EFFECTS OF MUSTARD OIL ON OXIDATIVE STRESS PARAMETERS OF MALE MICE

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ABSTRACT

Seeds of *Sinapis alba* Linn (commonly called yellow or white mustard) and their components have been reported to possess anticancer properties. The present study was performed to evaluate the effects of mustard oil on the liver and kidney functions as well as on the oxidative stress of male mice. Male mice were fed normal chow diet or corn oil-based diet or mustard oil-based diet for 3 weeks. Body and organ weights were measured at the end of experimental period. Biomarkers for liver and kidney functions were determined in serum. Lipid peroxidation levels, superoxide dismutase activity and glutathione levels were determined in liver and kidney. The results suggested the potential for MSO to reduce the LPO and to increase the GSH levels in liver and kidney tissues of mice. The consumption of MSO is associated with low body weight gain, increasing the LDH activity and total cholesterol level. The greater efficacy could be due to its radical scavenging property.

KEYWORDS: Mustard oil, fatty acid, corn oil, oxidative stress, antioxidant, mice

1. INTRODUCTION

The effect of dietary factors on health promotion and disease prevention has been an issue of interest since antiquity, and has become a subject of renewed research activity in recent years [1]. Many of the components involved are antioxidative in nature and include phenolic compounds. These phenolics exist in the free, esterified, glycosylated and polymeric forms. Scrutiny of the source materials, their subsequent extraction under optimized conditions, and evaluation of activity, followed by fractionation and structure elucidation of active components, is generally necessary. Meals obtained from oilseeds, such as canola, mustard, flax, borage, and evening primrose, after oil extraction, contained a variety of antioxidative factors. The antioxidative effect of source materials, their extracts or fractions thereof, depended on the seed type, the content and chemical nature of their active components [1].

The seeds of *Sinapis alba* L. (commonly called white or yellow mustard) have been used as a spice and herbal essence in alternative medicinal practices [2]. Mustard oil, which contains essential fatty acids, is widely used as cooking oil in Asia, especially in India. However, it also contains about 50% erucic acid (22:1), which is a concern for many individuals, as erucic acid levels higher than 7% in the oil of the diet are known to cause myocardial lipidosis and fibrosis in experimental animals [3].

In human study, dietary mustard oil consumption protected against oxidative stress [4]. In animal study, mustard products (seed or leaf) decreased lipid peroxidation and increased the activity of glutathione S-transferase, superoxide dismutase, and catalase against chromosomal damage and oxidative stress induced by gamma-radiation or cancer [5-7]. *In vitro*, mustard extracts scavenged free radicals, decreased low density lipoprotein peroxidation, and inhibited free radical-mediated protein damage against diabetic oxidative stress [8].

Mustard seeds, like all seeds of the Brassica family, including canola (rapeseed) and turnip, have high levels of omega-3 (6–11%) and are a common, cheap, mass-produced source of plant-based (therefore, vegetarian) omega-3 fatty acids. Flax (linseed) oil has 55% plant-based omega-3 but is uncommon as a table or cooking oil. Soybean oil has 6% omega-3 but contains over 50% omega-6, the fatty acid that competes with the omega-3 function [9].

Mustard oil is also used for rub-downs and massages, thought to improve blood circulation, muscular development and skin texture; the oil is also antibacterial. It is considered to be the oil that has low saturated fat as compared to other cooking oils. This pungent tasting oil is mostly used for cooking in parts of Gujarat, Bengal, Assam, Bihar, Orissa, Haryana, and some other parts of India. It basically consists of fatty acids (oleic, erucic and linoleic acid) [9].

An epidemiological study (http://en.wikipedia.org/wiki/Mustard_oil - cite_note-7) suggested that, in regions where mustard oil is still used in a traditional manner, mustard oil may afford some protection against cardiovascular diseases [10]. Whether this effect is due to the nature of erucic acid *per se* to make the blood platelets less sticky, or to the presence of a reasonably high percentage of α-linolenic acid, or to a combination of properties of fresh unrefined oil, is as yet uncertain. The fact that early asymptomatic coronary disease is absent in the mustard oil cohorts tends to add weight to the hypothesis that mustard oil is protective [11].

Oils in the diet are available to the body as fatty acids, which are excellent sources of dietary calorie intake. Fatty acids (FAs) are classified as saturated (SFA), monounsaturated (MUFA) and poly-unsaturated (PUFA) fatty acids [9]. Deficiency of essential fatty acids (EFA), such as linoleic (18:2), linolenic (18:3) and arachidonic acid (20:4), retards growth, and dermal symptoms appear.

The use of mustard oils in traditional societies for infant massage has been identified by one study as risking damaging skin integrity and permeability [11]. Other studies over larger samples have shown that massaging with mustard oil improved the weight, length, and mid arm and mid leg circumferences, as compared to infants without massage, although sesame oil is a better candidate for this than mustard oil [12].

Therefore, the present study was concerned to determine the different types of fatty acids in yellow mustard seed oil (MSO), and moreover, to study the effect of MSO on hepato-renal function as well as to evaluate the endogenous antioxidants status of mice.

