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Impact of Flax Seed and Canola Oils Mixture Supplementation on The Physiological and Biochemical Changes Induced by Monosodium Glutamate in Rats

M.M. Anwar and N.E. Mohamed*

*Plant Research Department and * Biological Applications Department, Nuclear Research Center, Atomic Energy Authority, Cairo, Egypt, P.O. 13759*

E-mail: **Dr_Mervatanwar@yahoo.com**

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ABSTRACT

One of the most important problems in the human health nutrition field is the use of food flavor. Monosodium glutamate is one of the main flavors used as an ingredient in various food products, however it produces physiological and biochemical changes. The main objective of this study is to evaluate the supplementation of flax seed and canola oils mixture against the physiological and biochemical changes induced by monosodium glutamate in rats. In addition to analyses the physical and chemical characteristics of flax seed and canola oil and fatty acids composition by using gas liquid chromatography. The results concerning that unsaturated fatty acids of flax seed oil were oleic (18:1) 22%, linoleic acid (18:2) 30 % and linolenic acid (18:3) 36%. Total unsaturated fatty acids percentage in flaxseed oil was 88% and total saturated fatty acids 12%. The unsaturated fatty acids of canola oil were oleic (18:1) 66%, linoleic acid (18:2) 18% and linolenic acid (18:3) 7%, total unsaturated fatty acids percentage in canola oil was 92% and total saturated fatty acids was 8%. On the other hand, treatment of rats with monosodium glutamate for ten consecutive days led to a decrease in RBCs, Hb, Hct % and increased platelet count with decrease in WBCs and undesirable changes in its differential count. There is also, high significant increase in testicular thiobarbituric acid reactive substances (TBARS) which is accompanied with significant reduction in catalase (CAT) activity, reduced glutathione (GSH) content and serum testosterone level. These disturbances were associated with significant increase in the liver enzymes ALT,AST and ALP and increase in the level of total biluribin and glucose. Also, significant increase in urea, creatinine and uric acid were recorded. The supplementation with mixture of flax seed and canola oils mixture for one month after the injection of monosodium glutamate caused noticeable amelioration in the damage occurred as a result of this flavor. To

eliminate the deleterious effects of this food flavor, the use of mixture of flax seed and canola oils is recommended.

Keywords: Monosodium glutamate, Flax seed oil, Canola oil, Blood picture, Biochemical parameters, Oxidative stress, Testosterone, Rats.

INTRODUCTION

Food additives are used to serve preservation or to improve food quality such as taste, color or appearance. They can be divided into six major categories: preservatives, nutritional additives, flavoring agents, texturing agents, coloring agents and miscellaneous. These compounds unfortunately were reported to cause health hazards to consumers [(1)].The best known and most widely used food additives and flavor enhancer in the developed world is monosodium glutamate (MSG)[(2)].The major use of MSG around the world is as a flavor enhancer in soup and broth, sauces, gravies flavoring and spice blend. MSG is also included in a wide variety of canned and frozen meats,poultry vegetables and combination diets. Recently, MSG has been extensively used as a flavoring agent in different child food products and in bouillons [(3)].

Monosodium glutamate (MSG) ($C_5H_8NO_4NaH_2O$), a sodium salt of naturally occurring (non- essential) L- form of glutamic acid, constituties about 20% of total amino acids found in natural protein source. Glutamate is found in two forms (bound) glutamate (like to other amino acids forming a protein molecule and peptides of the most tissues) and free glutamate (not linked to protein) that is effective in enhancing the flavor of food [(4)]. Glutamate is a normal neurotransmitter in the brain existing in very small concentrations acting as an excitatory neurotransmitter; it basically causes the nerve cells to discharge an electrical impulse and it use as a flavor enhancer [(5)]. When administrated at high doses, glutamate levels rise in the brain causing neurotoxic effects [(6)].

Several studies have shown that MSG administration to adult mice or rats caused change in the levels of biochemical parameters such as lipids and proteins, alterations in the levels of thiobarbituric acid reactive substances (TBARS) [(7,8)] and marked alteration in cholesterol and total protein levels [(9)]. Several studies confirmed that MSG induced hyperlipidemia, hyperglycemia and oxidative stress [(10)] and it has widely incriminated by various researchers for its neurotoxic effect [(11)]. However, it is still widely used in eastern and western foods as well as in baby´s foods.

Oil seeds are very important for human food and they have gained third position among crops next to cereals and legumes. Rape seed oil has been grown since the 16th century in Europe but it is only since the 1960 that has became a major world crop[12].

