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Effect of *Mucuna Sloanei* Seed Ethanol Extract on Some Haematological Indices in Monosodium Glutamate Intoxicated Albino Rats

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ABSTRACT
Some food additives have been known to disrupt the integrity of blood cells while some medicinal plants are presumed to combat these deleterious effects and *Mucuna sloanei* is one of such plants. In this study, we evaluated the blood profile of Monosodium glutamate (MSG) intoxicated albino rats treated with ethanolic seed extract of *Mucuna sloanei*. Thirty (30) female albino rats were divided into 6 groups, n= 5. Group 1 received 1 ml of clean water (normal control); Group 2 was intoxicated with MSG only (8000 mg/kg), Group 3 received extract only (400 mg/kg) while Groups 4, 5 and 6 were also intoxicated with MSG at the dose of 8000 mg/kg then treated with different doses of the extract (200, 400 and 800 mg/kg) respectively. They were treated for 28 days and their blood were collected for haematology using standard analytical procedures. Blood parameters evaluated include RBC, PCV, WBW, Hb, platelet and RBC indices. The results showed that rats intoxicated with MSG had the least values of erythrocytes 5.89±0.07($x10^{12}$/L), haematocrit, 45.33±1.45%, haemoglobin, 12.44±0.33 g/dl and leucocytes, 12.44±0.33 ($x10^{9}$/L) while the groups given only the extract together with those given both MSG with 800 mg/kg of the extract had significantly ($P<0.05$) higher values of the same parameters. Thus, *Mucuna sloanei* seed extract (MSSE) at the dose of 800 mg/kg b.w., can ameliorate the deleterious effects of MSG on the blood cells.

Keywords: Haematology, Monosodium glutamate, Mucuna sloanei, Intoxication, Albino rats

NTRODUCTION

Blood is a fluid made up of specialized cells enveloped in cell membranes with specific functions (Schwartz and Conley, 2016). Blood can easily be affected by various factors including metabolic and chemical agents like monosodium glutamate (MSG) which acts as an oxidant to the cell membranes including that of blood cells. Monosodium glutamate (MSG) (Fig 1) is commonly consumed unknowingly as flavor enhancer or food additive added in different food seasonings all over the world aimed at improving the taste of food with an umami taste that is neither sweet nor sour (Alao *et al*., 2020).

Monosodium glutamate (MSG) is sold as crystallized sodium salt of glutamic acid containing 78% of glutamic acid with 22% of sodium and water (Samuels, 1999). When consumed in excess, it is known to produce some adverse effects in humans and experimental animals called Chinese restaurant syndrome characterized by headache, chest discomfort and facial flushing (Schaumberg *et al*., 1969).

Fig. 1: Monosodium glutamate crystals in a pack

It has also been reported to produce oxygen free radicals causing oxidation which attack the membrane lipids, leading to disruption of the integrity of biological membranes, especially the actively dividing cells, blood cells inclusive (Singh and Ahluwalia, 2003). Some plants are known to possess medicinal properties and *Mucuna sloanei* seeds is one of them.

Fig. 2: Monosodium glutamate crystals

Mucuna sloanei (Figure 2) is a legume found in the tropical and sub-tropical regions of the world. It is known to originate from Asia and was introduced into the western hemisphere (Obochi *et al*., 2007). *Mucuna sloanei* is a high climbing woody plant of the vine species which produces pods containing buoyant seeds that are easily dispersed by the sea giving it the name "sea beans". It is also called the "hamburger bean" because of the three layer colour of the seed or "horse eye".

Fig. 3: *Mucuna sloanei* seeds

It is commonly called "ukpo" by the Igbos, "karasuu" by the Hausas, and "verepe" by the Yorubas (Nwosu, 2011; Obochi *et al*., 2007) The seed is cooked and eaten as a vegetable when they are young but when mature, is used as soup thickeners. The cooking is essential since the seeds are potentially toxic and contain L-DOPA in them known to be a potent precursor of the brain neurotransmitter, dopamine (Nagatsua and Sawadab, 2009). It has also been reported to have medicinal properties, sedative effects as well as blood agglutinating properties due to the presence of lecithin present in the seeds (Obochi *et al*., 2007). Owing to the oxidant effect of MSG on biological membranes, we seek to determine the effect of ethanol extract of M*ucuna sloanei* seeds on blood profile of MSG intoxicated wistar rats.