2. MATERIALS AND METHODS

2.1 Chemicals

Thiobarbituric acid (TBA), ethylenediaminetetraacetic acid (EDTA), reduced glutathione (GSH), Tris-HCl, 5,5′dithiobis-2-nitrobenzoic acid (DTNB), potassium dihydrogen o-phosphate (KH_2PO_4), sulfuric acid, butanol and sodium chloride (NaCl) were supplied by Sigma Chemical Co. (St. Louis, MO, USA). Other chemicals were procured from Merck Ltd., and SRL Pvt., Ltd., Mumbai, India. Commercial kits of lactate dehydrogenase (LDH), alanine aminotransferase (ALT) and total protein were obtained from Human diagnostic worldwide, Germany. Kits of total cholesterol (TC) were obtained from Biodiagnostic, B.D., Egypt.

2.2 Extraction of mustard oil

Extraction of edible oil using mechanical pressing is also recognized as a solvent-free alternative. Mechanical pressing process in the common practice consists of two stages, preparation and extraction. The preparation phase consists of cleaning, breaking and grinding, to make the material in optimum condition before being pressed [13]. The extraction is done by a screw press that can produce up to 71-82% oil recovery [14].

2.3 Determination of total fatty acids in mustard seed oil after extraction

Gas liquid chromatography was applied to identify the fatty acids present in mustard oil. Lipids were extracted by a modified method of Xu and Beardall [15]. The identification of the peaks was carried out by retention times.

2.4 Determination of peroxide value and acidity in mustard seed oil

The peroxide value is defined as the amount of peroxide oxygen per 1 kg of fat or oil. Traditionally, this was expressed in units of milli-equivalents (meq.), although if we are using SI units, then, the appropriate option would be in millimoles (mmol) per kg (N.B. 1 meq. = 0.5 mmol; because 1 meq of O_2 =1mmol/2 = 0.5 mmol of O_2, where 2 is valence). R-O-O-R peroxides oxidize Fe^{2+} ions. The Fe^{3+} ions resulting from oxidation are grouped, and form a red complex. Its colorimetric intensity, measured at 505 nm, is directly proportional to the concentration of peroxides in the sample. Results are expressed as meq O_2/kg [16]. The acidy of oil was determined using the method of Baker [17].

2.5 Experimental design

The study was conducted on adult albino male mice, obtained from King Fahd Center for Medical Research, King Abdulaziz University, Jeddah, Saudi Arabia (n = 30, divided into three sets) at an initial age of 5-6 weeks. Male albino mice were kept at room temperature (25 ± 2 °C) in a light-controlled room with an alternating 12 hrs light/dark cycle. The mice were maintained in solid-bottom shoe box type polycarbonate cages with stainless steel wire-bar lids, using a wooden dust-free litter as a bedding material. All animals were acclimatized to the above conditions for one week and were fed standard lab chow. All animals were cared for according to the guidelines of the Canadian Council on Animal Care. Animals were randomly divided into three groups with 10 animals in each.

Group I, mice were fed on a standard basal diet (control group). The basal diet was formulated according to Farag *et al*. [18] (Table 1), and water was available *ad libitum*.

Group III, mice were fed on normal basal diet supplemented with 5% of corn oil.

Group II mice were fed on normal basal diet supplemented with mustard oil (0.643 mg/kg b.w.) replacing 5% of corn starch (Table 1). The dose has been selected as described before by Choudhury *et al.* [19]. This dose showed no toxic effect on *Musmusculus* and has radical scavenging property.

All groups were on the experimental diets for a period of 3 weeks. Body weights and food intake were monitored routinely on a daily basis. After 3 weeks of feeding, all animals were fasted for 12 h overnight, and blood was collected for different biochemical analyses.

TABLE 1 - Diet composition (%) for the experimental mice groups.

Ingredients of diet (%)	Normal	MSO diet	Corn oil diet	MSO diet
Crude Proteins	20.0	20.0	20.0	20.0
Crude Fat	4.0	4.0	4.0	4.0
Corn oil	---	---	5.0	---
Mustard oil	---	0.5	---	0.5
Crude Fiber	3.5	3.5	3.5	3.5
Ash	6.0	6.0	6.0	6.0
Salt	0.5	0.5	0.5	0.5
Calcium	1.0	1.0	1.0	1.0
Phosphorus	0.6	0.6	0.6	0.6
Vitamin A, IU/g	20.0	20.0	20.0	20.0
Vitamin D, IU/g	2.2	2.2	2.2	2.2
Vitamin E, IU/g	70.0	70.0	70.0	70.0

2.6 Determination of body weight and coefficients of organs

Individual animal body weight was recorded at the beginning of the experiment and after 21 days for each group. After weighing the body and tissues, the coefficients of the liver and kidney to the body weight were calculated as the ratio of tissues (wet weight, mg) to body weight (g).

2.7 Collection of serum and organs

Blood samples of the fasted mice for 12 hrs were collected from the medial retro-orbital venous plexus immediately with capillary tubes (Micro Hematocrit Capillaries, Mucaps) under ether anesthesia [20]. Then, the blood was centrifuged at 4000 rpm for 15 min and serum was collected for different biochemical analyses.

