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Haematological effect of ethanolic extract of *Uvaria chamae* on monosodium glutamate (MSG)-induced toxicity in sprague-dawley rats

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ABSTRACT

Monosodium glutamate (MSG) is permitted as a safe food additive. However, there are concerns regarding its high intake. Using medicinal plants for treatment is an ancient practice, which still holds today. Uvaria chamae is one plant whose therapeutic activities are being investigated. MSG and phytochemicals have been reported to alter haematological parameters. 25 Sprague dawley rats, 16-18 weeks old (80g –120g) were grouped as follows; CONTROL, MSG, MSG+U.chamae(low dose), MSG+U.chamae(middle dose) and MSG+U.chamae(high dose). Animals in the experimental groups were orally administered MSG daily at 150mg/kg body weight for 30 days after which, the animals in the three MSG+U.chamae groups were treated with respective doses (LOW dose; 100mg/kg body weight; MIDDLE dose; 200mg/kg body weight, HIGH dose; 300mg/kg body weight) of the Uvaria chamae extract. Haematological parameters were evaluated; the white blood cell (WBC), red blood cell (RBC), haemoglobin (HB), haematocrit (HCT) and neutrophil (NEU) levels were significantly lowered while the mean cell volume (MCV), mean corpuscular haemoglobin (MCH) and lymphocyte (LYM) levels were significantly raised in the MSG-administered rats when compared with the CONTROL ($P < 0.05$). The three doses of the extract significantly raised the RBC, HB and HCT levels which were lowered by the MSG; the extract also significantly lowered the MCV, MCH levels raised by the MSG. However, the extract had no significant ($P < 0.05$) effect on the NEU and LYM levels. Monosodium glutamate has detrimental effect on haematological parameters but Uvaria chamae ethanolic extract is a potent remedy against MSG-induced toxicity.

Keywords: Monosodium glutamate, toxicity, haematological parameters and *Uvaria chamae*

INTRODUCTION

Monosodium glutamate (MSG) is the sodium salt of the non-essential amino acid (glutamic acid), one of the most abundant amino acids found in nature; glutamic acid is the main component of many proteins and peptides of most tissues. Monosodium glutamate contains 78% of glutamic acid, 22% of sodium and water. Glutamate is also produced in the body and plays an essential role in human metabolism. It is a major component of many protein-rich food products such as meat, fish, milk and some vegetables [1].

MSG is produced in many countries around the world through a fermentation process of molasses from sugar cane or sugar beets, as well as starch and corn sugar. MSG is commonly consumed as a flavour enhancer or food additive and both animal model experiment and human clinical reports has suggested harmful effects. In Nigeria, despite evidence of negative consumer response to MSG, reputable international organizations and nutritionist have continued to endorse Monosodium glutamate, and reiterate that it has no adverse reactions in humans. The Directorate of Regulatory Affairs of Food and Drug Administration and Control (FDA & C) in Nigeria, now NAFDAC has also expressed the view that MSG is not injurious to health [2].

The use of plants as source of medicine in treating disease (including chemical poisoning) is an ancient practice, however, in recent times; attention has been reverted back to plants as sources of therapeutic agents due to obvious reasons as reduced cost, relative lower incidence of adverse reactions compared to modern conventional pharmaceuticals and ready availability among others. A large number of plants (including *Uvaria chamae)* in different locations around the world have been extracted and semi-purified to investigate their individual therapeutic activity.

Uvaria chamae belongs to the family Annonaceae [3]. It is a climbing plant predominantly found in the tropical rain forest of West Africa. Literature has shown that ingestion of phytochemicals as drugs can alter normal range of haematological parameters [4].

Haematological parameters refer to levels of blood components; red blood cells (RBC), white blood cell (WBC), platelet (PLT), haemoglobin (HB), neutrophil count (NEU) and lymphocyte count (LYM). Due to the widespread consumption of MSG and use of herbal medicine, it is necessary to study the effect of MSG on blood, the tissue that transports substances in the body and the ameliorating effect of *Uvaria chamae*.

This study is aimed at evaluating the effect of MSG-induced toxicity on haematological parameters and examining the therapeutic effect of *Uvaria chamae* in correcting the adverse effect of MSG-induced toxicity on haematological parameters in Sprague-dawley rats.

