Original Research Article

REPRODUCTIVE TOXICITY & BIOMARKER RESPONSE TO A DAILY DOSE OF INDOMIE SEASONING IN MALE ALBINO RATS *(Rattus norvegicus)*

Abstract

The effect of Indomie seasoning containing the Monosodium glutamate (MSG) on Rat was evaluated in this study, The parameters investigated include; Alkaline aminotransferase (ALT), Aspartate aminotransferase– (AST). Hemoglobin (Hb), parked cell volume (PCV) white blood cell (WBC), protein, platelets, lymphocytes and Serum electrolytes; sodium (Na+), potassium (K+) chloride (Cl), bicarbonate (HCO_3 -). Sperm count was also investigated. The results revealed the following, the mean PCV was 29 and 25.13on week 1 and week 4, with an average control of 30.69, mean Hb was 10 in week 1 and 6.57 in week 4, RBC had an average control of 5.28 while week 1 had a mean of 4.77 and week 4 3.67, there was a significant difference (P<0.05) for PCV and Hb. The mean WBC and Lymphocyte were 6 and 61 in the first week, and 5.8 and 60.17 on the fourth week, with an average control of 5.28 for WBC and 77.53 for lymphocytes. Platelet had a mean of 251 on the first week and a mean of 532 on the fourth week with a significant difference across the group in WBC and platelets (P<0.05). The mean serum Na, K and Cl reduced from 140.67, 4.13 and 100.67 in week 1 to 116, 2.5 and 98 in week 4 with a significant difference (P<0.05) across the group when compared to the average control for Na and K. HCO_3 had a mean of 23.67 in week 1 and a mean of 22.67 in week 4 in the treated group. AST had a mean of 24 in week 1 which increased to 41.67 in week 4 while ALT increased from a mean of 4.00 in week 1 and 28 in week 4 with a significant difference (P<0.05) across the group. The mean serum protein was 51.93 in week 1 and a 74.29 in week 4. The mean sperm count was 800, 299.67, 450.67 and 501 for week 1, 2, 3 and 4 respectively. The results indicates that Indomie seasoning when continuously consumed causes liver damage, and kidney dysfunction and has negative effects on blood and sperm cells.

Formatted: Subscript

Formatted: Subscript

INTRODUCTION

Indomie noodles are commonly eaten as food for a meal after preparation with the attached seasoning which is a food additive. Food additives are mostly used in the world today in enhancing the taste of food, food value, food texture, and the colour of the food stuff (Imane *et al.*, 2011). Most food additives are made from Monosodium glutamate. Monosodium glutamate (MSG) has been used for more than a century and it is described as a white

crystalline powder, which is a sodium salt which occurs naturally as a non-essential amino acid and glutamic acid (Furst and Stehle, 2004). Monosodium glutamate has been approved by food and drug administration (FDA) to maintain or improve the texture, taste and quality of the nutrient of the food. MSG as a food additive is used by so many people and there is no daily specified dosage limit (Samuel, 1999), as a result of this people use this food additive (Monosodium glutamate) based on their own discretion. MSG is a sodium salt of glutamic acid and it composed 78% of glutamic acid and 22% of sodium and water (Adrienne, 1999). This Food Addictive (MSG) is being used in this modern time by many industries and cooks in production and preparation of various foods and food ingredients in order to enhance the flavour and taste of the food (Alao *et al.,* 2010). Food additives are also widely used in many occasions and for different purposes; some use this food additive in restaurant, some in household cooking while some in a commercial packed food (Schiffman, 2000), They are also extensively used in a wide variety of processed foods including prepared meals, flavoured chips and snacks, marinated meats, flavoured tuna, soups or sauces (canned, packed), bottled soy or oriental sauces, fresh sausages, and stuffed or seasoned chicken, vegetarian burgers, some hams, luncheon chicken and turkey and sausages (Bojanic *et al.,* 2009). Despite MSG being approved by FDA, there have been many negative effects of MSG reported by many authors. According to Abass and El-Haleem, (2011), The proximal tubules epithelial cell in the kidney which function is to carry out diverse regulatory and endocrine function where numerous transport are located was reported damaged, they observed cytoplasmic, nuclear vacuolations and tubular dilatation due to the excessive intake of MSG. In line with the above report, it was also observed that intake of higher dose of MSG produced series of damages in the kidney membrane and also in the cellular organelle (Bopanno *et al.,* 1999). It was also reported that there was an increase in the tubulo-interstitial fibrosis in the kidney of the rat that consumes this MSG over a long period of time (Sharma *et al.*, 2013) and also Oxidation stress which happens to be the main cause of injury to the kidney is caused by increase in production or decreased elimination of free radicals present in cell of which oxygen radical and other reactive species is the majority (Bashan *et al.,* 2009). Nwaopara *et al.* (2004) reported that Monosodium glutamate has some detrimental effect on the liver of rat at higher concentration and it also affects the functions of the liver as well. Most amino acids and their derivatives metabolize in the liver to some significant extent and the possible overload of ammonium ion may occur with Monosodium glutamate consumption and could lead to liver damage and consequently releasing the transaminases; with its observed elevation in the plasma (Onyema*, et al.,* 2006). Egbuonu *et al.,* (2009) who also