The animals were then dissected under ether anesthesia, tissue samples (liver, kidney) were collected, washed with 1.15 % of KCl and 0.5 mM of EDTA, and preserved at -20 °C for subsequent biochemical analyses.

2.8 Biomarkers of hepatic and renal functions

The determination of LDH activity was detected according to the method of Friedman and Young [21]. ALT activity was determined according to Reitman and Frankel [22]. The activities were expressed in international units (U/L). Protein was measured according to method of Lowry *et al.* [23] using bovine serum albumin as the standard.

Creatinine and uric acid were determined using the commercial kits that were purchased from Human Diagnostic Worldwide, Germany.

2.9 Assessment of an oxidative bioindicator and endogenous antioxidants in tissues

The thiobarbituric acid-reactive substances (TBARS) levels, as an index of malondialdehyde (MDA) production, were measured by the method of Ohkawa *et al*. [24]. Following incubation, samples were extracted with n-butanol and the reaction product was determined at 535 nm using a standard curve of 1,1,3,3-tetraethoxypropane.

Superoxide dismutase (SOD) activity was determined spectrophotometrically based on its ability to inhibit the auto-oxidation of epinephrine to adrenochrome at alkaline pH, according to Misra and Fridovich method [25]. SOD activity was expressed as the amount of enzyme that inhibits the oxidation of epinephrine by 50%, which is equal to 1 unit.

Reduced glutathione (GSH) level as non-enzymatic antioxidant was estimated based on the method of Beutler *et al.* [26] who reported that the 5,50-dithiobis-(2-nitrobenzoicacid) is reduced by SH group to form 1 mol of 2-nitro-5-mercaptobenzoic acid per mol of SH.

2.10 Statistical analysis

The results were evaluated using Student's t-test and analysis of variance (ANOVA) with the SPSS version 17.0 software packages for statistical evaluation. Values are presented as means ± S.E. (n = 8-10; each group).The percentage of change from the corresponding control was calculated (control value - treatment value/control value) X 100. A probability of less than or equal to 0.05 was considered to be significant [27].

3. RESULTS

3.1 Fatty acid composition (%) and peroxide value of yellow mustard seed oil

Oleic acid (C18:1) and linoleic acid (C18:2) were common in the unsaturated fatty acids of mustard oils (Table 2). It has been found in this study that mustard oil contained 10 FAs. Mustard oil contained two monounsaturated fatty acids (MUFAs), erucic (C22:1) and oleic (C18:1), that were predominant in yellow seed (48.8 and 15.7%, respectively).

Mustard oil contained a little amount of saturated fatty acids (6.36%) as compared to other editable oil. In contrary, the total unsaturated fatty acids were at highly considerable amount, representing about 73.2%. Moreover, linoleic acid is the third dominant unsaturated fatty acid in yellow mustard seed oil (about 13.0%). In addition, the mustard seed oil also contained a considerable amount of linolenic acid, which is considered to be the most important essential fatty acid. Linolenic acid was represented by about 6.2%. Furthermore, gadolic acid was found in adequate amount in the investigated mustard oil which presented about 6.8%. Table 3 shows the peroxide value and acidity of mustard seed oil. However, the corn oil was rich in oleic acid (18:1) and linoleic acid (18:2) (36.1% and 47.14%, resp.).

3.2 Body weight, coefficient of organs and general observations

Normal healthy control mice were found to be stable in their body weight (Table 4). Mice treated with corn or mustard oil showed a slight weight loss as compared to the initial weight (self-control).

At the time of euthanasia, no significant differences in body weights or weights of liver and kidney were observed between the MSO-treated and control mice (Table 4). In comparison, the mice fed control diets and those fed MSO-supplemented diets did not display any significant differences in food intake (data not shown).

TABLE 2 - Fatty acid composition of yellow mustard seed oil.

C:D	Common name of fatty acid	Fatty acids (%) in MSO	Fatty acids (%) in corn oil
	Saturated fatty acid		
C14:0	Myristic	**ND**	**ND**
C16:0	Palmitic	**1.87 ± 0.02**	**11.43 ± 0.30**
C17:0	Margaric	**0.03 ± 0.01**	**ND**
C18:0	Stearic	**1.52 ± 0.01**	**10.44 ± 1.01**
C20:0	Arachidic	**1.13 ± 0.03**	**0.30 ± 0.02**
C22:0	Behenic	**1.40 ± 0.02**	**0.51± 0.01**
C24:0	Lignoceric	**0.41 ± 0.01**	**ND**
			ND
	Mono unsaturated fatty acids		
C16:1	Palmitoleic	**0.10 ± 0.01**	**12.41 ± 2.12**
C17:1	Heptadecenoic	**0.08 ± 0.01**	**ND**
C18:1	Oleic	**15.70 ± 0.10**	**36.10 ± 2.41**
C20:1	Gadolic	**6.81 ± 0.20**	**ND**
C22:1	Erucic	**48.80 ± 2.10**	**ND**
C24:1	Nervonic	**1.71 ± 0.2**	**ND**
	Poly unsaturated fatty acid		
C18:2	Linoleic	**12.99 ± 1.20**	**47.14 ± 1.12**
C18:3	Linolenic	**6.18 ± 0.30**	**1.23 ± 0.22**
Total unknown		**1.21 ± 0.20**	**ND**

The results are presented as means ± S.E. (n=3). SFAs = Saturated fatty acids, MFA = monounsaturated fatty acid, PFAs = polyunsaturated fatty acids, ND = not detected.

