervani and Devi ,1981). In the present investigation the modulatory effect of the hydro alcoholic extract of *Moringa oliefera*, Lam drumstick has been evaluated on levels/ activities of phase I and phase II enzymes, anti-oxidant enzymes, -SH content and lipid peroxidation as a rational to acces its chemopreventive potential. Further, the modulatory influence of the extract on 7,12dimethylbenz(a)anthracene (DMBA) induced and croton oil promoted skin papillomagenesis in swiss female albino mice was investigated to quantitate the chemopreventive response.

Materials and Methods

Chemicals

7,12 dimethylbenz(a)anthracene (DMBA), 5, 5 –dithiobis-2- nitrobenzoic acid (DTNB), 1- chloro-2,4-dinitrobenzene (CDNB), reduced glutathine (GSH),oxidized glutathione (GSSG), Pyrogallol, Triton-X 100, ethylene diamine tetraacetic acid (EDTA), thiobarbituric acid (TBA), reduced nicotinamide adenine dinucleotide (NADH), reduced nicotinamide adenine dincleophosphate (NADPH) were obtained from Sigma Chemicals Co. (St. Louis, USA). The rest of the chemicals were obtained from local firms (India) and are of highest purity grade.

Test Material

The drumsticks of Moringa oliefera Lam were purchased from the local market. It was washed thoroughly with double distilled water. Then it was wiped dry with clean mushlin cloth, cut into small pieces and shade dried. A known quantity of the dried ground material (50 gm) were subjected to soxhlet extraction using 80% hydroalcoholic solvent (80% ethanol: 20% distilled water). The final extract was filtered and the remaining alcohol was allowed to evaporate. Finally the extract was weighed and stored at 4 ^{o}C until further use.

Animals

Swiss female albino mice of random bred, 6-8 weeks old were selected for the study. They were provided with standard pellet diet and tap water ad libitum, under hygienic conditions.

Experimental Design

The experiments were carried out separately with specific objectives as stated earlier. Experiment I studies the enzyme profiles while Experiment II aims the study of tumorigenesis.

Experiment I

Modulatory influence of Moringa oleifera, Lam on drug metabolism and antioxidant status in mice. The animals were randomly assorted into the following groups:

Group I (n = 10): This group of animals received a normal diet daily for 7 days and served as negative control.

Group II (n = 10): This group of animals received a normal diet and treated daily with 125 mg/ kg body weight of *Moringa oleifera*, Lam extract, in distilled water, 12.5ml, through oral gavage for 7 days.

Group III (n = 10): This group of animals received a normal diet and treated daily with 250 mg/ kg body weight of Moringa oleifera, Lam extract, in distilled water, 25ml, through oral gavage for 7 days.

Group IV (n = 10): This group of animals received a normal diet containing 0.75% butylated hydroxyanisole (BHA) for 7 days and served as positive control.

Group V (n = 10): This group of animals received a normal diet daily for 14 days and served as negative control.

Group VI (n = 10): This group of animals received a normal diet and treated daily with 125 mg/ kg body weight of *Moringa oleifera*, Lam extract, in distilled water, 12.5ml, through oral gavage for 14 days.

Group VII (n = 10): This group of animals received a normal diet and treated daily with 250 mg/ kg body weight of *Moringa oleifera*, Lam extract, in distilled water, 25ml, through oral gavage for 14 days.

Group VIII (n = 10): This group of animals received a normal diet containing 0.75% BHA for 14 days and served as positive control.

Body weights of the mice were recorded initially at weekly intervals and at the end of the experiment. Diet was withheld from the animals on the night prior to the day of termination of the experiment.

Preparation of Homogenates, Cytosol and Microsomal Fractions

The animals were sacrificed by cervical dislocation, and the entire liver was perfused immediately with 0.9% ice-cold NaCl solution and thereafter carefully removed, trimmed free of extraneous tissue and rinsed in chilled 0.15M Tris-KCl buffer (0.15 M KCl + 10 mM Tris-HCl, pH 7.4). acid microsomal fractions was prepared by the method of Fry and Bridges[8]. The liver was blotted dry, and 500 mg of liver was weighed quickly and homogenized in 2ml of ice-cold 1.15% (w/v) KCl (by three up and down stroke at maximum speed). The homogenate was then centrifuged at 10,000 g for 20 min. The supernatant was taken and the pH was adjusted to 5.4 with acetate buffer (pH 4.0). It was then centrifuged at 10,000 g for 10 minutes. The supernatant was discarded and the pellet was washed in 1.15% KCl : glycerol (4:1 v/v) and recentrifuged at 10,000 g for 10 minutes. The pellet was then suspended in 0.1M phosphate buffer (pH 7.4) : glycerol (4:1 v/v) by one complete stroke in the

homogenizer to obtain the acid microsomal fraction, which was used for the assay of cytochrome P_{450}, cytochrome b_5 and lipid peroxidation.

