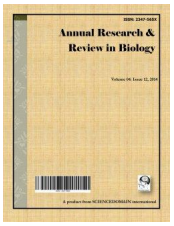

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Monosodium Glutamate Induced Haematological Alterations in Female Swiss Albino Mice *Mus musculus*

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Authors' contributions

This work was carried out in collaboration between both authors. Author TZ performed the experiment, carried out the statistical analysis, wrote the first draft of manuscript and prepared the art works. Author VKS carried out the analysis of data and critical evaluation of final draft of manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: The sodium salt of most abundant naturally occurring amino acid glutamic acid is a popular flavour enhancer used to generate savoury or umami taste in a variety of foods. Apart from various health implications, high doses of MSG are widely used in a variety of commercial, processed and junk foods. The objective of the present study is to observe haematological alterations in female mice after long-term oral exposure of high dose of MSG.
Methodology: Female Swiss albino mice have been divided into two groups named control and treatment for each duration. Mice were given 4 gram/kg/day MSG by oral gavage for thirty and sixty days respectively and then sacrificed for the assessment of haematological parameters.
Results: High dose of MSG consumption contributes significantly (p value ≤ 0.05), in the reduction of hemoglobin percentage (p value<0.05) red blood cells (p value ≤ 0.01) white blood cells count (p value ≤ 0.05) Serum bilirubin concentrations (p value ≤ 0.05) were elevated significantly in MSG

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treated groups after thirty day treatment. Over the time period severity of the implications became more significant (p value ≤ 0.01).
Conclusions: MSG consumption could cause haematological alterations. Authors strongly discourage the prolonged use of high doses of monosodium glutamate for better maintenance of health of young female population.

Keywords: Anemia; bilirubin; hemoglobin; monosodium glutamate; ajinomoto.

1. INTRODUCTION

Health of young female individuals of any society is a clear reflection of their dietary habits, dietary consumables and life style. Over past few decades' prevalence of many clinical conditions like metabolic syndromes, anemia, infections and jaundice become very common, among young female population [1-5]. In influence of modern lifestyle processed food consumption has significantly increased along with persistent use of high doses of flavour enhancers. The sodium salt of most abundant naturally occurring amino acid glutamic acid is a very popular food additive, generally known as monosodium glutamate (MSG). Monosodium glutamate (MSG) is a widely used flavour enhances with a huge consumer population worldwide. It is commercially available under many brand names like Ajinomoto, Sasa, Vetsin, Miwon and Weichaun. It is a popular ingredient of various Chinese, Japanese, South-Asian, soups or sauces (canned, packed), prepared meals, frozen foods, flavoured chips and snacks, marinated meats, fresh sausages, bottled soy or oriental sauces, and stuffed or seasoned chicken, manufactured meats, some hams, luncheon chicken and turkey, flavoured tuna, vegetarian burgers and sausages, noodles, soups, sauces, chips, packed, ready to eat, processed and branded foods. Extensive use of monosodium glutamate is not restricted to only ready to eat processed foods but it has also used in home made and restraunts made foods. Since many years safety status of MSG remained controversial, as many researchers have found it a cause of progression of various clinical disorders and syndromes. Despite of the excitotoxic nature of MSG it is widely use to enhance the savoury taste in many processed foods [6-8].

Food and drug administration (FDA) and Federation of American Societies for Experimental Biology (FASEB) recognize MSG in GRAS category (Generally recognize as safe) but the ADI value (advisable daily intake) is still not so specific over worldwide. High doses up to 4 g. per capita consumption is very common in various populations [9-10]. However Many researchers have been reported the adverse effect of high dose of monosodium glutamate in adults and infants. Many clinical and pathological conditions like asthma, urticaria, atopic dermatitis, ventricular arrhythmia, neuropathy and abdominal discomfort, degeneration of population of neurons, stroke, epilepsy, schizophrenia, anxiety, depression, Parkinson's disease, Alzheimer's disease, Huntington's disease, and amyotrophic lateral sclerosis were already known to have an association with MSG consumption [6,8,11-16]. The controversial status of MSG makes it more interesting molecule for possible haematological implications, as haematological profiling is a primary indicator, which is widely monitor by the clinicians and physicians to identify various clinical conditions. Focusing on the controversial status of MSG present study was conducted to monitor the possible haematological alterations associated with persistent use of high concentrations of MSG consumption.

