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CODEN: IAASCA **ORIGINAL ARTICLE**

Protective effect of *Nigella sativa* oil and Astaxanthin on Monosodium glutamate Induced dyslipidemia in Rats

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ABSTRACT
The present study was aimed to evaluate the possible ameliorative effect ofNigella sativa oil(NSO) andAstaxanthin(ASX) against Monosodium glutamate(MSG) (0.8g/Kg b.wt) induced dyslipidemia in rats. Forty eightpubertal male albino rats were divided into eight groups consisting of six each. Rats of groups 2, 3 and 4 were treated with MSG (0.8g/Kg b.wt), NSO (1ml/Kg b.wt) and ASX (25mg/Kg b.wt) orally for 28 days, respectively. Further, rats of groups 5, 6, 7 and 8 were co-administered with NSO + ASX, MSG + NSO, MSG + ASX and MSG + NSO + ASX orally, respectively. At the end of the experimentation, the blood samples were collected and the serum was used for biochemical analysis.Administration of MSG elevated the concentration of Total Cholesterol (TC),Triglycerides (TG),Low density lipoprotein (LDL) and Very low density lipoprotein (VLDL) with a decreased level of High density lipoprotein (HDL) in the serum of male albino rats, compared to control (group - 1). However, the concentration of all lipids in the serum was restored to normalcy in rats treated withNSO + MSG (group – 6),ASX + MSG (group-7),MSG + NSO+ ASX (group – 8), No significant change was observed in the Lipid profile in the serum of rats received NSO (group – 3), ASX (group – 4) alone and combination of NSO with ASX (group – 5). The results indicate the protective effect of NSO and ASX in the serum lipid profile in the MSG induced toxicity in male pubertal albino rats.
Keywords: *Dyslipidemia,Monosodium glutamate,Nigella sativa oil,Astaxanthin,Total cholesterol, Triglycerides, HDL, LDL, VLDL*

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INTRODUCTION

Monosodium glutamate (MSG), the sodium salt of glutamic acid (GA), is one of the most frequently used additives in the developed world [1] and Commonly used as a flavour enhancer especially in Chinese, Thainese and Japanese foods [2]. Glutamate is an excitatory amino acid neurotransmitter that acts as a chemical messenger that triggers the nerve cells to fire[3] which results in several chronic diseases like type 2 diabetes mellitus, hypertension, cardiovascular disease, dyslipidemia and several types of cancer [4,5], Chinese restaurant syndrome [6]. Further,Chronic administration of MSG induced oxidative stress in hepatic and cardiac tissues in experimental animals due to metabolic shifting[7,8] has been reported.

Recent studies have shown that many compounds of herbal origin are able to lower the levels of lipids and thereby reducing the risk of cardiovascular diseases [9]. *Nigella stativa*(NS)commonly known as black seed or black cumin belongs to family Ranunculaceae is an herbaceous plant. It has been shown that *Nigella sativa*has multiple beneficial actions including hypoglycemic, hypocholesterolemic, antibacterial, anti-tumor and antioxidant effects [10,11,12,13]. Further, ithas been reported that the biological activity of the seeds of *Nigella Sativa*is due to thymoquinone, the major component of the essential oil and fixed oil which has beneficial effect in hyperglycaemia and hyperlipidema[14,15]. The choleretic effect of NS seed is an important mechanism that could account for the lipid- lowering and athero protective properties of the plant [16,17,18].

Astaxanthin (ASX) is a lipophilic xanthophyll carotenoid and found in a variety of livingorganism, particularly in sea food such as microalgae, fungi, crustaceans, fishes- salmon andred fish[19]. The

primary natural source of astaxanthin for commercial uses is *Haematococcus pluvialis* (green microalgae) a single-celled aquatic organism. Astaxanthin was shown to have 500-fold stronger free radical antioxidant activity than vitamin E and 38-fold than ß-carotene [20] and this effect is due to its unique molecular structure which confers this natural product as a powerful antioxidant [21]. Further, various studies have shown that astaxanthin possesses a wide range of biological effects such as anti-cancer [22], anti-inflammatory [23], anti-diabetes,cardioprotective effect [24],hypolipidemic effect [25], positive effects on blood pressure [21]and neuroprotective actions [26].

