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Research Article

EFFECT OF MONOSODIUM GLUTAMATE ON LIPID PEROXIDATION AND VARIOUS LIPID FRACTIONS IN PLASMA OF HYPERCHOLESTREMIC ADULT MALE MICE

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ABSTRACT
Monosodium glutamate, a flavour enhancer was administrated subcutaneously at dose levels of 4 and 8mg/g body weight to hypercholestremic adult male mice for 6 consecutive days and its effect was observed on 31st day after the last injection for the genesis of atherosclerosis by evaluating the changes in plasma lipid peroxidation product, malondialdehyde (MDA) and various fractions of lipid and cholesterol. The animals were divided in four groups each comprising 6 mice. Group-I: Control, Group-II: Hypercholestremic animals, Group-III: 4mgMSG/g body weight + Hypercholestremic animals and Group-IV: 8mgMSG/g body weight + Hypercholestremic animals. Blood samples were obtained after the dose period for the measurement of lipid peroxidation, total lipid, phospholipids, triglycerides, free fatty acids, total cholesterol, esterified cholesterol and free cholesterol from all the groups. A significant increase was observed in lipid peroxidation, lipid fractions (total lipid, phospholipids, triglycerides & free fatty acids) and cholesterol fractions (total cholesterol, free cholesterol and esterified cholesterol) in all the study groups with respect to control animals and hypercholestremic animals not receiving monosodium glutamate. These observations suggested that administration of monosodium glutamate at dose levels of 4 mg/g body weight and above to hypercholestremic animals had no beneficial effect instead it further enhanced the lipid peroxidation, various lipid & cholesterol fraction levels and thereby being responsible for the initiation of coronary heart disease/atherosclerosis.

Key Words: *Monosodium Glutamate, Hypercholestremia, Malondialdehyde, Coronary Heart Disease And Atherosclerosis*

INTRODUCTION
Cardiovascular disease (CVD) remained one of the main causes of death in all over the World. Coronary artery disease (CAD) is the single most important disease entity, in terms of mortality and morbidity in the entire World population. Both men and women between the age group of 40-60 years of age are susceptible to CAD (Kuldip and Ahluwalia, 2010). Despite all round efforts, it remains a challenge to the healthy managers and scientists. It is predicted that by the years 2020, this disease would be persists as the major and the most common threat to human life. In developing countries like India, the incidences of CAD are increasing alarmingly (Gupta, 2005). The risk of CAD in Indian is three times higher than in the white Americans, six times higher than in the Chinese and 20times higher than in the Japanese. At the threshold of this millennium, CAD is looming large as a new epidemic afflicting Indians at a relatively younger age (Gupta, 2005 and Enas & Yusuf, 1999). The underlining cause of CAD is atherosclerosis. Atherosclerosis is a multifactorial disease and results from complex interactions among injurious stimuli & healing and reparative responses of the arterial wall occurring in a hyperlipidemia and dyslipoproteinemic environment. The increase in the scientific literature reflects the opinion that oxidative stress is likely to be involved in the pathogenesis of various diseases like atherosclerosis (Kuldip and Ahluwalia, 2010).
Monosodium glutamate (MSG), a sodium salt non essential L-form of glutamic acid used as flavour enhancer in various Chinese, Japanese, fast food and ready to serve foods like 2 minute noodles, soups,

sauces etc. Survey of literature revealed that a little work has been done on the adverse effects of MSG in adult population especially in India, where its use is wide spreading due to increased craze for Chinese, Japanese, fast food and ready to serve foods like 2 minute noodles, soups, sauces etc. all containing MSG and plays a critical role as a part of socioeconomic development. Concomitantly, there is a tremendous increase in the incidences of CHD/atherosclerosis in developed and developing nations like India.