Flax seed oil (Linum usitatissimum) is one of the natural products which may lead to this fatty acid balance since it is an excellent source of omega 3- and omega 6 fatty acids, fiber and lignans. It is considered the nature richest storehouse of omega 3 fatty acids which is necessary for a wide variety of biological processes. Flaxseed oil contain alpha linolenic acid (ALA 18:3 n-3) which is converted by the body into eicosapentaenoic acid (EPA) and docosahexaenoic acid(DHA). Diets rich in ALA significantly reduced the incidence of several diseses such as nonfatal infarction and overall mortality in men [13]. The benefits of EPA and DHA include protection from heart attack, fighting inflammation, boosting the immune system, reducing blood clots and pain in arthritis. DHA is one of the building blocks for brain growth and development and is noted for its effects on brain function, mood and behavior. Flaxseed oil is rich in lignans which are phytoestrogens (a plant like estrogen and an antioxidant). The main flax seed lignan is called secoisolariciresinol diglucoside(SDG) found in the hull flaxseed oil has small amount of lignans. Also, it is a platelet activating factor receptor antagonists [14]. Flaxseed and flaxseed oil are not a magic cure but they can play an important part in maintaining a healthy lifestyle [15].

Flax oil is commonly used in food due to high percentage of omega-3 fatty acid and omega 6 fatty acid. Flax seed or linseed which is a member of the Linaceae family is an important oil seed crop in the world. The plant is not a new crop and native to west Asia and the Mediteranean region. It is mainly grown in Canada, Argentina, America, China and India[16]. Flax is the canada´s third major oil seed crop after canola and soybean. Flax is an economically important oil seed crop, especially for Canada,which produced about 40% of world´s flax seed and is the world´s flax seed and is the world´s largest exporter representing about 75% of the global flax trade [17]. Flax seed is rich in fat, protein and dietary fiber. The composition of flax seed averaged 30-40 % fat, 20-25% protein, 20-28 % total dietary fiber,4-8% moisture and 3-4 % ash and the oil contains vitamins A,B,D and E.,minerals and amino acids[18].

Scaribsbrick and Ferguson[19] mentioned that canola oil extracted from (Brassica crops) will play an increasing role in supplying the world´s need for

human and animal food stuffs and industrials oils. In global terms, conventionally grown rape seed oil is the second most important vegetable oil seed after soybean, which accounting for 14% of total oil seed production at 40 Mt in 2000[20].

Brassica napus belongs to the so-called 16:3 plants, fatty acid composition of the leaf lipids is characterized by an appreciable content of hexadecartrienoic acid (16:3) which presented 15% of the total fatty acids. The other important fatty acids are (18:3) the major component, 16:0,16:1,18:0,18:1 and 18:2[21].

Canola seeds (Brassica napus L.) are used to produce edible oil that is fit for human consumption because it has lower levels of Erucic acid than traditional rapeseed oils and to produce livestock feed because it has reduced levels of the toxin glucosinolkates[22].

Canola oil is a natural product produced by pressing the seeds of the rapeseed plant. Typically and approximately, the fatty acid components of triglycerides present in canola oil consist of 6% saturated fatty acids, 62% similar mono unsaturated fatty acids and 30% polyunsaturated fatty acids. There is no recognized evidence that canola oil contains sinigrin or any allergic components [23]. Canola oils are widely used as cooking and salad oils in table spreads for baking and in a variety of other prepared foods. Canola oils low in Erucic acid and are considered favorable dietary oil because of the relatively high proportion of monounsaturated fatty acids, especially oleic acid[24]. Previous studies showed that consumption of canola oil decreased the total cholesterol and ratio of omega6/ omega3 polyunsaturated fatty acids relative to other oils [25]. Also, Gomaa et al., [26] indicated that total sterols of the unsaponifible matter of canola varietied ranged from 12.29 to 80.35% ß-sitosterol was the major sterol fraction of the total sterols followed by compesterol,brassica sterol and stigma sterol for pactol.

Rapeseed (canola) was introduced to Canada in 1950 and it has become a Cinderella crop. In Canada, consumption of canola oil represents about 63% of the total edible oils compared with soybean 24% and sunflower oil13% [27]. Egypt is particularly concerned with food security to find out an optimal degree of food self reliance. The country´s policy is directed towards increasing domestic production of different oil crops to overcome being largely dependent on imports of edible oils. The production of vegetable oils in Egypt is not sufficient to provide the people with edible oils. In Egypt we need detailed

information on the amount of fat, specific fatty acids composition including transunsaturated and polyunsaturated fatty acids, cholesterol and plant sterols in food as eaten.

The present work was undertaken to analyses the physical and chemical properties of flax seed and canola oils, determination of fatty acids composition of flax seed and canola oils as well as their nutritional effects and to assess the physiological and biochemical changes in response to treatment with monosodium glutamate (as food flavor) in male albino rats.

MATERIALS and METHODS

OILS

Flax seed oil was purchased from local market and canola oil was purchased from Arab company for Pharm Medicinal Plants (MEPACO) Egypt.

Analysis of flax seed and canola oils

Physical Analysis:

Refractive index

Refractive index was determined according to AOAC[28] using refractmeter,(NYRL-3Poland). The results were standardized to 25ºC.

Chemical Analysis:

Acid value, peroxide value, iodine number and saponification number were determined according to AOAC[28].

Determination of Fatty acids:

Methylation of fatty acids: An aliquot of fatty acids (about 10 mg) was dissolved in 2ml hexane and then 0.4 ml 2N KOH in anhydrous methanol was added[29]. After 3 min..3 ml water was added. The organic layer separated by centrifugations and dried on anhydrous sodium sulfate then concentrated with N_2 steam to around 0.5ml for gas chromatography analysis of fatty acids methyl esters(FAME).