TABLE 3 - Peroxide value and acidity of yellow mustard seed oil.

Parameters	Value
Peroxide value (meq O_2/kg oil)	0.9
Acidity (as oleic acid %)	0.22%
Color (LOvibond Inch 5.25)	12 yellow - 5 red

TABLE 4 - Effect of corn and mustard oil on mice body weight.

Groups/Weight	Control	Corn oil	Mustard oil
Initial (g)	26.06 ± 0.32	28.26 ± 1.27	30.14 ± 1.08
Final (g)	26.60 ± 0.27	26.66 ± 1.10	28.72 ± 0.88
Coefficients of livers	0.052	0.057	0.057
Coefficients of kidneys	0.018	0.018	0.017

The results are presented as means ± S.E. (n= 8-10).

Regardless of the diet treatment, none of the mice exhibited signs of toxicity, discomfort or behavioral anomalies.

3.3 Hepato-renal functions

Liver functions were determined by measuring serum activities of ALT and LDH as well as evaluating the cholesterol and protein contents. Table 5 demonstrates the effect of MSO and corn oil on enzyme activities of mice livers. Corn oil and MSO-treated mice decreased significant the leakage of ALT as compared to control, respectively. Corn oil significantly reduced the enzyme activity of LDH, while MSO increased LDH activity as compared to control.

The ability of MSO to affect levels of cholesterol in mice was also studied (Table 5). The levels of cholesterol was decreased significantly by 24.29% in corn oil-treated mice, while the total cholesterol content was increased significantly by 41.81% in MSO-treated animals. Total protein levels increased as the result of corn oil and MSO treatment by 1.51 and 1.63%, with regard to control (Table 5).

TABLE 5 - Effects of mustard seed oil on liver functions of mice as compared with corn oil.

Groups	Control	Corn oil	Mustard seed oil
ALT (U/L)	23.74 ± 1.64	2.61 ± 0.35[a]	3.49 ± 0.6[a]
LDH (U/L)	29.85 ± 0.96	26.77 ± 0.40[a]	37.16 ± 1.25[a]
Cholesterol (mg/dL)	66.79 ± 3.13	50.56 ± 3.93[a]	94.71 ± 4.67[a,b]
Total proteins (mg/dL)	3.46 ± 0.14	5.23 ± 0.06[a]	5.63 ± 0.11[a]

The results are presented as means ± S.E. (n= 8-10). [a] = significant difference as compared to control group, [b] = significant difference as compared to corn oil group.

TABLE 6- Effects of mustard oil on kidney function of mice as compared with corn oil.

Groups	Control	Corn oil	Mustard oil
Creatinine (mg/dL)	0.44 ± 0.00	2.60 ± 0.15[a]	3.15 ± 0.20 [a]
Uric acid (mg/dL)	0.92 ± 0.03	0.63 ± 0.05[a]	3.71 ± 0.26[a,b]

The results are presented as means ± S.E. (n= 8-10). [a] = significant difference as compared to control group, [b] = significant difference as compared to corn oil group.

As shown in Table 6, there was a slight change in uric acid levels in the mice treated with corn oil, and the level was 0.6-fold decreased as compared to control. The MSO treatment was associated with a 4.01-fold increase of uric acid level as compared to control animals. Supplementations of the diets with corn oil or MSO significantly elevated creatinine levels in affected mice (2.6- and 3.2-fold), compared to control, respectively (Table 6).

3.4 Oxidative/ antioxidant status

As shown in Fig. 1, LPO levels of liver tissue were found to be increased in male mice (2.28-fold) treated with corn oil, with respect to control. However, hepatic LPO level showed a significant decline in MO-treated mice by

47% as compared to corn oil group. LPO levels of kidney tissue were increased (1.17- and 3.62-fold) in MSO and corn oil groups, respectively, as compared to the control. LPO level of MSO was found to be significantly lower ($P \leq 0.001$) in kidney tissues, by 52.7%, as compared to corn oil mice.

The results shown in Fig. 2 revealed that the supplementation of corn oil for 21 days caused significant increase of SOD activity in the kidney and liver tissues as compared to control group. However, feeding the mice with MSO only increased SOD activity of liver tissue.

Compared with the control mice; animals treated with MSO revealed higher GSH content in liver and kidney (1.24- and 1.99-fold, respectively) (Fig. 3). The GSH contents of liver and kidney tissues were significantly increased ($P \leq 0.001$) in corn oil-treated group (1.53- and 1.89-fold) compared to the control mice (Fig. 3). Kidney GSH content of MSO was higher than that of corn oil-group. Hepatic GSH of MSO-treated mice decreased more than that of corn oil-treated ones (0.81-fold).