The remaining portion of the liver tissue was weighed quickly and homogenized in ice-cold 0.15M Tris-KCl buffer (pH 7.4) to yield 10% (w/v) homogenate. An aliquot of this homogenate (0.5ml) was used for assaying acid soluble Sulphydryl group (-SH) while the remainder was centrifuged at 10,000 g for 20 min. The supernatant (cytosol fraction), after discarding any floating lipid layer and appropriate dilution, were used for the assay of glutathione-S-transferase and antioxidant enzymes.

Assay Methods

Cytochrome P_{450} and Cytochrome b_5

Cytochrome P_{450} was determined using the carbon monoxide difference spectra. Both cytochrome P_{450} and cytochrome b_5 content were assayed in microsomal suspension by the method of Omura and Sato[9], using an absorption coefficient of 91 and 185 cm^2 M^{-1} m^{-1}, respectively.

Glutathione S-transferase

The cyclosolic glutathione S-transferase activity was determined spectrophotometrically at 37 °C by the method of Habig et al[10]. The reaction mixture (1ml) contained 0.334ml of 100mM phosphate buffer (pH 6.5), 0.033ml of 30mM CDNB and 0.033ml of 30mM of reduced glutathione. After preincubating the reaction mixture for 2 minutes, the reaction was started by adding 0.01ml of diluted cytosol and the absorbance was followed for 3 minutes at 340nm. The specific activity of glutathione S-transferase is expressed as µ moles of GSH-CDNB conjugate formed/min./mg protein using an extinction co-efficient of 9.6 mM^{-1} cm^{-1}.

Reduced Glutathione

Reduced glutathione was estimated as total non protein sulphydryl group by the method as described by Moron et al[11]. Homogenates were immediately precipitated with 0.1ml of 25% trichloroacetic acid and the precipitate was removed after centrifugation. Free -SH groups were assayed in a total volume of 3ml by adding 2ml of 0.6mM DTNB prepared in 0.2M Sodium phosphate buffer (pH 8.0), to 0.1ml of the supernatant and absorbance was read at 412nm using a Cintra-5 UV-VIS-double beam spectrometer of GBC, Australia make.

Glutathione Reductase

Glutathione reductase was determined by the procedure as described by Carlberg and Mannervik[12]. Reaction mixture (final volume 1ml) contained 0.2M sodium phosphate buffer (pH 7.0), 2mM EDTA, 1mM GSSG and 0.2mM NADPH. The reaction was started by adding 25µl of cytosol and the enzyme activity was measured indirectly by monitoring the oxidation of NADPH followed decrease in OD/min for minimum 3 min at 340nm. One unit of enzyme activity was defined as n moles of NADPH consumed/min/mg protein based on an extinction co-efficient of 6.22 mM^{-1} cm^{-1}.

Glutathione Peroxidase

Glutathione peroxidase activity was measured by coupled assay method as described by Paglia and valentine[13]. 1ml of the reaction mixture contained 50mM Sodium phosphate buffer (pH 7.0) containing 1mM EDTA, 0.24 v/ml yeast glutathione reductase, 0.3mM glutathione (reduced), 0.2mM NADPH, 1.5mM H_2O_2 and cytosol sample. Reaction was initiated by adding NADPH and its oxidation was monitored at 340nm by observing the decrease in OD/min for 3min. One unit of enzyme activity has been defined as n moles of NADPH consumed/min/mg. Protein based on an extinction co-efficient of 6.22 mM^{-1} cm^{-1}.

Catalase

Catalase was estimated in a Cintra 5, UV-VIS-double beam spectrometer as described by Aebi[14]. The reaction mixture (1ml, vol.) contained 0.02ml of suitably diluted cytosol in phosphate buffer (50mM, pH 7.0) and 0.1ml of 30mM H_2O_2 in phosphate buffer. The specific activity of catalase has been expressed as µ moles of H_2O_2 consumed/min/mg protein.

Superoxide Dismutase

Superoxide dismutase was assayed by the method of Marklund and Marklund[15], which involves inhibition of pyrogallol auto-oxidation at pH 8.0. A single unit of enzyme is defined as the quantity of Superoxide dismutase required to produce 50% inhibition of auto-oxidation.