2. MATERIALS AND METHODS

2.1 Animal Care and Handling

Adult female Swiss albino mice (Parke's strain) were brought from Jawaharlal Nehru Cancer Hospital and Research Center, Bhopal, India and maintained in the animal house of Department of Biosciences, Barkatullah University, Bhopal, India. Mice were kept in polypropylene cages on paddy husk bedding under controlled conditions of temperature (25-27°C) and light (14 hours light period: 10 hours dark period) along with standard mice feed and water *ad libitum* through out the experiment. Present study is a part of the research plan approved by institutional ethical committee with reference number 1885/GO/S/16 /CPCSEA/IAEC/BU/05. Animal care and handling were performed according to guidelines issued by CPCSEA, (Committee for the purpose of control and supervision of experiments on animals) New Delhi.

2.2 Experimental Design and Methods

Animals were treated with 4 g/kg body weight dose of monosodium glutamate (MSG) by oral gavage using a metallic feeding cannula, daily up to thirty and sixty days. At the end of each time point control and treated mice were sacrificed by cervical dislocation and immediately blood was collected from cardiac puncture in an ethylene di amine tetra acetic acid (EDTA) precoated vial for haematology as well as in plain vial for serum bilirubin estimation. Hemoglobin percentage was determined by Sahli's acid hematin method [17]. Red blood cells (RBCs.) and white blood cells (WBCs) counts were calculated using neubauer's chamber [18]. Serum Bilirubin was estimated using spectrophotometer. For serum bilirubin estimation blood sample was taken through cardiac puncture and then incubated at 37 ˚C for 1 hour without any blood anticoagulant. It was further incubated at 4°C for 2 hr. The sample was centrifuged at 10,000 g for 10 minutes at 4°C. The serum was aspirated from the cells/blood clot and processed for bilirubin estimation using previously described method [19].

2.3 Statistical Analysis

The collected data was subjected to statistical analysis using Excel–mac operating system software. Mean ± standard deviation and standard error of mean were calculated. Independent student's 't' test was used for statistical comparison and significance level determination between the control and treatment groups using Excel–mac operating system. Figures have been prepared using Excel–mac operating system.

3. RESULTS

At the end of each time point (thirty days and sixty days of oral MSG treatment) haematological values of treated groups were compared to their respective time point controls. Observation of haematological parameters reveals that hemoglobin percent and WBCs count reduced significantly (p value ≤ 0.05) after the treatment of monosodium glutamate for thirty days. The difference between treated and control groups become more significant (p value ≤ 0.01) when oral treatment of MSG was continued for sixty days (Fig. 1 and Fig. 2).

Prominent reduction was observed in count of RBCs and WBCs of MSG treated mice in compare to untreated healthy mice of control groups. However a more significant (p value ≤ 0.01) reduction in RBCs count of blood observed when the treatment continued for sixty days (Fig. 3). Serum bilirubin level elevated significantly (p value ≤ 0.05) after thirty days of oral MSG exposure, which increased further in duration dependent manner and became more significant after sixty days of MSG exposure (Fig. 4).

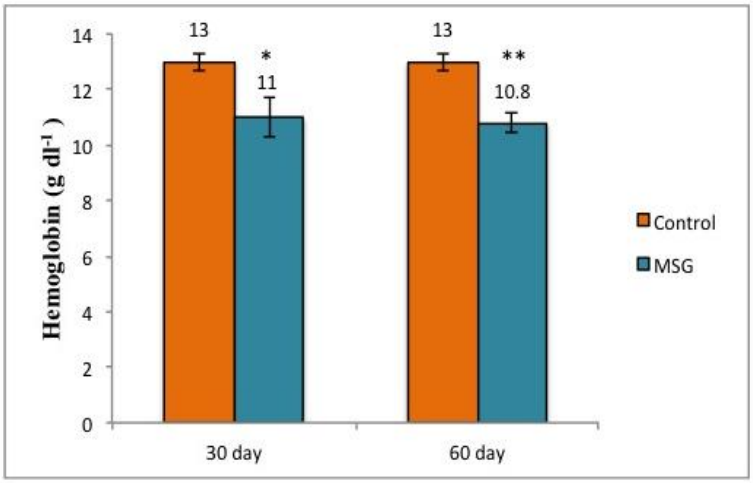

Fig. 1. Alterations in hemoglobin percentage associated with MSG consumption

** Represents p value ≤ 0.05 which is considered significant and **represents p value ≤ 0.01 which is considered more significant*