conducted an experiment on this, reported that there was an increase in the serum transaminases in the male albino rat due to increase in Monosodium glutamate, it was found that there was an increase in level of glutamate which resulted from the overload of possible ammonium ion. (Contini *et al.,* 2012) in their histological study of the liver section showed vacuolar degeneration of hepatocytes cords, nuclei pyknosis and blood vessel congestion. They also indicated that despite that the trabecular, portals and the lobular structure were preserved in the liver, there was steatosis characterized by cytoplasmic vacuoles fat throughout the liver lobule in the animals treated with different doses of monosodium glutamate. (Ochiogu *et al.,* 2011) reported that the inference monosodium glutamate may have impacted spermatogenesis through its disruption of the hypothalamic-pituitary-testis regulatory axis, and not through any direct toxic effect on the testis. Their study demonstrated administration of oral monosodium glutamate resulted in lowered serum testosterone levels and reduction in the cauda epididymal sperm reserves of male rats, but did not cause any overt pathological lesions in their testes. The process of spermatogenesis is a complex cyclic process in which germ cells undergo series of mitotic and meiotic cell divisions, followed by morphological differentiation in a delicately regulated spatiotemporal fashion in the seminiferous epithelium. In mammals, spermatogenesis is totally dependent upon testosterone (Pakarainen *et al.,* 2005; Wang *et al.,* 2009). Male infertility, testicular haemorrhage and alteration of sperm production and morphology, reduction of body growth, obesity and hypogonadism are the most often reported changes in cases of male infertility after administration of monosodium glutamate (Oforofuo *et al.,* 2006). There are so many controversies on the effects of monosodium glutamate. It was reported (Akanya *et al.,* 2015) that administration of different doses of monosodium glutamate (0.5, 1.0 and 5.0% per kg on diet) did not have any significant change in WBC, RBC and PCV when compared with the control group. But this result is contradicting with (Ashaolu *et al.,* 2011; Meraiyebu *et al.,* 2012) who reported that monosodium glutamate has toxic effect on the RBC and also have deleterious changes in the haematological parameters. An experiment conducted by Eweka, (2007) indicates that administration of monosodium glutamate has a significant effect on the neutrophil and lymphocyte count, indicative of a compromised immune status and poisoning respectively in the treated animals while alterations in counts of PCV, HB, RBC, MCV and MCH were all indicative of anaemic conditions in the treated animals. This food additive (Indomie noodle seasoning containing MSG amidst other ingredients) is widely used by many people including adult for the preparation of Indomie noodles and sometimes this food additives are used in exceeding concentrations. This research is therefore aimed at unveiling

the potential effect of Indomie noodle seasoning on the haematological, renal function, liver function, sperm parameter of a male Albino rat (*Rattus norvegicus*).