Lipid Peroxidation

Lipid peroxidation in microsomes prepared from liver, was estimated spectrophotometrically by thiobarbituric acid-reactive substances (TBARS) method as described by Varshney and Kale[16] and is expressed in terms of malandialdehyde (malondialdehyde) formed/mg protein. In brief, 0.3ml of microsomal sample was mixed with 1.7ml of 0.15M Tris KCl buffer to which 0.5ml of 30% TCA was added. Then 0.5ml of 52 mM TBA was added and placed in a water bath for 45 minutes at 80 °C, cooled in ice and centrifuged at room temperature for 10 min at 3000rpm. The absorbance of the clear supernatant was measured against reference blank of distilled water at 531.8nm.

Protein

Protein was estimated by following the method of Lowry et al17 using bovine Serum albumin (BSA) as standard at 750nm.

Experiment II

Chemopreventive action of the extract on DMBA-induced mouse skin papillomagenesis.

Preparation of Chemicals and Modulator

The carcinogen 17,12-dimethylbenz(a)anthracene (DMBA) was dissolved in acetone at a concentration of 50µg/50µl. Croton oil was mixed in acetone to give a solution of 1% dilution. The modulator was diluted appropriately in acetone to give a dose level of 5mg/kg body weight/day.

Sixty (60) female swiss albino mice (6-8 weeks old) were taken at the onset of the experiment. The hairs on the dorsal interscapular region of the mice were clipped off before the commencement of the experiment. Three days later, 48

animals in the resting phase of hair growth cycle were selected for the study. The animals were assorted into the following groups:

Group I (n = 12): A single dose of 50µg of DMBA in 50µl of acetone was applied topically over the shaven area of the skin of the mice. Two weeks later, croton oil (100µl of 1% croton oil in acetone) was applied three times per week until the end of the experiment (15 weeks).

Group II (n = 12): Animals received a topical treatment (on shaven area of the skin of the mice) of an ethanolic extract of the whole plant of *Moringa oleifera*, Lam (5.0mg/ kg body weight per day) in 100µl acetone for 14 days i.e., 7 days before and 7 days after the application of DMBA. Croton oil was given as in Group I. The experiment was carried out for 15 weeks.

Group III (n = 12): Animals received a topical treatment of *Moringa oleifera*, Lam extract (5.0mg/ kg body weight per day) in 100µl acetone, starting from the time of croton oil treatment and continued till the end of the experiment (15 weeks). DMBA was given as in Group I.

Group IV (n = 12): Animals were treated topically with *Moringa oleifera*, Lam extract (5.0mg/ kg body weight per day) throughout the experimental period, i.e., before and after DMBA application and also at the promotional stage. Croton oil was given as in Group I. The experiment was carried out for 15 weeks.

Papillomas appearing on the shaven area of the skin were recorded at weekly intervals. Only those papillomas that persisted for two weeks or more have been considered for final analysis of the data. Animals were sacrificed 15 weeks after commencement of the experiment.

Statistical analysis

In the enzymatic studies, statistical analysis was performed using ANOVA test. A value of $p < 0.05$ was considered to indicate a significant difference between the groups. In skin papillomagenesis, the significant level of difference between control and experimental values were statistically analyzed using chi-square test at 5% probability level.

Results

Findings of the present investigations are summarized in Tables 1-3. There were no adverse effects on the animals at the given dose levels (125 and 250mg/ kg body weight/ day for 7 and 14 days respectively). BHA was used as a positive control in the present study. The liver somatic index, which is the ratio of liver weight to final body weight and microsomal and cytosolic protein profiles remained comparable to the control indicating a favourable effect of the modulator on general body metabolism.

Phase I Microsomal Haemproteins: Cytochrome P_{450} and Cytochrome b_5

Table 2 shows a significant elevation in the specific activity of cytochrome P_{450} by 1.21 fold ($P < 0.01$) in Group II and 1.57 fold ($P < 0.01$) in Group III when treated for 7 days with the low dose and high dose respectively. During 14 days treatment with low dose the activity of Cytochrome P_{450} has increased by 1.01 fold ($P < 0.01$) in Group VI while against a high dose, it was recorded to be 1.46 fold ($P< 0.01$) in Group VII. In the positive control group the specific

Table 1. Modulatory Influence of *Moringa oleifera*, Lam and BHA on Weight Gain Profiles and Hepatic Protein Levels in Mice