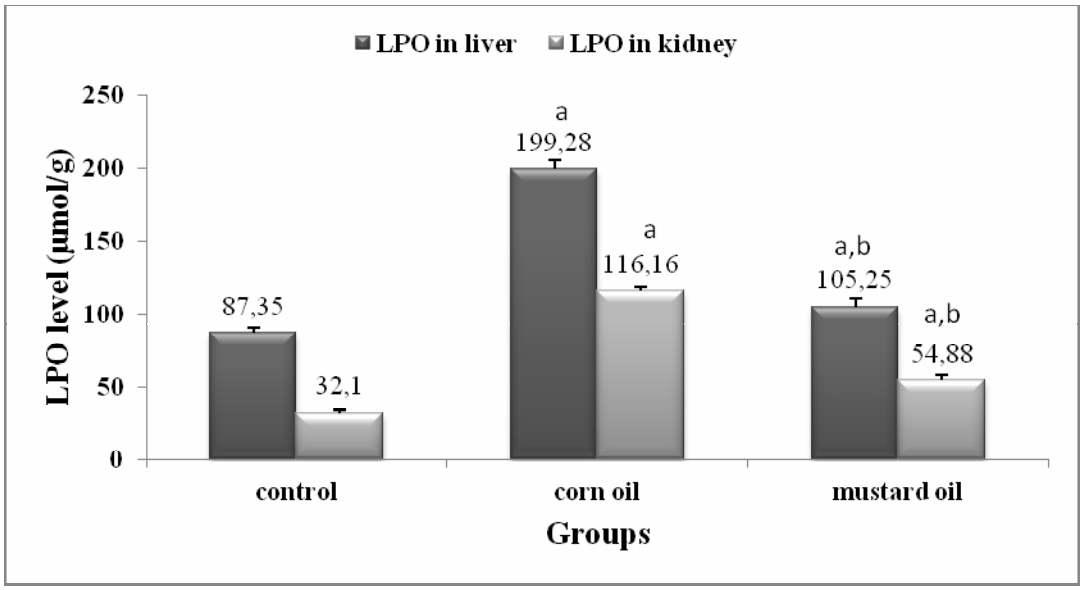

FIGURE 1 - Effect of mustard oil on the LPO level of liver and kidney tissues of male mice. The results are presented as means ± S.E. (n= 8-10). [a] = significant difference as compared to control group, [b] = significant difference as compared to corn oil group.

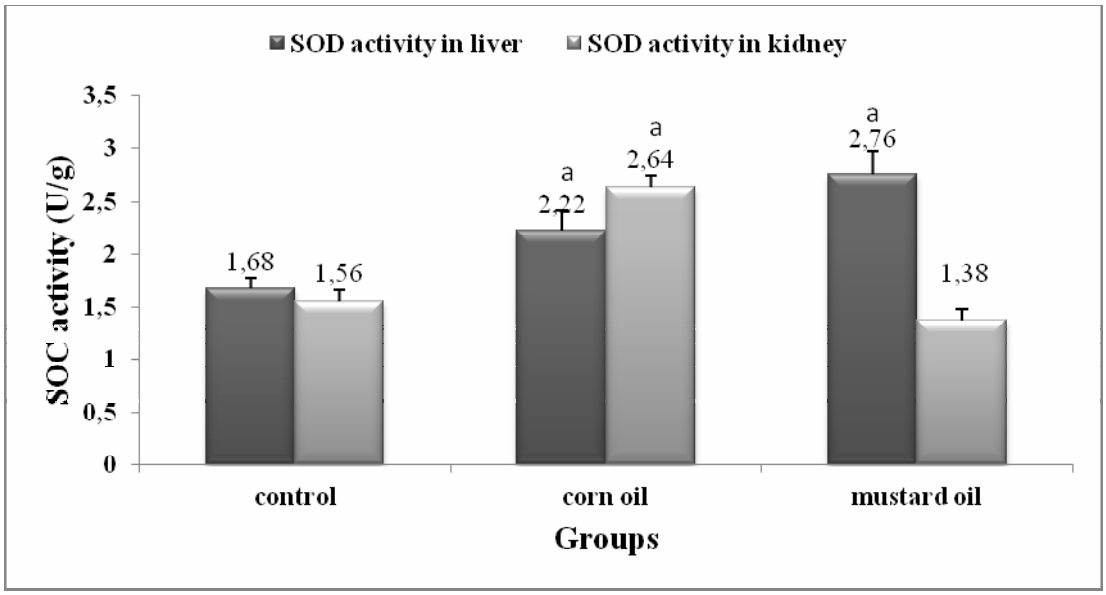

FIGURE 2 - Effect of mustard oil on the SOD level of liver and kidney tissues of male mice. The results are presented as means ± S.E. (n= 8-10). [a] = significant difference as compared to control group, [b] = significant difference as compared to corn oil group.

