

INTRODUCTION

Depending on the brand, instant noodles are precooked, dry noodles made from wheat flour that are packaged with oil, seasoning, flavoring powder, and other components. (Amin and others, 2010). These noodles still exist today in many types of instant noodles, and some of its ingredients include refined flour made from weed, salt, and oil (Kim, 1996a). The great majority of supplements have been shown to work remarkably well with noodles. Most people call them junk food (Hope, 2009). According to Joseph *et al.* (2017), a specific meal consisting of these instant noodles is stated to be poor in vital nutrients including vitamins, protein, fiber, and minerals and high in added sugar, sodium, and other chemicals. These noodles are particularly popular because they are readily available, inexpensive, and simple to prepare—they only take two to three minutes. In recent times, there have been multiple brands of instant noodles available in the market. (Shin, 2003). Around the world, there are a plethora of distinct instant noodle options (Seller *et al.*, 2007). Indofood's Indomie instant noodles were the first instant noodle brand to be introduced in Nigeria in 1988. Numerous other nations throughout the world also distribute instant noodles (Seller *et al.*, 2007). Nigeria has been producing instant noodles since 1995, however Indonesia is still the world's largest producer of indomie (World Instant Noodle Association (WINA, 2016).

Ever since the first instant noodle brand was introduced into Nigeria, rich people, middle-class people, and the impoverished have accepted it into many homes in both urban and rural areas. Children and even some adults referred to all types of instant noodles as "INDOMIE" as the indomie instant noodle developed a household word over time (Alabi *et al.*, 2014). However, because of the intricate advertisements for many brands of these noodles, as well as their accessibility and ease of consumption, they have drawn people from a variety of social groups in both urban and rural areas, including children, adults, students, and the working class. 2010 saw Indofood release.

In routine clinical evaluations of the state of health, haematological measures are of diagnostic value and have been linked to health indices (Saliu *et al.*, 2012). It is well known that long-term illnesses have a negative impact on blood cells. Blood parameter analysis is important for assessing the risk of changes to the human hematological system (Olson *et al.*, 2000). Free radical damage causes oxidative DNA, protein denaturation, lipid peroxidation, disruption of membrane fluidity, and changes in platelet functions. These effects have been connected to a number of chronic health issues, including diabetes, cancer, inflammation, aging, and atherosclerosis. Despite the fact that nearly all organisms have evolved antioxidant defense and repair systems to shield them from free radicals, these systems are not enough to shield them entirely from oxidative damage (Nwanna and Oboh, 2007).

Glutamate consumption has been connected, independently of calorie intake and physical exercise, to obesity and metabolic syndrome. Additionally, several researchers have documented glutamate's neurotoxic consequences. Scientists generally agreed that glutamate is safe for use in children, expectant mothers, and nursing mothers. It has been demonstrated that, under typical situations, glutamate has a very low acute and chronic toxicity profile. More research is required to determine how this seasoning affects albino rats' organosomatic and haematological parameters, given the contradictions in the literature and the growing concerns about the safety of using monosodium glutamate.

MATERIALS AND METHODS

Indomie Seasoning

Indomie noodles (Chicken flavor) manufactured February 2023 by De United Foods industries Limited, Idiroko road, Ota, Ogun State Nigeria was bought at Nyong Essien Market Uyo Akwa Ibom State and the seasoning powder containing iodized salt, sodium polyphosphate, potassium bicarbonate, sodium bicarbonate, flavor enhancer (E621, E627, E631), Sugar, yeast extract, soy powder, ginger powder, garlic powder, chicken flavor was used for experiment.

Experimental Animals

The Laboratory Animal House, Department of Pharmacology, Faculty of Pharmacy, University of Uyo, Nigeria, provided sixteen (16) normal, healthy female albino rats weighing between 83 and 140 g. They were housed in a clean, well-ventilated laboratory setting. The rats were given unlimited access to clean water and conventional rat food pellets after four weeks of acclimatization. The National Institute of Health's (1985) Guidelines for the Care and Use of Laboratory Animals and the University of Uyo's (Nigerian) Ethical Guidelines for the Use of Laboratory Animals were followed in all experimental methods.