Gas chromatography(GC) analysis FAME:

Agilent 6890 series GC apparatus provided with DB-23 column (60X0.32mmX0.25µm). Fatty acids methylesters were directly injected into the GC carrier gas was N_2 with a flow rate of 2.2 ml/ min., splitting ratio of 1:80.

The injector temperature was 250 °C and that of FID detector was 270 ºC. The temperature were as follows:150ºC to 225ºC at 5ºC/ min, and then held at 225ºC for 20 min.[30]

Chemicals

Monosodium glutamate as accent Brand Flavor Enhancer low in sodium packed by pet Incorporated ST. Louis., MO USA an IC Industries Company .

Experimental design:

Forty eight male albino rats of average weight (120-140 g) were obtained from the Animal House of Nuclear Research Center,Atomic Energy Authority, Inshas ,Egypt. The animals divided into equal groups. All groups were fed with normal diet for two weeks for acclimation. The diet includes protein, minerals, vitamins, energy resources and other beneficial dietary constituents as recommended by the National Research Council [31].

Group 1 : rats given a diet without any additional source and served as control

Group 2: rats injected subcutaneously with (4mg/g body weight / rat) with monosodium glutamate for ten consecutive days and fed on normal basal diet [32].

Group 3: rats fed on the normal laboratory diet mixed with an equal mixture volume of flaxseed and canola oils in ratio 1:1 (25 g/ kg) diet allover one month .

Group 4: rats fed on the normal laboratory diet mixed with a mixture of flax seed and canola oils in ratio 3:1 (25g / kg diet)allover one month .

Group 5: rats fed on the normal laboratory diet mixed with a mixture of flaxseed and canola oils in ratio 1:3 (25 g/ kg) diet allover one month

Group 6: rats injected subcutaneously with (4mg/g body weight / rat) with monosodium glutamate for ten consecutive days then fed on the normal laboratory diet mixed with an equal mixture volume of flax seed and canola oils in ratio 1:1 (25 g/ kg diet) allover one month.

Group 7: rats injected subcutaneously with (4mg/g body weight / rat) with monosodium glutamate for ten consecutive days then fed on the normal laboratory diet mixed with mixture of flaxseed and canola oils in ratio 3:1 (25g / kg) diet allover one month .

Group 8: rats injected subcutaneously with (4mg/g body weight / rat) with monosodium glutamate for ten consecutive days then fed on the normal laboratory diet mixed with a mixture of flaxseed and canola oils in ratio 1:3 (25 g/ kg diet)allover one month.

The feeding experiments continuous for one month after injection with monosodium glutamate. Then rats were slaughtered after overnight fasting to follow up the changes that might take place in hematological and biochemical parameters. Blood samples collected on three types of tubes, the first contains (EDTA) ethylendiethylene tetraacetic acid for hematological parameters [33] .The second contains sodium fluoride for estimation of the glucose level and other part collected on plain tube to separate serum for the biochemical analysis. Testis was excised washed with saline solution, weighted and homogenized in phosphate buffer pH7.4 the homogenate kept frozen for the pending of biochemical assays.

Assessment of Biochemical Parameters:

The serum enzyme activities of liver functions, aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) were assessed according to Reitman and Frankel [34] and Belfield and Goldberg [35] .Total bilirubin was estimated according to Walters and Gertade [36].The level of glucose determined according to the method of Tietz [37] Serum urea, creatinine and uric acid were estimated according to the Biodiagnostic kit described. Serum total testosterone was estimated by radioimmunoassay technique using kit purchased from Siemens Healthcare Diagnostics Inc., Los Angeles, USA.In testicular tissues lipid peroxidation was assessed as thiobarbituric acid reactive substances (TBARS) following methods of Yoshioka et al., [38]. Estimation of catalase (CAT) activity and glutathione (GSH) content were estimated according to the methods of Johansson and Borg [39] and Beutler et al,[40] respectively.

Statistical analysis

The data were expressed as mean ±SE using one way analysis of variance (ANOVA) .The significant difference between means of groups was done according to Duncan [41].

RESULTS

As illustrated in Table (1) the refractive index were 1.22and1.46, acid value were 1.82 and 0.72, iodine value were 180 and 112, saponification value

were 224and 176 and peroxide value were 4.2 and 1.34 respectively for flax seed and canola oils.

The results in Table (2) showed that the fatty acid composition of flax seed oil, the percentage of saturated fatty acids in flaxseed oil were palmitic acid (16:0) was 7.58% and stearic acid (18:0) was 4.17%. The percentage of unsaturated fatty acid were oleic acid (18:1) was 21.26%,linoleic acid(18:2) was 30.07% and linolenic acid (18:3) 36.14%.

The fatty acid composition of canola oil was identified by GLC and the results showed that the saturated fatty acids in canola oil were palmitic and stearic acid(Table 3). The percentage of palmitic acid (16:0) was 4.46%, while stearic acid(18:0) percentage was 2.19% ,unsaturated fatty acid were oleic acid(18:1) was 66.01%, linoleic acid (18:2) was 18.02%, linolenic acid (18:3) was 6.81%, eicosaesnoic acid acid (20:1) was 1.24% and erucic acid (22:1) was traces.