Previously, we reported that MSG at dose levels of 4 mg/g body weight and 8 mg/g body weight for consecutive 6 days induced hyperlipidemia and oxidative stress in various tissues of normal adult male mice's, well known risk factors for atherosclerosis (Malik & Ahluwalia, 1994; Ahluwalia *et al*., 1996; Chaudhary *et al*., 1996; Kuldip & Ahluwalia, 2003; Kuldip & Ahluwalia, 2005; Ahluwalia *et al*., 2005 and Sharma *et al*., 2009). So, in the present work we studied the effect of MSG on hypercholestremic adult male mice to observe whether MSG has beneficiary effects or not in hypercholestremic adult animals.

MATERIALS AND METHODS

Animals: Normal adult male mice (LAKA, US) weighing 25-30g in body weight were procured from the animal house of Panjab University, Chandigarh - India. Animals were maintained on standard pellet diet (Hindustan Lever Ltd., Bombay) with free access to water.

Grouping: MSG was subcutaneously administered at dose levels of 4 and 8 mg/g weight for consecutive 6days that is 31st to 36th day to 1% cholesterol fed animals and continuing the feeding of cholesterol (1%) for 67 days. These animals were divided into following four groups and each group containing 6 mice;

Group-I (Control): 0 mg MSG/g body weight.
Group-II : 0 mg MSG/g body weight + 67days of 1%cholesterol fed animals
Group-III : 4 mg MSG/g body weight + 67days of 1%cholesterol fed animals
Group-IV : 8 mg MSG/g body weight + 67days of 1%cholesterol fed animals

This experimental design was approved by the Animal Experimental Ethics Committee of Panjab University, Chandigarh and conducted according to Indian National Science Academy Guidelines for the use & care of experimental animals.

Sample Preparation: After the dose period, animals were fasted overnight and blood was drawn from the eye of mice and collected in sterile tubes containing ethylene diamine tetra acetic acid (EDTA). These blood samples were centrifuged by using cold centrifuge at 4°C for 10minutes at 3000rpm and supernatant (plasma) obtained was used for various biochemical assays.

Biochemical Assays*:*

1. ***Lipid peroxidation (LPO):*** The LPO levels were assayed by measuring the pink color chromophore formed by the reaction of thiobarbituric acid with malondialdehyde (MDA) according to the method of Beuge and Aust, 1978.
2. ***Determination of Lipid fractions:***

i. **Total lipids:** Total lipid levels were estimated by the method of Frings and Fendly, 1972.
ii. **Phospholipids:** Phospholipids were estimated by applying the methods of Fiske and Suba Row, 1925.
iii. **Triglycerides:** The concentration of Triglycerides was determined by the method of Mc Gowan *et al*., 1983.
iv. **Free fatty acids:** Free fatty acids were estimated by applying the methods of Lowry and Tinsley, 1976.
v. **Total cholesterol:** Total cholesterol levels were assayed by the methods of Zlatkis *et al*., 1953.
vi. **Free Cholesterol:** Free Cholesterol levels were estimated by the method of Cour Chaine *et al*., 1959.
vii. **Esterified Cholesterol:** Esterified Cholesterol levels were calculated by subtracting the value of free cholesterol from total cholesterol.

Statistical Analysis: Results of biochemical analyses are presented as mean value ± standard deviation (S.D.). The difference between control and test groups was analyzed by using Student "t" test (significant

difference at $p < 0.05$ confidence level). Correlation between the investigated groups was performed using test ONE-WAY ANOVA (one-way variance analysis).

RESULTS

Effect of cholesterol on various lipid and cholesterol fractions:

The results of various lipid fraction and cholesterol fractions are summarized in Table-1, Table- 2, Table-3 and Table- 4. Oral ingestion of cholesterol (1%) for 67 days significantly increased the plasma total lipids levels by 47.76% ($p<0.001$, from 226.10 ± 12.97mg/dl to 334.09 ±12.85 mg/dl), phospholipid levels by 33.78% (from 51.15±3.10mg/dl to 68.43±2.78mg/dl), triglycerides by 41.81% ($p<0.001$, from 92.81±4.99 mg/dl to 131.62±4.61 mg/dl) and free fatty acids by 36.58% ($p<0.001$, from 144.01±5.71mg/dl to 196.70±5.95 mg/dl) respectively with respect to control (Group-I) animals. A significant increase was also observed in total cholesterol by 42.06% ($p<0.001$, from 58.00±2.37 to 82.40±2.57), free cholesterol by 41.29% ($p<0.001$, from 23.10±1.42 to 32.64±1.04) and esterified cholesterol by 42.57% ($p<0.001$, from 34.90±1.86 to 49.76±2.51) in cholesterol ingested adult male mice's with respect to control (Group-I) animals (Table-1, 2, 3 and 4).

