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Adverse Effects of Monosodium Glutamate on Serum Lipid Profile, Cholesterol Status and Blood Glucose in Adult Rats.

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ABSTRACT

Monosodium glutamate (MSG) is one of the world's most widely used food additives and flavor enhancer. MSG has been implicated to cause some adverse effects in human and experimental animals. The study aimed to investigate possible adverse effecs of MSG on serum lipids, cholesterol, lipoproteins and blood glucose. The experiment was conducted on 40 adult male rats. They were divided into 4 equal groups. First group represented the control animals and only received drinking water. Second, third, and fourth groups were given 2, 4, and 8 mg/g of MSG respectively in drinking water. After 8 weeks, blood was prepared from each rat individually for biochemical assay. The results indicated that MSG treatment significantly increased the level of serum lipids, fatty acids, triglycerides and phospholipids in MSG-treated animals. The findings of current work also revealed that oral adminstration of MSG significantly elevated the concentration of serum cholesterol, LDL and VLDL in treatment groups. A significant reduction in HDL level was also observed. There was also a marked increase in the levels of blood glucose in treated animals. Thus, MSG consumption produced hyperlipidemia, hyperlipoproteinemia and hyperglycemia. This suggests that MSG could be a risk factor for initiation of atherosclerosis and associated diseases.

Keywords: MSG, serum, lipid profile, cholesterol, lipoproteins, glucose

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INTRODUCTION

Several food additives have been implicated as causing harmful effects on health. Monosodium glutamate (MSG) is one of the world's most widely used food additives and flavor enhancer [1], which added to various food products such as Chinese food, canned vegetables, soups, sauces, processed meats, crackers, salad dressing etc. Monosodium glutamate is the sodium salt of glutamic acid, which is one of the most abundant naturally occurring non-essential amino acid. Glutamate is one of the main components of many proteins and peptides of most tissues and can be found in many protein-rich food products such as meat, fish, cheese, milk, tomato, and mushroom. The human body also produces glutamate and it plays an important role in normal body metabolism. MSG is approximately contains 78% glutamic acid and 22% sodium and water [2]. Glutamate is transformed into alanine in intestinal mucosa and lactate in liver [3]. It is metabolized in liver and eliminated by kidney [4]. Glutamate is an excitatory neurotransmitter in the central nervous system of mammals, plying a vital role in several physiological and pathological processes [5]. Its receptors scattered throughout the central nervous system in areas where they regulate various metabolic functions [6].

While MSG benefits to the food industry are quite clear, this food additive could be slowly and silently doing major damage to our health. It is known to have some adverse effects in human and experimental animals. MSG induces the appetite positively and stimulates weight gain due to its irritation of the ornosensory receptors and enhancing the palatability of food [7]. Despite MSG improves taste enhancement and stimulates appetite, many studies indicated that MSG is toxic and causes some adverse effects in human and experimental animals [8]. It was reported that MSG could produce various symptoms such as headache, dizziness, flushing, numbness, weakness and sweating. In addition, MSP has been alleged to be associated with causing several conditions, such as neuropathy, atopic dermatitis, abdominal discomfort, asthma and ventricular arrhythmia [9]. Administration of MSG could develop neurotoxic effects leading to brain cell damage, retinal degeneration, and can cause several pathological conditions such as brain trauma, strok, endocrine disorders, addiction, schizophrenia, neuropathic pain, epilepsy, depression, anxiety, amyotropic lateral sclerosis, and Parkinson's disease [2, 10].

MSG has been implicated to produce oxygen derived free radicals and induce oxidative stress in experimental animals [11]. In MSG-sensitive individuals, the adverse effects are observed even at doses recommended in food [12]. Alterations in liver parenchyma around central vein, inflammatory cells, and dilated sinusoids have been observed in MSG-treated mice [3]. MSG has adverse effects on testis of rats by causing oligozoospermia and increase in abnormal sperm morphology in a dose-dependent manner [13]. MSG has also been implicated in male infertility by causing testicular haemorrhage, degeneration and alteration of sperm cell population and morphology [14,15]. Therefore, the current study was designed to investigate the possible adverse effecs of monosodium glutamate that may occur on the concentration of serum lipid profile, cholesterol, various lipoprotein fractions, and the level of blood glucose.

