

Effect of Ethanolic Extract of Pulverized *Mangifera indica* (Mango) Seed Kernel on Some Hematological Parameters in Normal and Monosodium Glutamate-Intoxicated Rats

Anthony Cemaluk C. Egbuonu

Department of Biochemistry, College of Natural Sciences, Michael Okpara University of Agriculture Umudike, Nigeria

***Corresponding Author:** Anthony Cemaluk C. Egbuonu, Department of Biochemistry, College of Natural Sciences, Michael Okpara University of Agriculture Umudike, Nigeria

Abstract: The effect of ethanolic extract of pulverized mango (*Mangifera indica*) seed kernel (MSKE) on some hematological parameters in normal and monosodium glutamate (MSG)-intoxicated rats was investigated, using standard protocols. Higher ($p < 0.05$) red blood cell (RBC) count in MSKE-exposed or in MSG plus high MSKE-exposed rats but non-significantly higher ($p > 0.05$) RBC count in MSG-exposed or in MSG plus low MSKE-exposed group compared to the control. The relative change was highest in the MSKE group compared to rats control (18.36 %) and MSG (14.85 %) rats. The white blood cell (WBC) count in rats in the various treatment groups was higher ($p < 0.05$) compared to that in the control. However, the WBC count relative to control (55.80 %) and to MSG-treated (16.02 %) groups was (in both cases) highest in the rats concomitantly exposed to MSG and high MSKE. The higher packed cell volume (PCV) value compared to the control and MSG-treated rats was significant ($p < 0.05$) in the MSKE-treated rats but not statistically significant ($p > 0.05$) in the rats exposed to MSG together with either low or high concentration of MSKE. Thus, MSKE improved, and at highest tested concentration only mitigated MSG-induced influence on, the studied hematological parameters of rats warranting further studies.

Keywords: Red blood cell, White blood cell, Packed cell volume, anti-anemic, erythropoiesis, leukopoietic

1. INTRODUCTION

Monosodium Glutamate (MSG), a food additive taken together with especially commercially processed foods^[1] could dissociate to glutamate, an amino acid that could enter systemic circulation via related metabolic routes. Reports of MSG-intoxication in animals abound^[2-11]. The non-fully understood biochemical basis of MSG-intoxication heightened research activities on particularly the possibilities of using hitherto agro waste to modulate any adverse effect of MSG in animals^[12-14].

Mango, genus *Mangifera*^[15] and family *Anacardiaceae*^[16] has potential applications in nutrition and medicine owing to the phytoconstituents in its various parts^[6,17-24]. Thus, mango fruit is extensively utilized resulting to the generation of mango seed (hence seed kernel) as waste^[25]. Promising results were obtained in similar studies using mango seed kernel^[14,26,27]. However, to the knowledge of the author, there is little or no literature on the effect of mango seed kernel on the hematological parameters of normal and MSG-intoxicated animals.

These warranted the present study aimed at determining the effect of pulverized ethanolic extract of mango seed kernel on the hematological parameters in normal and monosodium glutamate-intoxicated rats. Blood functions in the maintenance of physiological homeostasis^[28]. Thus, hematological parameters including red blood cell count, white blood cell count and packed cell volume value were of diagnostic value^[29-35]. For instance, red blood cell is involved in the transport of oxygen and carbon dioxide in the body and a reduced red blood cell count implies a reduction in the level of oxygen that would be carried to the tissues as well as the level of carbon dioxide returned to the lungs^[36,37,28].

2. MATERIALS AND METHODS

Sample procurement and identification

A commercial brand of MSG (99 % purity) procured from *Ubani* market, a daily food condiments market in Umuahia, south east Nigeria was used in this study. Chemicals and solvents used in the present study were products of reputable companies procured from reputable chemical dealers in Nigeria. They were used without further purification.

This study was conducted between June and August, 2016. Fresh mango fruits collected from a particular mango tree by the owner were purchased in June, 2016 at *Orie ugba*, a fruit and foodstuff market in Umuahia, Abia state, Nigeria. The mango fruits were identified and authenticated as *Mangifera indica* (German variety) by a taxonomist in the department of Plant Science and Biotechnology, Michael Okpara University of Agriculture Umudike, Nigeria.

Sample preparation and extraction

The mango fruits were thoroughly washed using tap water and the fleshy part of each of the fruits was removed to obtain the seed stones which were sun-dried for three days. The seed kernels were obtained by carefully cutting the sun-dried seed stones with clean table knife and removing the stony seed coat. The kernels thus obtained were chopped with home choice knife into bits and sun-dried for one week (seven days). The dried mango seed kernels were pulverized using Arthur Thomas Laboratory Mill, Crypto Model, USA. The pulverized mango seed kernel was extracted with ethanol (98 %) as described earlier^[38] and stored in a refrigerator at 4 °C until used.