Group	Treatment	Duration of Treatment(days)	Body Weight (g) Initial	Body Weight (g) Final	Liver weight × 100/final body weight	Protein mg/ml Microsome	Protein mg/ml Cytosol
I	Control (only vehicle distilled water)	7	19.1 ± 0.74 (1.00)	20.1 ± 0.74 (1.00)	4.26 ± 0.59 (1.00)	5.28 ± 0.48 (1.00)	5.5 ± 0.24 (1.00)
II	M. oleifera (125 mg/kg body wt in distilled water)	7	19.1 ± 0.57 (1.00)	20 ± 0.67 (1.00)	5.05 ± 0.79 (1.19)	5.46 ± 0.34 (1.03)	5.33 ± 0.27 (0.97)
III	M. oleifera (250 mg/kg body wt in distilled water)	7	19.2 ± 0.79 (1.01)	20.2 ± 0.61 (1.00)	4.59 ± 0.75 (1.08)	5.58 ± 0.19 (1.06)	5.61 ± 0.17 (1.02)
IV	BHA (0.75% in diet; positive control	7	19.3 ± 0.22 (1.01)	19.6 ± 0.47 (0.98)	5.81 ± 0.62 (1.36)	4.67 ± 0.52 (0.88)	4.59 ± 0.49 (0.83)
V	Control (only vehicle distilled water)	14	19.2 ± 0.63 (1.00)	20.6 ± 0.84 (1.00)	4.49 ± 0.79 (1.00)	5.2 ± 0.16 (1.00)	5.25 ± 0.24 (1.00)
VI	M. oleifera (125 mg/kg body wt in distilled water)	14	19.2 ± 0.79 (1.00)	20.2 ± 1.03 (0.98)	4.67 ± 0.88 (0.86)	5.26 ± 0.43 (1.01)	5.37 ± 0.51 (1.01)
VII	M. oleifera (250 mg/kg body wt in distilled water)	14	19.2 ± 0.42 (1.00)	20.2 ± 0.63 (0.98)	4.73 ± 0.68 (0.88)	5.18 ± 0.38 (0.99)	5.37 ± 0.35 (1.01)
VIII	BHA (0.75% in diet ; positive control)	14	19.2 ± 0.67 (1.00)	20.3 ± 0.82 (0.99)	6.61 ± 0.83 (1.22)	4.49 ± 0.19 (0.99)	5.13 ± 0.57 (0.85)

Values are expressed as mean ± S.D of 8-10 animals. Values in parenthesis represent relative changes in parameters assessed (i.e. levels of parameter assessed in livers of mice receiving test substance to that of control mice). Abbreviation: BHA – Butylated hydroxyanisole.

activity of Cytochrome P_{450} was enhanced by 1.26 fold (P < 0.01) in Group IV and 1.92 fold (P < 0.01) in Group VIII when treated for 7 days and 14 days respectively. The specific activity of cytochrome b5 has been found to be increased by 1.28 fold (P < 0.05) in Group II and 1.56 fold (P < 0.05) in Group III when treated with both doses i.e. low dose and high dose respectively for 7 days. The same has been recorded to be increased by 1.41 fold (P < 0.05) in Group VI against a low dose and 1.64 fold (P < 0.05) in Group VII against high dose when treated for 14 days. The BHA treated groups showed an increase by 1.72 fold (P < 0.05) in Group IV and 3.18 fold (P < 0.05) in Group VIII when treated for 7 days and 14 days respectively. The results obtained are in comparison with the control group.

Phase II Enzymes and Anti-oxidant Parameters:

Glutathione-S-transferase

The specific activity of GST was significantly augmented by 1.18 fold (p < 0.01), only with a high dose of the modulator treatment, relative to the control basal value. In the BHA treated groups, the activity of GST remained comparable to that of the control group except for Group VII where there is an increase of 4.48 fold (p < 0.01).

Reduced Glutathione

Table 3 shows a significant elevation of reduced glutathione measured as acid soluble sulphydryl (-SH) group, relative to the control group, by 1.04 fold (P < 0.01) in Group II and 1.92 fold (P < 0.01) in Group III when treated with low and high dose of the extract respectively for 7 days. The same has been found to be increased by 1.65 fold (P < 0.01) in Group VI against low dose and 2.39 fold (P < 0.01) in Group VII against high dose, compared to control. The BHA treated group recorded an increase by 1.95 fold (P < 0.01) in Group IV against 7 days treatment and 2.48 fold (P < 0.01) in Group VIII against 14 days, in comparison to the control group.

Glutathione Peroxidase

Both the doses of *Moringa oleifera*, Lam has elevated the specific activity of glutathione peroxidase significantly compared to the control value. Group II and III have exhibited augmented levels of GP specific activity by 1.53 fold (P < 0.01) against low dose and 1.74 fold (P < 0.01) against high dose when treated for 7 days. On the other hand, the activity level increased by 1.91 fold (P < 0.01) in Group VI against low dose and 1.99 fold (P < 0.01) against a high dose when treated for 14 days. The BHA treated animals also recorded a significant increase by 1.69 fold (P < 0.01) in Group IV and 1.96 fold (p < 0.01) in Group VIII when fed for a period of 7 and 14 days respectively. The elevations are in comparison with the control basal level.