MATERIALS AND METHOD

Experimental Design: A total number of twenty-four (24) male adult Albino rats weighing 200g -225g were used for the experiment. The rats were housed in a wooden cage. The 24 rats were randomly divided into a six group ~~of six~~ (6) labelled A, B, C, D, E, F, and each group contains four rats and were acclimatized for one week before the commencement of the experiment and kept in a wooden cage. The weekly average body weights were 200, 225, 225 and 225. Based on this body weights the treatment (Indomie seasoning) was administered to all the rats in the treated group orally 0.13g/ml directly into the esophagus~~oesophagus~~ of the animals with the aid of 1000µl syringe. The measurement of the treatment administered was determined in relation to the average intake of Indomie Seasoning by an average human weighing 60kg.

Biochemical Analysis: Standard procedures were ensured during the collection of the blood, sperm and liver samples prior to biochemical analysis. Semen was collected and the epididymal sperm count was done with a Neubauer haemocytometer (Deep 1/10 mm, LABART, Munich, Germany) with a light microscope at 40× magnifications. The plasma activity of Alkaline Phosphatase (ALP) was determined using Radox kit (colorimetric method) of Rec (1972). Biuret method was used to determine the level of total protein in the samples according to the method of Flack and Woollen (Flack and Woollen, 1984). The plasma activity of aspartate transaminase AST and alanine transaminase ALT was determined using Reitman and Frankel method (Reitman and Frankel, 1957). The serum electrolytes were determined using ISO 4000 Automated electrolyte analyzer~~analyser~~. SFRI, France.

Method of Data Analysis: Data were analyzed using the Tukey test~~Tukey test~~ at a level of 5% probability, using Assitat Software Version 7.7 en (2017).

RESULTS

The result of Haematological Analysis is shown in Table 1; Mean PCV for the treated group was 29, 32.83,36.7 and 25.13 in weeks 1, 2, 3 and 4, the control group had 26.67, 32.56, 32.87 and 39.07 in weeks 1, 2, 3 and 4 with an average control of 30.69 with a significant difference ($P<0.05$) across the week. The mean Hb level in the treated group was 10, 9.67,

8.33 and 6.57 in weeks 1, 2, 3 and 4 while, the control group had 9, 9.90, 10.37 and 13.87 in weeks 1, 2, 3 and 4 with an average control of 9.75. There was a significant difference (P<0.05) across the week. The RBC and WBC in the treated group was 4.77 and 6.0 in week 1, 6.9 and 5.43 in week 2, 6.84 and 6.01 in week 3, 3.67 and 5.8 in week 4, the control group had a mean of 4.37 and 9.0 in week 1, 4.23 and 9.87 in week 2, 6.04 and 7.47 in week 3, 6.90 and 6.27 in week 4 with an average control of 5.28 and 5.28. There was no significant difference (P>0.05) across the week. The blood platelet and lymphocyte had a mean of value of 251 and 61 in week 1, 495.67 and 83.90 in week 2, 237.33 and 86.67 in week 3, 532.67 and 60.17 in week 4 in the treated group, while the control group had a mean value of 270 and 70 in week 1, 335.66 and 84.40 in week 2, 423 and 78.2 in week 3, 416.67 and 84 in week 4. The average control was 309.67 and 77.53 for the blood platelets and lymphocytes respectively, with a significant difference (P<0.05) across the week. The results for Hepato-renal analysis Table 2 indicate a mean value for Na 140.67 in week 1, 148.33 in week 2, 148.33 in week 3 and 116.00 in week 4 with a control of 134 in week 1, 157.67 in week 2, 157.67 in week 3 and 149.67 in week 4, the average control was 147.33. There was a significant difference (P<0.05) across the week. The mean potassium in the treated group was 4.13 in week 1, 4.50 in week 2, 3.73 in week 3 and week 4 had 2.5, the control group had a mean of 4.03 in week 1, 5.60 in week 2, 4.33 in week 3 and 5.10 in week 4. The average control was 5.44. There was significant difference (P<0.05) across the group when compared to the average control. A mean value of 100.67 was recorded for Cl in week 1, 98 in week 2, 73.33 in week 3, and 98 in week 4 in the treated group, and the control group had a mean of 100.67 in week 1, 109.67 in week 2, 86.67 in week 3 and 106 in week 4 having an average control of 100.75. There was no significant difference (P>0.05) across the week. The mean value of Bicarbonate in the treated group was 23.67 in week 1, 27.33 in week 2, 20.33 in week 3 and 22.67 in week 4. The control group had a mean value of 23.67 in week 1, 23.67 in week 2, 24.67 in week 3 and 23.00 in week 4 with an average control of 24.33. There was also no significant difference (P>0.05) across the week. The AST and ALT mean values were 24 and 4 in week 1, 24.33 and 8.67 in week 2, 30.67 and 15 in week 3, 41.67 and 28 in week 4 in the treated group with the control group having a mean of 17.67 and 9 in week 1, 34.66 and 10.0 in week 2, 23.67 and 11.00 in week 3, 23.00 and 13.00 in week 4 with an average control of 25.67 and 10.67 respectively. There were significant difference (P<0.05) in both AST and ALT across the week. A mean value of 51.93, 82.67, 67. 87 and 73.27 were recorded for serum protein in week 1, 2, 3 and 4 respectively in the treated group. While the control group 66. 04, 72.31, 69.27 and 73.27 in weeks 1, 2, 3 and 4 respectively, with an