Table (1): Physicochemical properties of flax seed and canola oils

Varieties	Refractive index	Acid value mgKOH/g	Iodine value	Saponification value	Peroxide value meq/kg
Flaxseed oil	1.22	1.82	180.0	224.0	4.2
Canola oil	1.46	0.72	112.0	176.0	1.34

Table (2): Fatty acid composition of flax seed oil

Fatty acid	Concentration
C16:0(Palmitic acid)	7.58
C16:1(Palmitolic acid)	0.07
C17:0(Hepatdecanoic acid)	Trace
C18:0(Stearic acid)	4.17
C18:1(Oleic acid)	21.26
C18:2(Linoleic acid)	30.07
C18:3(Linolenic acid)	36.14
C20:0(Eicosanoic acid)	0.19
C20:1(Eicosaenoic acid)	0.16
C22:0(Behenic acid)	0.20

Table (3): Fatty acids composition of canola oil.

Fatty acid	Concentration
C16:0(Palmitic acid)	4.46
C16:1(Palmitolic acid)	0.23
C18:0(Stearic acid)	2.19
C18:1(Oleic acid)	66.01
C18:2(Linoleic acid)	18.02
C18:3(Linolenic acid)	6.81
C20:0(Eicosanoic acid)	0.60
C20:1(Eicosaenoic acid)	1.24
C22:0(Behenic acid)	0.30
C22:1(Erucic)	Traces

Comparing to control, Table (4) illustrated that MSG exhibit significant reduction in the RBCs, Hb, Hct% and increased platelet. The animals showed nearly the control pattern for all values after feeding on oils mixture for one month.

Table (4): Flax seed and canola oils mixture effects on RBCs (10^6/mm^3), Hb (g/dl), Hct (%), platelet (N$x10^3$/µl) and its indices in male rats treated with monosodium glutamate.

Groups	RBCs	Hb	Hct	Platelet	MCV (fl)	MCHC (g/dl)	MCH (pg)
Control	5.15±0.41^{a}	10.12±0.56^{b}	29.35±1.27^{a}	391.33±32.88^{e}	55.66±3.35^{a}	35.02±0.87^{a}	19.72±1.31^{a}
MSG	3.75±0.24^{c}	6.92±0.74^{c}	20.93±2.66^{b}	776.33±77.65^{a}	45.16±0.70^{b}	33.00±0.26^{b}	16.48±0.58^{b}
Flax:canol1:1	5.1±0.35^{a}	12.26±0.83^{ba}	31.23±3.17^{a}	389.0±66.73^{ed}	55.80±1.51^{a}	35.20±0.47^{a}	20.78±0.66^{a}
Flax:canola3:1	4.33±0.09^{a}	10.21±0.53^{b}	28.33±1.38^{a}	333.5±22.01^{e}	57.33±0.80^{a}	35.10±0.68^{a}	22.53±1.17^{a}
Flax:canola1:3	4.38±0.08^{a}	11.62±0.57^{ba}	29.60±1.63^{a}	353.33±65.79^{e}	55.83±0.95^{a}	35.46±0.70^{a}	21.56±0.95^{a}
MSG and Flax :canola 1:1	5.1±0.11^{ba}	11.90±0.36^{a}	30.66±0.92^{a}	533.2±45.90^{da}	55.33±4.01^{a}	35.90±1.20^{a}	22.15±0.78^{a}
MSG and Flax :canola 3:1	4.38±0.09^{a}	11.08±0.65^{ba}	28.25±1.59^{a}	594.33±61.64^{ba}	55.50±2.01^{a}	34.63±0.53^{a}	22.87±1.39^{a}
MSG and Flax :canola 1:3	4.53±0.18^{a}	11.80±0.55^{ba}	32.66±1.52^{a}	585.67±53.23^{cad}	55.0±1.83^{a}	35.30±1.08^{ab}	22.83±1.44^{a}

Data are presented as mean ±SE of six animals
The different small letters in the same column are significantly different at P.<0.05.

Data presented in Table (5) showed that MSG treatment induced significantl decrease in leucocytes and its differential lymphocytes and monocytes. While neutrophils showed a significant increase compared to the control. The supplementation of oils mixture after MSG treatment discerned significant recovery compared to the corresponding mixture level of control group.

Table (5): Flax seed and canola oils mixture effects on WBCs ($10^3/mm^3$), and its absolute differential counts in male rats treated with monosodium glutamate.