Animal study

Animal procurement and exposure groups

Twenty adult male albino rats (weight range, 104-170 g) were used in this study as procured from the animal house of the Faculty of Biological Sciences, University of Nigeria, Nsukka. The animals were randomized based on weight (after acclimatization for 2 weeks) to five experimentation groups with sample size of four rats.

Rats in the control group were sham-dosed with distilled water (without either the extract or MSG) while rats in the MSG group were fed intoxicating dose (8000 mg/kg body weight) of MSG based on previous studies^[10,14,26,27, 39-42]. Rats in the extract group were fed mango seed kernel extract at 300 mg/kg body weight while rats in the MSG + low extract group were concomitantly fed the mango seed kernel extract (200 mg/kg body weight) and intoxicating dose of MSG (8000 mg/kg body weight) whereas rats in the MSG + high extract group were co-administered 400 mg/kg body weight of the mango seed kernel extract and intoxicating dose of MSG (8000 mg/kg body weight). The exposure was *per oral* and daily for 14 days.

Sacrifice, blood sample collection and preparation

After 2 weeks (14 days) exposure, the rats were sacrificed the next day after overnight fast *via cardiac* puncture and the blood sample of the respective rats was collected individually into clean polystyrene tubes. The blood samples thus collected were stored in deep freezer for the determination of some hematological parameters viz: red blood cells (RBC), white blood cells (WBC) and packed cell volume (PCV).

Ethical consideration

This study which is a continuation of our line of studies on the evaluation of biochemical effects of mango seed kernel extract on normal and monosodium glutamate-challenged experimental models considered and adhered to the standard ethical use of experimental animals. Throughout out the experimentation (acclimatization and exposure periods), all rats were housed at 25 °C in stainless steel cages under normal daylight/dark cycle and humid tropical conditions. The rats were allowed free access to rat feed (Vital feed, Jos Nigeria) and tap water, and generally received humane care in accordance with the guidelines of the National institute of Health, USA for ethical treatment of laboratory animals as approved by the various (departmental and college) ethical committees of Michael Okpara University of Agriculture Umudike, Nigeria.

Determination of the hematological parameters of rats' whole blood

The RBC count was determined by the haemocytometric method described by Ochei and Kolhatkar [43]. The blood specimen was diluted to 1:200 with RBC diluting fluid (Sodium citrate) and the red blood cells were counted under high power (40X) objective using a counting chamber. The number of cells was calculated and presented as the number of red blood cells per whole blood.

The total leucocyte or WBC count was determined by haemocytometry based on the method described by Ochei and Kolhatkar [43]. An aliquot (0.02 ml) of whole blood was added to 0.38 ml of diluting fluid (Acetic acid, tinged with gentian violet) and mixed. The counting chamber was charged with the well-mixed diluted blood (after discarding the first five drops) with the aid of a pipette. White blood cells were allowed to settle in a moist chamber for 3 minutes, counted under low power microscope using a counting chamber and calculated using the relation:

$$\text{Total WBC count} = \text{Num of Cells} \times \text{diluted factor} \times \text{area counted} \times \text{depth of fluid}$$

The number of cells was reported as total WBC per whole blood.

The PCV of the samples was estimated as described by Ochei and Kolhatkar [43]. Blood sample was taken with anti-coagulated (heparinised) capillary tube, cleaned and sealed with plasticine. The filled tubes were placed in the microhematocrit centrifuge, spun at 12,000 rotor per minute for 5 minutes and placed in a specially designed scale. The PCV (%) was calculated from the relation:

$$\text{PCV (\%)} = \frac{\text{Packed RBC column height} \times 100}{\text{Total blood volume height}}$$

Calculation of change relative to control

The calculation of change relative to control was as developed earlier [44] and severally used [5,6, 14, 26, 27,39,45,46], using the relation:

$$\text{Change relative to K (\%)} = \frac{(V - K) \times 100}{K}$$

where *K* represented the constant group hence constant value and *V* represented the variable groups hence variable values.

Statistical analysis

All numerical data collected were analyzed by one way analysis of variance (ANOVA) using the statistical package for Social Science (SPSS version 17; SPSS Inc., Chicago.IL.,USA). Results were presented as means ± standard error of the mean (Mean ± SEM) at 95 % significance level (p < 0.05).