Experimental Procedure

Following a month of acclimation, the rats were split into four groups, each consisting of four rats. As the control group, Group A was given normal saline (3 ml/kg). For 21 days, the seasoning powder dosages of 25 mg/kg, 50 mg/kg, and 75 mg/kg were administered to Groups B, C, and D, respectively. Every seasoning was taken orally. Diethyl ether was used to anesthetize the rats following their 21-day treatment. Every animal was killed by cervical dislocation, the heart was carefully accessed by cutting up the thoracic cage, and a cardiac puncture was used to get a blood sample. To avoid blood clotting, the blood from each rat was taken and placed into labeled 5ml heparinized Ethylene Diamine Tetra-acetic acid 8.5% (EDTA) vials.

Blood Sample: Rats were weighed and fasted for an entire night following their 21-day exposure before having blood samples taken. After that, the rats were killed. We collected all blood samples between 7 and 9 am in order to minimize variations resulting from circadian rhythm. Blood samples were quickly combined with the anticoagulant in EDTA (Ethylene Diamine Tetra-Acetic Acid 8.5%) anticoagulant tubes. After being labeled, every blood sample was taken right away to the lab for analysis.

Organosomatic index of Experimental rats

Throughout the experiments, weekly records of body weight changes were made. At the conclusion of the trial, the experimental rats' body weight gain and a few key organs were weighed. Using the Sellers *et al.* (2007) approach, the liver and kidney (left and right) were excised and weighed in order to determine the organosomatic index.

$$\text{Organ – body weight ratio} = \frac{\text{Weight of the organ}}{\text{Weight of the whole animal}} \times 100$$

Haematological parameters

The improved Nuebauer hemocytometer was used to count blood, and an automatic hematological assay analyzer was used to determine several haematological parameters, including packed cells (PCV), hemoglobin concentration (Hb), total red blood cell (RBC) count, mean cell volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), total white blood cell (WBC), and total platelets count. The following formula was used to determine the haematological indices, such as MCHC, MCH, and MCV, Dacie and Lewis (2001)

$$MCHC (gdl - 1) = \frac{Hb(gdl - 1)}{PCV(\%)} \times 100$$

$$MCH (pgcell - 1) = \frac{Hb(gdl - 1)}{RBC \text{ count in millions } mm - 3} \times 100$$

$$MCV (flcell - 1) = \frac{PCV(\%)}{RBC \text{ count in millions } mm - 3} \times 100$$

Haematological Analysis

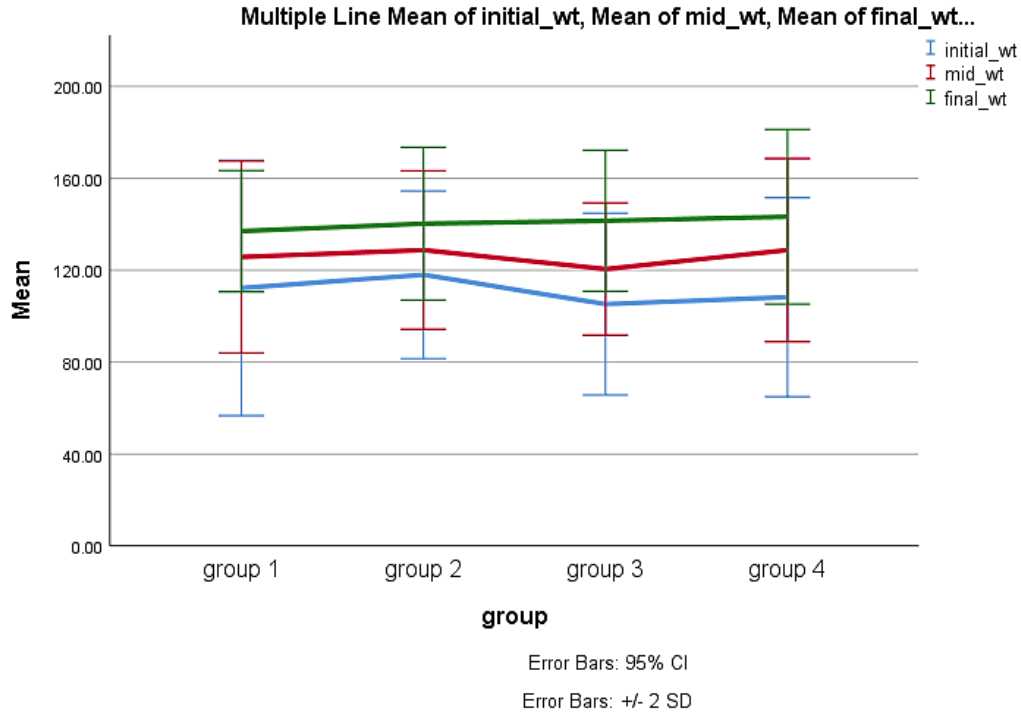
Haematological parameters determination was carried out at the Haematology unit, Ibom Specialist Hospital Uyo-Ikot Ekpene road, Ekit Itam Akwa Ibom State, Nigeria, using Automated Hematology Analyzer (Mindray BC-5380, UK –manufactured in 2017).