171 average control of 69.11. There was a significant difference (P<0.05) across the week. A
172 mean value for sperm count (Table 3) 800.67, 299.67, 450.0 and 501 were recorded in week
173 1, 2, 3 and 4 respectively in the treated group while the control group had a mean of 475, 575,
174 475 and 650 in week 1, 2, 3 and 4 respectively with a significant difference across (P<0.05)
175 the week.

Table .1: Effects of Indomie Seasoning on PCV, Hb, RBC, WBC, Platelets and Lymphocytes Levels in Albino Rats

		PCV (%)	Hb	RBC ($x10^{12}$)	WBC ($x10^{9}$)	Platelet ($x10^{9}$)	Lymphocytes ($x10^{9}$)
Week 1	Treatdest	29.00±5.29^{aAB}	10.00±1.0^{aA}	4.77±3.11^{aA}	6.00±3.61^{aA}	251.00±5.0^{bB}	61.00±3.61^{aB}
	Control	26.67±1.53^{a}	9.00±0.30^{a}	4.37±0.15^{a}	9.00±2.50^{a}	270.00±0^{a}	70.00±5.0^{a}
Week 2	Treatedest	32.83±2.73^{aAB}	9.67±2.08^{aAB}	6.90±1.59^{aA}	5.43±1.30^{aA}	495.67±5.13^{aA}	83.90±5.88^{aA}
	Control	32.56±2.95^{a}	9.90±0.90^{a}	4.23±0.70^{a}	9.87±5.65^{a}	335.66±105.5^{a}	84.40±1.4^{a}
Week 3	Treatedest	36.70 ±3.11^{aA}	8.33±0.85^{aAB}	6.84±2.04^{aA}	6.01±0.71^{aA}	237.33±8.74^{bB}	86.67±4.97^{aA}
	Control	32.87±3.95^{a}	10.37±1.15^{a}	6.04±0.64^{a}	7.47±2.85^{a}	423.00±108^{a}	78.20±1.4^{b}
Week 4	Treatedest	25.13±3.41^{bB}	6.57±1.01^{bB}	3.67±1.93^{aA}	5.80±1.54^{aA}	532.67±4.51^{aA}	60.17±5.01^{bB}
	Control	39.07±2.35^{a}	13.87±0.45^{a}	6.90±1.60^{a}	6.27±0.06^{a}	416.67±3.51^{b}	84.00±0.7^{a}
	Average control	30.69±1.22^{AB}	9.75±0.78^{AB}	5.28±0.50^{A}	5.28±3.67^{A}	309.67±71.12^{B}	77.53±2.6^{A}

$^{a-b}$ Different letters in the same column indicate significant difference (P<0.05) within the weeks

$^{A-B}$Different letters in the same column indicate significant difference (P<0.05) across the weeks