MATERIALS AND METHODS

Animals and MSG treatment

The experiment was conducted on 40 normal adult male rats with an average weight of 160 g. the animals were kept in standard neat metallic and well-ventillated cages. They maintained on standard healthy laboratory conditions at temperature of 18-24°C and an appropriate humidity and lighting. They had access to 12 hr of darkness and 12 hr of daylight. The rats were acclimatized to the new environment for 14 days before commencement of the experiment. All animals received humane care in accordance with the guidelines of the national institute of health, USA, for ethical treatment of laboratory animals. All rats had free access to drinking water and food, *ad libitum*, during the experimental period. They were fed with standard pellet diet (LabDiet, Missouri, USA) consisting of 60% starch, 20% casein, 10% cotton seed oil, 4% salt mixture, 5% cellulose, and 1% vitamin mixture.

After the adaptation period of 14 days, the rats were distributed into 4 equal groups, each contained 10 rats. Group I represented the healthy control animals and received distilled water. The second, third, and fourth groups were marked as group II, III, and IV, and given 2, 4, and 8 mg/g body weight of monosodium glutamate (Sigma-aldrich Ltd., UK) respectively in their daily supply of drinking water for 8 weeks.

Blood collection and samples preparation

At the end of the experimental period of 8 weeks, blood was collected from each rat individually for biochemical assay. The animals were fasted for twelve hours prior to blood collection. All animals were anesthetized by chloroform and blood samples were collected immediately from their heart using heart puncture technique with the aid of disposable sterile syringe and needle (Sigma). Blood samples were then transferred into capped tubes with no anticoagulant (Greiner Bio-One, Frickenhausen, Germany). The blood was allowed to clot at room temperature for 30 minutes prior to centrifugation at 2500 rpm for 20 minutes using centrifuge 5418 R (Eppendorf, Ontario, Canada) to obtain the serum for biochemical analysis. The yellow serum supernatant was removed without disturbing the white buffy layer. Samples were tested immediately or frozen at -80°C for storage.

The Roche Hitachi 704 Chemistry Analyzer (Roche Diagnostics Corporation, Indianapolis, USA) was used to determine the concentration of serum total lipids, triglycerides, fatty acids, phospholipids, total cholesterol, lipoproteins, and the level of blood glucose. This machine is a fully automated and composed of an analytical unit which produces chemical reactions in samples, an operation unit which inputs and outputs analytical conditions, and a control unit which controls each function required for operation of the instrument.

Biochemical assay protocols

Determination of total lipids

Lipid quantification kit (Cell Biolabs Inc., California, USA) was used to measure serum total lipids, resulting in a simple colorimetric readout amenable to multi-well plate detection. Following the manufacturer's instructions, Samples (15 µl each) were applied to a 96-well plate prior to adding 150 µl of 18M sulfuric acid. They were incubated at 90°C for 10 min to solubilize and prime the total lipid sample. The reaction mix 100 µl was transferred into a clean 96-well plate and incubated at 4°C for 5 min, followed by the addition of vanillin in an acid solution. Thus, the lipids react with vanillin in the presence of the acids to form a colorimetric product that is easily detected by the machine. Absorbance was read at 540 nm to determine signal.

Determination of Triglycerides

The process of triglycerides (TGs) measurement was carried out using the triglycerides quantification kit (Roche Diagnostics) in accordance to the manufacturer's instructions. Triglycerides were measured enzymatically in blood samples in a series of coupled reactions in which triglycerides were hydrolyzed to produce glycerol. Glycerol was then oxidized using glycerol oxidase and hydrogen peroxide, one of the reaction products, was measured quantitatively in a peroxidase catalyzed reaction that produced a color. Absorbance was measured at 500 nm. The following reaction mix was used for each reaction; PIPES buffer 50 mmol/l, pH 6.8, magnesium 40 mmol/l, Sodium cholate 0.20 mmol/l, aminophenazone 0.13 mmol/l, 4-Chlorophenol 4.7 mmol/l, Potassium hexacyanoferrate 1 µmol/l, fatty alcohol polyglycolether 0.65%, lipoprotein lipase 5.0 U/ml, glycerolkinase 0.19 U/ml, glycerophosphate oxidase 2.5 U/ml, and peroxidase 0.10 U/ml.