Evidences are available to suggest that the beneficial effect of NSO and ASX when they were given alone. However,the information on the protective effect of NSO and ASX combination on the MSG induced toxicity is lacking. Therefore, the present investigation is aimed to study the effect of NSO and ASX on the MSG induced toxicity in the serum lipid profiles of male pubertal albino rats.

MATERIALS AND METHODS

Chemicals

Monosodium glutamate was purchased from the Local available market, *Nigella Sativa* oil was purchased from Greenish (India) trades Pvt.Ltd, Astaxanthin ≥97% (HPLC), from *Haematococcuspluvailis* (CAS 472-61-7) were purchased from Sigma–Aldrich Chemicals, Saint Louis, MO, USA and All other chemicals used were of analytical grade.

Animals

Pubertal male albino rats (Wistar), 130-140g in body weight was procured from Tamil Nadu Veterinary and Animal Science University (TANUVAS), Madhavaram, Chennai, India. The animals were housed in clean polypropylene cages lined with paddy husk with a temperature-controlled environment of 25ºc ± 1ºc, 50±10% humidity and an automatically controlled cycle of 12/12 h light and dark. The animals were fed with standard commercial diet pellets (M/s Hindustan Foods Ltd., Bangalore, India) *ad libitum*. The animals were maintained and handled as per the guidelines given by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India (845/GO/ac/04/2004/CPCSEA) and Institutional Animal Ethic Committee (IAEC).

Experimental Design

The Pubertal male rats were divided into eight groups consisting of six rats each.

Group 1: The rats were given distilled water as vehicle orally, daily for 28 days (Control rats).

Group 2: The rats were treated with 0.8g/Kg b.wt of MSG orally (1/20 of rats oral LD50 which equals 16gm/kg b.wt) for 28 days.

Group 3: The rats were treated with 1ml/Kg b.wt of *Nigella sativa* oil (NSO) for 28days.

Group 4: The rats were treated with 25mg/Kg b.wt of Astaxanthin (ASX) which was mixed with olive oil (1ml/Kg.b.wt) orally for 28 days.

Group5: The rats were Co-administered with NSO (1ml/Kg b.wt) and ASX (25mg/Kg b.wt) for 28 days.

Group6: The rats were Co-administered with MSG (0.8g/Kg b.wt) and NSO (1ml/Kg b.wt) for 28 days.

Group7: The rats were Co-administered with MSG (0.8g/Kg b.wt) and ASX(25mg/Kg b.wt) for 28 days.

Group 8: The rats were Co-administered with MSG (0.8g/Kg b.wt) and Combination of NSO (1ml/Kg b.wt) +ASX(25mg/Kg b.wt) for 28 days.

After the experimental period the Blood samples were collected in sterile tubes. Each blood sample was centrifuged for 10 minutes at 1000rpm at 15°C to separate the serum which was stored at 4°C and used for various biochemical analyses.

Biochemical analysis

Lipid concentrations were estimated spectrophotometrically. Serum total cholesterol level has been estimated according to Allain*et al*. (1974) [27], **S**erum triglycerides were estimated by the method of McGowam (1983) [28]. The method of Grillo and Izzo (1985) [29] was followed to estimate the level ofHDL-cholesterol in the serum. Serum LDL- Cholesterol was calculated by using the empirical equation [Total Cholesterol - (HDL+VLDL)] of Friedewald*et al*. (1972) [30] and VLDL-Cholesterol in serum was evaluated by Friedwald equation VLDL = Triglyceride/5.