Table 2: Effects of Indomie Seasoning on Na, K, Cl, Bicarbonate, AST, ALT and Protein of a Male Albino Rats

		Na(mmol/l)	K(mmol/l)	Cl(mmol/l)	Bicarbonate (mmol/l)	AST(U/L)	ALT(U/L)	Protein
Week 1	Treatedest	140.67±5.69^{aAB}	4.13±1.91^{aA}	100.67±5.51^{aA}	23.67±4.73^{aA}	24.00±4.36^{aB}	4.00±1.73^{aC}	51.93±6.96^{aC}
	Control	134.00±2^{a}	4.03±0.25^{a}	100.67±4.51^{a}	23.67±0.58^{a}	17.67±3.51^{a}	9.00±1.53^{a}	66.04±12.21^{a}
Week 2	Treatedest	148.33 ±5.13^{aA}	4.50±2.10^{aA}	98.00±5.57^{aA}	27.33±3.79^{aA}	24.33±3.21^{bB}	8.67±1.53^{aBC}	82.67±6.12^{aA}
	Control	157.67±22.5^{a}	5.60±2.55^{a}	109.67±18.50^{a}	23.67±1.53^{a}	34.66±3.51^{a}	10.00±2.0^{a}	72.31±3.36^{a}
Week 3	Treatedest	148.33 ±8^{aBC}	3.73±2.14^{aA}	73.33±3.06^{aA}	20.33±4.16^{aA}	30.67±4.93^{aAB}	15.00±4.36^{aB}	67.87±5.45^{aB}
	Control	157.67 ±10.5^{a}	4.33±0.60^{a}	86.67±4.51^{a}	24.67±3.51^{a}	23.67±5.51^{a}	11.00±4.0^{a}	69.27±4.05^{a}
Week 4	Treatedest	116.00±5.29^{bC}	2.5±1.18^{bB}	98.00±4.0^{bA}	22.67±4.16^{aA}	41.67±4.51^{aA}	28.00±3.61^{aA}	74.29±4.51^{aB}
	Control	149.67±0.58^{a}	5.1±0.10^{a}	106.00±1.0^{a}	23.00±1^{a}	23.00±1.0^{b}	13.00±1.0^{a}	73.27±2.16^{a}
	Average control	147.33±11.67^{A}	5.44±1.13^{A}	100.75±10.08^{A}	24.33±1.87^{A}	25.67±4.18^{B}	10.67±2.51^{BC}	69.11±6.54^{AB}

$^{a-b}$ Different letters in the same column indicate significant difference (P<0.05) within the weeks

$^{A-B}$ Different letters in the same column indicate significant difference (P<0.05) across the weeks

Table 3: Effects of Indomie Seasoning on the Sperm Parameter of an Albino rat

		Sperm count(x^6)
Week 1	Treated~~est~~	800.67±4.16 ^{aA}
	Control	475.00±25^{b}
Week 2	Treated~~est~~	299.67±2.31^{bD}
	Control	575±25^{a}
Week 3	Treated~~est~~	450.67 ±5.86 ^{aC}
	Control	475.00±175 ^{a}
Week 4	Treated~~est~~	501±4.5 ^{bBC}
	Control	650±50 ^{a}
	Average control	566.67±57.74^{B}

$^{a-b}$ Different letters in the same column indicate significant difference (P<0.05) within the weeks

$^{A-B}$Different letters in the same column indicate significant difference (P<0.05) across the weeks

DISCUSSION

The PCV, Hb, RBC, WBC and lymphocyte in treated rats decreased when compared with the control group for week 1 and then for week 4 and this decrease was significant for PCV, Hb and Lymphocyte and is due to the adverse effect of MSG which is present in the Indomie seasoning on the haematology of rats, this result is in agreement with Rasha, *et al.*(2014) who stated that rat treated with MSG for 30 successive days showed significant decrease in RBCs count, Hb and WBCs when compared to the control and also Ashaolu *et al.,* (2011) and Meraiyebu *et al.*,(2012) who reported that monosodium glutamate has toxic effect on the RBC and also have deleterious changes in the haematological parameters, this indicates a possible anaemic condition. The significant decrease in lymphocyte recorded is in concord with the work of Alao, *et al.,* (2010) and Eweka, (2007) who reported that there was a significant effect on the lymphocyte count which indicated compromised immune status in the treated animals. The level of Na was higher than the control in the first –week when