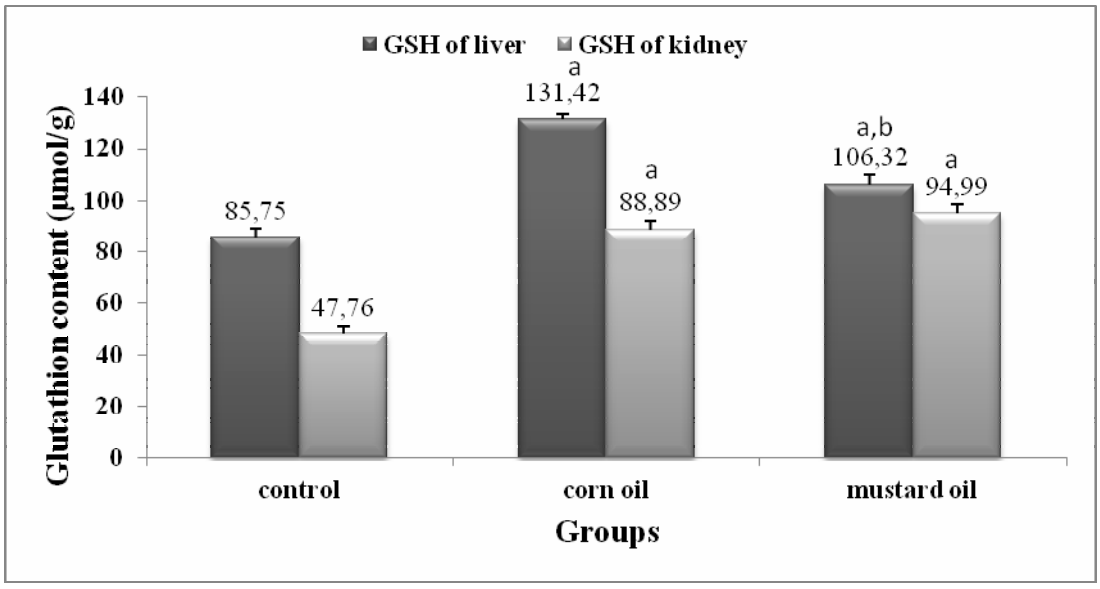

FIGURE 3 - Effect of mustard oil on the GSH level of liver and kidney tissues of male mice. The results are presented as means ± S.E. (n= 8-10). [a] = significant difference as compared to control group, [b] = significant difference as compared to corn oil group.

4. DISCUSSION

There is little information regarding the health benefits of MSO. The present study was undertaken to examine the effect of mustard oil in comparison to the effects of corn oil on liver and kidney function as well as the endogenous antioxidant.

Content ratio of unsaturated or polyunsaturated fatty acids to saturated fatty acids was higher (14.7), mainly due to the high content of erucic and linoleic acids in the MSO. However, the content ratio of polyunsaturated fatty acids to saturated fatty acids was 3.2. The limiting factor of mustard seed oil for use in human food application or animal feed formulation has been its high content of erucic acid which is indigestible for human and animal organisms. Chowdhury *et al.* [9] found two varieties with respect to erucic acid composition of mustard oil (5 % and 14-34 %).

In this study, the results of composition in corn oil were in agreement with some published data [28, 29]. The high content of monounsaturated fatty acids (MUFAs), especially oleic acid (18:1), is associated with a low incidence of coronary heart disease (CHD) because it decreases total cholesterol (10%) and low-density lipoprotein cholesterol [30]. Unsaturated (especially polyunsaturated) fatty acids are also more prone to oxidation. In contrast, dietary intake of certain unsaturated fatty acids, particularly conjugated linoleic and fat-soluble antioxidants (e.g., α-tocopherol, carotenoids), has been linked to potential health benefits [31].

Fatty acids do not show a great variation in their acidities, as indicated by their respective pK_a. Nonanoic acid, for example, has a pK_a of 4.96, being only slightly weaker than acetic acid (4.76).

The data on total body weight did not indicate accelerated growth in the mice under corn oil treatment when compared with the other dietary groups. The mice fed with MSO showed a decreased body weight gain in spite of a distinct flavor and taste as observed before by Dwivedi *et al*. [32]. These results confirmed that MSO has potential anti-obesity effect by regulating body weight gain [33]. Moreover, increased consumption of PUSF-rich diet prevents the development of obesity both in animals as well as in humans when exposed to high fat diet [34, 35]. Therefore, it is possible to speculate that decreased body weight gain observed in mice herein may be due to increase of PUFA ratio in the diet.

The hypocholesterolaemic activity of corn oil diet (Table 5) observed in the present study may be due to the presence of a high level of omega-6-fatty acids, e.g. linoleic acid, known to be hypocholesterolaemic [32]. There was significant hypercholesterolaemic activity in MSO diet fed mice, and this may be because the mustard oil contains 6.18% linolenic acid (an omega-3-fatty acid).

The possible harmful effect of hypercholesterolemia is that it leads to increased plasma total cholesterol, plasma non-HDL cholesterol, and plasma triglyceride levels.

There is a positive correlation between atherosclerosis and hypercholesterolemia [36]. The present results demonstrated that mustard oil contains 48.8% eurcic acid which may cause accumulation of fat as the total cholesterol level increased in mice. This observation is supported by Kramer *et al.* [37] who reported that erucic acid may cause lipidosis in rat by accumulation of fat in cardiac muscles. The present results are in contrary with other studies which have shown that mustard oil has protective effects against cardiovascular diseases in regions where it is consumed traditionally [10, 33].