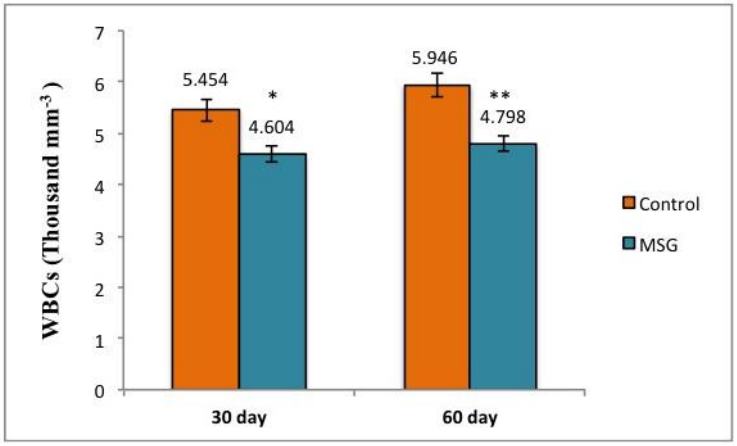

Fig. 2. Alterations in white blood cells count associated with MSG consumption
**p value ≤ 0.05 is considered significant and **p value ≤ 0.01 is considered more significant*

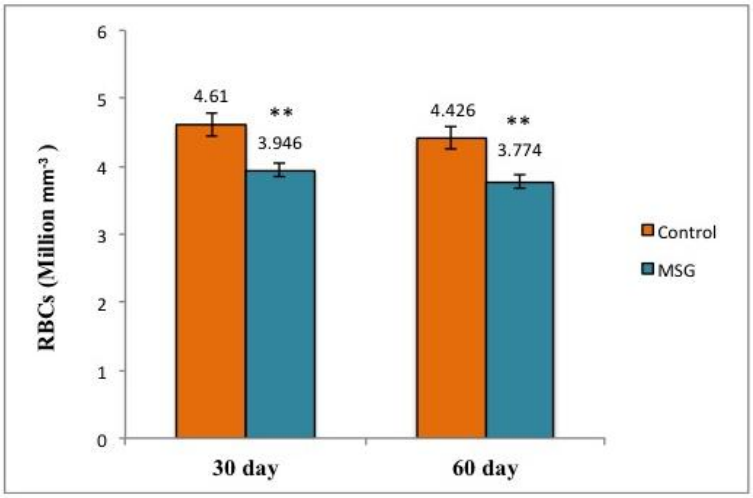

Fig. 3. Alterations in red blood cells count associated with MSG consumption
**p value ≤ 0.05 is considered significant and **p value ≤ 0.01 is considered more significant*

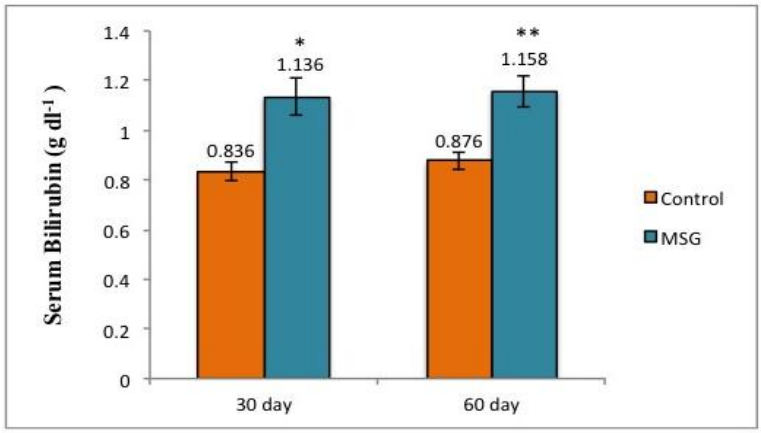

Fig. 4. MSG induced increment in serum bilirubin level
**p value ≤ 0.05 is considered significant and **p value ≤ 0.01 is considered more significant*