Glutathione Reductase

A significant induction of the specific activities of GR has been observed in Group II and III by 1.61 fold (P < 0.05) against low dose and 2.09 fold (P < 0.05) against high dose when treated for a period of 7 days over that of control. When treated for 14 days, the elevation was found to be

Table 2. Modulatory Influence of *Moringa oleifera*, Lam and BHA on Mouse Hepatic Phase I and Phase II Drug Metabolising Enzyme Levels and Lipid Peroxidation

Group	Treatment	Duration of Treatment(days)	Cyt P_{450} (n mole/mg protein)	Cyt b_5 (n mole/mg protein)	GST (µ mole of CDNB-GSH conjugate formed/ min /mg protein)	LPO (n mole malondialdehyde formed/mg protein)
I	Control (only vehicle distilled water)	7	2.34 ± 1.03 (1.00)	0.22 ± 0.03 (1.00)	3.46 ± 0.78 (1.00)	1.31 ± 0.42 (1.00)
II	M. oleifera (125 mg/kg body wt in distilled water)	7	2.6 ± 0.99[a] (1.21)	0.24 ± 0.16[b] (1.28)	3.7 ± 1.1 (1.07)	0.75 ± 0.12[a] (0.39)
III	M. oleifera (250 mg/kg body wt in distilled water)	7	3.37 ± 0.54[a] (1.57)	0.28 ± 0.05[b] (1.56)	3.9 ± 0.73 (1.13)	0.41 ± 0.04[a] (0.22)
IV	BHA (0.75% in diet; positive control	7	2.7 ± 0.85[a] (1.26)	0.31 ± 0.14[b] (1.72)	8.02 ± 3.07 (2.14)	0.33 ± 0.03[a] (0.17)
V	Control (only vehicle distilled water)	14	2.67 ± 0.55 (1.00)	0.24 ± 0.06 (1.00)	3.34 ± 0.85 (1.00)	0.89 ± 0.08 (1.00)
VI	M. oleifera (125 mg/kg body wt in distilled water	14	2.7 ± 0.89[a] (1.01)	0.31 ± 0.19[b] (1.41)	3.46 ± 1.08[a] (1.05)	0.57 ± 0.07[a] (0.41)
VII	M. oleifera (250 mg/kg body wt in distilled water	14	3.9 ± 0.74[a] (1.46)	0.36 ± 0.14[b] (1.64)	3.89 ± 1.45[a] (1.18)	0.34 ± 0.05[a] (0.24)
VIII	BHA (0.75% in diet ; positive control)	14	4.2 ± 1.06[a] (1.92)	0.70 ± 0.12[b] (3.18)	14.77 ± 2.94[a] (4.48)	0.32 ± 0.03[a] (0.23)

Values are expressed as mean ± S.D of 8-10 animals. Values in parenthesis represent relative changes in parameters assessed (i.e. levels of activity in livers of mice receiving test substance to that of control mice). [a] (P < 0.001); [b] (P < 0.01) represent significant changes against control. Abbreviations: BHA – butylated hydroxyanisole; Cyt P_{450} – Cytochrome P_{450}; Cyt b5 – Cytochrome b_5; GST – Glutathione S-transferase; LPO – Lipid peroxidation.

Table 3. Modulatory Influence of *Moringa oleifera*, Lam and BHA on Mouse Hepatic Antioxidant Related Parameters

