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RESEARCH ARTICLE

Monosodium Glutamate Effects on Inflammation Status in Male Rats: Molecular and Physiological Study

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Abstract

The current study included the effect of Monosodium Glutamate (MSG) on male rats by measuring the level of Physiological parameter by determining the level of total serum protein, albumin and by measuring the level of Molecular parameter by determining the level of IL-6, NF-κb, IKK and TNF-a. The study was conducted at in the Department of Biology, College of Science, University of Wasit from November, 2017 until April, 2018. Study was used nigh teen adult wister rats male weighing 150-250 g and 10-14 weeks age were used and then randomly divided into three group six animal in each group. The first group was control; while the second group was orally dose the MSG 100g/kg dissolved in distilled water and third group take orally dose from MSG 200g/kg dissolved in distilled water via orogastric gavages for 30 days. The results were as follows: weight gain significant increase ($p \leq 0.05$) of for group that treatment with 100 mg/kg and 200 mg/kg when compare with control group and no a significant ($p \leq 0.05$) in concentration of total serum protein, albumin and globulin in male rat that treated with MSG 100 mg /kg . Whereas serum total protein, albumin and globulin show a significant decreases in rats that treated with 200 mg /kg of MSG compere with control group, increases of Nf- κB treated with MSG as 100 mg/kg and 200 mg /kg when compere with control group, increases of IL-6 treated with MSG as 100 mg/kg and 200 mg /kg when compere with control group, increases of TNF-a treated with MSG as 100 mg/kg and 200 mg/ kg when compere with control group and decreases of IKK treated with MSG as 100 mg/kg and 200 mg/kg when compere with control group. Conclusion: MSG as food additive can cause weight increase, soluble total protein decrease significant, MSG increases gene expression

Keywords: *MSG, Gene expression, Obesity*

Introduction

Food additives are various chemical substances added to foods to produce specific desirable effects [1]. Today, MSG has become one of the world's most widely used food additives [2]. MSG is a common glutamic acid salt. It compose of 78%glutamic acids, 22%sodium salt and water [3]. The chemical name is monosodium L-glutamate monohydrate ($C_5H_8NNaO_4.H_2O$) [4]. MSG is toxic to the various organs such as the liver, brain, thymus and kidneys [5].

Since the detection of MSG sensitivity, a range of studies have claimed the association between MSG and several health outcomes including, asthma, diabetes, obesity and allergic rhinitis. Moreover, the long-term intake of MSG results in an excess of appetite, obesity, asthma, poor memory, and damage to nerve cells, at the same time, researches have shown that MSG can cause brain damage in infants [6].

The MSG increased mRNA expression of IL-6, TNFa, resistin and leptin, but adiponectin did not exhibit any changes. In addition, impaired glucose tolerance, increased levels of insulin, resistin, and leptin were observed in serum. Both PPARa and PPARc were activated in MSG-induced obese mice [7].

MSG increased the expression of several genes implicated in adipocytes differentiation, elevated serum free fatty acids, triglycerides, insulin and bile synthesis [8]. Aimed to understanding the possible effect of MSG on weight body and organ, concentration of protein, albumin and globulin, gene expression related to metabolism and inflammation.

Materials and Methods

Design of the Study

Used nighteen adult Wister rats male weighing 150-250 g and 10-14 weeks age were used. The rats were housed under appropriate controlled room in approved cages with the standard temperature ranging between (26.5-20℃) and maintained under standardized conditions away from any stressful conditions with 12/12 light and dark cycle with free access to humidity and were fed standard rat diet for experimental animals, with a constant source of water and then randomly divided into three group six animal in each group. The first group was control; while the second group was orally dose the MSG 100g/kg dissolved in distilled water via orogastric gavage for 30 days and third group take orally dose from MSG 200g/kg dissolved in distilled water via orogastric gavage for 30 days.

Relative Body and Organ Weight

Body weight of each animal was determined before treatments and before sacrifice. Liver and intestinal of each animal were dissected out and weighed.

Sample Collection

At the end of the experiment, animals were sacrificed and taken liver, intestinal and blood samples were collected from the heart directly. Blood samples were withdrawn from each animal ranging from 3-5 ml of blood divided into two parts. The first was placed in test tubes containing the EDTA to perform the blood tests, while the remaining of the blood was placed in a in a plain test tube until the coagulation occurred. The serum samples were then separated by centrifugation 2500-3000 cycles /min for 15 min. Serum was separated, frozen at–20 until used, also for liver and intestinal samples at same temperature.