MATERIALS AND METHODS

Experimental Animals: A total of 25 female Sprague dawley rats, 16-18 weeks old and weighing between 80g – 120g were obtained from the laboratory animal centre, College of Medicine, University of Lagos, Idi-araba. The rats were bred for one month in the animal house of the College of Medicine, University of Lagos, Idi-araba to allow them acclimatize to their new environment. The rats were feed with rat pellets (purchased from Ladokun Feeds, Ibadan) and with water *ad labium* throughout the experiment period. The rats were then picked at random and divided into five groups; a CONTROL group, and four other experimental groups which were all administered MSG. Each of the rats were painted at the tail with indelible dyes of different colours and put into different cages, according to their group.

The rats were grouped as follows;

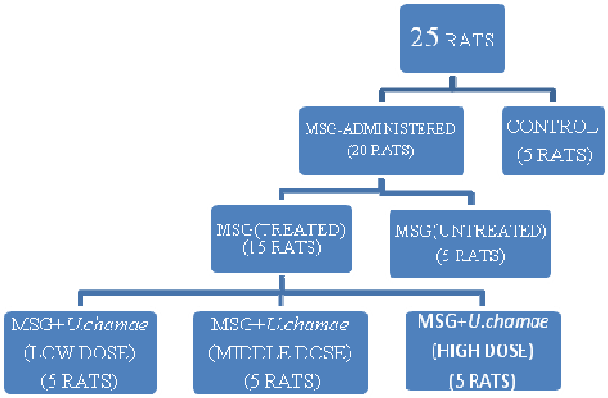

Figure-1: Experimental design

Administrations of Monosodium Glutamate (MSG): After one month of acclimatization, the 20 rats in experimental group were administered monosodium glutamate orally by means of calibrated syringe with attached rubber cannula at a daily dose of 150mg/kg body weight for 30 days. The rats in the CONTROL group were given distilled water. The administration was carried out for 30 days after which, the animals in the MSG+*U.chamae* groups were administered with the ethanolic plant extract as treatment.

Collection of Plant Samples: About 2kg of *Uvaria chamae* root, locally called 'eruju', were bought from Ojuwoye market, Mushin Local Government area of Lagos State. Botanical identification and authentication of the plant was done at the herbarium of the Botany Department of University of Lagos, Akoka.

Preparation of Ethanolic Plant Extract: The plant's root bought, were chopped into small pieces and air dried for 2 weeks. The dried samples were grounded almost to powder using a Thomas Wiley mill machine (Model Ed – 5 USA) in Pharmacognocy laboratory of the College of Medicine, University of Lagos. 2kg of this was weighed and then soaked in 70% ethanol for 72hours after which it was sieved using muslin cloth. The plant extract was concentrated in the same laboratory using rotary evaporator and further evaporation was done with oven at 50°c. Steam evaporation using water bath set at 50°c was also carried out to get a very thick jelly-like extract. The ethanolic extract was then prepared into three different doses (Low dose; 100mg/kg body weight dose; Middle dose; 200mg/kg body weight, High dose; 300mg/kg body weight) for administration. The average weight of the animals as at the beginning of treatment was 100g.

Administration of Plant Extract: After 30 days of administration of the monosodium glutamate, the animals in the MSG+*U.chamae* groups were administered orally with the ethanolic plant extract (as treatment) according to their respective daily doses; those in the MSG group were sacrificed before this administration started. The administration was carried out for 21 days after which all the animals were sacrificed.

Collection of Blood Samples: After 21 days of the administration of the ethanolic plant extract, the rats were fasted over night. The following morning, the rats were weighed and collection of blood sample was done through ocular puncture i.e. using Na-Heparinized haematocrit tubes inserting through optical sinus and collected into both ethylene diamine tetraacetate (EDTA) bottles for evaluation of haematological parameters.

Evaluation Haematological Parameters: The whole blood in the EDTA bottles was homogenized to allow for proper mixing of the blood components. Evaluation of the haematological parameters; haemoglobin concentration (HB), packed cell volume (PCV), red blood cell count (RBC),mean cell volume (MCV), white blood cell count (WBC), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), neutrophil count (NEU)and Lymphocyte count (LYM) was carried out using automated haematological Analyzer K-X- 21 made by SYSMEX, Kobe, Japan. The machine uses impedance and colorimetric method in which haemoglobin form a complex by the action of lyase, one of the reagents in the machine and then analyses the sample by measuring the concentration of the haemoglobin and other blood components in the medium [5].

Statistical Analyses: Data were analyzed by comparing mean values from the CONTROL group and the MSG group (being the positive and negative controls respectively) by student t-test. Mean values from different treatment (MSG+*U.chamae*) groups were also compared with mean values from MSG group, (negative control) by analysis of variance (One-way ANOVA) using SPSS Version 20 software. Results are expressed as Mean ± Standard Deviation (SD) and are regarded as significant at $P \leq 0.05$.

