Original Research Article

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

Abstract

The effect of a daily consumption of Instant noodle seasoning containing the Monosodium glutamate (MSG) on rat was evaluated, The parameters investigated include; Alkaline aminotransferase (ALT), Aspartate aminotransferase (AST). Hemoglobin (Hb), packed 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 continuous consumption of Instant noodle seasoning may cause liver damage, and kidney dysfunction and has been discovered to have negative effects on blood and sperm cells.

INTRODUCTION

Instant noodles are commonly eaten as food for a meal after preparation with a separately included seasoning which contains food additives. 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). Instant noodle seasoning contains monosodium glutamate and most food additives are made from MSG. 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. Food additives are 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) at their own discretion. Most food additives contain sodium salt and glutamic acid in the ratio of 78% of glutamic acid and 22% of sodium and water (Adrienne, 1999). Food additives are widely used for different purposes; some use food additives in restaurant, some in household cooking while some in a commercially packed food (Alao *et al.,* 2010, Schiffman, 2000, Bojanic *et al.,* 2009). It has been observed that intake of high doses of food additives containing MSG produced series of damages in the kidney membrane, Oxidative stress, and damages in the kidney cellular organelles (Bopanno *et al.,* 1999, Bashan *et al.,* 2009 Abass and El-Haleem, 2011, Sharma *et al.*, 2013). Nwaopara *et al.,* (2004), Onyema*, et al.,* (2006), Egbuonu *et al.,* (2009) and Contini *et al.,* 2012, have reported that Monosodium glutamate has some detrimental effect on the liver at higher concentrations and may induce vacuolar degeneration of hepatocytes cords. (Ochiogu *et al.,* 2011) reported that monosodium glutamate impacted spermatogenesis through its disruption of the hypothalamic-pituitary-testis regulatory axis, and not through any direct toxic effect on the testis. In mammals, spermatogenesis is totally dependent upon testosterone (Pakarainen *et al.,* 2005; Wang *et al.,* 2009). Male infertility, testicular haemorrhage, 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). Akanya *et al.,* (2015) stated that administration of different doses of monosodium glutamate did not have any significant effect in WBC, RBC and PCV when compared with the control group. But this result is contradicts works of Eweka, (2007), 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. This research is therefore aimed at evaluating the potential effect of Instant noodle noodle seasoning a food additive containing MSG on the haematological, renal function, liver function, sperm count of male Albino rats (*Rattus norvegicus*).

MATERIALS AND METHOD

Experimental Design: A total number of twenty-four (24) male eight (8) weeks old albino rats weighing 200g -225g were used for the experiment. The 24 rats were randomly divide into a 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 cages. Rats were maintained on daily rat feed before and during the experiment. The weekly average body weights were 200, 225, 225 and 225. Based on this body weights the treatment (Indomie brand noodle seasoning) was administered to all the rats in the treated group orally 0.13g/ml directly into the esophagus of the animals with the aid of 1000µl syringe. The measurement of the treatment administered was determined in relation to the average intake of Instant noodle 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. SFRI, France.

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

Table .1: Effects of Instant noodle 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	Treated	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	Treated	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	Treated	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^{a}
Week 4	Treated	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	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}
	Control						

[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 Instant noodle 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	Treated	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	Treated	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	Treated	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	Treated	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 Instant noodle Seasoning on the Sperm Parameter of an Albino rat

		Sperm count(x^6)
Week 1	Treated	800.67±4.16 ^{aA}
	Control	475.00±25^{b}
Week 2	Treated	299.67±2.31^{bD}
	Control	575±25^{a}
Week 3	Treated	450.67 ±5.86 ^{aC}
	Control	475.00±175 ^{a}
Week 4	Treated	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

This study was specifically on the responses of albino rats to a daily dose of Instant noodle seasoning which contains monosodium glutamate as a key component. The PCV, Hb, RBC, WBC and lymphocyte in treated rats decreased when compared with the control group for week 1 and for week 4 and this decrease was significant for PCV, Hb and Lymphocyte and may be attributed to the adverse effect of additives of the Instant noodle seasoning. This result is in agreement with Rasha, *et al.,* (2014) who stated that rat treated with MSG a known key component of food additive 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 Instant noodle 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 Instant noodle 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 Instant noodle 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 Instant noodle seasoning had negative effect on the sperm count. This negative effect on Sperm count might be due to the indirect effect of instant noodle seasoning components 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 indicate that instant noodle seasoning had negative effects on parameters studied in rats which are mammals. Since the primary consumption of instant noodle seasoning is by humans which are mammals having similar though higher and more advanced anatomical and physiological responses with rats, it is advised that consumption or use of flavour enhancers containing MSG should be reduced by using less of such flavouring agents.

COMPETING INTERESTS DISCLAIMER:

Authors declare that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

Ethical Approval:

A university ethical clearance was sought for and obtained.

As per international standard or university standard ethical approval has been collected and preserved by the authors.

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