Statistical Analysis

Data were expressed as mean SD. The comparisons between groups were performed

with analysis of variance (ANOVA) by using computerized SPSS program (Statistical Program for Social Sciences). $P \leq 0.05$ was considered to be the lest limit of significance. Used past 3.31 for data analysis for analysis of molecular study and use qPCRsoft 2.17 for PCR analysis.

Results

Showed that there were a significant increase ($p \leq 0.05$) in final body weights of animals in groups MSG 100mg/kg and MSG 200mg/kg compared with final weight of animals in control group, also there were a significant increase ($p \leq 0.05$) in final body weight of group MSG 200mg/kg compared to the final weight of animals in group MSG 100 mg/kg. While no asignificant ($p \leq 0.05$) in concentration of total serum protein, albumin and globulin in male rat that treated with MSG 100 mg /kg.

Where as serum total protein,albumin and globulin show asignificant decreases in rats that treated with 200 mg /kg of MSG compere with control group, The concentration for total serum protein of control rats was 110.2 ±11.2 g/dl, Where as group treated with MSG 100 mg/ kg mean concentration was 98.2±13.1g/dl and group treated with MSG 200 mg/ kg mean concentration is 85±5.9 g/dl.

The MSG 200 mg/kg reduced serum total protein the statistical significance level. Mean concentration for albumin control rats was 48.98±5.9 g/dl, group treated with MSG as 100 mg/ kg mean concentration was 39.78±6.65 g /dl and group treated with MSG 200 mg/ kg mean concentration was 31.36±9.04g/dl. While mean concentration for globulin control in rats was 61.23 ±13.19 g/dl, group treated with MSG as 100 mg/ kg mean concentration was 58.42±9.28 g /dl and group treated with MSG 200 mg/ kg mean concentration was 53.71±7.41g/dl.

Table 1: Effect of dietary MSG (100 mg / kg of diet and 200 mg / kg of diet for 30 days) on male rats body weights

Group	Initial Animal Weight (gr)	Final Animal Weight (gr)	Gain Weight(gr)
Control	152.5± 22.6	255.3±30.44 a	103.3±28 .7 b
MSG 100mg/kg	203.8 ±72.2	356.8 ±85.1 bc	153±30 ac
MSG 200mg/kg	172.0 ±22.5	289.6±28 ac	117.6±29.7 bc
LSD	-	34.33	14.5

Table 2: Changes Concentration of Total Serum Protein, Albumin and Globulin on male rat that dietary MSG (100 mg / kg of diet and 200 mg / kg of diet for 30

Prameter / group	Total Protein(g/dl)	Albomin(g/dl)	Globulin(g/dl)
Control	110.2±11.2 a	48.98±5.96 a	61.23 ±13.19 a
MSG 100mg/kg	98.2±13.1 a	39.78±6.65 ac	58.42±9.28 a
MSG 200mg/kg	85.08±5.9 b	31.36±9.04 bc	53.71±7.41 a
LSD	13.1266	9.2000	--

Results of IL-6 showed in 100 mg/kg MSG treatment and sample in liver IL-6 increased in range between (1.3-1.6) folds which represent vast change in expression level. Sample showed less change in expression in intestinal range from (0.2-0.3) fold, while blood sample showed (0.7-1) fold increases of IL-6 expression. While 200 mg/kg MSG treatment and sample in liver IL-6 increased in range between (0.3-0.6) folds which represent large change in expression level.

Sample showed change in expression in intestinal range from (0.3-0.6) fold, while blood sample showed (0.4-0.7) fold increases of IL-6 expression. Results of TNF-a showed that100 mg/kg MSG treatment and sample in liver TNF- a increased in range between (1.4-1.8) folds increases of TNF- a expression. Sample showed less change in expression in intestinal range from (1.2-1.6) fold, while blood sample showed (1.5-1.9) fold which Exemplify huge change in expression level.

While in 200 mg/kg MSG treatment and sample in liver TNF- a increased in range between (0.4-0.8) folds which represent vast change in expression level. Sample showed change in expression in intestinal range from (0.1-0.4) fold, while blood sample showed (0.6-0.8) fold increases of TNF- a expression.

Result NF-κB showed that 100 mg/kg MSG treatment and sample in liver NF-κB increased in range between (1.2-1.6) folds which represent huge change in expression level. Sample showed less change in expression in intestinal range from (0.1-0.3) fold, while blood sample showed (0.8-1) fold increases of NF-κB expression.while in 200 mg/kg MSG treatment and sample in liver NF-κB increased in range between (0.9-1.2) folds which represent large change in expression level.

Sample showed change in expression in intestinal range from (0.1-0.3) fold, while blood sample showed (0.8-1) fold increases of NF-κB expression. Result of IKK showed that 100 mg/kg MSG treatment and sample in intestinal IKK decreases in range between(0.5-0.7) folds and blood range (0.7)fold. While sample liver showed less change in expression in range from (0.5-0.6) fold.