Statistical Analysis

Using the SPSS-PC software package (Version 26.0 SPSS Inc. Chicago, USA), the group means \pm SEM for each parameter was computed, and significant differences were identified by Analysis of Variance (ANOVA). Means were separated using Turkey's Post hoc test at 5% level of significance. A clustered chart was utilized to evaluate the impacts on several days.

RESULTS

Figure 1 displays the body weights at the start of the trial (starting weight), at midpoint (2 weeks), and at final (3 weeks) following the oral administration of indomie seasoning to all experimental groups. When the experimental rats were given oral doses of the seasoning powder at 25 mg/kg, 50 mg/kg, and 75 mg/kg, their body weight increased significantly in comparison to the control group.



The liver's organ weight (hepatosomatic index) increased significantly ($p < 0.05$) in all groups when compared to the control, according to the findings in Table 1. Additionally, it demonstrates that there was no discernible difference in the organosomatic index in the liver and kidney between the control group and the groups that received 25%, 50%, and 75% of their protein from Indomie noodles (groups A, B, and C). On the other hand, the organosomatic index differed significantly ($p < 0.05$) between the groups given indomie seasoning orally, and the weight of the right and left kidneys increased significantly ($p < 0.05$).

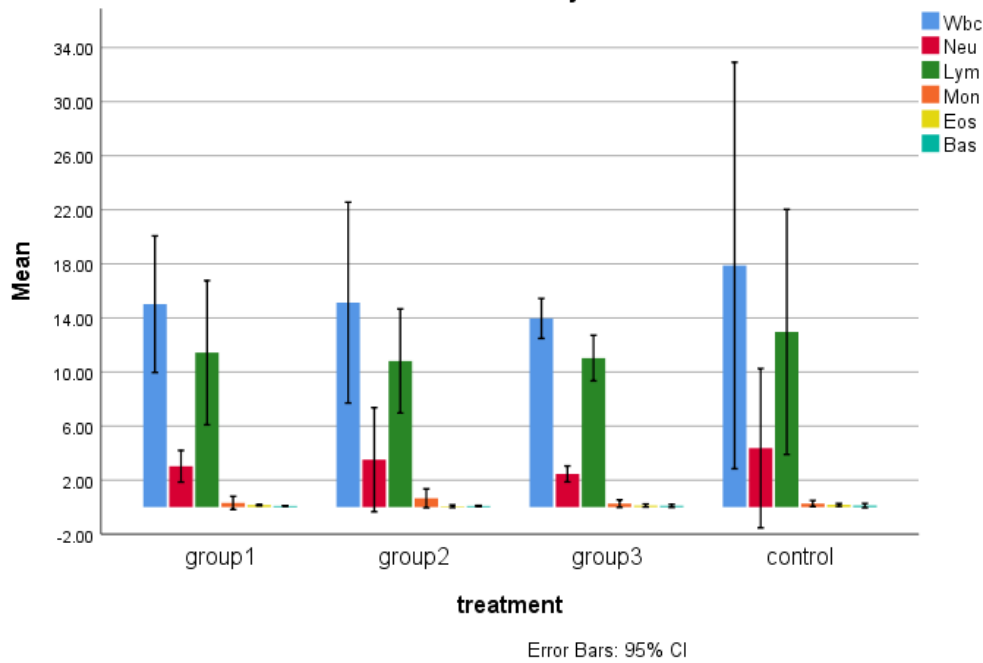
Table 1: Effects of indomie seasoning on some organosomatic index in female albino rats