4. DISCUSSION

Many adverse effects of MSG consumption are available in literature. However in few reports MSG was found safe for consumption, still the literature available in support of deleterious effects of MSG consumption is not ignorable. MSG intake was known to have associated with various adverse effects including induction of oxidative stress, formation of reactive oxygen species, brain damage, renal damage, abnormal liver function, altered nerve function etc [20-25]. Very low concentrations of MSG were used for safety assesment previously by a few researchers, where MSG was found to have no effect on hematological parameters. In year 2013 a study performed by Maluly *et al.* reported no metabolic changes in MSG treated diabetic induced rats. In contrast to previously drawn conclusions present study reported a significant alteration in hematological parameters when higher concentrations of MSG were used for relatively longer duration. Present findings are in connection with the findings reported by Ashaulo *et al.* where MSG was found to alter mean corpuscular volume, mean corpuscular hemoglobin concentration, spleen and thymus damages etc. MSG was found capable to induce the formation of micro nucleated polychromatic erythrocytes (MNPCEs) by developing oxidative stress in tissue. Increament in oxidative stress markers were previously reported by many reseachers in various tissue systems as an after effect of MSG treatment. Decreased number of WBCs in the circulation could be an indicator of thymic lymphocyte toxicity which results in low lymphocytes count, due to spleen and thymus damage by monosodium glutamate. Elevated glutamate concentrations could impair lymphocyte functions and induce secondary immunopathological consequences. This effect of high glutamate production leads to the suppression of mitogenin iduced proliferation and is mediated by glutamate receptors. A significant decrement in haematological parameters along with elevation in serum bilirubin indicates that prolonged use of high monosodium glutamate intake is not good to haematopoietic system [21,22,25,26-30].

Erythropoiesis is the process of production of red blood cells, which is modulated by presence of many factors including erythropoietin, vitamin B_{12} etc. RBCs are consists of a very important red colored respiratory pigment hemoglobin, which is responsible for oxygen transport from lungs to tissues. Under normal conditions RBCs have a life span of about 120 days. When RBCs die its dissociation results in the release of heme prosthetic group along with globin protein. Heme is an iron containing pigment, which carries oxygen from lungs to distant peripheral parts via blood. These constituents result in elevation of a yellow colored pigment, bilirubin via metabolic pathways. MSG consumption is responsible to induce destruction of red blood corpuscles either in premature or in young phase. This shortened life span of RBCs results in breakdown, which is confirmed by less number of RBCs in blood. After the destruction of RBCs unbound bilirubin is released in the serum, which is converted in its conjugated form by liver. Presence of significantly high amount of bilirubin in serum of MSG treatment groups is an indication that MSG consumption contributes in higher rate of degradation of RBCs and low capabilities of liver to detoxify respective amount of bilirubin. MSG is also supposed to alter the rate of WBCs production, as the number of WBCs were reported low in MSG treated groups in compare to untreated controls. Decreased number of WBCs in blood of MSG treated animals is an indication of low production of WBCs in bone marrow which could be a result of adverse changes in haematopoietic stem cells or mild bone marrow toxicity. WBCs are the principal cells of immune system. Low production of WBCs could possibly result in a sluggish immune response (Fig. 5). Less number of monocytes and neutrophils adversely affect the first line of body defense. Deterioration of immune system could be very dangerous in such cases. Presence of significantly less number of WBCs and associated bone marrow cells indicates that after the consecutive consumption of MSG at high doses immune system become mildly sluggish [31-33]. Less active immune system along with less number of different blood cells contributes in low peripheral oxygen supply and results in progression of various clinical conditions including poor health, anemia and jaundice. This condition makes the individual more prone to variety of infections available within surroundings.

MSG is already well known to induce oxidative glutamate toxicity in neuronal hippocampal cells, which could be a result of increment of anaerobic respiration by cells [34-37]. A low concentration of hemoglobin and RBCs could affect the oxygen supply within the body and brain. This could make the implications more sensitive. Persistent use of high dose of monosodium glutamate suppresses the immune system somehow, which

will possibly make individual prone for all the available surrounding infections. Naturally occuring glutamate within the body is not harmfull but, excess consumption of high dose of monosodium glutamate via various sources adversely effects many *in vivo* mechanisms [38]. In the light of present findings authors strongly discourage the used of MSG for prolonged duration, as it could be detrimental to health in a silent manner (Fig. 6). Authors strongly recommend the restricted and controlled use of monosodium glutamate as a flavour enhancer under strict monitoring.