Groups	WBCs	Monocytes	Lymphocytes	Neutrophils	Eosinophiles
Control	10.37±1.01^{a}	7.67±0.88^{a}	66.0±2.96^{c}	25.0±2.93^{c}	1.83±0.40^{a}
MSG	6.51±0.56^{b}	5.0±0.45^{b}	49.66±4.12^{f}	37.0±3.94^{a}	1.0±0.37^{a}
Flax:canol1:1	9.65±0.98^{a}	6.85±0.37^{a}	74.33±1.89^{b}	23.66±1.28^{cd}	1.67±0.34^{a}
Flax:canola3:1	9.0±0.58^{a}	6.67±0.56^{a}	67.0±1.59^{ce}	24.33±1.17^{c}	1.67±0.42^{a}
Flax:canola1:3	9.65±1.11^{a}	7.0±0.37^{a}	73.60±1.05^{b}	22.80±0.31^{c}	1.67±0.21^{a}
MSG and Flax :canola 1:1	10.48±1.88^{ab}	7.0±0.37^{a}	76.0±1.47^{abd}	26.0±0.37^{bdc}	1.33±0.42^{a}
MSG and Flax :canola 3:1	10.86±1.27^{a}	7.0±0.58^{a}	67.50±1.57^{ce}	24.50±1.28^{c}	1.50±0.22^{a}
MSG and Flax :canola 1:3	12.44±2.47^{a}	6.33±0.76 ^{ab}	72.67±1.69^{cbd}	22.83±1.80^{cb}	1.33±0.56^{a}

Legends as in Table 4

The activities of AST, ALT, ALP and levels of total bilirubin in the serum of various rat groups are presented in Table (6). MSG administration significantly increased the activities of this tested enzymes comparing with the corresponding enzyme activities of the control group. Significant increase of glucose after MSG injection was recorded. Feeding rats on mixture of flaxseed and canola oils revealed a significant decrease in these liver enzymes and glucose level. Moreover, the MSG group showed the highest level of total bilirubin. Supplementation of oils mixture led to a decrease in the level of total bilirubin as compared with the corresponding values of MSG group.

Table (6): Flax seed and canola oils mixture effects on serum AST, ALT, ALP, bilirubin and glucose in male rats treated with monosodium glutamate.

Groups	AST (U/ml)	ALT (U/ml)	ALP (IU/L)	Total Bilirubin (mg/dl)	Glucose (mg/dl)
Control	95.11±5.36^{b}	49.11±4.74^{e}	105.91±6.60^{b}	0.95±0.16^{ab}	124.69±6.84^{b}
MSG	110.0±1.89^{a}	77.18±2.64^{a}	168.94±9.99^{a}	1.35±0.14^{a}	161.20±5.76^{a}
Flax:canol1:1	100.83±2.50^{bcd}	48.29±3.24^{efg}	104.78±6.45^{b}	0.71±0.14^{b}	119.02±4.17^{b}
Flax:canola3:1	98.66±0.92^{bc}	48.33±3.99^{efg}	102.97±7.73^{b}	0.75±0.22^{b}	121.40±7.14^{b}
Flax:canola1:3	99.0±2.62^{bc}	49.28±2.05^{ef}	107.38±3.09^{b}	0.91±0.13^{b}	122.88±4.42^{b}
MSG and Flax :canola 1:1	106.33±2.43^{bae}	59.16±1.35^{ed}	115.76±6.23^{b}	0.71±0.06^{b}	129.21±7.43^{b}
MSG and Flax :canola 3:1	104.16±2.98^{bacde}	63.66±1.33^{c}	112.88±5.48^{b}	0.79±0.03^{b}	116.86±7.37^{b}
MSG and Flax :canola 1:3	109.0±3.95^{bade}	65.16±2.04^{bfgc}	111.87±5.52^{b}	0.80±0.16^{b}	130.51±5.04^{b}

Legends as in Table 4

The present results depicted that MSG injection induced significant increase in urea, creatinine and uric acid levels as shown in Table (7).

Table (7): Flax seed and canola oils mixture effects on serum urea(mg/dl), creatinine(mg/dl) and uric acid(mg/dl) levels in male rats treated with monosodium glutamate.

Groups	Urea	Creatinine	Uric acid
Control	47.05 ± 0.91^{d}	0.76 ± 0.06^{c}	3.54 ± 0.32^{c}
MSG	56.39 ± 1.57^{a}	1.30 ± 0.04^{a}	5.71 ± 0.57^{a}
Flax:canol1:1	41.80 ± 0.68^{ef}	0.81 ± 0.05^{cb}	3.52 ± 0.27^{cd}
Flax:canola3:1	44.64 ± 1.21^{df}	0.82 ± 0.03^{c}	3.17 ± 0.12^{cd}
Flax:canola1:3	47.26 ± 0.52^{d}	0.86 ± 0.05^{cb}	3.53 ± 0.48^{cb}
MSG and Flax :canola 1:1	50.05 ± 1.98^{dcb}	0.90 ± 0.01^{b}	4.21 ± 0.28^{cb}
MSG and Flax :canola 3:1	50.18 ± 1.83^{db}	0.79 ± 0.03^{c}	4.24 ± 1.30^{cadb}
MSG and Flax :canola 1:3	48.96 ± 1.72^{dbcf}	0.85 ± 0.05^{cb}	4.29 ± 0.71^{cadb}

Legends as in Table 4

As shown in Table (8) the testicular level of TBARS in MSG group exhibited significant increase accompanied by significant decrease in CAT activity ,GSH content and serum testosterone level as compared with the control group. The oil mixture in the diet significantly reduced TBARS levels and significantly increased CAT activity, GSH content and testosterone level.