While in 200 mg/kg MSG treatment and sample in liver IKK increased in range between (0.7-1) folds. Sample showed change in expression in intestinal range from (0.9-1.2) fold, while blood sample showed (0.7-0.8) fold which represent less change in expression level.

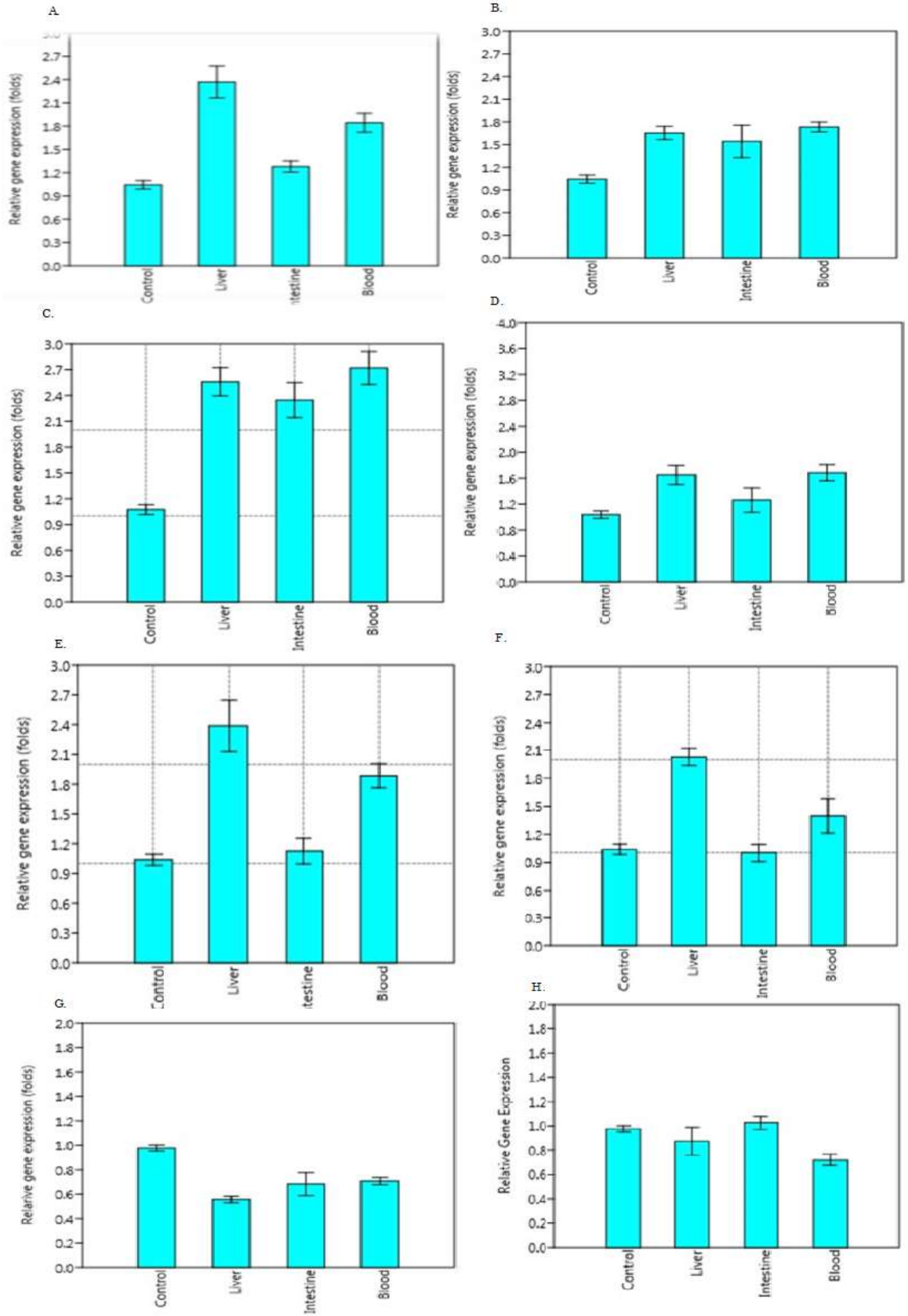

Figure 1: Effect of inflammatory factors gene expressions at transcriptional levels in adipose tissue of obese rat. The expression level mRNA was normalized to that of GAPDH reference gene A. IL-6 gene expression MSG-induced(100 mg/kg), B. IL-6 gene expression MSG-induced(200 mg/kg), C. TNF-a gene expression MSG-induced(100 mg/kg),D. TNF-a gene expression MSG-induced(200 mg/kg), E. NF-κB gene expression MSG-induced(100 mg/kg), F. NF-κB gene expression MSG-induced(200 mg/kg), G.IKK gene expression MSG-induced(100 mg/kg), H. IKK gene expression MSG-induced(200 mg/kg)

Discussion

Differentiation final body weight from initial body weight in all group revealed a significant difference and therefore increase was considerable in group that treatment with MSG 100mg/kg and 200mg/kg compared with control. The most frequent cause which leads to the development of obesity is a misbalance between energy intake and energy expenditure [9].

