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Monosodium glutamate-containing flavour enhancer: effect on liverand kidney in experimental animal

Abstract

The safety of monosodium glutamate-containing flavour enhancer at 15 ml/Kg (0.6g/Kg) body weight was investigated on the liver and kidney function, antioxidant/lipid peroxidation and lipid profile of albino rats. Twenty eight (28) male albino rats were randomly assigned into four groups, of seven (7) rats each. Animals in group A (the control)were administered normal saline orally while animals in groups B, C, and D were administered orally with 15ml/Kg (0.6g/Kg) body weight of *Vedan*, fermented *Locust Bean (Iru)* and Maggi cubes (all are monosodium glutamate-containing flavour enhancer) respectively for 32days. Results showed that there was no significant *(P<0.05)* difference in the activities of alanine transaminase (ALT) and aspartate transaminase (AST), creatinine and uric acid level, lipid profile and level of TBARS of the liver and kidney of the animals when compared with those of the control. *Vedan*, fermented Locust Bean Seed (Iru) and Maggi significantly *(P<0.05)* increased catalase activity of the liver and kidney of the animals. Results from this study showed that *Vedan*, fermented *Locust Bean (Iru)* and Maggi cubes consumed at 15ml/Kg (0.6g/Kg) pose no adverse effect on the liver and kidney functions, generated no reactive species, increased enzymic antioxidant system and does not increase cholesterol deposition in the liver and kidney in rats.

Keywords: Monosodium glutamate, liver and kidney functions, antioxidant, lipid peroxidation, lipid profile, TBARS

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Introduction

Monosodium glutamate (MSG), also known as sodium glutamate, is the sodium salt of glutamic acid, one of the most abundant naturally occurring non-essential amino acids.[1] Trade names of monosodium glutamate include *Ac'cent*, *Aji-No-Moto* and *Ve-Tsin*. MSG was classified by the United StatesFood and Drug Administration and the European Union as a food additive.[2] The L-glutamate form of MSG confers the same taste of free L-glutamate naturally found in foods.[3] Industrial food manufacturers market and use MSG as a flavour enhancer because it balances, blends and rounds the total perception of other tastes.[4] Natural foods, especially soy sauce, strong cheeses, tomatoes and mushrooms, can contain high amounts of free glutamates that are concentrated by processing. So if a snack product has a strong flavour, some of the free glutamate is likely to be in highly flavoured additives such as concentrated soy sauce, tomato, cheese, mushroom or vegetable powder although a form of MSG such as yeast extract or hydrolysed vegetable protein can be probably also found.[5] It is impossible to tell the number of free glutamates a product contains because an FDA proposal to list this on the label was defeated by the food industry.[6]

MSG has been used for more than 100 years to season food. During this period, extensive studies were conducted to elucidate the role, benefits and safety of MSG. At this point, international and national bodies for the safety of food additives consider MSG safe for human consumption as a flavour enhancer.[7] The "MSG symptom complex" originally termed "Chinese Restaurant Syndrome" as reported by Robert Ho Man Kwok after an American-Chinese meal. Kwok suggested multiple reasons behind the symptoms, including alcohol from cooking with wine, the sodium content, or the MSG seasoning[8] and ever since, MSG had been the focus and the symptoms have been associated with it.

Food condiment (popularly called *iru*) obtained from processing fermented locust bean are taken among the Yoruba people of Nigeria and it is used as spice and flavour enhancer to meal.[9] The increased nutritive value and flavour enhancing properties of locust bean proteins was attributed to fermentation and increase in amino acids profile.[10] The fermented condiment utilizes the flavour richness associated with the high level of glutamate, among which the sodium form (MSG) has the most prominent flavour enhancing capacity.[11]

MSG was released into the market; it has been produced by three methods: hydrolysis of vegetable proteins with hydrochloric acid to disrupt peptide bonds (years 1909–1962), direct chemical synthesis with acrylonitrile (1962–1973), and bacterial fermentation, the current method.[12] MSG is freely soluble in water but not hygroscopic and practically insoluble in common organic solvents such as ether.[13] In general, MSG is stable under the conditions of regular food processing. During cooking, MSG does not decompose: Like other amino acids, browning or Maillard reactions will occur in the presence of sugars at very high temperatures.[14] Although, previous studies have reported consumption of MSG to have deleterious effects in virtually all organs which will apparently affect their functions, hence this research work focused on the effect of monosodium glutamate-containing flavour enhancer on function, antioxidant/lipid peroxidation and lipid profile of liver and kidney of experimental animals.

Materials and methods

Food additives: MSG-containing flavour enhancer: *Maggi* cubes were product of Nestlé Nigeria PLC, *Vedan* was product of Vedan

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Enterprise Corp., Taiwan and fermented *Locust Bean* seed was obtained from Awolowo Market, Sagamu, Nigeria.

Reagents and Chemicals: Sodium hydroxide, sodium chloride, sodium dihydrogen phosphate, disodium hydrogen phosphate and sodium chloride were products of Sigma Aldrich Chemicals, UK, 2-thiobarbituric acid (TBA), 5,5'-dithio 2-nitrobenzoic acid (BTNB), sulphuric acid (H_2SO_4), potassium dichromate solution ($K_2Cr_2O_7$) and hydrochloric acid were purchased from BDH Chemical Limited, Poole, England. All other chemicals are of analytical grade.

Animal Groupings: Twenty eight (28) male albino rats were purchased from the Physiology Department of the University of Ibadan, Ibadan, Nigeria. The animals were housed in individual well ventilated cages, acclimatized for two (2) weeks during which they were allowed free access to commercial pelleted rat chow (Oyedokun Feeds Limited, Ibadan, Nigeria) and weighed at the end of each week. The research adhered strictly and conformed to the Principles of Laboratory Animals Care (NIH Publication, No 85-23).