Creatinine levels were elevated in corn oil and MSO-treated mice inducing renal toxicity when compared to control animals. The MSO could be considered to be unsuitable for human consumption.

One more important observation in the present study was the effect of MSO on the endogenous antioxidant of mice. Results demonstrated that dietary MSO significantly decreased the LPO of liver and kidney as compared to the dietary corn oil. Dietary mustard oil did not alter the kidney SOD activity in experimental animals.

Benson and Devi [38] reported that treatment of rats with mustard oil increased SOD and decreased LPO significantly. Therefore, the MSO plays an important role in reducing the free radicals. These results are confirmed by increasing GSH levels in liver and kidney of mice fed with MSO (Fig. 3). To our knowledge, no single study has been carried out addressing the efficacy of MSO on GSH of mice.

CONCLUSION

In conclusion, the results from the present study suggest the potential for MSO to reduce the LPO and increase the GSH levels in liver and kidney of mice. The greater efficacy could be due to its radical scavenging property. The consumption of MSO is associated with low body weight gain, increasing the LDH activity and total cholesterol level, and its negative effects deserve further investigation.

The authors have declared no conflict of interest.

REFERENCES

[1] Shahidi, F. (2000) Antioxidant factors in plant foods and selected oil seeds. Biofactors. 13(1-4),179-185.

[2] Eskin, N.A.M., Raju, J. and Bird, R.P. (2007). Novel mucilage fraction of *Sinapis alba* L. (mustard) reduces azoxymethane-induced colonic aberrant crypt foci formation in F344 and Zucker obese rats. Phytomedicine 14(7-8), 479-85.

[3] Beare-Rogers, J.A. (1997) Docosaenoic acid in dietary fats. In: Holman RT, editor. Progress in chemistry of fats and other lipids, vol. 15. Paris: Pergamon Press; p. 29-56.

[4] Singh, R.B., Niaz, M.A., Sharma, J.P., Kumar, R., Rastogi, V. and Moshiri, M. (1997). Randomized, double-blind, placebo-controlled trial of fish oil and mustard oil in patients with suspected acute myocardial infarction: the Indian experiment of infarct survival--4. Cardiovascular Drugs Therapy. 11(3), 485-91.

[5] Khan, B.A., Abraham, A. and Leelamma, S. (1997). Antioxidant effects of curry leaf, Murrayakoenigii and mustard seeds, *Brassica juncea* in rats fed with high fat diet. Indian Journal Experimental Biology. 35(2), 148-50.

[6] Gagandeep, A., Dhiman, M., Mendiz, E., Rao, A.R., Kale, R.K. (2005). Chemopreventive effects of mustard (*Brassica compestris*) onchemically induced tumorigenesis in murine forestomach and uterine cervix. Human Experimental Toxicology. 24(6), 303-312.

[7] Tiku, A.B., Abraham, S.K. and Kale RK. (2008). Protective effect of the cruciferous vegetable mustard leaf (*Brassica campestris*) against *in vivo* chromosomal damage and oxidative stress induced by gamma-radiation and genotoxic chemicals. Environmental Molecular Mutagen. 49(5), 335-342.

[8] Yokozawa, T., Kim, H.Y., Cho, E.J., Yamabi, N. and Choi, J.S. (2003) Protective effects of mustard leaf (*Brassica juncea*) against diabetic oxidative stress. Journal Nutrition ScienceVitaminology (Tokyo). 49(2), 87-93.

[9] Chowdhury, K., Banu, L.A., Khan, S. and Latif A. (2007). Studies on the fatty acid composition of edible oil. Bangladesh Journal Scientific and Industrial Research. 42(3), 311-316.

[10] Rastogi, T., Reddy, K.S., Vaz, M., Spiegelman, D., Prabhakaran, D. and Willett, W.C. (2004). Diet and risk of ischemic heart disease in India. American Journal of Clinical Nutrition. 79 (4), 582–592.

[11] Darmstadt, G.L., Mao-Qiang, M., Chi, E., Saha, S.K., Ziboh, V.A., Black, R.E., Santosham, M. and Elias, P.M. (2002). Impact of topical oils on the skin barrier: possible implications for neonatal health in developing countries. Acta Paediatrica. 91(5):546-54.

[12] Agarwal, K.N., Gupta, A., Pushkarna, R., Bhargava, S.K., Faridi, M.M., Prabhu, M.K. (2000) Effects of massage and use of oil on growth, blood flow and sleep pattern in infants. Indian journal Medical Research. 112, 212-217.

[13] Fils, J.M. (2000). The production of oils. In: Hamm, W. and R.J. Hamilton, (Eds.), Edible oil processing. Sheffield: Sheffield Academy Press, U.K.

[14]. Gunstone, F.D. (2004). The chemistry of oils and fat: sources, composition, properties and uses. CRC Press LLC, Boca Raton.

[15]. Xu, X. and Beardall, J. (1997). Modification of fatty acid composition in halophilic anthracitic micro algea. Phytochemistry. 49, 1249-1252.