Group	Treatment	Duration of Treatment(days)	GSH (n mole GSH/g tissue)	GPx (n mole of NADPH consumed/min/ mg protein)	GR (n mole of NADPH consumed/min/ mg protein)	SOD (specific activity expressed as µ mole/mg protein)	CAT (µ mole H_2O_2 consumed/min/ mg protein)
I	Control (only vehicle distilled water)	7	1.89 ± 0.20 (1.00)	10.09 ± 3.49 (1.00)	13.47 ± 3.39 (1.00)	8.62 ± 3.43 (1.00)	73.8 ± 20.76 (1.00)
II	M. oleifera (125 mg/kg body wt in distilled water)	7	2.02 ± 0.38[a] (1.04)	15.84 ± 5.41[a] (1.53)	21.72 ± 6.63[b] (1.61)	8.8 ± 4.58 (1.18)	128.75 ± 23.85[b] (1.74)
III	M. oleifera (250 mg/kg body wt in distilled water)	7	3.73 ± 0.09[a] (1.92)	17.99 ± 6.18[a] (1.74)	28.26 ± 2.59[b] (2.09)	9.01 ± 0.95 (1.20)	132.91 ± 4.31[b] (1.80)
IV	BHA (0.75% in diet; positive control	7	3.37 ± 0.39[a] (1.95)	17.59 ± 3.71[a] (1.6)	17.44 ± 3.5[b] (1.29)	8.74 ± 1.1 (1.17)	140.03 ± 30.88[b] (1.89)
V	Control (only vehicle distilled water)	14	1.84 ± 0.35 (1.00)	15.77 ± 2.16 (1.00)	20.06 ± 6.26 (1.00)	8.31 ± 0.13 (1.00)	124.42 ± 26.47 (1.00)
VI	M. oleifera (125 mg/kg body wt in distilled water	14	2.93 ± 0.46[a] (1.65)	22.30 ± 3.71[a] (1.19)	24.33 ± 7.55[a] (1.08)	10.06 ± 1.59 (1.24)	133.19 ± 23.48[b] (1.23)
VII	M. oleifera (250 mg/kg body wt in distilled water	14	4.25± 0.03[a] (2.39)	34.14 ± 9.65[a] (1.99)	47.1 ± 2.09[a] (2.08)	10.61 ± 1.46 (1.31)	152.26 ± 21.06[b] (1.40)
VIII	BHA (0.75% in diet ; positive control)	14	4.41 ± 0.39[a] (2.48)	30.27 ± 7.18[a] (1.96)	27.62 ± 3.82[a] (0.98)	9.82 ± 2.24 (1.21)	141.09 ± 36.26[b] (1.29)

Values are expressed as mean ± S.D of 8-10 animals. Values in parenthesis represent relative changes in parameters assessed (i.e. levels of activity in livers of mice receiving test substance to that of control mice). [a] ($P < 0.001$); [b] ($P < 0.01$) represent significant changes against control. Abbreviations: BHA – butylated hydroxyanisole; GSH – Reduced glutathione; GPx – Glutathione peroxidase; GR – Glutathione reductase; SOD – Superoxide dismutase; CAT – Catalase.

1.08 fold ($P < 0.01$) in Group VI against low dose and 2.08 fold ($P < 0.01$) in Group VII against high dose when treated for 14 days relative to control. An increase of 1.29 fold ($P < 0.05$) in Group IV has been found when the animals were fed with a diet containing 0.75% BHA for 7 days compared to control group.

Superoxide Dismutase

There was no any significant alteration in the SOD activity compared to the negative control group, both in the modulator treated groups and in the positive control groups, treated with BHA.

Catalase

The specific activity of catalase has been recorded to be increased by 1.74 fold ($P < 0.05$) in Group II and 1.8 fold ($P < 0.05$) in Group III when treated for 7 days with the low dose and high dose of the extract respectively compared to control. On the other hand, treatment for 14 days with both high and low dose, the catalase activity was found to be increased by 1.23 fold ($P < 0.05$) in Group VI and 1.4 fold ($P < 0.05$) in Group VII, relative to control group. An increase by 1.89 fold ($P < 0.05$) in Group IV and 1.29 fold ($P < 0.05$) in Group VIII has been recorded in the BHA treated group for 7 days and 14 days respectively.

Lipid Peroxidation

In comparison to the control group, Table 2 showed a significant inhibition in the lipid peroxidation (LPO) measured as the formation of malondialdehyde production by 0.39 fold ($P < 0.01$) in Group II and 0.22 fold ($P < 0.01$) in Group III when treated for 7 days with low dose and high dose of the extract respectively. The same has been observed in Group VI against low dose and in Group VII against high dose when treated for 14 days as the LPO activity has decreased by 0.41 fold ($P < 0.01$) and 0.24 fold ($P < 0.01$) respectively. The BHA treated group for 7 days and 14 days also showed a significant inhibition in the LPO activity by 0.17 fold ($P < 0.01$) in Group IV and 0.23 fold ($P < 0.01$) in Group VIII respectively relative to control value.