Effect of MSG on various lipid and cholesterol fractions in hypercholestremic animals (Table-1, Table-2, Table-3 and Table-4):

A highly significant increase was found in total lipids (71.13% & 76.67%), phospholipid (58.98% &75.09%), triglycerides (71.89% & 89.95%) and free fatty acid (66.30% &82.02%) levels in plasma of MSG treated hypercholestremic (Group-III & Group-IV respectively) adult male mice with respect to control animals (Table-1, Table- 2, Table- 3 and Table- 4) and a highly significant increase by 15.81% ($p<0.05$) &19.56% ($p<0.01$) in total lipids, 18.83% ($p<0.01$) & 30.87% ($p<0.001$) in phospholipids, 21.21% ($p<0.01$) & 32.53% ($p<0.001$) in triglycerides and 21.75% ($p<0.01$) & 33.26% ($p<0.001$) in free fatty acids levels was observed in group-III and group-IV respectively as compared to group-II (Table-1, Table- 2, Table- 3 and Table- 4).

Effect of cholesterol on lipid peroxidation:

The results of lipid peroxidation levels summarized in Table-5 and Table-6. The lipid peroxidation levels were significantly increased by 37.54 % ($p<0.001$, from 3.01±0.230 nmol of TBARS/mg protein to 4.14±0.133 nmol of TBARS/mg protein) in hypercholestremic (Group-II) adult male mice with respect to control (Group-I) animals.

Effect of MSG on lipid peroxidation in hypercholestremic animals:

Lipid peroxidation levels were significantly increased by 72.42% ($p<0.001$, from 3.01±0.230 nmol of TBARS/mg protein to 5.19±0.246 nmol of TBARS/mg protein) and 73.08% ($p<0.001$, from 3.01±0.230 nmol of TBARS/mg protein to 5.21±0.200 nmol of TBARS/mg protein) was observed in 4mgMSG/g body weight (Group-III) and 8mgMSG/g body weight (Group-IV) treated hypercholestremic adult male mice's respectively with respect to group-I (Table-5 and Table-6). A significant increase by 15.81% ($p<0.05$) and 19.56% ($p<0.01$) was also observed in MSG treated hypercholestremic (Group-III and Group-IV) animals with respect to hypercholestremic animals not receiving MSG (Group-II), Table-5 & Table-6.

DISCUSSION

The oral ingestion of cholesterol (1%) for 67days to adult male mice induced hypercholestremia as in the present study, we observed a significantly increase in various lipid fractions (Total lipids, phospholipid, triglycerides and free fatty acids) and cholesterol fractions like total cholesterol, free cholesterol and esterified cholesterols with respect to control (Group-I) animals (Table-1, Table- 2, Table- 3 and Table-4). It is well reported that hypercholestremia is a well known risk factor for the initiation of atherosclerosis (Hasler-Rapacz *et al*.,1995; Knight-Lozano *et al*.,1995 and Csont et al., 2007). Subcutaneous administration of MSG at dose levels of 4mg/g body weight (Group-III) and 8mg/g body weight (Group-IV) to hypercholestremic adult male mice's was also significantly increased the levels of

various lipid fractions and cholesterol fractions with respect to group-I and group-II (Table-1, 2, 3 & 4). These observations indicated that MSG act in synergism with cholesterol and had no beneficial effect on hypercholestremic animal to reduce the levels of lipid and cholesterol fractions.