Parameters	Mean±standard deviation			
	Group A (25%)	Group B (50%)	Group C (75%)	Control
Hepatosomatic index	0.80±0.00	0.80±0.00	0.80±0.10	0.84±0.20
Renosomatic index (Right)	4.20±0.40	3.81±0.30	4.24±0.40	4.14±0.50*
Renosomatic index (Left)	4.15±0.40	3.57±0.20	4.16±0.45	3.98±0.40*

Significance levels are indicated as follows: *indicates significance at the 5 % level

The data presented in figure 2 demonstrated a significant difference ($p>0.05$) between the WBC counts of albino rats given different amounts of indomie seasoning and the control groups.

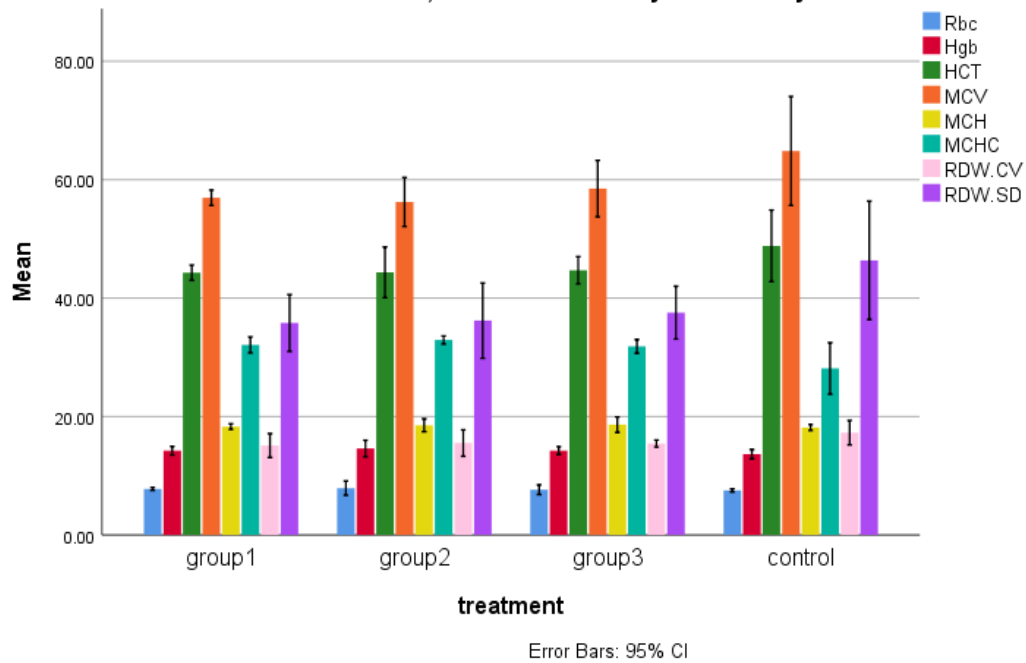
Clustered Bar Mean of Wbc, Mean of Neu, Mean of Lym, Mean of Mon, Mean of Eos, Mean of Bas by treatment by INDEX



Keys: Wbc – White Blood Cell, Neu – Neutrophil, Lym – Lymphocyte, Mon – Monocyte, Eos – Eosinophils, Bas – Basophils