RESULTS AND DISCUSSION

Haematological parameters, which include: white blood cell (WBC), red blood cell (RBC), haemoglobin (HB), haematocrit (HCT) [which is also packed cell volume (PCV)], mean cell volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), neutrophil (NEU) and lymphocytes (LYM) were analyzed.

Table-i: Comparison of the levels of hematological parameters of normal controls and MSG-administered Rats

Haematological Parameters	CONTROL Mean ± S.D	MSG Mean ± S.D
WBC (10^9/L)	14.92 ± 4.35	*9.10 ± 2.08
RBC (10^{12}/L)	7.83 ± 0.25	*3.18 ± 0.21
HB (g/dl)	15.92 ± 0.33	*12.02 ± 1.10
HCT (%)	45.50 ± 1.40	*36.06 ± 3.30
LYM (%)	42.30 ± 4.21	*61.00 ± 2.59
NEU (%)	45.44 ± 3.19	*31.32 ± 3.77
MCV (pg)	58.20 ± 2.01	*114.11 ± 15.46
MCH (FL)	20.32 ± 0.72	*38.04 ± 5.15
MCHC (g/dl)	34.96 ± 0.87	33.33 ± 0.00

**Statistically significant when compared with the CONTROL group at P ≤ 0.05*

The above table shows that MSG had significant effects on haematological parameters; the WBC, RBC, HB, HCT and NEU levels were significantly reduced in the MSG-administered rats when compared with the CONTROL group; the MCHC level was slightly lowered (but not statistically significant), while the LYM count, the MCV, MCH levels were raised significantly by the administration of MSG.

Table-ii: Effect of different doses of ethanolic extract of *Uvaria chamae* on hematological parameters in MSG-administered Rats

Haematological Parameters	CONTROL Mean ± S.D	MSG (Untreated) Mean ± S.D	MSG + *U.chamae* (Low Dose) Mean ± S.D	MSG + *U.chamae* (Middle Dose) Mean ± S.D	MSG + *U.chamae* (High Dose) Mean ± S.D
WBC (10^9/L)	14.92 ± 4.35	*9.10 ± 2.08	6.95 ± 0.55	6.30 ± 3.16	7.32 ± 2.31
RBC (10^{12}/L)	7.83 ± 0.25	*3.18 ± 0.21	**7.14 ± 0.05	**7.93 ± 0.42	**8.07 ± 0.58
HB (g/dl)	15.92 ± 0.33	*12.02 ± 1.10	**12.80 ± 0.58	**14.92 ± 1.21	**14.48 ± 0.56
HCT (%)	45.50 ± 1.40	*36.06 ± 3.30	**41.85 ± 0.94	**45.24 ± 4.20	**43.72 ± 2.05
LYM (%)	42.30 ± 4.21	*61.00 ± 2.59	53.40 ± 2.54	58.66 ± 5.14	60.78 ± 6.21
NEU (%)	45.44 ± 3.19	*31.32 ± 3.77	34.35 ± 1.52	30.34 ± 2.47	28.56 ± 4.61
MCV (pg)	58.20 ± 2.01	*114.11±15.46	**58.78 ± 0.61	**57.00 ± 2.53	**55.96 ± 2.02
MCH (FL)	20.32 ± 0.72	*38.04 ± 5.15	**17.95 ± 0.97	**18.76 ± 0.61	**18.56 ± 1.03
MCHC (g/dl)	34.96 ± 0.87	33.33 ± 0.00	30.55 ± 1.95	32.96 ± 0.49	33.02 ± 0.96

**Statistically significant when compared with the CONTROL group at P ≤ 0.05*
*** Statistically significant when compared with the MSG (Untreated) group at P ≤ 0.05.*

As shown in table above, the three doses of the extract had significant effect on some of the haematological parameters in the MSG-administered rats; the ethanolic extract of *Uvaria chamae* significantly raised the RBC and HB levels which were lowered by the administration of MSG; this effect is found to be dose dependent. However, the extract further lowered the WBC level but this was not statistically significant.

The HCT level which was lowered by the administration of MSG was significantly raised by the extract in a dose dependent manner. Although the LOW and MIDDLE doses of the extract tend to lower the LYM count which was elevated by the administration of the MSG, the HIGH dose sustained the elevated level; this effect was however not statistically significant. The NEU count also reduced insignificantly as the extract dose increases. MCV and MCH levels which were raised by the MSG were significantly lowered by the extract. However, the mean corpuscular hemoglobin concentration MCHC levels seem unaffected by the extract.