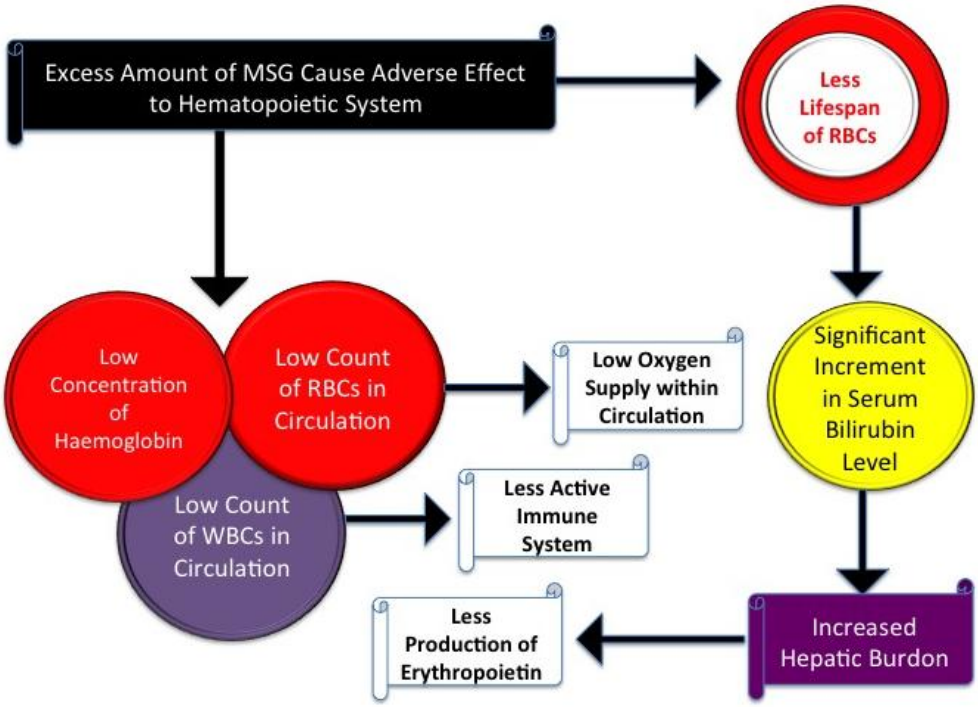

Fig. 5. Progression of MSG induced hematological alterations over time period

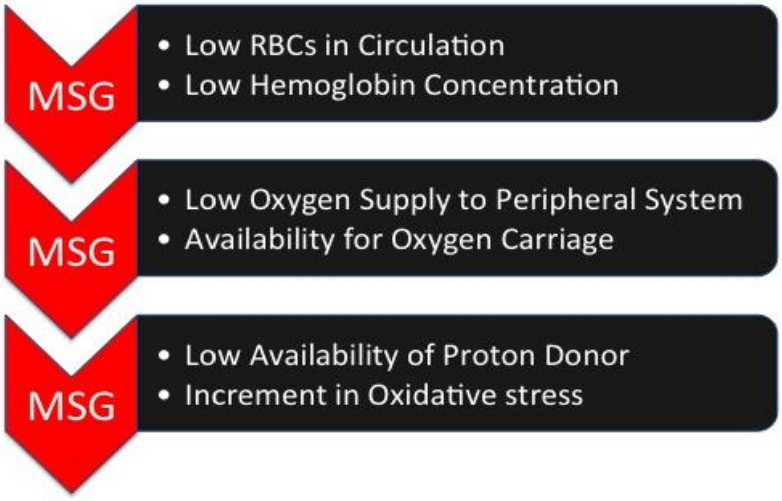

Fig. 6. Consequences of MSG induced hematological alterations due to persistent oral consumption of MSG

5. CONCLUSIONS

A shift in the balance of homeostasis followed by monosodium glutamate administration appeared to impair haematological parameters. It is concluded that frequent intake of monosodium glutamate for prolonged duration is detrimental to health.

ETHICAL APPROVAL

Animal care and handling were performed according to guidelines issued by CPCSEA, (Committee for The Purpose Of Control and supervision of experiments on animals) New Delhi, India. Present study is a part of the research plan approved by ethical committee with reference number 1885/GO/S/16/CPCSEA/IAEC/BU/05.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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