Determination of fatty acids

The concentration of fatty acids in serum samples was determined using fatty acid quantitation Kit (Sigma) in accordance to the manufacturer's instructions. In this kit, the concentration of fatty acids is determined by a coupled enzyme assay, which results in a colorimetric product, proportional to the fatty acids present. Assay reaction started by adding Acyl-CoA synthetase 2 µl to each sample well prior to incubation for 30 min at 37°C. The Master reaction mix 50 µl was added to each well. The master mix composed of the following components: fatty acid assay buffer 44 µl, fatty acid probe 2 µl, enzyme mix 2 µl. This followed by incubation for 30 min at 37°C and the plate was protected from light during the incubation. Absorbance was then measured at 570 nm.

Determination of phospholipids

The phospholipid assay kit (Sigma) was used to measure phospholipid concentration in blood samples. This kit provides a simple, direct, and high throughput assay for measuring cholinecontaining phospholipids in biological samples. In this assay, phospholipids were enzymatically hydrolyzed to release choline, which is determined using choline oxidase and hydrogen peroxide specific dye. This resulted in a simple colorimetric product directly proportional to the phospholipid concentration in the sample. Absorbance was measured at 570 nm.

Determination of total Cholesterol

The process of cholesterol determination was carried out in accordance to the manufacturer's instructions using the total cholesterol quantification kit (Roche Diagnostics). Cholesterol was measured enzymatically in serum samples in a series of coupled reactions that hydrolyze cholesteryl esters and oxidize the 3-OH group of cholesterol. One of the reaction byproducts, hydrogen peroxide was measured quantitatively in a peroxidase catalyzed reaction that produced a color. Absorbance was measured at 500 nm. The color intensity is proportional to cholesterol concentration. For each reaction, the following reaction mix was prepared and used; PIPES buffer 75 mmol/l, pH 6.8, Magnesium 10 mmol/l, Sodium cholate 0.2 mmol/l, aminophenazone 0.15 mmol/l, phenol 4.2 mmol/l, Cholesterol esterase 0.5 U/ml, Cholesterol oxidase 0.15 U/ml, peroxidase 0.25 U/ml, and fatty alcohol polyglycol ether 1%.

Determination of various lipoprotein fractions

High density lipoprotein (HDL) cholesterol was measured directly in serum specimens. The reagents were purchased from Roche Diagnostics. The basic principle of the method is as follows, the apoB containing lipoproteins in the specimen are reacted with a blocking reagent that renders them non-reactive with the enzymatic cholesterol reagent under conditions of the assay. The apoB containing lipoproteins are thus effectively excluded from the assay and only HDL cholesterol is detected under the assay conditions. This method uses sulfated cyclodextrin in the presence of magnesium, which forms complexes with apoB containing lipoproteins, and polyethylene glycol-coupled cholesteryl esterase and cholesterol oxidase for the HDL measurement. Absorbance was measured at 600 nm. The reagents contained the following components: cyclodextrin 0.5 mmol/l, dextran sulfate 0.5 g/l, magnesium sulfate 7.0 mg/ml, morpholino propane sulfonic acid buffer 10 mmol/l, pH 7.0, polyethylene glycol (PEG)-modified cholesterol esterase 1 kU/l, cholesterol oxidase 5.6 kU/l, peroxidase 30 kU/l, and 4-aminophenazone 0.5 g/l. Low density lipoproteins (LDL) cholesterol was calculated from measured values of total cholesterol, triglycerides and HDL according to the relationship: LDL equal to total cholesterol minus HDL minus TG/5 where TG/5 is an estimate of very low density lipoproteins (VLDL).

