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HAEMATOLOGICAL RESPONSE OF ADULT MALE WISTAR RATS EXPERIMENTALLY EXPOSED TO ORAL MONOSODIUM GLUTAMATE

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ABSTRACT

Excessive use of monosodium glutamate has been shown to alter several ranges of haematological parameters. This study evaluated the effect of sub-chronic oral doses of monosodium glutamate on the haematological profiles of male Wistar rats. Sixty-four adult male Wistar rats weighing 170 to 230 g were procured and allowed to acclimatize to their new environment for two weeks. They were randomly separated into two groups; monosodium glutamate-treated and non- monosodium glutamate-treated (control) groups of 32 rats each. The monosodium glutamate-treated group was given aqueous solution of monosodium glutamate at 5 g/kg body weight and concentration of 500 mg/ml; while rats in the control group were administered distilled water throughout the eight weeks period of the experiment. Feed and water were provided ad libitum to both groups. Four rats from each group were humanely sacrificed weekly and blood sample routinely collected for haematological analysis. The results showed that total red blood cell counts, packed cell volume, haemoglobin concentration, neutrophil and total white blood cell counts decreased significantly (P < 0.05) while lymphocyte counts were increased. The mean corpuscular volume and mean corpuscular haemoglobin concentration of the two groups were similar (P > 0.05). It was concluded that sub-chronic oral monosodium glutamate administration could lead to adverse effects on the haematological profiles of rats which may ultimately result in anaemia.

Keywords: *Haematology, Monosodium glutamate, Wistar rats.*

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INTRODUCTION

Monosodium glutamate (MSG) is a naturally occurring sodium salt of glutamic acid which was initially synthesized from wheat gluten but now produced in commercial quantities by bacterial fermentation.

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Monosodium glutamate is marketed in Nigeria as *Ajinomoto;* other trade names include *Vetsin*, *Ac'cent* and *Tasting powder* [1]. It is composed of white colorless and odorless crystals that exist in two forms called enantiomers, although only the L forms are used as seasoning agents [1]. Monosodium glutamate has been documented as one of the most extensively researched food additives in the world [2]. Results of studies continue to support the findings that at levels normally consumed as a food additive, MSG is safe for the general population [3].

Assessment of haematological parameters has been used in determining the extent of deleterious effects on blood constituents of animals [4]. Evaluation of haematological parameters has also been used to explain blood related effects of other chemical compounds or plant extracts [5]. This is because blood plays roles in physiological, nutritional and pathological states of an organism [6]. Under normal physiologic conditions, the concentrations of blood components and metabolites may oscillate within a narrow range, whereas wide variations denote incidences of pathologic conditions [7]. Despite the numerous beneficial effects of MSG, literature is still full of controversies, hence the need for further research so as to have more scientific facts on the safety of this important food additive.

MATERIALS AND METHODS

Experimental animals

Sixty-four adult male Wistar rats weighing 170 to 230 g procured from the Experimental Animals Unit, Department of Human Anatomy, Faculty of Medicine, Ahmadu Bello University, Zaria, Nigeria were used for the study. They were housed in aluminum cages covered with wire mesh, under ambient temperature (24℃-27℃) and humidity in the Department of Veterinary Pathology of the same University. The rats were fed pelletized commercially growers feed (Vital feed®, Grand cereals Jos, Nigeria). Water was provided *ad libitum*.

Experimental design

After two weeks of acclimatization, the rats were divided into two groups of 32 rats each; MSG-treated and non-MSG-treated (control) groups. The MSG-treated group was orally administered aqueous solution of MSG daily at 5 g/kg body weight, while the non-MSG-treated group was given distilled water throughout the experimental period of 8 weeks.

Preparation and administration of monosodium glutamate

Food grade MSG (Ajinomoto® brand, containing 99+ % of MSG; marketed by West African Seasoning Company, Nigeria) in dry form was used for the experiment. Aqueous solution of MSG was prepared by dissolving 16 g in 32 ml of distilled water to obtain a concentration of 500 mg/ml. This procedure was carried out few minutes prior to the administration and the rats were dosed by oral gavages every 24 hours, using a graduated syringe and a stainless steel intubation cannula.

Sample collection and analyses

Four rats were humanely sacrificed weekly from each experimental group. Two millilitres of blood were collected and dispensed into EDTA sample bottles for haematological analyses at the Clinical Pathology Laboratory, Department of Veterinary Pathology, Ahmadu Bello University, Zaria, Nigeria.