[16] IFRA Analytical Method: Determination of the Peroxide Value (October 17th, 2011).

[17] Baker, D., Neustadt, M.H. and Zeleny, L. (1957). Application of the fact acidity test as an index of grain deterioration. Cereal Chemisty. 34, 226-233.

[18] Farag, R.S., Mahmoud, E.A., Basuny, A.M. and Ali, R.F.M. (2006). Influence of crude olive leaf juice on rat liver and kidney functions. International Journal Food Technology. 41, 790-798.

[19] Choudhury, A.R., Das, T. and Sharma, A. (1997). Mustard oil and garlic extract as inhibitors of sodium arsenite-induced chromosomal breaks *in vivo*. Cancer Letter. 121(1), 45-52.

[20] Boussarie, D., (1999). Hématologie des *Rongeurset Lagomorphes* de Compagnie. Bulletin de l'Academie Veterinaire de France. 72, 209-216.

[21] Friedman, R.B. and Young, D.S. (2001). Effects of Disease on Clinical Laboratory Tests, 4th Edition, AACC Press, Washington, D.C.

[22] Reitman S, Frankel S. A colorimetric method for the determination of glutamic oxaloacetic and glutamic pyruvic transaminases. American Journal of Clinical Pathology. 1957; 28: 56–58.

[23] Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951). Protein measurement with the Folin phenol reagent. The Journal of Biological Chemistry. 193(1), 265-275.

[24] Ohkawa, H., Ohishi, N. and Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Analytical Biochemistry. 95, 351-358.

[25] Misra, H.P. and Fridovich I. (1972). The role of superoxide anion in the autooxidation of epinephrine and a simple assay for superoxide dismutase. Journal of Biological Chemistry. 247, 3170-3175.

[26] Beutler, E., Olga, D. and Kelly, M. (1963). Improved method for determination of blood glutathione. From the department of medicine, City of Hope Medical Centre.Journal LaboratoryClinicalMedicin. 61, 882-888.

[27] Hill, A.B. (1971). Principles of medical statistics, 9th ed. Oxford University Press Oxford UK.

[28] Juyoung, K., Deok, N.K., Sung, H.L., Sang-Ho, Y. and Suyong, L. (2010). Correlation of fatty acid composition of vegetable oils with rheological behavior and oil uptake. Food Chemistry. 118, 398-402

[29] Dauqan, E.M.A., Abdullah Sani, H., Abdullah, A. and Kasim, Z.M. (2011). Fatty acids composition of four different vegetable oils (Red *Palm olein, Palm olein*, Corn Oil and Coconut Oil) by Gas Chromatography. 2nd International Conference on Chemistry and Chemical Engineering. IPCBEE vol. 14 IACSIT Press, Singapore.

[30] Dennys, E.C.C., Andre, G.V.C., Maria do, C.G.P., Sergio Matta, D.S., Marco, T.C.S. and Neuza, M.B.C. (2006) Lipid profile of rats fed high-fat diets based on flaxseed, peanut, trout, or chicken skin. Nutrition. 22, 197– 205

[31] Gillian, B., Jacob, H.N., Tina, S., Chris, S., Mick, D.E., Roy, S. and Carlo, L. (2008). Fatty acid and fat-soluble antioxidant concentrations in milk from high- and low-input conventional and organic systems: seasonal variation. Journal Science Food Agriculture. 88, 1431-1441.

[32] Dwivedi, C., Muller, L.A., Goetz-Parten, D.E., Kasperson, K. and Mistry, V,V. (2003). Chemopreventive effects of dietary mustard oil on colon tumor development. Cancer Letters. 196, 29-34.

[33] Malik, Z.A., Goyal, A. and Sharma, P.L. (2011). Mustard oil based high fat diet is associated with decreased body weight gain, less adiposity and improved glucose and lipid homeostasis in Wistar rats. Asian Journal of Clinical Nutrition. 3 (2), 43-52.

[34] Sahin, H., Fatma, U. and Neriman, I. (2008). Effects of conjugated linoleic acid on body composition and selected biochemical parameters in obese women. Pakistan Journal of Nutrition. 7: 546-549.

[35] Buckley, J.D. and Howe, P.R. (2009). Anti-obesity effects of long-chain omega-3 polyunsaturated fatty acid. Obesity Reviews. 10, 648-659.

[36] Sengupta, A. and Ghosh, M. (2011). Basic nutritional investigation: Hypolipidemic effect of mustard oil enriched with medium chain fatty acid and polyunsaturated fatty acid. Nutrition.27, 1183-1193.

[37] Kramer, J.K., Saver, F.D., Wolynetz, M.S. Farnworth, E.R and Johnston, K.M. (1992). Effect of dietary saturated fat on erucic acid induced myocardial lipidosis in rats. Lipids. 27, 619-623.

[38] Benson, K.M and Devi, K. (2009). Influence of W-6/W-3 rich dietary oil on lipid profile and antioxidant enzymes in normal and stressed rats. Indian Journal of experimental Biology. 47: 98-103.

Received: March 06, 2014
Accepted: April 07, 2014

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