Determination of blood glucose

Glucose assay kit (Sigma) was used for the quantitative determination of glucose in blood samples. The basic principle of the method is as follows, glucose is oxidized to gluconic acid and hydrogen peroxide by glucose oxidase. Hydrogen peroxide reacts with o-dianisidine in the presence of peroxidase to form a colored product. Oxidized o-dianisidine reacts with sulfuric acid to form a more stable colored product. The intensity of the pink color measured at 540 nm is proportional to the original glucose concentration. At zero time, the reaction was started by adding 2 ml of assay reagent to 1 ml of each sample. The reaction mix was allowed to react for 30 min at 37°C, followed by adding 2 ml of 12N sulfuric acid to stop reactions. Absorbance was read for each tube against the reagent blank at 540 nm.

Statistical analysis

Data provided in this investigation were analyzed using one way analysis of variance. Comparisons between different groups were made using the student's t-test. Data were presented as mean values ± standard errors. All statements of significance were based on probability of less than 0.05 confidence levels.

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RESULTS AND DISCUSSION

Effects on serum lipid profile

The results of this investigation showed that the treatment with MSG at dose level of 4 and 8 mg per gram body weight, significantly (P<0.05) increased the level of serum total lipids by 10.76 percent and 22.52 percent, free fatty acids by 17.44 percent and 22.80 percent, triglycerides by 21.81 percent and 28.91 percent, and phospholipids by 29.59 percent and 42.14 percent respectively in the treated animals compared to the control ones. In addition, administration of MSG at dose level of 2 mg/g body weight did not alter any of these parameters as seen in table 1. Therefore high doses of MSG (4 and 8 mg/g) given orally induced hyperlipidemia in adult rats.

Hyperlipidemia observed in the present work following the administration of MSG might be attributed to the fact that glutamate favors lipogenesis by converting to glutamine [16,17]. It has been reported that MSG treatment elevated the concentration of serum triglycerides which indicated an obvious breackdown in the metabolism of triglycerol that might be induced mobilization of free fatty acids from the peripheral fat depots [18], as triglycerides regulation is driven by the availability of free fatty acids [19]. Since the risk of atherosclerosis is elevated by hyperlipidemia, this suggests that MSG probably act as a causative agent in the initiation of atherosclerosis.

The results of present investigation concur with previous study indicated that MSG remarkedly elevated the concentration of serum triglycerides and free fatty acids in the absence of increased exogenous dietary lipid [20]. The process of lipid mobilization and storage has been shown to be affected in liver of MSG-treated rats [21]. MSG has been observed to increase the expression of a number of genes implicated in adipocytes differentiation, elevated serum triglycerides, free fatty acids, insulin, and bile synthesis in mice [22]. In addition, MSG was also seen to increase hepatic lipid catabolism through upregulation of oxidative genes and induce genes involved in the bile acid pathway such as cholesterol-7-hydroxylase [23]. The significant elevations observed in the serum concentrations of total lipids, free fatty acids, triglycerides, and phospholipids, which represent main alterations found in obesity, are in consonance with the findings recorded in previous works, which stated that high doses of MSG produce hypophagia and obesity [24]. When high doses of MSG given subcutaneously to adult rats, this induced hypertriglyceridemia and led to significant increases in the serum concentrations of free fatty acids, phospholipids, and glucose [25]. Simillar results have been demonstrated when doses of MSG given orally to a dult male rats [26].

Table 1: Effects of MSG on serum lipid profile and blood glucose after 8 weeks of treatment in adult rats