Table (8): Flaxseed and canola oils mixture effects on TBARS concentration (µmol/g tissue), GSH content (mg/g tissue), CAT activity (U/ g tissue) in testicular homogenate and serum testosterone (ng/dl) levels in male rats treated with monosodium glutamate.

Groups	TBARS	GSH	CAT	Testosterone
Control	8.72 ± 1.35^{b}	26.66 ± 4.10^{a}	11.48 ± 0.69^{a}	160.25 ± 4.29^{c}
MSG	14.42 ± 1.47^{a}	17.33 ± 0.64^{b}	8.28 ± 0.49^{b}	48.87 ± 6.18^{d}
Flax:canol1:1	8.76 ± 0.56^{b}	27.57 ± 2.31^{a}	11.80 ± 1.03^{a}	161.37 ± 4.13^{c}
Flax:canola3:1	8.14 ± 0.92^{b}	28.15 ± 3.45^{a}	13.27 ± 0.57^{a}	172.63 ± 4.91^{acb}
Flax:canola1:3	8.78 ± 0.71^{b}	27.21 ± 3.16^{a}	12.55 ± 0.43^{a}	171.89 ± 1.96^{b}
MSG and Flax :canola 1:1	9.23 ± 0.78^{b}	23.13 ± 2.27^{a}	12.63 ± 0.89^{a}	153.01 ± 7.0^{c}
MSG and Flax :canola 3:1	9.58 ± 0.65^{b}	21.94 ± 1.83^{a}	13.84 ± 1.05^{a}	152.18 ± 3.43^{c}
MSG and Flax :canola 1:3	10.90 ± 1.46^{ba}	24.82 ± 3.13^{a}	13.73 ± 0.97^{a}	164.98 ± 4.78^{cb}

Legends as in Table 4

DISCUSSION

Monosodium glutamate (MSG), the sodium salt of L- glutamic acid, is one the main flavor enhancers needed as an ingredient in various food

products[42]. It is widely used as a food additive to improve the taste of food because it potentiates the activities of gustatory nerve [43] which mediate sweet taste[44].

The results of physicochemical properties of flax seed and canola oils agree with Gomaa et al.,[26] who found that refractive index , acid value, peroxide value and iodine value of canola oil was ranged between 1,4610-1.4723 at 25ºC, 0.25-1.61 mgKOH /g oil, 0.00-10 m.eq/kg oil and 96.22-126, respectively.Bozan and Temelli[45] found that fatty acid composition of flax seed oil consisties of palmitic acid (16:0)was 6.86% and stearic (18:0) was 4.59 %. The percentage of unsaturated fatty acid were oleic acid (18:1) was 15.0%, linoleic acid (18:2) was 13.96% and linolenic acid (18:3) was 58.31%. Similarly the obtained results of fatty acid composition of canola oil was in agreement with Gomaa et al., [26] who found that fatty acid composition of different varieties of canola seed oil consists of olic ranged from 29.46 to 80.44 % , linoleic from 1.5 to 43.46% and linolenic from 1.14 to 12.54%.

The decrease in erythroid series (RBCs, Hb, Hct%) with the consequent variation in blood indices may be due the increase in red blood destruction or / and decrease in red blood cell formation and release from bone marrow .This is in agreement with Anand et al., [46]. These findings indicate that anemia may occur as a result of MSG consumption. The decrease in leucocytic ,and lymphocytic count with increase in neutrophils and platelet are often associated with a large number of inflammatory reactions[47].These finding indicate that MSG may cause some element of inflammation leading to disturbance in platelet and differential count.

MSG rats showed a significant increase of glucose level when compared to control rats suggesting that MSG develop glucose intolerance and / or an insulin resistant state . It has been demonstrated that oils with a high n-3 to n-6 fatty acid content may improve insulin action [48] .

The improved blood glucose level and blood picture after feeding on oils mixture due to the free radical scavengers such as reduced glutathione and vitamin E prevent haemolysis of red blood cells and lipid peroxidation[49] ,however flaxseed oil contains vitamins A,B,D and E[18] .

In the present study, it has been found that ALT, AST ,ALP as well as the concentration of total bilirubin were significantly increased in rats injected subcutaneously with MSG as compared with control. These increase may be

indicative to the hepatic cell damage. These results are in agreement with Farombi and Onyema [50] and Ortiz et al., [32]. The increase in glutamate has been the result of diminished tissue uptake of the amino acid or of delayed catabolism of the amino acid and the increase in ALP activity related to damage of the liver membranes. The increase in the level of bilirubin might be attributed to defective conjugation of bilirubin in the liver to form a water soluble diglucuronide leading to impair its secretion into the bile canaliculi resulted in the accumulation of bilirubin[51]. This increase may be also due to the decrease in RBCs due to it destruction and release of bilirubin also from it.