Mouse Skin Papillomagenesis

The findings of the present study have been furnished in Table-4. The application of the plant extract did not affect the body weight of the animals during the experimental period. In Group I (control group), treated with a single dose of DMBA and two weeks later promoted by repeated application of croton oil, 91.67% mice developed skin tumors. The average number of papillomas per mouse was 5.17 and the papillomas per papilloma bearing mouse was 5.64. On the other hand, the animals of Group II, receiving similar treatment of DMBA and croton oil when subjected to a topical application of the extract of *Moringa oleifera*, Lam for 14 days (i.e., 7 days before and 7 days after the treatment of DMBA), the percentage of tumor incidence, the average number of papillomas per mouse and papillomas per papilloma bearing mouse were found to be 66.67, 2.08 and 3.13 respectively. In Group III, the percentage of tumor incidence, the average number of papillomas per mouse and

Table 4. Chemopreventive Action of *Moringa oleifera*, Lam on DMBA-induced Skin Carcinogenesis in Mice

Groups	Total number of animals		Body weight(g) [Mean ± SD]		Papillomas per papilloma bearing mouse	Mice with papillo-mas(%)	Average number of papillomas per mouse
	Initial	Effective	Initial	Final			
I	12	12	13.25±1.14	15.75±2.99	5.64	91.67	5.17
II	12	12	12.83±1.26	18.83±1.99	3.13*	66.67*	2.08*
III	12	12	13.5±1.57	17.66±2.01	3*	25*	0.75*
IV	12	12	13.66±1.66	18.25±1.22	1.5*	16.67*	0.25*

*P<0.05

papillomas per papilloma bearing mouse were found to be 25, 0.75 and 3 respectively, when the mice received the application of the extract for 91 days (i.e., from the time of application of croton oil). When the mice of Group IV were treated with the *Moringa oleifera*, Lam extract continuously for 105 days (i.e., 7 days prior to DMBA application and continued till the end of the experiment) the percentage of tumor incidence, the average number of papillomas per mouse and papillomas per papilloma bearing mouse were recorded to be 16.67, 0.25 and 1.5 respectively. The difference in the values of the results of Group II, III and IV were statistically analysed and found to be significant in comparison to the control group (P< 0.05).

Cumulative number of papillomas and percentage inhibition of tumor multiplicity in control and experimental groups during the observation period are shown in Figures 1 and 2 respectively.

Discussion

Cancer chemoprevention involves the use of either natural or synthetic compounds to delay, inhibit or reverse the development of cancer in normal or pre-neoplastic conditions. Recent upsurge in identifying natural products of dietary origin associated with high degree of safety margins has been found to be beneficial as potent cancer chemopreventive agents (Pezzuto, 1997). Comprehensive reviews provide strong evidence that high intake of vegetables and fruits are associated with reduced cancer incidence (Steinmetz and Potter, 1991; Block et al., 1992). It is known that balance between Phase I and Phase II enzymes can afford protection against numerous chemical carcinogens, and the induction of antioxidant enzymes facilitates their degradation from the body (Miller, 1998). 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 drumstick extract.

The findings of the present study reveals that administration of the hydroalcoholic extract of *Moringa oleifera*, Lam at both dose levels (125mg/kg body weight and 250mg/kg body weight) for 7 and 14 days daily have enhanced the levels of hepatic cytochrome b_5, cytochrome P_{450} and glutathione-S-transferase, elucidating that *Moringa oleifera*, Lam acts as bifunctional inducer as it induces both Phase-I and Phase-II system enzymes that furnish the balance of xenobiotic metabolism towards detoxification.

The beneficial effect of vegetables may be due to either individual or combined effects of their constituents, includs large number of inhibitors of carcinogenesis such as indoles, phenols, aromatic isothiocyanates, methylated flavones and sterols (Wattenberg, 1983). Among these, indol-3-carbanol, a chief component of cruciferous vegetables acts as a blocking agent and reduces cancer incidence through modulation of cytochrome P450 dependent metabolism (Keloff et al., 1996). Though the exact mechanism underlying the co-ordinated increase of cytochrome P_{450} monooxygenase system by the Moringa oleifera, Lam drumstick extract has not been fully understood, it may be inferred that it may have acted as a "blocking agent" and augmented the sequential reduction of xenobiotic substrates preparing it for Phase-II metabolism.

Several workers have reported that phytochemicals

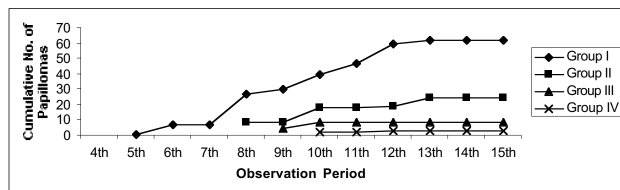

Figure 1. Effect of *Moringa oleifera* on Cumulative Number of Papillomas in the Treated Groups (Group II, III and IV) in Contrast to the Control (Group I) Mice.