Parameters	Group I (control)	Group II (2 mg/g)	Group III (4 mg/g)	Group IV (8 mg/g)
Total lipids (mg/dl)	212.01 ± 3.04	215.13 ± 2.91 (+ 1.47)	234.84 ± 4.22 (+ 10.76)*	259.76 ± 4.80 (+22.52)**
Phospholipids (mg/dl)	48.93 ± 2.76	51.24 ± 3.64 (+ 4.72)	63.41 ± 5.01 (+ 29.59)**	69.55 ± 4.36 (+ 42.14)***
Free fatty acids (mg/dl)	144.21 ± 4.36	145.38 ± 5.23 (+ 0.81)	169.36 ± 3.58 (+ 17.44)*	177.09 ± 3.69 (+ 22.80)**
Triglycerides (mg/dl)	96.74 ± 3.58	97.06 ± 2.92 (+ 0.33)	117.84 ± 4.67 (+ 21.81)**	124.71 ± 5.25 (+ 28.91)**
Glucose (mg/dl)	61.38 ± 3.27	70.81 ± 2.11 (+ 15.36)**	84.15 ± 3.72 (+ 37.09)**	86.29 ± 4.23 (+ 40.42)***

Values are expressed as mean ± SE where n=10, Values in brackets represent percentage changes relative to control
*P<0.05, **P<0.01, ***P<0.001

Table 2: Effects of MSG on serum cholesterol and various lipoprotein fractions after 8 weeks of treatment in adult rats

Parameters	Group I (control)	Group II (2 mg/g)	Group III (4 mg/g)	Group IV (8 mg/g)
Total cholesterol (mg/dl)	67.45 ± 4.61	69.15 ± 3.73 (+ 2.52)	72.37 ± 5.16 (+7.29)*	76.73 ± 4.08 (+ 13.76)**
HDL (mg/dl)	14.65 ± 1.34	11.42 ± 1.54 (- 22.05)*	10.27 ± 2.89 (- 29.9)**	8.31 ± 1.44 (- 43.28)***
LDL (mg/dl)	33.46 ± 2.66	38.32 ± 2.31 (+ 14.52)*	38.54 ± 2.08 (+ 15.18)*	43.48 ± 3.27 (+ 29.94)***
VLDL (mg/dl)	19.34 ± 3.72	19.41 ± 2.15 (+ 1.00)	23.56 ± 3.09 (+ 21.82)**	24.94 ± 3.68 (+ 28.95)**

Values are expressed as mean ± SE where n=10, Values in brackets represent percentage changes relative to control
*P<0.05, **P<0.01, ***P<0.001

Effects on cholesterol and lipoprotein fractions

The findings of current work also revealed that MSG treatment significantly (P<0.05) increased the serum cholesterol concentration, LDL level, and VLDL level in group II, III, and IV compared to the control. In addition, a significant reduction in HDL level was observed following exposure to MSG (table 2). These observations are consistent with the findings of earlier studies which reported marked increases in the concentrations of serum cholesterol, LDL, and VLDL in adult rats [26,27]. The increased levels of serum cholesterol under the influence of MSG, probably indicates an impairment of cholesterol metabolism and attendant risk of coronary heart disease in rats [28].

Hyperlipoproteinemia seen in current study perhaps due to hyperinsulinemia, which caused remarkable increase in the levels of VLDL in rats following MSG exposure [29]. The activity of lipoxygenase, and lipid peroxidation products have been observed to be stimulated by hyperglycemia. The lipoxygenase catalyzes the conversion of arachidonic acid to leukotriene and othr products which induce the onset of atherosclerosis via stimulating LDL oxidation and enhancing growth and migration of vascular smooth muscle cells [30,31,32]. It has been reported that exposure to different doses of MSG increased oxidative stress and then could stimulate oxidation of LDL [11,33].

Therefore, the probable upregulation of cholesterol and other lipid fractions under the effect of MSG leading to their elevation in blood serum may predispose animals to numerous health hazards such as atherosclerosis [34], coronary heart disease [35], and diabetes mellitus [19].

Effects on blood glucose

The oral administration of MSG resulted in a marked increase (P<0.05) in the levels of blood glucose in group II, III, and IV by 15.36, 37.09, and 40.42 percent respectively relative to the control (table 1). The raised levels of triglycerides and cholesterol indicate hyperlipidemia, which was accompanied by insulin resistance and type-2 diabetes mellitus [36,37,19] and therefore lead to hyperglycemia [38]. This hyperglycemia might be attributed to the inhibitory action of MSG on growth hormone, and therefore reducing hepatic glycogenesis and deactivating the gluconeogenesis from amino acids.