compared to the control but it later reduced significantly as the week progressed, similar pattern was also observed for K, Cl and Bicarbonate although in Bicarbonate it wasn't significant (P>0.05). This shows that the Indomie seasoning had a negative effect on the sodium and potassium level of the rats and also on the chloride and bicarbonate levels in the rats and it's not in agreement with the work of Meldrum, (1993) and Choi *et al*, (2004) which showed that MSG does not alter the serum potassium and sodium level, it also doesn't agree with the findings of Zhang *et al,* (1996) and Mozes *et al*, (2004). This negative effect as seen in the result might be due to damage of kidney because high dose of MSG has been reported to damage the kidney membrane and also the cellular organelle (Bopanno *et al.,* 1999). The level of AST and ALT increased significantly from the first week to the last week even after 7 days of withdrawal, this indicates that Indomie seasoning caused some considerably level of damage to the liver cells which leads to the release of transaminases from the liver into the blood stream which will in turn increase the level of AST and ALT (Al-mamary *et al.,* 2002; Onyema*, et al.,* 2006). This result is also consistent with the reports of Egbuonu *et al.,* (2009) who reported that there was an increase in the serum transaminases in the male albino rat due to increase in Monosodium glutamate. This liver damaging ability or hepatotoxic property of MSG have been reported by many authors, A study conducted by Tchaou *et al.,* (2013) showed that MSG consumption is hepatotoxic, and another work done by Diniz *et al.,* (2004) found out that administration of MSG was associated with oxidative stress in hepatic tissues. The result was also in agreement with the work of Bopanna *et al.,* (1999) who observed adverse effect on the liver of rats fed with food contaminated with monosodium. The serum protein level was irregular with a drop in the first week and increase in the second week of treatment compared to the control but decreased on the third week, the value was fairly equal to the control on the fourth week which is the 7^{th} day after withdrawal. This indicates that the Indomie seasoning also affected the serum protein but unlike in AST and ALT, the level normalized after withdrawal. The reason for the irregularity in serum protein might be due to liver damage, as hepatic cells loss the ability to make proteins when damaged and this usually leads to a drop in serum protein which is not easily detected because protein produced earlier may stay in the blood for about two weeks (Pagana and Pagana, 2010), the normalizing of serum protein in week 4 might be because the liver may be recovering from the possible damage. The low sperm count recorded in the experiment indicates that Indomie seasoning had negative effect on the sperm count. This negative effect on Sperm count might be due to the indirect effect of MSG on spermatogenesis through interfering with serum testosterone and a reduction in cauda epididymal sperm reserves of male rats as proposed by Pakarainen

et al.,(2005) and Wang *et al.,*(2009). Oforofuo *et al.,* (2006) and Ochiogu *et al.,* (2011) also reported possible negative effect of monosodium glutamate on spermatogenesis.

CONCLUSION

The results clearly indicates that Indomie seasoning has a negative effect on the body and individuals who consume Indomie or use flavour enhancers containing MSG should reduce the consumption rate by using less of the flavouring agent.

REFERENCE

Abass, M.A., and El-haleem, M.R. (2011). Evaluation of MSG induced neurotoxicity and nephrotoxicity in adult male albino rat. *Journal of American science, vol. 9(8),* pp. 264-276.

Adrienne T. (1999). The toxicity of MSG: A study in suppression of information. *Accountability in research, vol. 6(4),* pp. 259-310.

Akanya, H.O., Peter, S., Ossamulu1, F. I., Oibiokpa, F.I., and Adeyemi, H. Y. (2015). Evaluation of the Changes in Some Liver Function and Haematological Parameters in MSG Fed Rats. *International Journal of Biochemistry Research and Review, vol. 6(3),* pp. 113-120.