MATERIALS AND METHODS

Plant material

Mucuna sloanei seeds were obtained from Amokwo Ugwu Nkpa in Bende LGA in Abia State and authenticated at the Department of Crop Science, College of Crop and Soil Science, Michael Okpara

University of Agriculture, Umudike, Abia State, Nigeria. A voucher specimen was deposited in the herbarium of Veterinary Physiology and Pharmacology laboratory, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria (MOUAU/VPP/2019/045).

Preparation of the extract

The seeds were toasted, de-hulled and ground. A portion (500 g) of the powder was extracted in 2.0 litres of 98 % ethanol for 24 hours and agitated intermittently every 3-hours using cold maceration method, after which it was sieved using Whatman's® filter paper (No 4.) and dried using hot air oven set at 40°C extract was stored as MSSE in the refrigerator at 4 °C until use. A calculated amount of the extract was weighed and reconstituted in distilled water to give the required different doses of 200, 400 and 800mg/kg body weight and given per os using oral gavage daily for 28 days. The acute toxicity study (LD_{50}) showed that *Mucuna sloanei* extract was not toxic with LD_{50} of 4000mg/kg b.w. (Oguwuike *et al*., 2017)

Monosodium glutamate intoxication

Monosodium glutamate (Ajinomoto®) was procured from Ubani market, Umuahia, Abia State. A toxic concentration of 500 mg/ml was formulated to be given to all the groups at the dose of 8000 mg/kg except Group I (Plain water only) and Group III (Extract only) per os using oral gavage daily for 28 days.

Experimental Animals

Thirty female albino rats (*Rattus norvegicus*) of Wistar strain with an average weight of 113 g were obtained from the Animal house of Veterinary Physiology and Pharmacology, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria. They were housed in aluminum cages under standard conditions (temperature 26 °C; photoperiod- 12 hours day light and 12 hours darkness; humidity: 45-50%) and allowed to acclimatize for two weeks before the experiment. They were given free access to feed (Grower pellets from Vital Feeds®, Nigeria) and clean tap water. All the animals received humane treatment in accordance with the guidelines of the National Institute of Health, USA for ethical treatment of laboratory animals.

Experimental design

The thirty (30) female albino rats were randomly divided into six groups of five rats per group and dosed once per day for 28 days as follows: Group A received 1ml of clean water as normal control; Group B received 8000 mg/kg b.w of MSG only (negative control); Group C received 400 mg/kg b.w of the extract only and Groups D-F received 8000 mg/kg b.w of MSG with different doses of the extract (200, 400 and 800 mg/kg b.w) respectively. Dosing was done *per os*. On day 29, the animals were all sacrificed by inhalation anaesthesia using chloroform.

Blood collection and analysis

Blood samples were collected from the rats in all the groups from the heart through abdominal dissection under chloroform anaesthesia. Blood for haematological analysis were collected in 5 ml vials containing potassium EDTA as anticoagulant Haematological analysis made include red blood cell count (RBC), packed cell volume (PCV), haemoglobin levels (Hb), total white blood cell count (WBC), platelet count, mean cell volume (MCV), mean cell haemoglobin levels (MCH) and mean cell haemoglobin concentration (MCHC).

RBC and WBC counts were done using haemocytometer method (Thrall and Weiser, 2002) while PCV count was done using microhaematocrit method (Bull *et al*., 2001). Haemoglobin levels were determined using cyanomethaemoglobin method (Higgins, 2016). Red blood cell indices were calculated using standard formulae.

Statistical analysis

The data obtained from blood analysis were subjected to statistical analysis using one way analysis of variance (ANOVA) with significance accepted at ($P<0.05$). Post Hoc multiple comparisons for

differences within groups were established using least significant difference (LSD). Results are presented as Mean ± S.E.M.

RESULTS

Red blood cell (RBC)

Monosodium glutamate caused a significant (P<0.05) reduction in Red blood cell (RBC) count (5.89±0.07 x10^{12}/L) of rats. Rats given MSG and extract (800 mg/kg) had significantly higher red blood cell count when compared with the control (8.00±0.05 x10^{12}/L), while those given only the extract had an RBC count of 8.23±0.33 x10^{12}/L-(Table 1).

Packed cell volume (PCV) count

There was a significant (P<0.05) decrease in the PCV (45.33±1.45 %) of the group fed with MSG only when compared to the normal control (57.33±3.18%). However, rats given MSG and extract at 800 mg/kg had significantly higher PCV value of 55.33±1.76 % than the negative control (P<0.05) (Table 1).