Packed cell volume (PCV) was determined using the microhaematocrit method while erythrocytes (RBC) and leukocytes (WBC) were enumerated using the Neubaur haemocytometer [8]. Thin blood smears stained with Giemsa stain were used to enumerate differential leukocytes [9].

Data Analysis

Data obtained from the study were summarized as means ± standard errors of means and analyzed using Graph-pad prism version 5.00 [10]. Differences between the two groups were tested using students't-test and values of $P \leq 0.05$ were considered significant.

RESULTS

Table 1 shows the effects of MSG on haematological parameters of the experimental rats. The PCV, RBC, Hb, and neutrophil levels were significantly ($P < 0.05$) reduced in the MSG-treated rats compared with the untreated (control) group while the white blood cell (WBC) counts, mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH) levels remained unchanged ($P > 0.05$). On the other hand, lymphocyte counts were raised significantly ($P < 0.05$) in the MSG-treated rats.

The mean PCV in the MSG treated rats decrease from week 1 value of 41 $\pm$ 4.8 to 26 $\pm$ 3.7 % at week 8 post treatment. The decreases were only significant ($P < 0.05$) at weeks 2, 3, 5 and 7. The mean RBC counts were decreased following treatment with MSG and the decrease became significantly ($P < 0.05$) lower than those of the untreated group from week 6 post treatment. Mean haemoglobin concentration significantly decreased ($P < 0.05$) in the MSG-treated rats in a similar pattern as the RBC, when compared with the control. The MCV and MCH levels remained unchanged in both treated and control groups of rats ($P > 0.05$).

The mean WBC counts were consistently lower in the MSG-treated rats than in the control although the differences were not statistically significant ($P > 0.05$). There were significant ($P < 0.05$) decreases in mean neutrophil counts of the MSG-treated rats at weeks 3, 4 and 6 post-treatment. Compared to the control group, the lymphocyte counts of the MSG-treated rats increased significantly ($P < 0.05$) from week 2 post-treatment till the termination of the experiment in week 8.

DISCUSSION

The MSG-induced decrease in PCV conformed with the report of Orooba *et al*. [11] who attributed the decrease to possible MSG induction of generation of reactive oxygen species, which caused lysis of RBC. Ibukun *et al.* [12] recorded similar results with a lower dose of MSG than that used in the present study and attributed the decrease in PCV to the direct toxic effect of MSG on the haematopoietic stem cells of the bone marrow that may have affected erythropoiesis. Additionally, George and Kumaran, [13] implicated the effect of MSG-induced renal cortical necrosis to the observed decrease in PCV; considering the endocrine role of the kidney in erythropoiesis.

The remarkable decrease in RBC count in the MSG-treated rats further suggests that MSG could have reduced the life span of RBCs in the blood resulting from direct toxic effects [14]. This might also have been mediated through its deleterious effect on the haemopoietic stem cells in the bone marrow [15]. It has also been suggested that MSG could induce oxidative stress in the bone marrow of the animals resulting in the formation of micro nucleated polychromatic erythrocytes [11].

Similarly, the observed decrease in haemoglobin in the MSG-treated rats could be attributed to lysis of RBC by exposure effect of MSG [13]. This may not be unconnected to the observed decreases in both RBC and PCV in this and earlier studies. An increase in MCV indicates the presence of macrocytic cells [15] which may be seen in pernicious (normochromic) anaemia and megaloblastic (hypochromic) anaemia while increase in MCH were observed in macrocytic anaemia [16]. The relative increases in the mean values of the MCV and MCH in the MSG-treated rats agree with the findings of George and Kumaran [13].

The reduction in WBC counts in the MSG-treated rats could be due to metabolic changes induced by the MSG, thus reducing immunologic function while increasing the risk of infection [17]. Other mechanisms of MSG toxicity such as cell lyses and bone marrow effect could also contribute to the observed decrease in WBC counts.