3. RESULTS

Table 1. Effect of pulverized mango (*M. indica*) seed kernel extract, MSK, on red blood cell, RBC, count ($\times 10^{12}/L$) of normal and monosodium glutamate-challenged rats' whole blood

Groups	RBC ($\times 10^{12}/L$)	Change relative to the Control (%)	Change relative to MSG group (%)
Control (distilled water 2 ml/kg b.w)	245.00±2.88	0.00	-2.97
MSG 8000 mg/kg b.w)	252.50±4.79	+3.06	0.00
Extract 300 mg/kg b.w)	290.00±5.77	+18.36	+14.85
MSG 8000 mg/kg b.w) + low Extract 200 mg/kg b.w)	255.00±5.00	+4.08	+0.99
MSG 8000 mg/kg b.w) + high Extract 400 mg/kg b.w)	270.00±5.77	+10.20	+6.93

Value presented as mean ± SEM of sample size, n = 4 rats. + denotes higher by; - denotes lower by. Significant difference at p<0.05.

The result as shown on Table 1 revealed higher (p<0.05) RBC count in rats exposed to MSK alone or to MSG plus high extract as compared to the control. However, the higher RBC count in the rats exposed to MSG alone or to MSG plus low extract group was not significant (p>0.05) compared to the control. The relative change was highest in the MSKE group compared to rats in the control (18.36 %) or in the MSG (14.85 %) groups.

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Table 2. Effect of pulverized mango (*M. indica*) seed kernel extract, MSK, on white blood cell, WBC, count ($\times 10^9/L$) of normal and monosodium glutamate-challenged rats' whole blood

Groups	WBC ($\times 10^9/L$)	Change relative to the Control (%)	Change relative to MSG group (%)
Control (distilled water 2 ml/kg b.w)	22.40 \pm 0.75	0.00	-25.53
MSG 8000 mg/kg b.w)	30.08 \pm 0.47	+34.28	0.00
Extract 300 mg/kg b.w)	34.45 \pm 0.33	+53.79	+14.52
MSG 8000 mg/kg b.w) + low Extract 200 mg/kg b.w)	30.85 \pm 1.12	+37.72	+2.55
MSG 8000 mg/kg b.w) + high Extract 400 mg/kg b.w)	34.90 \pm 0.60	+55.80	+16.02

Value presented as mean \pm SEM of sample size, n = 4 rats. + denotes higher by; - denotes lower by. Significant difference at $p < 0.05$.

From Table 2, the WBC count in rats exposed to the various treatment groups was higher ($p < 0.05$) compared to control. However, the observation relative to control was highest in the rats concomitantly exposed to MSG and high extract (55.80 %) followed by that in the rats exposed to the Extract alone (53.79 %) and MSG plus low extract (37.72 %) while the least was in the rats exposed to MSG alone (34.28 %). The observations relative to MSG exposure was highest in the group co-exposed to MSG and high extract concentration.

Table 3. Effect of pulverized mango (*M. indica*) seed kernel extract, MSK, on packed cell volume, PCV (%) of normal and monosodium glutamate-challenged rats' whole blood

Groups	PCV (%)	Change relative to the Control (%)	Change relative to MSG group (%)
Control (distilled water 2 ml/kg b.w)	58.75 \pm 0.48	0.00	0.00
MSG 8000 mg/kg b.w)	58.75 \pm 0.75	0.00	0.00
Extract 300 mg/kg b.w)	64.75 \pm 0.48	+10.21	+10.21
MSG 8000 mg/kg b.w) + low Extract 200 mg/kg b.w)	60.00 \pm 0.82	+2.12	+2.12
MSG 8000 mg/kg b.w) + high Extract 400 mg/kg b.w)	62.50 \pm 0.96	+6.38	+6.38

Value presented as mean \pm SEM of sample size, n = 4 rats. + denotes higher by; - denotes lower by. Significant difference at $p < 0.05$.

As depicted on Table 3, the higher PCV value compared to the control and MSG treated rats was significant ($p < 0.05$) in the Extract-treated rats but not statistically significant ($p > 0.05$) in the rats exposed to MSG together with either low or high extract concentration.

4. DISCUSSION

Blood functions in the maintenance of physiological homeostasis [28]. Thus, hematological parameters including red blood cell count, white blood cell count and packed cell volume value were of diagnostic value [29-34]. This study investigated the effect of ethanolic extract of pulverized mango (*Mangifera indica*) seed kernel (MSKE) on some hematological parameters in normal and monosodium glutamate (MSG)-intoxicated rats, using standard protocols. In addition, the corresponding change relative to controls (normal control and monosodium glutamate intoxicated/negative control) was computed to provide a better understanding of the magnitude and statistical significance (relative change of up to and above ten percent) of any seemingly negligible numeric change between the compared groups.