Hyperinsulinemia has also been shown to be produced in mice due to insulin resistance following MSG treatment. It is known that insulin plays an important role in increasing glucose intake by cells through inducing the translocation of glucose transporter GLUT4 from intracellular sites to the cell surface. Therefore, hyperglycemia might be attributed to impaired glucose intake by cells due to decreased GLUT4 expression despite hyperinsulinemia [39,40]. When 2 mg/g of MSG was injected subcutaneously in rats, this elevated serum insulin levels and it also impaired glucose tolerance [41]. It has been reported that MSG ingestion increased body weight and caused an increase in fasting blood glucose relative to standard chow, and also caused increased insulin resistance during insulin tolerance test in mice [42]. The insulin response in 75 min postprandially positively correlated with the plasma glutamate concentration during the oral glucose tolerance test, which indicates that glutamate can participate in the insulin response to nutrients during food intake [43]. This suggests that MSG may influence on insulin release, as the efferent pancreatic branch of vagus nerve could stimulate insulin secretion during the cephalic phase following MSG feeding in rats [44].

CONCLUSION

MSG is one of the world's most widely used food additives and flavor enhancer which added to various food products. MSG has been implicated to cause some adverse effects in human and experimental animals. It could be slowly and silently doing major damage to our health. Data provided in this investigation showed that oral administration of MSG at different dose levels produced hyperlipidemia, hyperlipoproteinemia and hyperglycemia. All the above observations suggest that MSG consumption could be a risk factor for the onset of atherosclerosis, coronary heart disease and diabetes mellitus. It is recommended that the mechanism of MSG-action and its toxic effects have to be investigated further in order to corroborate the findings of current work.