Haemoglobin Hb concentration

The haemoglobin concentration (12.44±0.33g/dl) of the group fed with MSG was significantly (P<0.05) reduced when compared to that of the normal control (14.65±0.57 g/dl). Rats given MSG and the extract had significantly higher haemoglobin concentration 15.43±0.37 g/dl than the rats in the untreated group (Table 1).

Total White blood cell (WBC) count

The group fed with MSG had a significantly (P<0.05) reduced total WBC count (12.44±0.33 x10^{9}/L) when compared to those of the control (14.27±0.90 x10^{9}/L) and the group fed with the extract only (14.04±0.40 x10^{9}/L). Rats given MSG and treated with 800 mg/kg of extract had white blood cell count of 15.43±0.37 x10^{9}/L (Table 1).

Platelet

Rats intoxicated with MSG had a reduced platelet count (484.67±9.13 x10^{9}/L) which was significantly (P<0.05) different from the control (556.33±31.31 x10^{9}/L) and the group fed with the extract only (561.00±50.33 x10^{9}/L) (Table 1).

Mean cell volume (MCV)

The MCV of the group fed with only MSG was significantly (P<0.05) higher (76.93±1.51*f*l) than those of the normal control (71.59±3.50 *f*l) and the rats given extract only (64.74±1.00 *f*l) (Table 2).

Mean cell haemoglobin (MCH)

The MCH of rats given MSG was significantly (P<0.05) increased (21.13±0.52 pg) when compared to that of the control (18.30±0.58 pg) and the extract only group (17.07±0.38 pg) (Table 2).

Mean cell Haemoglobin concentration (MCHC)

The MCHC (27.48±0.81 g/dl) of the MSG group was also significantly (P<0.05) increased when compared to the control group (25.61±0.45 g/dl) and the extract only group (26.37±0.61 g/dl) (Table 2).

Table 1: Haematological profile of MSG intoxicated albino rats treated with *Mucuna sloanei* seed extract (MSSE).

	MCV (*fl*)	**MCH (pg)**	**MCHC (g/dl)**
Control	71.59±3.50^{ab}	18.30±0.58^{cd}	25.61±0.45^{b}
MSG only	76.93±1.51^{a}	21.13±0.52^{a}	27.48±0.81^{ab}
Extract only (400mg/kg)	64.74±1.00^{c}	17.07±0.38^{d}	26.37±0.61^{ab}
MSG + MSSE (200mg/kg)	73.27±0.74^{a}	19.94±0.22^{ab}	27.91±0.42^{a}
MSG + Extract (400mg/kg)	70.74±1.47^{abc}	19.16±0.50^{bc}	27.22±0.57^{ab}
MSG + Extract (800mg/kg)	66.70±1.81^{bc}	18.61±0.38^{bc}	27.09±0.38^{ab}

Mean±SEM; Means with different superscript letters (a-d) in the same column are significantly different from each other (P<0.05). RBC = Red blood cell; PCV = Packed cell volume; Hb = Hemoglobin; WBC = White blood cell

Table 2: Red blood cell indices of MSG intoxicated albino rats treated with *Mucuna sloanei* seed extract (MSSE)

	RBC (x10^{12}/L)	**PCV (%)**	**Hb (g/dl)**	**WBC (x10^{9}/L)**	**PLATLETS (x10^{9}/L)**
Control	8.00±0.05^{ab}	57.33±3.18^{a}	14.65±0.57^{a}	14.27±0.90^{a}	556.33±31.31^{a}
MSG only	5.89±0.07^{c}	45.33±1.45^{b}	12.44±0.33^{b}	12.44±0.33^{b}	484.67±9.13^{a}
MSSE only (400mg/kg)	8.23±0.33^{a}	53.33±2.60^{a}	14.04±0.40^{a}	14.04±0.40^{a}	561.00±50.33^{a}
MSG + Extract (200mg/kg)	7.44±0.27^{b}	52.67±2.33^{a}	14.25±0.52^{a}	14.25±0.52^{a}	532.67±6.01^{a}
MSG + Extract (400mg/kg)	7.55±0.07^{b}	55.33±0.33^{a}	15.06±0.29^{a}	15.06±0.29^{a}	533.67±25.14^{a}
MSG + Extract (800mg/kg)	8.29±0.04^{a}	55.33±1.76^{a}	15.43±0.37^{a}	15.43±0.37^{a}	542.00±7.81^{a}