The higher ($p < 0.05$) RBC count in rats exposed to MSKE alone or to MSG plus high MSKE indicated the overriding capacity of MSKE either alone or together (at the highest test dose) with MSG, as against either the control or MSG, to improve the RBC count, enhance physiological transport of oxygen and carbon dioxide and prevent anemia, perhaps *via* improved erythropoiesis (RBC synthesis) or diminished RBC destruction in the rats. Increased RBC count suggested potential stimulation of erythropoietin releases from the kidneys with a resultant increase in RBC synthesis (erythropoiesis) or diminished destruction that could result to polycythemia (instead of anema) and enhanced oxygen-carrying/delivery capacity via the blood in the rats [28,36,37,47-49]. This further indicated higher MSKE-

related benefit and protection on the hematology of the rats [47,49-51]. However, the higher but non-significant ($p>0.05$) RBC count in the rats exposed to MSG alone or to MSG plus low MSKE groups compared to the control indicated that MSG at the tested concentration either alone or together with low MSKE concentration did not cause measureable destruction of matured RBC's or change in the rate of production of RBCs (erythropoiesis) due probably to inability to stimulate erythropoietin (the humoral regulator of RBC production) release in the kidney [52]. The relative change was highest in the MSKE group compared to rats in the control (18.36 %) or in the MSG (14.85 %) groups seemingly indicating overriding benefit of MSKE on RBC production resulting to the observed highest count in MSKE-treated rats. Contrary to the present study, MSG intoxication caused a significant ($p<0.05$) reduction in RBC count in the rats as compared to control [10,53], indicating inconsistent adverse response on the RBC count of rats following MSG-intoxication.

The WBC count in rats exposed to the various treatment groups was higher ($p<0.05$) compared to that of the control. However, the observation relative to control (55.80 %) and exposure to MSG (16.02 %) was highest in the rats concomitantly exposed to MSG and high MSKE. This suggested probable synergistic interactive potential of MSKE and MSG to improve the WBC count, and perhaps the overall immunity of the rats. Higher white blood cells suggested enhanced immune system activity hence diminished possibility of infection of the rats [48,54] or probably a manifestation of increased WBC production due to increased defense mechanism activity in response to toxic assault in the rats. Thus, the observed elevated WBC count reflected possible increased leukopoietic and immunomodulatory potentials of MSKE in rats [55,56]. White blood cell (WBC) count measures the number of white blood cells in ones blood and higher value reflected higher capacity to enhance the immune system [37,50,57,58]. The observation of lower WBC count in MSG-exposed rats compared to control in a similar study [42] was not significant and the difference may be due to different methods used in the determination. Contrary to the present study, MSG intoxication caused a significant ($p<0.05$) reduction in WBC counts in the rats as compared to control [10,53], indicating inconsistent adverse response on the WBC of rats exposed to intoxicating concentration of MSG. The implication of altered immune response following reduced WBC count have been reported [59-61], thus further studies are warranted to determine the basis for the high WBC count observed in this study.

The higher PCV value compared to the control and MSG treated rats was significant ($p<0.05$) in the MSKE-treated rats but not statistically significant ($p>0.05$) in the rats exposed to MSG together with either low or high MSKE concentration. This indicated that the overriding potential of MSKE to improve the PCV value could not affect the MSG-induced effect on the PCV value of the rats. Increased PCV value suggested potential increase in RBC production or erythropoiesis resulting to polycythaemia [47,48,51,62,63] as noted in this study; decreased thrombotic events or cardiovascular mortality [50]; higher protection on the integrity of erythrocytes by boosting their volume percentage in the blood [50]; improved transport of oxygen and absorbed nutrients [28,30]. The PCV value of the control in this study compared with that in earlier studies [48,62]. In agreement with the present study, MSG intoxication did not alter the PCV value in the rats as compared to control [10,53,64,65], indicating a consistent response on the PCV value of rats exposed to intoxicating dose of MSGs.

5. CONCLUSION

Thus, MSKE improved, and at highest tested concentration only mitigated MSG-induced influence on, the studied hematological parameters of rats. The possibility that the MSKE could increase red blood cells production in the bone marrow or posses anti-anemic properties warranted further studies hence are recommended.

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Citation: A. C. C. Egbuonu, " *Effect of Ethanolic Extract of Pulverized *Mangifera indica* (Mango) Seed Kernel on Some Hematological Parameters in Normal and Monosodium Glutamate-Intoxicated Rats* ", *International Journal of Research in Environmental Science*, vol. 4, no. 1, p. 47-55, 2018. <http://dx.doi.org/10.20431/2454-9444.0401006>

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