Table-ii above proves that ethanolic extract of *Uvaria chamae* is effective in correcting some anomalies in haematological parameters caused by the administration of MSG in rats; this corrective effect could be said to be dose dependent.

This study indicates that MSG administration significantly lowered neutrophil levels; this might be that MSG has a direct toxic effect on the neutrophils in the blood or it has a deleterious effect on blood production in the bone marrow, especially on the progenitor cells (aplasia). Neutrophils along with monocytes provide the first line of defence against invading micro organism, toxic substances, and foreign substances emphasizing the important role neutrophils play in the body defence [6]; this might be indicative of the deterioration of immune status [7] in the treated rat groups in response to the toxic effect of MSG. The observed increased lymphocyte levels in the MSG-administered animals might be due to the fact that MSG is perceived as a toxic agent in the administered animals or probably due to a considerable increase in granulocytes or could be a consequence of the interaction between MSG

and the gastrointestinal macrophages which serve as antigen presenting cell and the antigenic products (polypeptides) to the helper T cells and the B lymphocytes bringing about their activation [8]. Macrophages secrete substances called interleukin-1 /-cytokines, which brings about the activation, proliferation and increase in the lymphocyte count [9,10]. The count increase (from 42.30% to 61%) seen in the MSG group indicate that MSG does not have a destructive effect on the lymphocytic cells. This also implies that MSG has a residual and prolonged effect within the body system [11]. The extract of *Uvaria chamae* did not to have significant effect on the lowered neutrophil and the raised lymphocyte counts. This could be due to the immune enhancing effect of *Uvaria chamae*, thus maintaining the elevated lymphocyte level.

The reduction effects of MSG on the packed cell volume, PCV (same as hematocrit, HCT) (from 45.50% to 36.06%), haemoglobin concentration, HB (from 15.92% to 12.02%) and the lowered RBC count suggests that it probably reduces the life span of red blood cells in the blood which might be as a result of direct toxicity. This might also have been mediated through a deleterious effect on the haemopoietic stem cells in the bone marrow [12]. MSG might cause increased oxidative stress (a function of anaerobic respiration) in the tissues of the animals. At the daily dose of 150mg/Kg body weight used, MSG may have induced the formation of micronucleated polychromatic erythrocytes (MNPCEs) [13]. These toxic effects characterized by cell and nuclear condensation, could occur in the absence of either DNA fragmentation or mitochondrial *cytochrome c* release [14]. The extract significantly raised the lowered haematocrit HCT, red blood cell RBC and haemoglobin HB levels back to normalcy, and this could be due to the antioxidant activities of the phytochemicals of *Uvaria chamae* thereby protecting the hemopoietic stem cells that produce the red blood cells in the bone marrow from oxidative damage which may have been induced by MSG toxicity [15].

MCV and MCH values were observed to be significantly increased (from 58.20pg to 114.11pg and 20.32FL to 38.04FL respectively) by the MSG, this is indicative of anaemia as increase in MCV is seen in pernicious anaemia (normochromic) and megaloblastic anaemia (hypochromic) [16]. While MCH levels is significantly raised (which is indicative of macrocytic anaemia [10]), there was a slight reduction in the levels of MCHC (from 34.96g/dl to 33.33g/dl) which was statistically significant. Thus, macrocytic normochromic anemia (pernicious anaemia) was more specifically indicated. This might be due to the atrophy of the gastric mucosa (gastritis) caused by the MSG which is acidic, resulting in reduced synthesis of the intrinsic factor, and thus poor absorption of vitamin B_{12} which is the main cause of pernicious anaemia [10]. The extract was able to lower the raised levels of MCV and MCH to levels close to that of the CONTROL group, this could be as a result of the corrective effect *Uvaria chamae* has on the epithelial tissues of the gastrointestinal tract, thus correcting the atrophy of the gastric mucosa (gastritis) caused by the MSG thereby allowing for absorption of vitamin B_{12} and preventing anaemia.

CONCLUSION

This study shows MSG administration has a significant effect on the neutrophil and lymphocyte count, indicative of a compromised immune status in the MSG-administered animals, while alterations in counts of haematocrit HCT, haemoglobin HB, red blood cell RBC, mean corpuscular volume MCV and mean corpuscular haemoglobin MCH were all indicative of anaemic conditions in the MSG-administered animals. The study also proves that ethanolic extract of *Uvaria chamae* is effective in correcting some anomalies in haematological parameters caused by the administration of MSG in rats. Hence, these findings support the fact that monosodium glutamate, despite its flavouring functions may be detrimental to health and also propose that ethanolic extract of *Uvaria chamae* is a potent remedy against MSG-induced toxicity.

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