Table 1. Means (± SEM) of haematological parameters in MSG-treated and control adult male Wistar rats

Indices	Group	Weeks of Experiment							
		1	2	3	4	5	6	7	8
PCV (%)	Treated	41 ± 4.8	35 ± 0.82*	32 ± 1.1*	32 ± 2.0	29 ± 0.91*	29 ± 0.41	28 ± 2.00*	26 ± 3.70
	Control	41 ± 1.6	48 ± 2.90	50 ± 2.0	41 ± 2.4	47 ± 3.30	41 ± 2.50	43 ± 2.00	38 ± 0.41
Hb (g/dl)	Treated	14 ± 1.60	11 ± 0.71*	10 ± 0.44*	10 ± 0.51	9.8 ± 0.80*	9.1 ± 0.14*	8.8 ± 0.64*	6.2 ± 0.87*
	Control	14 ± 0.59	15 ± 0.61	15 ± 0.48	13 ± 1.20	14 ± 0.94	14 ± 0.36	14 ± 0.61	13 ± 0.34
RBC	Treated	7.0 ± 0.31	6.4 ± 0.46	6.1 ± 0.37	5.2 ± 0.22	5.0 ± 0.18	4.4 ± 0.25*	3.7 ± 0.29*	3.7 ± 0.55*
($x10^{12}$/L)	Control	7.5 ± 0.28	7.6 ± 0.39	7.5 ± 0.50	6.5 ± 0.60	6.1 ± 0.13	7.1 ± 0.60	7.0 ± 0.34	7.3 ± 0.38
MCH (pg)	Treated	20±2.3	20±0.4	21±0.8	20±0.4	23±1.9	21±1.4	24±0.80	18±3.30
	Control	18±0.4	17±0.8	17±1.5	19±0.5	20±1.4	20±1.3	20±1.70	18±1.40
MCV (fl)	Treated	59±7.1	64±5.2	69±7.9	65±4.6	77±6.6	64±3.2	76±2.60	73±12.0
	Control	52±2.2	56±4.8	54±4.8	61±3.0	59±2.1	59±1.2	61±0.63	53±3.0
WBC	Treated	13 ± 2.1	12 ± 3.0	12 ± 1.3	11 ± 3.0	9.8 ± 0.82	6.7 ± 0.48	6.3 ± 0.96	4.5 ± 1.0
($x10^9$/L)	Control	14 ± 1.1	14 ± 1.3	15 ± 2.4	14 ± 0.82	13 ± 1.70	13 ± 0.95	12 ± 0.89	12 ± 1.10
Neutrophil	Treated	0.20 ± 0.00	0.27 ± 0.02	0.27 ± 0.03	0.2 ± 0.03*	0.2 ± 0.04*	0.16 ± 0.03*	0.13 ± 0.02	0.09 ± 0.01
($x10^9$/L)	Control	0.24 ± 0.02	0.41 ± 0.03	0.32 ± 0.06	0.3 ± 0.03	0.31 ± 0.03	0.39 ± 0.03	0.31 ± 0.01	0.27 ± 0.01
Lymph.	Treated	0.6 ± 0.06	0.6 ± 0.01*	0.7 ± 0.01*	0.7 ± 0.03*	0.7 ± 0.01*	0.8 ± 0.01*	0.8 ± 0.03*	0.9 ± 0.0*
($x10^3$/µl)	Control	0.6 ± 0.02	0.5 ± 0.02	0.5 ± 0.01	0.5 ± 0.05	0.5 ± 0.02	0.5 ± 0.02	0.4 ± 0.04	0.5 ± 0.03

Means (± SEM) of haematological parameters in MSG-treated and control rats; Means with superscript * differs significantly from their corresponding control values ($P < 0.05$). PCV= packed cell volume, Hb= haemoglobin, RBC= red blood cell, WBC= White blood cell, MCH= mean cell haemoglobin, MCV= Mean corpuscular volume.

The significant decrease in neutrophil counts in the MSG-treated rats agrees with Ashaolu *et al.* [15] who reported that MSG has a toxic effect on neutrophils and also exerts deleterious effect on blood production in the bone marrow, especially on the progenitor cells (aplasia). This might also be indicative of the deterioration of immune status in the MSG-treated rat group in response to the toxic effect of MSG. The observed increase in lymphocyte count in the MSG-treated rats could probably be due to considerable increase in granulocytes or a consequence of interaction between MSG and gastrointestinal macrophages when it is perceived as a toxic substance [16].

CONCLUSIONS AND RECOMMENDATION

Alterations in the levels of all the analyzed haematological parameters in the MSG-treated rats at the dose used in this study suggest a possible MSG toxicological effect, thus pointing toward anaemia. Therefore, caution must be observed in feeding on MSG so as to prevent any associated cumulative toxic effect.

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