Heamatology values= Mean±SEM; Means with different superscript letters (a-d) in the same column are significantly different from each other (P<0.05). MCV = Mean corpuscular volume; MCH = Mean corpuscular hemoglobin; MCHC = Mean corpuscular hemoglobin concentration;

DISCUSSION

Monosodium glutamate induced oxidative stress is evident in significant reductions in RBC and WBC counts, as well as in packed cell volume and haemoglobin concentration (Table 1). Oxidative stress is an imbalance initiated by excessive production of free radicals leading to oxidative damage to biomolecules found in cells, especially on cell membranes. (Minibayeya *et al*., 2009). Studies have shown that MSG induces its oxidative damage when combined with food by increasing the foods palatability, hence increasing food consumption, this leads to increased oxidative phosphorylation by the electron transport system, where biochemical free radicals such as superoxides, hydrogen peroxides and hydroxyl radicals are produced (Diniz *et al*., 2002; Diniz *et al*., 2004; Diniz *et al*., 2005). Therefore, in MSG intoxication, MSG is broken down to sodium and glutamate which enters the Citric acid cycle through its intermediate alpha ketoglutarate. This Citric acid cycle intermediate produce electron carriers that are fed into the electron transport chain (ETC), which when in excess will bombard the ETC with electron carriers and since oxygen is used to fuel

oxidative metabolism will lead to excess production of these free radicals and reactive oxygen species (ROS). This excess production of free radicals and ROS will lead to an imbalance between oxidant and antioxidant systems leading to oxidative stress that can affect cells and the membranes in the body (Feuers, 1998).

Oxidative stress is usually ameliorated by endogenous and exogenous antioxidants. *Mucuna sloanei* extract induced a dose-dependent reversal of the reductions in blood parameters caused by MSG intoxication, with the highest dose causing the highest increases in RBC, WBC, Haemoglobin and packed cell volume (Table 1). A previous study using toasted *Mucuna sloanei* seeds reported reductions in haemoglobin (Hb), the packed cell volume and the red blood cells possibly as a result of the short period of toasting the seeds.

Rats intoxicated with MSG had leucopenia, due largely to a possible neutropenia, which was ameliorated by MSSE. Previous work by Ashaolu *et al*., 2011 showed that all doses of MSG administered showed significant decrease (A1-19.76%, A2-56.80%, B1-38.89%, B2- 66.67%) in neutrophil count and the decrease is higher in the groups that received treatment for longer days. Thus, MSG has a toxic effect on the neutrophils, which leads to formation of toxic or degenerate neutrophils in the blood.

The increase in platelet count seen in rats dosed with the extract could be as a result of the modulatory effect of anti-oxidants present in the extract on platelet function, an indication of its vaso-protective effect. Obochi *et al.,* 2007 reported that the extract has agglutinating properties, thus can be used by individuals suffering from haemophilia (Graham, 2014).

Increased mean cell volume (MCV) and normal mean cell haemoglobin (MCH) seen in rats given only MSG (Table 2) is indicative of a macrocytic and normochromic anaemia (Sembulingham, 2005).This type of anaemia indicates a regenerative nature, and suggests that there was no bone marrow hypoplasia. Therefore, a reversal would be possible and the extract at the highest dose brought about a reversal of the anaemia in the treated rats.

There is also a possibility that MSG destroyed most of the RBCs as seen in low RBC count in the MSG treated group thus increasing the MCH value. Exceptionally low MCH values strongly suggest true iron deficiency is present. Since the MSG group did not have a low MCV and MCH value, one can rule out the possibility of iron deficiency (Graham, 2014).
In conclusion, MSG intoxication deleteriously affected the blood cells, possibly due to their oxidative damage, while the extract ameliorated those effects and protected the blood cells from oxidative damage by MSG.

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REFERENCES

Alao, O. A., Ashaolu, J. O., Ghazal, O. K. and Ukwenya, V. O. (2010): Histological and biochemical effects of monosodium glutamate on the frontal lobe of adult Wistar rats, *International Journal of Biomedical and Health Sciences*, 6(4): 197-203.