Lipid peroxidation (representing malondialdehyde) levels were found to be significantly in hypercholestremic (Group-II) animals as compared to control adult male mice's (Table-5 and Table-6). High cholesterol might lead to an increased production of oxygen free radicals, which in turn might lipid peroxidation, causing endothelial cell damage. High blood cholesterol may damage the endothelial cell in various ways. Hypercholestremia may increase the cholesterol content of platelets. Cholesterol-rich platelets have been shown to increase the release of arachidonic acid from the platelets and to enhance the production of thromoboxane A_2 (Stuart *et al*., 1980). Cholesterol enhances platelet function and cholesterol-rich platelets release histamine, ADP and serotonin (Shattil *et al*., 1975 and Henry, 1977). Histamine and ADP are known to increase phospholipase A2 activity (Ruzicka and Printz, 1984). Phospholipase A2 acts on phospholipids to form arachidonic acids (Vanden, 1980). Another possibility for an increase in the arachidonic acid in hypercholestremia may be an increase in the calcium influx in endothelialcells. Phospholipase A2 activity is calcium dependent (Vanden, 1980). Hence an increase in the intracellular calcium would increase phospholipase A2 activity. Cholesterol is known to increase membrane fluidity (Shattil and Cooper, 1978). Increase in the membrane fluidity would increase the calcium influx. It has also been suggested that cholesterol can affect phospholipase A2 through alteration in lipid water interphase (Stuart *et al*., 1980).

Table 1: Effect of subcutaneous administration of MSG at dose levels of 0, 4 and 8mg/g body weight (for 6 consecutive days) on total lipids, phospholipids, triglycerides and free fatty acids in plasma of hypercholestremic adult male mice's.

Groups	Total lipids (mg/dl)	Phospholipids (mg/dl)	Triglycerides (mg/dl)	Free Fatty Acids (mg/dl)
Group-I (Control)	226.10 ± 12.97[a]	51.15±3.10	92.81±4.99	144.01±5.71
Group-II [0mg MSG/g b.wt. + Cholesterol (1% for 67days)]	334.09 ±12.85	68.43±2.78	131.62±4.61	196.70±5.95
Group-III [4mg MSG/g b.wt. + Cholesterol (1% for 67days)]	386.94±12.28	81.32±3.89	159.54±5.59	239.49±6.72
Group-IV [8mg MSG/g b.wt. + Cholesterol (1% for 67days)]	399.46±15.28	89.56±3.06	174.44±5.95	262.14±6.17

a - values are expressed as Mean ± S.D. of 6 observation

Table 2: Percentage change in total lipids, phospholipids, triglycerides and free fatty acids upon subcutaneous administration of MSG (for consecutive 6 days) to hypercholestremic adult male mice's.

Groups	Total lipids	Phospholipids	Triglycerides	Free Fatty Acids
Group-I *vs* Group-II	+5.90	+18.10*	+20.15*	+12.34*
Group-I *vs* Group-II	+71.13***	+58.98***	+71.89***	+66.30***
Group-I *vs* Group-IV	+76.67***	+75.09***	+89.95***	+82.02***
Group-II *vs* Group-III	+15.81*	+18.83*	+21.21**	+21.75*
Group-II *vs* Group-IV	+19.56**	+30.87***	+32.53***	+33.26**

** p≤ 0.005, **p≤ 0.01 and *** p≤ 0.001*

Table 3: Effect of subcutaneous administration of MSG at dose levels of 0, 4 and 8mg/g body weight (for 6 consecutive days) on total cholesterol, free cholesterol and esterified cholesterol in plasma of hypercholestremic adult male mice's.