After feeding of oils mixture ,there is a decrease in the activities of AST,ALT and ALP and decrease the level of total bilirubin in treated group with MSG. This may be due to the effect of alpha linolenic acid(ALA), oleic acid and some polyunsaturated fatty acids the ingredient of flax seed and canola oils which decrease conjugation of bilirubin content of rat liver as illustrated by Hargreaves [52] ameliorate the increase of these enzymes .

There are some parameters used to evaluate renal functions including urea, creatinine and uric acid [53]. Flax seed oil contain lignan, seciosolaricir-esinol diglucoside(SDG) which are implicated in attainment of health and treatment of renal injury and osteoporosis[54], similarly Sankaran [55] reported that linseed oil slowed down early fibrosis in renal injury of mice.

It is clarified from Table (8) that treatment with MSG caused an elevation of lipid peroxidation end product (TBARS) accompanied with significant decrease in the antioxidant defense(CAT activity and GSH content)in the testicular homogenates. This became in accordance with Farombi and Onyema [50] and Manivasagam and Subramaniam[9]. The increase in TBARS levels reflects the increase in lipid peroxidation supporting the previous results [56,57] that MSG achieve its deleterious effect through oxidative stress. The wide MSG distribution in modern nutrition enables a continuous intake of this substance resulting in accumulation and rise of the glutamic acid concentration in blood [58]. Glutamine could initiate the lipid peroxidation by changing the redox potential of cell and thus favoring the lipogenesis [42].

In the current investigation, the increase in TBARS and decrease in reduced glutathione and catalase is indicative of enhanced oxidative stress. The oxidative stress play an important role in the development of the

pathophysiology of many disease such as cancer ,diabetes[59].Many studies have reported a correlation between oxidative stress and MSG treatment [8,50]. The significant increase in testicular TBARS indicated the over production of free radicals and hence oxidative stress may account for testicular injury associated with MSG treatment which was associated with the significant decrease in serum testosterone level. The increased TBARS concentration may be due to the decreased formation of antioxidants in MSG treated tissues in consequence of the augmented activity of reactive oxygen species. The level of lipid peroxidation in cells is controlled by various cellular defense mechanisms consisting of scavenger enzymatic system (SOD,CAT ,glutathione peroxidase and glutathione reductase) and non enzymatic (vit. C,E, uric acid). So, the decrease in the activity of CAT in the present study could be due to less availability of NADPH as MSG favor lipogenesis by increasing the level of glutamine[8]. The significant decrease in testicular GSH as a result of MSG treatment may be due to the formed aldehyde during peroxidation of lipids can bind to sulfhydryl group of proteins to form stable products in tissues which is associated with loss of protein function [59].

Feeding on oils mixture significantly decrease the TBARS and increase the CAT activity and GSH content in testicular tissue this result is in agreement with Bhatia et al., [60] who reported that pretreatment with linseed oil significantly lowered radiation induced damage in term of the increased malondialdehyde level . This explain the potential beneficial effects of a diet high in mono unsaturated fatty acids (oleic acid –OA) from canola oil and alpha linolenic acid(ALA) from flax seed oil in reducing markers of oxidative stress . It also indicates the importance of dietary OA and ALA as important modulators of membrane lipid compositions. They suggested that flax seed oil has the potential effect to scavenge free radicals formed during oxidative stress, through increase the GSH content which diminish oxidative stress through catalyses the detoxification of H_2O_2 by the action of glutathione peroxidase with net results of GSSG and H_2O[60]. Also, TBARS decrease after the supplementation on the oils mixture may be attributed to the constituents of the oil of omega-3- essential fatty acids and phytoestrogenic lignans that play an important role in free radical scavenging [61]. It is well known that CAT is responsible for breakdown of hydrogen peroxide an important ROS produced during metabolism [62] and the glutathione dependent enzymes like glutathione reductase, glutathione peroxidase and glutathione transferase have been shown to protect against MSG cytotoxicity[63].

In the present study, animals subcutaneously injected with MSG resulted an increased in the testicular levels of TBARS which reduces reproductive ability. This alteration in the testicular lipid peroxidation became in accordance with Ahluwalia et al., [7] and Choudhary et al., [8] in adult mice during MSG treatment. The levels of antioxidants which declined in testis as a consequence of MSG injection were significantly restored to normal values after treatment of rats with mixture of flax seed and canola oils mixture .This is due to enrichment of oils mixture with omega-3-polyunsaturated fatty acids as flax seed oil feeding produced hepatic and renal enrichments of n-3 PUFA. Consumption of linssed oil based products may provide health benefit [64].

The rats treated with MSG had significantly lower serum concentration of testosterone. This result was in agreement with [65] who reported that male rats treated with MSG had significantly smaller accessory sexual organs (seminal vesicles, prostate, testes) and significantly lower serum FSH and testosterone levels than sex matched controls. This reduction in gonadal steroids levels and inappropriately low gonadotrophin levels may be due to deficient of feed back regulation in the hypothalamic pituitary gonadal axis in MSG treated rats. This may be due to the direct effect of MSG mediated through oxidative stress on testicular tissues as a cause of testosterone level reduction as evidenced by increasing testicular TBARS levels in rats treated with MSG Nemeroff et al., [65] .