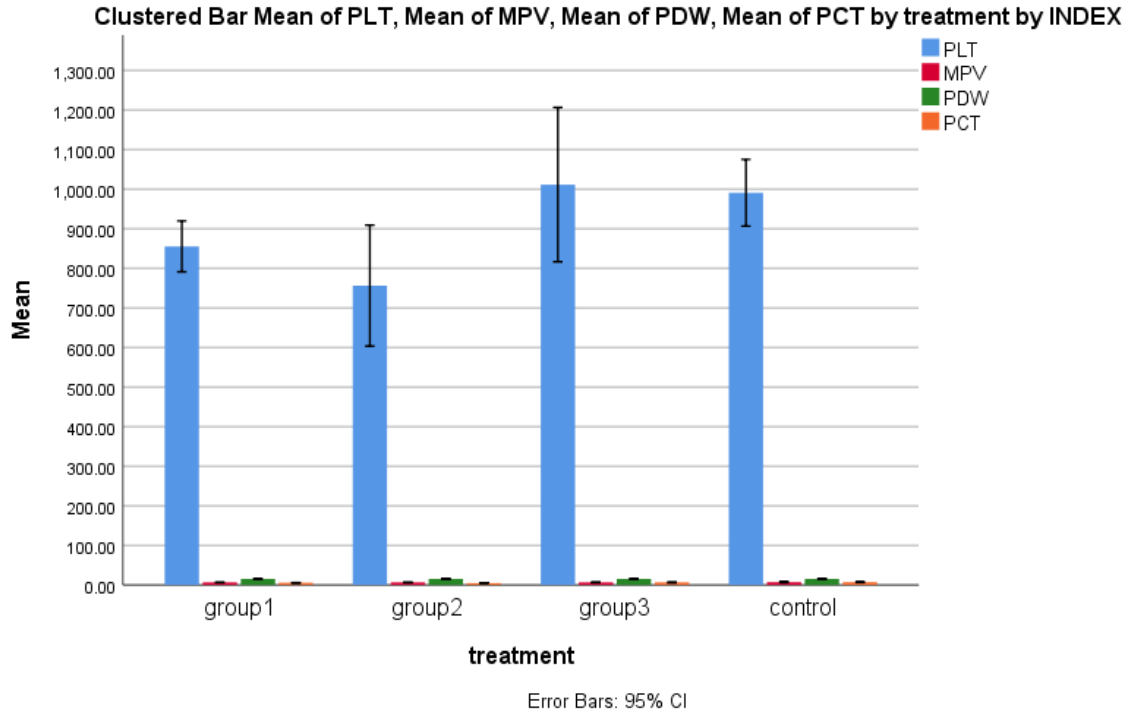
The results, which are displayed in figure 3, demonstrated that the albino rats given Indomie Seasoning experienced a substantial shift in packed cell volume and a significant drop in mean hemoglobin as compared to the control group. While not statistically significant, the mean corpuscular hemoglobin concentration dropped when compared to the control.

Clustered Bar Mean of Rbc, Mean of Hgb, Mean of HCT, Mean of MCV, Mean of MCH, Mean of MCHC, Mean of RDW.CV, Mean of RDW.SD by treatment by INDEX



Keys: RBC – Red Blood Cells; HGB – Haemoglobin Concentration, HCT – Haematocrit Concentration, MCV – Mean Cell Volume, MCH – Mean Corpuscular Haemoglobin, MCHC – Mean Corpuscular Haemoglobin Concentration, RDW.CV – Red Cell Distribution Width RDW.SD- Red Cell Distribution Width

There was no discernible difference between the treated groups and the control when indomie seasoning was examined for its effect on albino rats' platelet levels. Groups 1 and 2 showed very little differences between the treatment and the control, while group 2 demonstrated notable differences among the treated, as indicated in figure 4.



Keys: PLT – Platelets Concentration, MPV – Mean Platelet Volume, PDW – Platelet Distribution Width, PCT – Plateletcrit

DISCUSSIONS

The study's findings demonstrated that the experimental rats' body weights increased significantly across all groups when they were fed indomie seasoning orally. This is consistent with research by Hermanussen *et al.* (2006), who found that rats' body weight increased when they consumed monosodium glutamate, suggesting that the substance may be responsible for obesity and metabolic diseases. The results of this investigation are at odds with those of studies conducted by Mululy *et al.* (2013) and Tordoff *et al.* (2012), which found that consuming monosodium glutamate did not affect adult rats' or mice's body weight and was therefore unlikely to contribute to obesity.