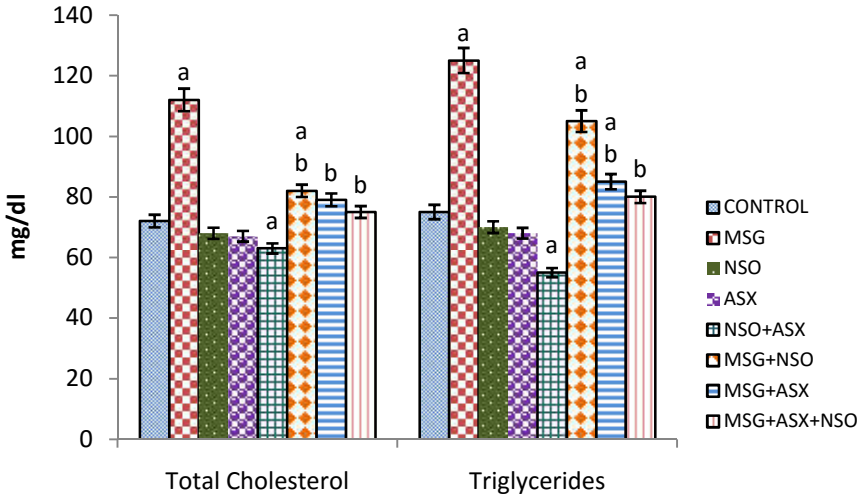
Statistical Analysis

The data obtained in the present study were analysed by using the Single way Analysis of Variance (ANOVA) according to [31] Zar (1974). If the 'F'ratio was significant the Student-Neuman-Keul's (SNK) test was followed. All statistical analyses were performed by using IBM SPSS 20.0 statistical software.

RESULTS

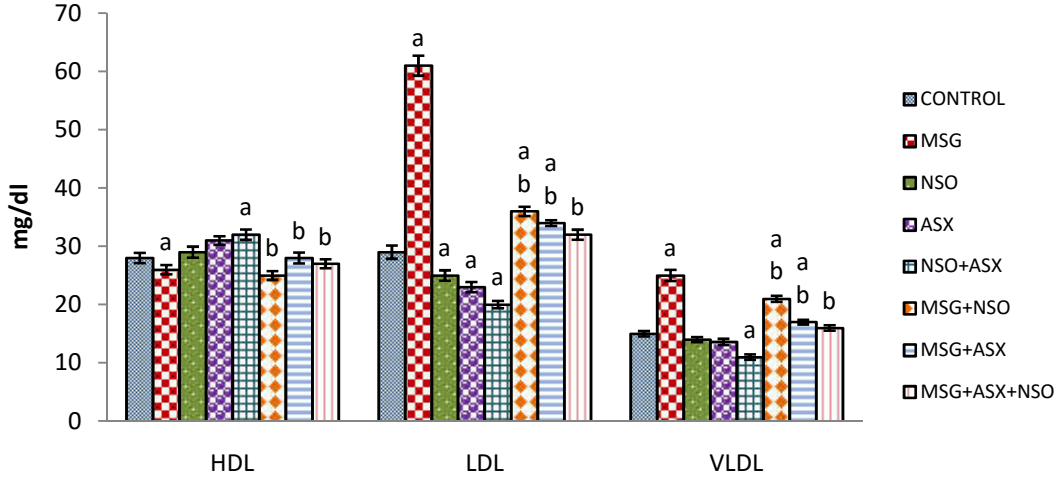
The data obtained in the present study have been represented in figure 1 and figure 2. Administration of MSG (0.8g/kg b.wt)(Group-2) orally for 28days elevated the levels of TC, TG, LDL and VLDL significantly (P<0.05) in the serum of pubertal male rats, compared to control (Group-1). However, the level of HDL was found to be decreased significantly (P<0.05).The concentration of TC, TG, LDL, VLDL and HDL was found to be normal in the rats treated with MSG+NSO (Group-6), MSG+ASX (Group-7) and MSG+NSO+ASX (Group-8). Co-administration of NSO+ASX (Group-5) lowered the level of TC, TG, LDL and VLDL significantly (P<0.05) when compared to control. However, it is interesting to observe that the NSO (Group-3) and ASX (Group-4) had no significant effect on the lipid parameters studied, when they administered alone.

Figure : 1: Effect of MSG treatment, NSO alone,Co-administration of MSG with NSO, ASX alone,co-administration of MSG with ASX,Co-administration of ASX with NSO and administration of MSG with ASX and NSO on the Total Cholesterol and Triglycerides levels of Serum in Pubertal Male rats



Each value is Mean± SEM of 6 Animals. [a]and[b] represent statistical significant at P<0.05 Compared with Control and MSG respectively. Control Vs other groups; MSG Vs MSG + NSO; MSGVs MSG + ASX; MSG Vs MSG+ASX+NSO.