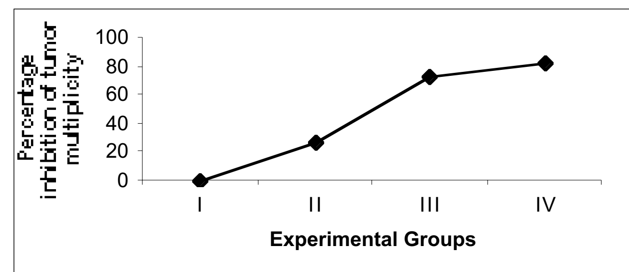

Figure 2. Effect of *Moringa oleifera* on Percentage Inhibition of Tumor Multiplicity in the Treated Groups (Group II, III and IV) in Contrast to the Control (Group I) Mice.

having chemopreventive potential can induce GST activity (Keloff et al., 1996; Nakamura et al., 2000). In the present study, wherein CDNB was used as a non-specific substrate for assaying total cytosolic GST,(as CDNB is active for different degrees with all the isoenzymes of GST) (Habig et al., 1974), the hepatic GST activity was found to be enhanced by the *Moringa oleifera*, Lam drumstick extract significantly in a dose and time dependent manner. The elevated level of GST by the extract may have facilitated the conjugation reaction of xenobiotic metabolism and may have increased the availability of non-critical nucleophiles for inactivation of electrophiles and therefore might be playing a major role in chemoprevention.

Active oxygen species and free radicals are involved in a variety of pathological events including cancer. The anti-oxidant defense enzymes have been suggestive of playing an important role in maintaining physiological levels of oxygen and hydrogen peroxide and eliminating peroxides generated from inadvertent exposure to xenobiotics and drugs. Any natural compound with anti- oxidant properties may help in maintaining health when continuously taken as components of dietary foods, spices or drugs (Singh, 2000). The increase in the levels of antioxidant profiles i.e. GPx, GR, SOD and Catalase by *Moringa oleifera*, Lam drumstick extract may be attributed to have biological significance in eliminating reactive free radicals that may affect the normal functioning of cells.

The elevated level of GSH protects cellular proteins against oxidation through glutathione redox cycle and also directly detoxifies reactive species (Ketter, 1998) while the increased level of glutathione reductase helps in maintaining the basal level of cellular GSH (Lopez-Barea et al., 1990). It has been proposed that glutathione peroxidase is responsible for the detoxification of hydrogen peroxide in low concentration whereas catalase comes into play when glutathione peroxidase is saturated with the substrate (Gaetom et al., 1989).

Naturally occurring antioxidants present abundantly in vegetables and fruits are the carotenoids (Villa, 1993; Nishimo, 1995; Tanaka et al., 1995; Ashmawy, 1999). Vitamin A has also been found to inhibit the formation of DNA adduct by Aflatoxin B_1, a potent hepato-carcinogen in a dose dependent manner (Firozi et al., 1987). β-carotene, the major component reported from the drumsticks of the plant (Geervani and Devi, 1981) and vitamin A and C present in *Moringa oleifera*, Lam serve as an explanation for their mode of action in the induction of antioxidant profiles in the present investigation, however, the exact mechanism is yet to be elucidated. The decreased lipid peroxidation as MDA formation in the present study is in correlation with the induction of antioxidant enzymes above basal level by the *Moringa oleifera*, Lam extract.

A significant inhibition in tumor burden as well as tumor incidence has been observed in the skin papillomagenesis studies. Literature suggest that naturally occurring substances have been known to cause inhibition of tumorigenesis either by preventing the formation of active carcinogen from their precursors or by suppressing the expression of neoplacia (Wattenberg, 1983; Wattenberg, 1985). The possible involvement of either of the above mechanisms observed in the present investigation cannot be ruled out. In DMBA induced tumorigenesis on mouse skin the free radical scavenging mechanism pathway may be operating (Jannu et al., 1990) while Dasgupta et al., 2001 has suggested that β-carotene and sterols present in plant acts as potent inhibitors of formation of reactive oxygen intermediates, a pre-requisite for tumorigenesis. The biochemical basis of the chemopreventive potency of *Moringa oleifera*, Lam extract may be attributed to the synergistic action of the constituents of the extract and the induction of Phase-II enzymes (GSTs) and antioxidant enzymes,which might be implicated in the antitumorigenic activity.

The induction of both Phase-I and Phase-II enzymes by the *Moringa oleifera*, Lam extract represents a promising chemopreventive strategy as a bifunctional inducer, along with the enhancement of antioxidant system enzymes which affords protection against cellular damage and inhibit tumor promotion. Since most of the phytochemicals and micronutrients having chemopreventive properties occur in low concentration in vegetables and fruits, it may be possible that selective interactions among these dietary constituents may have a long lasting, potent and effective modality for cancer chemoprevention.

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