When compared to the control group (group 4), the organosomatic index significantly changed in all three treated groups (25 mg/kg, 50 mg/kg, and 74 mg/kg). According to Nelson *et al.* (2002), an increase in the organ-body weight ratio is a sign of inflammation. According to reports, an increase in an animal's liver weight is frequently indicative of induced toxicity brought on by the stimulation of liver enzymes; this toxicity may be brought on by a notable growth of the smooth endoplasmic reticulum (Anozie and Onwurah, 2001). Nephropathy may be indicated by the rise in kidney weight (Ijeh and Obidoa, 2001). Additionally, as observed in the cases of hyponatremia and hypernatremia, the cellular transporters activity is roughly altered by up or down regulates as brain tissue swells or shrinks. On the other hand, a variety of factors, such as acute hyponatremia, drug poisoning, water intoxication, and hypoxia, can result in either cell shrinkage or swelling. Brain enlargements have the potential to cause significant cytotoxic oedema as well as a noticeable reduction in the size of the basal cisterns and ventricular system (Tordoff *et al.* 2012).

Animal blood is a biological fluid that takes metabolic waste products out of the cells and provides essential elements like nutrition and oxygen to the cells. Red blood cells, white blood cells, platelets, and plasma—the liquid component of blood that comprises water, proteins, salts, lipids, and glucose—are among the components of blood (Alberts, 2012). Blood is essential for sustaining homeostasis and controlling the body's functions. It performs a wide range of functions within the body, including the following: feeding tissues with oxygen (bound to hemoglobin, which is carried by red blood cells), supplying nutrients and fatty acids either dissolved in blood or bound to plasma proteins, eliminating waste, immune functions, including the circulation of white blood cells and the detection of foreign material by antibodies, coagulation, which is a component of the body's self-repair mechanism (blood clotting by the platelets after an open wound to stop bleeding), messenger functions, including the transport of hormones and the signaling of tissue damage, regulating body pH, regulating core body temperature, and hydraulic functions, among others (Martini and Nath, 2009).

Most hematological indicators showed significant variations between treatment groups. The addition of Indomie noodles in the meals did not cause any pathological effects, as indicated by the total leucocyte count, which suggests that the rats' health status was normal (Paul *et al.*, 2012). In comparison to the control group, there was a substantial ($p < 0.05$) rise in the percentage of lymphocytes and a decrease in the percentage of monocytes and granulocytes. Examining the red blood cell indices revealed lower MCV, MCH, and MCHC levels. This demonstrates that all blood parameters showed differences between the three levels of Indomie noodles (25, 50, and 75%) and the control values. The reduced ability of the bone marrow to produce hemoglobin is most likely the cause of the corpuscles' decreased hemoglobin levels (Sharma, 2015). When compared to the control group, a 75% rise in platelet count may indicate secondary thrombocytosis. It was proposed that this rise might be the result of consuming excessive amounts of salt and glutamate, two ingredients in Indomie seasoning that have an impact on the body's water balance and blood and bodily fluid compartments. They go on to say that it might have something to do with the thrombopoietin hormone's biosynthesis, which increases platelet production (Tordoff *et al.*, 2012).

The evaluation of haematological parameters can be employed to ascertain the degree of detrimental impact that foreign substances, such as plant extract, have on activities associated to blood. In comparison to their respective control groups, Yakubu *et al.* (2007) found that a prolonged administration of the alcoholic bitters significantly reduced the levels of hemoglobin, red blood cells, packed cell volume, platelets, and white blood cells, among other differential counts.

According to the current study, the neutrophil count decreased significantly at all seasoning doses, and the decrease was greater in the groups who got the highest dose. Human neutrophil counts typically range from 3000 to 6000 / cu.mm of blood.