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REFERENCES

[1] Alao O, Ashaolu J, Ghazal O, Ukwenya V. Intern J Biomed and Health Sci 2010; 6: 197-203.
[2] Samuels A. Account Res 1999; 6: 259-310.
[3] Bhattacharya T, Bhakta A, Ghosh SK. Nepal Med College J 2011; 13: 11-16.
[4] Schwerin P, Bessman SP, Waelsch H. J Biol Chem 1950; 184: 37-44.
[5] Mattson MP. Ann N Y Acad Sci 2008; 1144: 97-112.
[6] Meldrum BS. J Nut 2000; 130: 1007S-1015S.
[7] Moore KL. Congenital malformations due to environmental factors: Developing Humans. W.B. Saunders co. Ltd Philadelphia, 2003, 2nd ed. pp 173-183.
[8] Biodun D, Biodun A. Nat conc 1993; 4: 5-7.
[9] Geha RS, Beiser A, Ren C, Patterson R, Greenberger PA, Grammer LC, Ditto AM, Harris KE, Shaughnessy MA, Yarnold PR, Corren J, Saxon A. J Nut 2001; 130: 1058S-1062S.
[10] Eweka A, Adjene J. Electron J Biomed 2007; 2: 9-13
[11] Singh K, Ahluwalia P. J Nutr Sci Vitaminol 2003; 49: 145-148.
[12] Schaumburg H, Byck R, Gerstl R, Mashman J. Science 1969; 163, 826-828.
[13] Onakewhor JU, Oforofuo IA, Singh SP. Afr J Reprod Health 1998; 2: 190- 197.
[14] Oforofuo IA, Onakewhor JU, Idaewor PE. Bios Resch Comms 1997; 9: 12-14.
[15] Das RS, Ghosh SK. Nepal Med College J 2011; 13: 77-83.
[16] Malik VT, Ahluwalia P. Toxicol Lett 1989; 45: 195-198.
[17] Malik VT, Ahluwalia P. Toxicol Lett 1994; 74: 69-77.
[18] Bopama KN, Kanna J, Sushma G, Balaraman R, Rathod SP. Ind J Pharmacol 1997; 29: 162-167.
[19] Schummer CM, Werner U, Tennagels N, Schmol D, Haschke G, Juretschke H, Patel MS, Gerl M, Kramer W, Herling AW. Am J Physiol Endocrinol Metab 2008; 294: 88-96.
[20] Collison KS, Maqbool Z, Saleh SM, Inglis A, Makhoul NJ, Bakheet R, Al-johi M, Al-Rabiah R, Zaidi FA. J lipid Res 2009; 50: 1521-1537.
[21] Roden M. Nat Clin Pract Endocrinol Metab 2006; 2: 335-48.
[22] Collison KS, Maqbool ZM, Inglis AL, Makhoul NJ, Saleh SM, Bakheet RH, Al-Johi MA, Al-Rabiah RK, Zaidi MZ, Al-Mohanna FA. Obesity 2010; 18: 1122-34.
[23] Davis RA, Miyake JH, Hui TY, Spann NJ. J lipid Res 2002; 43: 533-543.
[24] Lorden JF, Caudle A. Toxicol Teratol 1986; 8: 509-519.
[25] Nakia T, Tamai T, Takai H, Hayashi S. Life Sci 1986; 38: 2009-2013.
[26] Singh K, Sharma J, Kaur A, Ahluwalia R. J Life Sci 2011; 3: 17-21.
[27] Inyang B, Ojewunmi O, Ebuehi O. Asian J Biochem Pharma Res 2012; 3: 266-273.
[28] Egbuonu AC, Obidoa CA, Ezeokonkwo PM, Ejikeme, Ezeanyika LU. Toxicol and Environ Chem 2010; 92: 1331-1337.
[29] Oida K, Nakai T, Hayashi T, Miyabo S, Takeda R. Int J Obesity 1984; 8: 385-391.
[30] Antonipillai I, Nadler J, Vu E, Bughim S, Natarajan R, Horton RA. J Clin Endocrinol Metab 1996; 81: 1940-1945.
[31] Natarajan R, Rosdahl J, Gonzales N, Bai W. Hypertension 1997; 30: 873-879.
[32] Sacks FM, Alaupovic P, Moye LA, Cole TG, Sussex B, Stampfer MJ, Pfeffer MA, Braunwal E. Circulation 2000; 102: 1886-92.
[33] Singh K, Ahluwalia P. Ind J Clin Biochem 2005; 20: 43-46.
[34] American Association of Clinical Endocrinologists (AACE). Lipid guidelines. Endocrine Practice 2000; 6: 162-213.
[35] LaRosa JC. Arch Intern Med 1997; 157: 961-968.
[36] Huponen RK, Viikari JS, Saarimam H. Diabetes Care 1984; 7: 575-578.
[37] Nikkila EA. Plasma Lipid and Lipoprotein Abnormalities in Diabetes. In: Jarret RF editor Diabetes and Heart Diseases. Amsterdam, the Netherlands. Elsevier Science Publishers BV. 1984, pp134-167.
[38] Diniz YS, Faine LA, Galhardi CM, Rodrigues HG, Ebaid GX, Burneiko RC, Cicogna AC, Novelli LB. Nutrition 2005; 21: 749-755.
[39] Machado UF, Shimizu Y, Saito M. Horm Metab Res 1993; 25: 462-465.
[40] Saltiel AR, Kahn CR. Nature 2001; 414: 799-806.
[41] Roman-Ramos R, Almanza-Perez JC, Garcia-Macedo R, Blancas-Flores G, Fortis-Barrera A, Jasso EI, Garcia Lorenzana M, Campos-Sepulveda AE, Cruz M, Alarcon-Aguilar FJ. Basic Clin Pharmacol Toxicol 2011; 108: 406-413.

[42] Collison KS, Makhoul NJ, Zaidi MZ, Al-Rabiah R, Inglis A, Andres BL, Ubungen R, Shoukri M, Al-Mohanna FA. Nut Metab 2012; 9: 58.
[43] Chevassus H, Renard E, Bertrand G, Mourand I, Puech R, Molinier N, Bockaert J, Petit P, Bringer J. Br J Clin Pharmacol 2002; 53: 641-643.
[44] Niijima A, Togiyama T. Adachi A. Physiol Behav 1990; 48: 905-8.