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*, *vol. 6*, pp. 197-203.

Al-Mamary, M., Al-Habori, M., Al-aghbari, A.M, and Basker, M.M. (2002). Investigation into the toxicological effects of Catha edulis leaves. A short term study in animals. *Phytotheraphy Research, vol. 16(2),* pp. 127-132.

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

Bashan, N., Kovsan, J., Kachko, I., Ovadia, H., Rudich, A. (2009). Positive and negative regulation of insulin signalling by reactive oxygen and nitrogen species. Physiological Reviews, vol. 89(1), pp. 27–71.

Björndahl, L., Söderlund, I and Kvist U. (2003). Evaluation of the one-step eosin- nigrosin staining technique for human sperm vitality assessment. *Hum Reprod*. 18(4):813–6.

Bojanic, V., Bojanic, Z., Najman, S., Savic, T., Jakovljevic, V., *et al.* (2009). Diltiazem prevention of toxic effects of monosodium glutamate on ovaries in rats. *General Physiology Biophysics, vol. 28* Spec No: 149-154.

Bopanna, K., Balaraman, R. and Noding, R. (1999). Antioxidant status of S- allyl cysteine sulphoxide on monosodium glutamate potentiated atherosclerosis. Indian J.Pharmacol., 30: 73-81

Bopanna, K.N., Balaraman, R., and Nadig, R.S. (1999). Organotropic ultrastructural changes produced by monosodium glutamate in rats on Atherogenic diet: effect of S-allyl cysteine sulphoxide. *Indian journal of pharmacology, vol. 31,* pp. 266-274

Choi, D., Duff, D.A., Snell, K. and Hing, S.A. (2004). Late effect of postnatal monosodium glutamate on insulin action in adult rats physiol Res 49: 79 – 86.

Contini, M.D., Millen, N., Riera, L., and Mahieu, S. (2012). Kidney and Liver Functions and Stress Oxidative Markers of Monosodium Glutamate Induced Obese Rats. *Food and Public Health, vol. 2(5),* pp. 168-177.

Diniz Y, Fernandes A, Campos K and Novelli E (2004): Toxicity of hyper caloric diet and monosodium glutamate oxidative stress and metabolic shifting in hepatic tissue. Food Chem. Toxicol., 42 (2): 319- 325.

Egbuonu, A.C., Obidoa, O., Ezeokonkwo, C.A., Ezeanyika, L.U. and Ejikeme, P.M. (2009). Hepatotoxic effects of low dose oral administration of monosodium glutamate in male albino rats. *African Journal of Biotechnology, vol. 8(13),* pp. 3031-3032.

Eweka, A.O. (2007). Histological studies of the effects of monosodium glutamate on the kidney of adult Wistar rats. *Internet Journal of Health, vol. 6(2),* pp. 14-18.

Flack, C. P. and Woollen, J. W. (1984). Prevention of interference by dextran with biuret-type assay of serum proteins. *Clinical Chemistry*, 30(4). 559-561.

Furst, P., and stehle, P. (2004). What are the essential elements needed for the determination of amino acid requirement in humans? *The journal of nutrition, vol. 34,* pp. 1558-1565.

Imane, H., Said, B., Faiza, S., Fatima, B., Mohammed, A., Mohamed, B., Jouhar, Z., *et al.* (2011). A 90 day oral toxicity study of Tartrazine, a synthetic food dye, in wistar rat. *International Journal of Pharmacy and Pharmaceutical Science, vol. 3(3)* pp. 159-169.)

Meldrum, B. (1993). Amino acids as dietary excitotoxins: a contribution to understanding neurodengerative disorder. Brian Research Reviews. 18: 293-314

Meraiyebu, A., Akintayo, C.O., Uzoechi, A.C., Okere, S. (2012). The effects of orally administered monosodium glutamate (MSG) on blood thrombocyte, blood coagulation and bleeding in rats. *International Journal of Pharmacy and Biological Sciences, vol. 4(1),* pp. 4-8.