This could mean that the seasoning has a time-dependent, direct toxic effect on blood neutrophils or that it negatively affects bone marrow blood formation, particularly on progenitor cells (aplasia). The initial line of defense against foreign chemicals, poisonous substances, and invasive microorganisms is provided by neutrophils and monocytes, highlighting the critical function neutrophils play in the body's defense (Hall, 2011). This could be a sign of the treated rat groups' immunological condition declining due to the seasoning's harmful effects. All treated animals showed an observed increase in lymphocyte count (normal value = 1500 to 2700 / cu.mm of blood). This increase could be attributed to the animals' perception of the seasoning as a toxic agent, a

significant increase in granulocytes, or the interaction between MSG and gastrointestinal macrophages. Macrophages function as antigen-presenting cells, delivering antigenic components (polypeptides) to activate B cells and helper T cells (Sembulingham, 2005). Additionally, macrophages emit chemicals known as interleukin-1 / -cytokines, which cause the lymphocyte count to rise and the lymphocytes to proliferate (Sembulingham, 2005; Barrett *et al.*, 2010). MSG did not have a deleterious impact on lymphocytic cells, as evidenced by the steady increase in counts observed in all withdrawal groups as well as in Groups B1 and B2, which received therapy for extended periods of time. This suggests that MSG has an ongoing, lingering impact on the body's systems.

The seasoning had a decreasing effect on both the concentration of hemoglobin and the packed cell volume. Humans typically have packed cell volume of 38–42%, hemoglobin concentration of 14–16 g%, and red blood cell count of 4–5.5 million/cu.mm, respectively. RBC count indicates that it most likely shortens red blood cell life in the blood, maybe due to direct toxicity. This could have potentially been mediated by negatively affecting the bone marrow's hematopoietic stem cells. Increased oxidative stress, a byproduct of anaerobic respiration, may be brought on by MSG in the animal tissues. Micronucleated polychromatic erythrocytes (MNPCEs) were considerably ($p < 0.01$) stimulated to develop by 4 mg/g of MSG (Farombi and Onyema, 2006). Elphick *et al.* (2008) used HT22 murine hippocampal cells as a model for oxidative stress-induced neuronal death to examine the oxidative glutamate toxicity. According to Elphick *et al.* (2008), these harmful effects were typified by nuclear and cell condensation but did not result in DNA fragmentation or the release of cytochrome c from the mitochondria. Significant increases were seen in the MCV and MCH levels (normal values = 27 to 32 pg and 78 to 90 cu. micron, respectively). Although there was no discernible ameliorating effect, the elevated values are more suggestive of anemia.

Macrocytic cells are indicated by an increased mean corpuscular volume (MCV) (Sembulingham, 2005). While elevated MCH is suggestive of macrocytic anemia, high MCV is reported in pernicious anemia (normochromic) and megaloblastic anemia (hypochromic) (Richards, 1993). Nonetheless, both the treatment and control groups have normal mean corpuscular hemoglobin concentrations (MCHCs; typical values for humans are between 30 and 38%). More precisely, macrocytic normochromic anemia, or pernicious anemia, was recommended. This could be because of the acidic MSG (L-form of glutamic acid) that causes the gastric mucosa to atrophy (gastritis). This leads to a decrease in the production of the intrinsic factor and poor absorption of vitamin B12, which is the primary cause of pernicious anemia (Sembulingham, 2005).

The current study demonstrates that the administration of indomie seasoning significantly affects the neutrophil and lymphocyte counts, which are suggestive of poisoning and weakened immune systems, respectively, in the treated animals. While changes in the treated animals' PCV, Hb, RBC, MCV, and MCH values were all suggestive of anemia. Therefore, our results agrees with Eweka, (2007) who stated that monosodium glutamate is unhealthy even when it serves as a flavoring agent.

Conclusion: Due to its association with Chinese Restaurant Syndrome (CRS), this study showed that indomie noodle seasoning containing monosodium glutamate may be harmful to human health. Long-term, frequent use of seasonings containing monosodium glutamate can cause diseases like obesity, kidney impairment, fibroids, and hepatotoxicity. To educate people about

the dangerous effects of the indomie noodle seasoning, more awareness should be raised, and natural substitutes for the seasoning should be encouraged.

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