Figure : 2: Effect of MSG treatment, NSO alone, Co-administration of MSG with NSO, ASX alone,co-administration of MSG with ASX,Co-administration of ASX with NSO and administration of MSG with ASX and NSO on HDL,LDL,VLDL levels of Serum in Pubertal Male rats .



Each value is Mean± SEM of 6 Animals. [a]and[b] represent statistical significant at P<0.05 Compared with Control and MSG respectively. Control Vs other groups; MSG Vs MSG + NSO; MSGVs MSG + ASX; MSG Vs MSG+ASX+NSO.

DISCUSSION

MSG has been consumed in large amounts as a food additive throughout the world to create a palatable taste sensation when co-administered with other food stuffs [32].In the present study oral ingestion of MSG (0.8G/Kg b.wt) for 28 days to pubertal rats induced hypercholestremia by increasing the concentration of total cholesterol, triglycerides, LDL and VLDL and decreasing the HDL cholesterol in the serum.

Our findings are in agreement with the earlier reports[33,34,35].They suggested that the dyslipidemia in rats treated with MSG might be due to destruction of hypothalamic arcuate nucleus and ventro-medial hypothalamus region which leads to multiple organ damage probably resulted in compromised functional capacity of the major organs to regulate sterol metabolism leading to the significant increase in the serum Cholesterol and Triglyceride concentration.The increased total cholesterol level and hypertriglyceridemia in MSG treated animals is associated with insulin resistance, type 2 diabetes mellitus [36]and coronary artery disease (CAD) [37]which constitute components of the metabolic syndrome that leads to altered cholesterol metabolism perhaps the main cause of dyslipidemia,which is a major risk factor for cardiovascular diseases[38,39]. In the present study also, the dyslipidemia observed in MSG treated rats might be due to altered cholesterol metabolism. Further the increasedlevel of LDL in MSG treated rats observed in the present study indicates that MSG increased oxidative stress which could induce oxidation of LDL [40]. Most of the cholesterol in the mature lesion originates from circulating LDL particles which cross the endothelium into the intimal of blood vessels and return to circulation due to unfavourable uptake into intimal macrophages. After oxidative modification the LDL particles are rapidly taken up into macrophages via the scavenger receptor. Subsequent loading with cholesteryl esters forms so called foam cells [41], which might be responsible for the initiation of atherosclerosis[42], Thus, the study suggests that the administration of 0.8g/Kg b.wt of MSG may alter lipid status in animals by damaging vital organs like liver and heart. Hyperlipoproteinemia also observed in the present work might be due to the increased hepatic VLDL synthesis. It has been reported that the VLDL synthesis might have been induced by hyperinsulinemia[43,44].

Nigella Sativa seeds are used in Ayurvedic system of medicine and Tibbe-Nabvi (Prophet's Medicine) throughout the world. It has been demonstrated that the volatile oil of NS has two main constituents i.e. nigellone and thymoquinone both playing key roles in the antioxidant defence and prevention of heartdisease [45].In addition, many pharmacological effects of *Nigella sativa* and its active principles have been identified, such as immune stimulation, anti-inflammatory, anti-cancer and antimicrobial activity [46].

Oil extracted from *Nigella sativa* seeds is rich in phytosterols such as beta-sitosterol, stigmasterol, campestral, flavonoids, fiberes, polyunsaturated fatty acids, mainly linoleic acid, oleic acid, eicosadienoic acid and dihomolinolenic acid all known to exert hypolipidemic and atheroprotective functions [12,47]. Aside from phytosterols, thymoquinone may also be responsible for the observed hypolipidemic and cardioprotectiveeffects.Thymoquinone is abundantly in the NS seed oil and is perhaps the most well-known phytochemical of the plant. Administration of thymoquinone has been shown to ameliorate hyperlipidemia and protect against development of atherosclerosis [48,49].