Ashaolu J. O., Ukwenya V. O., Okonoboh A. B., Ghazal O. K., Jimoh A. A. G.(2011) Effect of monosodium glutamate on hematological parameters in Wistar rats. *International Journal of Medicine and Medical Sciences* Vol. 3(6), pp. 219-222

Bull, B. S, Koepke, A., Elkin, S. M and Onno W. van Assendelft (2001). Procedure for determining Packed cell volume by the Microhematocrit method; Approved standard- Third Edition Vol 20, No 18 ISBN 1-56238-413-9

Diniz Y.S, Faine L.A, Galhardi C.M, Rodrigues H.G, Ebaid G.X et al (2005). Monosodium glutamate in standard and high- fiber diets: metabolic syndrome and oxidative stress in rats. *Nutrition*, 21: 749-755.

Diniz Y.S, Fernandes A.A, Campos K.E, Mani F, Ribas B.O, Novelli E.L (2004). Toxicity of hypercaloric diet and monosodium glutamate: oxidative stress and metabolic shifting in hepatic tissue. *Food Chemical Toxicology*, 42: 319-325.

Diniz Y.Z, Faine L.A, Almeida J.A, Silva M.D.P, Ribas B.O, Novelli E.L.B (2002). Toxicity of dietary restriction of fat enriched diets on cardiac tissue. *Food Chemical. Toxicology*, 40: 1892-1899.

Feuers R.J (1998). The effects of dietary restriction on mitochondrial dysfunction in aging. *Ann. NY Academy Science,* 854: 192-200.

Graham, B. (2014) Interpreting blood results. *Introduction to Clinical Biochemistry*. Pp 24-29

Higgins, C. (2016). Urea and the clinical value of measuring blood urea concentration, *acutecaretesting.org/en/articles/urea-and-the-clinicalvalueof-measuring-blood-urea-concentration*.

Minibayeya F, Kolesnikov O, Chasov A, Beckett RP, Lüthje S, Vylegzhanina N, Buck F, Böttger M (2009). Wound-induced apoplastic peroxidase activities: their roles in the production and detoxification of reactive oxygen species. *Plant Cell Environment,* 32(5): 497-508.

Nagatsua T, Sawadab M (2009). L-dopa therapy for Parkinson‟s disease: past, present, and future. *Parkinsonism Related Disorders* 15 (Suppl. 1): S3 – S8.

Nwosu J (2011). The Effect of Storage Condition on the Rheological/Functional Properties of Soup thickner *Mucuna sloanei* (Ukpo). *Researcher* 3:6-10.

Obochi G.O, Malu S.P, Effiom E.O, Bassey G.A (2007). Efficacy and suitability of lectin from Mucuna sloanei seeds extracts as a cell receptor signal inducer. *Research Journal of Biological Science* 2(6):667-669.

Oguwuike F. N., Eluke B. C., Offor C. C., Emenuga V. N., Onubeze D., Ozoekwe C. N.(2017) Study on The Effect of Aqueous Extract of Mucuna sloanei (Ukpo) On Haematological and Haemostatic Mechanisms of Albino Wistar Rats. *Imperial Journal of Interdisciplinary Research (IJIR)* Vol-3, Issue-9, Page 1051

Samuels, A. (1999): The Toxicity/Safety of MSG: A Study in Suppression of Information. *Accountability in Research,* 6(4): 259-310

Schaumberg H. H, Byck R, Gerstl R, Mashman J. H. (1969) Monosodium L-Glutamate: Its pharmacology and role in Chinese restaurant syndrome. *Science* 163: 826- 8.

Schwartz, R. S and Conley, C. L. (2016) Blood Biochemistry. *Encyclopaedia Britannica, Inc.*

Sembulingham K (2005): Essentials of medical Physiology 2nd ed. pp. 46-56

Singh K, and Ahluwalia P. (2003). Studies on the effect of Monosodium Glutamate (MSG) administration on some antioxidant enzymes in the arterial tissue of adult male mice. *Journal of Nutritional Science and Vitaminology (Tokyo)*; 49: 145-8.

Thrall, M.A. and Weiser, M.G., (2002). Haematology. In: C.M. Hendrix (ed), *Laboratory procedures for veterinary technicians,* 4th edition, (Mosby Inc, Missouri), 29–74.

Abbreviations

MSG - Monosodium glutamate,
MSSE - *Mucuna Sloanei* seed extract,
RBC - Red blood cells,
PCV - Packed cell volume,
Hb - Hemoglobin,
WBC - White blood cells,
MCV - Mean cell Volume,
MCH - Mean cell Hemoglobin,
MCHC -Mean cell Hemoglobin concentration