MSG is a flavour enhancer that increases the savory experience of foods and is the prototypical chemical related with the 'umami' taste because of its role as a flavour enhancer, it was firstly believed that MSG increased intake and appetite. However, initial enhanced intake tends to decrease over time and recent research suggests that MSG may also delay hunger recovery in the short term too, particularly when in combination with a food rich in protein [10].

Activities of liver enzymes total serum protein and albumin levels were significantly decreased. The synthetic function of liver was altered by MSG, total serum protein and albumin level decreased. Liver is the primary site of the synthesis of plasma proteins [11].

A disturbance of protein synthesis occurs as a consequence of impaired hepatic function which will lead to a decrease in their plasma concentration. The reduction of the protein concentration in the MSG treated rats could indicate a reduction in the synthetic function of the liver or increase rate of protein degradation [12]. The concentration of proteins and albumin in the serum can be used as indicators for the state of the liver and can be used to differentiate between different types of liver damage.

The observed reduction in albumin concentrations in serum indicated liver damage, arising from the uptake of the chemical compound. This may be an indication of diminished synthetic function of the liver which may consequently lead to enhanced retention of fluid in the tissues spaces [13].

Obesity and the metabolic syndrome is the development of a chronic, low-grade inflammatory state. In obesity, adipose tissue does not only change quantitatively, in other words, an increase in adipocyte size and number, but increased fat mass is also associated with recruitment and activation of T lymphocytes and macrophages in fat tissue [14]. This will leads to increases of proinflammatory cytokines expression in adipose tissue. This so-called 'metainflammation' is characterized by a systemic overabundance of inflammatory cytokines. The signaling cascades activated by these inflammatory cytokines can directly interfere with insulin signaling in insulin target tissues such as adipose tissue, liver, skeletal muscle, and even in the central nervous system [15].

TNF-a, the first inflammatory adipokine identified to be increased in obesity [16]. Increases level of TNF-a in liver and blood lead to increase inflammatory and eventually get increases weight one can, easy note that result conisid with that result of weight changing in compare with control group. Inflammation decreases in high dose of MSG who showed in animal that treatment with 200 mg/kg. This results agree with [17] Increased levels of TNF-a in obese subjects were reported, which supports the notion that obesity is a chronic inflammatory disease.

Evidence also indicates that TNF-a expression is genetically determined and polymorphic sites linked to the TNF-a locus are associated with different TNF-a expression levels [18]. Nf–κB nuclear translocation (activation), a major component of the inflammatory pathway [19].

Result indicate that 100mg/kg lead to increases in expression of the gene on about (1.2-2.6) this large expression will defiantly lead to increases of many other gene involving in synthesis and energy production, so will cause weight gain and also is increases in synthesis of IL-6 and is responsible for inflammation process and lipid are assimilator, which all consider with the gain of weight expression.

IKK is cytokines able to activate inflammatory signaling cascades such as inhibitor of nuclear factor kappa-B kinase 2(IKK_2) [20]. Result illustration that IKK lead decreases in transcription quaintly at a noticed level. This alteration will cause decrease of NF-κB level as mention before and this explains the increase of NF-κB level. This result agree with [21] who find atorvastatin significantly decreased the expression of IKK-ß and NF-κB increased

that the expression of the inhibitor of NF-κB, IκB-α. IKK-ß is an important kinase that can affect insulin signaling through serine phosphorylation of Insolin Receptor Substance (IRS-1) and by phosphorylation of IκB-α, which leads to stimulation of the NF-κB pathway. IκB -α inhibits the transcriptional activity of NF-κB in the cytoplasm by preventing the nuclear translocation of NF-κB. In the nucleus, it dissociates NF-κB from DNA and transports it back to the cytoplasm.

Conclusions

MSG as food additive can cause weight increase in lab animal. Soluble total protien decrease signifeantly in treated animals in compare with corresponding control. MSG causes (presumbly indirectly) significant increase of NFκB which lead to enhance transcription of deffenet genes including those involved in metabolism and energy. This up negulation result of NFκB is fortified by IKK down negulation which is decraese in treated animals.

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