The data obtained in the present study reveals that the co-administration of MSG and NSO significantly decreased the total cholesterol, Triglycerides, LDL and VLDL and increased the HDL level, compared to MSG alone treated rats which clearly demonstrates that NSO prevents the hypercholesterolemic effect of MSG. It is quite consistent with the previous reports [11,50,51].

The observed lipid-lowering properties of NSO could be due to synergism among several compounds present in NSO that leads to the favourable effects such as lowering the de novo cholesterol synthesis in hepatocytes and decreased dietary cholesterol absorption from small intestine [52]. Indeed, lipid lowering activity of *Nigellasativa*was probably contributed from its dietary soluble fibres [53]and sterols [11]which stimulates the primary bile acid synthesis and its faecal losses. NSO also makes liver cells more efficient to remove LDL-C from blood by increasing LDL-C receptor densities in liver and binding to apolipoprotein, apo B and decreasing intracellular cholesterol, which leads to rapid clearance of LDL-cholesterol from blood circulation[54,55,56].Another mechanism involved probably through up-regulation of LDL-C molecules through receptor mediated endocytosis. The endocytosed membrane vesicles fused with lysosomes and in which the apoproteins were degraded and the cholesterol esters were hydrolyzed to yield free cholesterol,these free cholesterol are then incorporated into plasma as necessary and excreted from the body [49].

In the present investigation, the effect of NSO caused a significant increase in HDL cholesterol perhaps the main transport form of cholesterol from peripheral tissue to liver which is later excreted through bile.

The level of HDL in serum is inversely related to the incidence of myocardial infarction as it is anti atherogenic and known as good cholesterol.[11,57].High level of HDL may compete with LDL receptor sites on arterial smooth muscle cells and thus partially inhibit uptake and degradation of LDL. Also, HDL could protect LDL against oxidation in vivo, because the lipids in HDL are preferentially oxidized before those in LDL [58].These mechanisms further supports our observation in the present study which clearly indicates that *Nigella sativa* oil has a favourable effect on lipid profile.

In the present study, administration of ASX along with MSG shows remarkable decrease in the level of Total cholesterol, Triglycerides, LDL and VLDL when compared to MSG alone treated rats, which undoubtedly contributes the beneficial effect of ASX. ASX has hypocholesterolemic and hypotriglyceridemic effects [59] that are potentially mediated by increasing LDL uptake and fatty acid β-oxidation, respectively in the liver and ASX has been shown to suppress the expression and activities of numerous hepatic fatty acid syntheses such as fatty acid synthase (FAS), malic enzyme and glucose 6-phosphate dehydrogenase [60,61,62]and hence decrease fatty acid synthesis in liver. On the other hand, ASX sharply enhances hepatic peroxisomal and mitochondrial fatty acid oxidation rate by increasing the expression and activities of a series of fatty acid oxidation enzymes which results in hypocholesterolemic effect [61]. In addition, the hypocholesterolemic effects of ASX are likely owing to elevated hepatic expression of LDL receptor [63,64]and HMGCR (3-hydroxy-3- methyl-glutaryl-CoA reductase) expression in response to low cellular cholesterol via SREBP-2 (sterol regulatory element binding protein), a key transcription factor that regulates the expression of genes important for cholesterol metabolism [65,25]as well as declined cholesterol biosynthesis [66,67]which results in preventing dyslipidemia.

In the present study,co-administration of NSO, ASX along with MSG restored the normal levels of lipids, which clearly demonstrates that the combination of NSO and ASX is more effective than the other co-administered groups (MSG + NSO), and (MSG + ASX).This effectmay be due to synergisticeffect of compounds of NSO and ASX which prevents the deleterious effect of MSG from altering the Lipid profile.

CONCLUSION

In Conclusion, the present study revealed that combination of NSO (1ml/Kg b.wt) and ASX (25mg/Kg b.wt) is capable of ameliorating against the MSG (0.8g /Kg b.wt) induced toxicity as it alleviates the alteration in lipid profile parameters (TC, TG, HDL, LDL and VLDL). The present study suggests that NSO and ASXhave a protective effect on the MSG induced toxicity in the pubertal male rats and they could be used as a food adducts to prevent hyperlipidemia.

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