Groups	Total Cholesterol (mg/dl)	Free Cholesterol (mg/dl)	Esterified Cholesterol (mg/dl)
Group-I (Control)	58.00±2.37[a]	23.10±1.42	34.90±1.86
Group-II [0mg MSG/g b.wt. + Cholesterol (1% for 67days)]	82.40±2.57	32.64±1.04	49.76±2.51
Group-III [4mg MSG/g b.wt. + Cholesterol (1% for 67days)]	91.70±3.68	35.42±1.11	56.28±2.62
Group-IV [8mg MSG/g b.wt. + Cholesterol (1% for 67days)]	100.60±1.29	40.52±1.29	60.08±3.19

a - values are expressed as Mean ± S.D. of 6observation

Table 4: Percentage change in total cholesterol, free cholesterol and esterified cholesterol upon subcutaneous administration of MSG (for consecutive 6 days) to hypercholestremic adult male mice's.

Groups	Total Cholesterol	Free Cholesterol	Esterified Cholesterol
Group-I *vs* Group-II	+1.24	+0.73	+2.14
Group-I *vs* Group-III	+58.10***	+53.33***	+61.26***
Group-I *vs* Group-IV	+73.44***	+75.41***	+72.14***
Group-II *vs* Group-III	+11.28*	+8.51	+13.10*
Group-II *vs* Group-IV	+22.08**	+24.14**	+20.73*

** p≤ 0.005, **p≤ 0.01 and *** p≤ 0.001*

Table 5: Effect of subcutaneous administration of MSG at dose levels of 4 and 8mg/g body weight (for 6 consecutive days) on lipid peroxidation levels in plasma of hypercholestremic adult male mice's.

Groups	**LPO (nmol of TBARS/mg protein)**
Group-I (Control)	3.01±0.230[a]
Group-II [0mg MSG/g b.wt. + Cholesterol (1% for 67days)]	4.14±0.133
Group-III [4mg MSG/g b.wt. + Cholesterol (1% for 67days)]	5.19±0.246
Group-IV [8mg MSG/g b.wt. + Cholesterol (1% for 67days)]	5.21±0.200

a - values are expressed as Mean ± S.D. of 6 observation

Table 6: Percentage change in lipid peroxidation levels upon subcutaneous administration of MSG (for consecutive 6 days) to hypercholestremic adult male mice's.

Groups	**LPO**
Group-I *vs* Group-II	+37.54**
Group-I *vs* Group-III	+72.42***
Group-I *vs* Group-IV	+73.08***
Group-II *vs* Group-III	+15.81*
Group-II *vs* Group-IV	+19.56**

* $p \leq 0.005$, ** $p \leq 0.01$ and *** $p \leq 0.001$

The increase in the arachidonic acid through these mechanisms would increase the formation of prostaglandins and leukotrienes. It is known that the intermediate steps in the biosynthesis of prostaglandins from arachidonic acid produce oxygen free radicals (Egan *et al*., 1976 and Panganamala *et al*., 1976). Leukocytes will produce leukotrienes through arachidonic acid metabolism. One of the leukotriene B4 (LTB4) has been found to be a potent inducer of leukocyte chemotaxis, aggregation and degranulation (Ford-Hutchinson, 1980). Leukotriene B4 would activate polymorphonuclear leukocytes (PMN) to secrete oxygen free radicals (Ford-Hutchinson, 1980). Oxygen free radicals thus produced would induce endothelial cell damage by causing peroxidation of membrane phospholipids. The first step in lipid peroxidation is the initiation reaction, which begins by taking out hydrogen atom from polyunsaturated fatty acid (PUFA) by oxygen radical. The second step is the propagation and the final step is termination. The extent of lipid peroxidation has often been determined by the thiobarbituric acid (TBA) test, which has also been considered for the determination of malondialdehyde. A significant increase in lipid peroxidation levels in hypercholestremic (Group-II) and MSG treated hypercholestremic (Group-III and Group-IV) animals was observed in the present study might lead to susceptibility of the biomembrane, which ultimately leads to tissue injury/damage.

Inclusion, a aforementioned observations suggested that administration of MSG at dose levels of 4mg/g body weight and above along with cholesterol produced hyperlipidemia and enhanced the oxidative stress by further increasing the levels of lipid peroxidation thereby MSG along with cholesterol had additive effect. Hence could act as an additional factor for the initiation of atherosclerosis.

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