Mozes S., Sefeikova I, and Lenhardt L. (2004). Obesity and changes of alkaline phosphatase activity in small intestine of 40 – and 80 – day old rats subjected to early postnatal overfeeding or monosodium glutamate physiol REs 53: 177-186.

Nwaopara, A. O., Anyanwu, L. C., Oyinbo, C. A., and Anaikot, I. C. (2004). The histological changes in pancreas of wistar rats fed with diets containing Yaji (local meat sauce). *Journal of Experimental and Clinical Anatomy, vol. 3,* pp. 44- 47.

Ochiogu, M. I., Ihedinihu, B. C., Ikokide, J. E., Igwebuike, I. K., Ochiogu, I. S., Ihedinihu, B. C., and *et al.* (2011). The effects of oral administration of monosodium glutamate (MSG) on the testicular morphology and cauda epididymal sperm reserves of young and adult male rats. *Veterinarski Arhiv, vol. 81 (4)*, pp. 525-534.

Oforofuo, I.A., Onakewhor, J.U., Idaewor, P.E. (2006). The effect of chronic administration of MSG on the histology of the adult Wistar rat testis. *Bioscience Research Communications, vol. 9*, pp. 1-2.

Onyema, O.O., Farombi, E.O., Emerole, G.O., Ukoha, A.I., and Onyeze, G.O. (2006). Effect of vitamin E on monosodium-glutamate induced hepatotoxicity and oxidative stress in rats. *Indian Journal of Biochemistry and Biophysics, vol. 43(1),* pp. 20-24

Pagana, K. D. and Pagana, T. J. (2010). Mosby's Manual of Diagnostic and Laboratory Tests, 4th ed. St. Louis: Mosby Elsevier.

Pakarainen, T., F. Zhang, S., Mäkelä, M., Poutanen I., and Huhtaniemi (2005). Testosterone replacement therapy induces spermatogenesis and partially restores fertility in luteinizing hormone receptor knockout mice. *Endocrinology. Vol. 146*, pp. 596-606.

Rasha, A. Elsabagh, Reham, Amin, A. and Aziza, A. (2014). Health Risks of Some Meat Additives on Male Rats. *World Journal of Dairy & Food Sciences, vol. 9 (2),* pp. 285- 298.

Rec, G. S. C. C. (1972). Colorimetric Method for Serum Alkaline Phosphatase Determination. *Journal of Clinical Chemistry and Clinical Biochemistry,* 10(2): 182

Reitman, S. and Frankel, S. (1957). A colorimetric method for determination of serum glutamate oxaloacetate and glutamic pyruvate transaminase. *American Jounal of clinical pathology*. 28: 56-58.

Richard, C. H. (2006). Monosodium glutamate Amulti Billion dollar flavor enhancer. *Biomedicine and Pharmacotherapy, vol. 60*, pp. 86-91.

Schiffman, S.S. (2000). Intensification of sensory property of foods for elderly. *Journal of nutrition, vol. 130* pp. 927-930.

Sharma, A., Prasongwattana, V., Cha'on, U., Selmi, C., Hipkaeo, W., boonnate, P. and *et al.*(2013). Monosodium glutamate (MSG) consumption is associated with urolithiasis and urinary tract obstruction in rats. PLoS One 8:e75546.

Tchaou, M.N., Lamboni, C., Eklu-Gadegeku, K., Abalokoka, E. and Aklikokou, K. (2013). Effect of food flavour enhancer (Monosodium glutamate and maggi poulet) supplementation on glucose tolerance in Sprague Dawley Rat. International Journal of Biological Sciences, 7(1):161-171.

345 Wang, R.S., Yeh, S. Tzeng, C.R., and Chang, C. (2009). Androgen receptor roles in
346 spermatogenesis and fertility. Lessons from testicular cell-specific androgen receptor
347 knockout mice. *Endocrinology Reviews, vol. 30,* pp. 119-132.

348 Zhang, Y. Shao J-s, Thie a-m, Alpers D.H. (1996). Effect of postnatal overfeeding on
349 intestinal alkaline phosphatase activity in tissue and serum of rat treated with MSG
350 AM. J. Physiol 241: G 461-G468.