The increase in testosterone level after supplementation on flax seed and canola oils mixture may be due to the effect of flax seed oil as , it has a chemo -protective effects on the mammalian cells and affects the endogenous hormone production and metabolism [66] and omega-3- essential fatty acids and phytoestrogenic lignans that play an important role in free radical scavenging[61].

The mechanism of MSG actions has been suggested to be mediated through oxidative stress [57]. Although the mechanisms are not fully elucidated, the deleterious effects of oxygen metabolites have been shown to be due to destruction of biomembrans and sub cellular organelles due to the presence of polyunsaturated fatty acids in such membranes, thus inducing lipid peroxidation [67]. The obtained results indicated the different ratio of flax seed oil : canola oil (1:1,1:3or3:1) had the same obvious result this may be due to the high content of unsaturated fatty acid of flax seed oil(88%) and the unsaturated fatty

acids of canola oil was (91%) i.e the percentage near to each other .

CONCLUSION

The current study proved the potentiality of the flax seed and canola oils mixture in abrogation of the deleterious effect of MSG on the physiological and biochemical parameters studied most probably due to its high content of omega-3, omega- 6 and polyunsaturated fatty acids. So, it is recommended to use flax seed and canola oils in our diet as a protection against deleterious effects of MSG especially in children which now consume many foods containing MSG as a food additive and flavors to improve the taste of foods.

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مجلة البحوث الإشعاعية
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تـأثير أضـافه خلـيط مـن زيـت الكتـان والكـانولا علـى التغيـرات الفسـيولوجية والبيوكيميائيه المستحدثة بأحادى جلوتامات الصوديوم في الجرذان

مرفت محمد أنور- نجلاء الشحات محمد *

*قسم البحوث النباتية– مركز البحوث النووية – هيئـه الطاقة الذرية –مصر- *- قسم التطبيقات البيولوجية - مركز البحوث النووية- هيئه الطاقة الذرية- مصر ص ب .13759*

أحد المشاكل الهامة في مجال تغذيه الإنسان هو استخدام نكهات مكسبات الطعم للغذاء ويعتبر أحادى جلوتامات الصوديوم من مكسبات الطعم المستخدمة والذي ينتج عنه أضرار فسيولوجية وبيوكيميائيه لـذا اسـتهدفت هـذه الدراسـة تقيم أضـافه خلـيط مـن زيـت الكتـان والكـانولا لتقليـل التغيـرات الفسـيولوجية والبيوكيميائيه المستحدثة بأحادي جلوتامات الصوديوم في ذكور الجـرذان بنسبه 4 مللى جرام / جرام من وزن الجسم وذلك لارتفاع محتواهم من الأحماض الدهنيه الغير مشبعه والاسترولات والتكوفيرولات حيث تم تقدير الصفات الطبيعية والكيميائية لزيت الكتان (الزيت الحار) وزيت الكانولا وكذلك تم تقدير الأحماض الدهنيـه المشـبعة والأحمـاض الدهنيـه الغيـر مشـبعه فـي كـلا الـزيتيين وذلـك باسـتخدام جهـاز التحليـل الكروماتوجرافى الغازي وأوضـحت النتـائج أن زيت الكتـان (الزيـت الحـار) يحتوى علـى حمـض الاوليـك بنسبه 22% وحمض اللينوليك بنسبه 30% وحمض اللينولينيك بنسبه 36% اى انـه يحتـوى علـى 88% أحماض دهنيه غير مشبعه وأحماض مشبعه بنسبه 12%وان زيت الكـانولا يحتوى علـى حمـض الاوليـك بنسبه 66% وحمض اللينوليك بنسبه 18% وحمض اللينولينيك بنسبه 7% اى انـه يحتـوى علـى 92% أحماض دهنيه غير مشبعه وأحماض دهنيه مشبعه 8% . أوضحت النتـائج المتحصل عليها عن استخدام أحادى جلوتامات الصوديوم فى حقن الجرذان لمده عشره أيام متصلة أدت إلى حدوث نقص في عدد كرات الدم الحمراء والبيضاء والهيموجلوبين والهيماتوكريت وزياده فى عدد الصفائح الدموية وكذلك زيادة الدهون فوق المؤكسدة مصاحبا بنقص معنوي في نشاط إنزيم الكتاليز ومحتوى الجلوتاثيون في الخصية ونقص هرمون التستوستيرون في مصل الدم. صاحب هذه الاضطرابات زيادة معنوية في مستوى نشاط إنزيمات الكبد الالنين ترانس امينيز والاسبرتات ترانس امينيز واالفوسفاتيز القاعدي وارتفاع الصفراء والجلوكوز والبولياناو الكرياتينين وحمض البوليك في مصل الدم. وقد ثبت أن أضافه خليط من زيت الكتان والكانولا بنسب مختلفه الى العليقه والتغذيه عليها بعد الحقن باحادى جلوتامات الصوديوم لمده شهر أدى إلى تحسن واضح للاضرار التي نتجت عن استخدام أحادى جلوتامات الصوديوم .ولذلك لتقليل التأثيرات الضارة لهذه الماده نوصى باستخدام خليط من زيت الكتان والكانولا.