Preparation of MSG-containing flavour enhancer: The solutions of the food additives were prepared by weighing and dissolving 8g of ground *Vedan*, fermented *Locust Bean (Iru)* and Maggi cubes respectively in 200ml of distilled water.

Treatment Administration: After acclimatization, the rats were randomly assigned into four groups, each containing seven (7) rats. Animals in group A (the control) were orally administered normal saline while animals in groups B, C, and D were orally administered with 15ml/Kg (0.6g/Kg) body weight MSG-containing flavour enhancer, i.e., *Vedan,* fermented *Locust Bean (Iru)*and Maggi respectively for 32days.

Sample Collection: After 32 days, the rats were sacrificed by cervical decapitation under the influence of diethyl ether anaesthesia. Blood samples were collected from each animal by cardiac puncture with sterile needle and transferred to heparinised tubes, centrifuged at 5,000rpm for 10minutes and plasma collected and stored at -4°C until it was required for assay. The kidney and liver were excised, rinsed in ice-cold saline, damp with filter paper and weighed. The organs were homogenized in normal saline (0.85% NaCl) solution in ratio 1:4 (1mg of tissue to 4ml of normal saline solution), the homogenates centrifuged at 4000rpm for 5minutes while the supernatants were separated and stored frozen at -4°C to ensure maximum release of enzymes (i.e. alanine transaminase and aspartate transaminase) until it was required for assaying.[15]

Biochemical Analysis: Alanine transaminase (ALT) and aspartate transaminase (AST) activities were determined with commercial kits from Randox Laboratory, U.K. using Kodac Autoanalyzer machine.[16] Triglyceride level was determined,[17] total cholesterol and HDL-cholesterol levels were determined by the method of De Hoff et al.[18] and LDL-cholesterol level was calculated by the method of Nauck et al.[19] Catalase activity was determined by the method of Johansson and Borg[20] and TBARS were determined by the method of Buege and Aust.[21] Serum creatinine concentration was assayed by the method of Blass and Thibert.[22]

Statistical Analysis: All the results were expressed as mean±standard error of mean (SEM) for animals in each group. Data obtained were statistically evaluated using SPSS 15.0 software. Hypothesis testing methods included One-way Analysis of Variance (ANOVA) and subsequent comparisons among groups were made using Duncan's Multiple Range Test (DMRT). Statistical significance was set at $p< 0.05$.

Results

Effect of monosodium glutamate-containing flavour enhancer on liver and kidney function

There was no significant *(P<0.05)* difference in the activities of alanine transaminase (ALT) and aspartate transaminase (AST) of the liver of the animals administered *Vedan*, fermented *Locust Bean (Iru)* and *Maggi* when compared with those of the control (Table 1). Similarly, the kidney fuction tests showed there was no significant *(P<0.05)* difference in the level of creatinine and uric acid of the animals administered *Vedan*, fermented *Locust Bean (Iru)* and *Maggi* when compared with those of the control (Table 1).

Table 1 Effect of MSG-containing flavour enhancer on liver and kidney fucntion test

Group	Liver		Kidney	
	AST	ALT	Creatinine	Uric acid
Control	21.97±0.31^{a,b}	20.67±3.21^{a}	2.73±0.07^{a}	7.55±1.07^{a}
Vedan	20.07±2.31^{a}	21.50±0.76^{a}	2.83±0.07^{a}	6.44±0.34^{a}
Locust Bean (Iru)	22.07±0.56^{a,b}	22.90±1.89^{a}	2.89±0.09^{a}	6.99±0.45^{a}
Maggi	24.17±0.58^{b}	23.57±0.07^{a}	2.80±0.09^{a}	7.39±1.15^{a}

All values are expressed as mean ± SEM. Values with different superscript indicate significant difference at $p<0.05$.

Effect of monosodium glutamate-containing flavour enhancer on liver and kidney oxidative stress markers: *Vedan*, fermented *Locust Bean (Iru)* and *Maggi* significantly *(P<0.05)* increased catalase activity in the liver and kidney of the animals when compared with those of the control (Table 2). There was no significant *(P<0.05)* difference in the catalase activity in the liver of the animals administered *Vedan*, fermented *Locust Bean (Iru)* and *Maggi.* The catalase activity of the kidney of the animals administered *Vedan* significantly *(P<0.05)* decreased when compared to those of fermented *Locust Bean (Iru)* and *Maggi*. There was no significant difference in the catalase activity of the kidney of animals fed with fermented *Locust Bean (Iru)* and *Maggi*. Similarly, there was no significant *(P<0.05)* difference in the level of TBARS in the liver and kidney of the animals administered *Vedan*, fermented *Locust Bean (Iru)* and *Maggi* when compared with those of the control (Table 2).There was no significant difference *(P<0.05)* in the level of TBARS in the kidney of the animals fed with *Vedan*, fermented *Locust Bean (Iru)* and *Maggi*.

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Table 2 Effect of MSG-containing flavour enhancer on liver and kidney oxidative stress Markers

Group	Liver		Kidney	
	Catalase	TBARS (x10^{-6})	Catalase	TBARS (x10^{-6})
Control	8.20±0.20^{a}	1.77±0.52^{a,b}	7.93±0.13^{a}	2.40±0.06^{a}
Vedan	8.70±0.05^{b}	3.07±0.81^{a}	8.47±0.05^{c}	2.80±0.21^{a}
Locust Bean (Iru)	8.70±0.04^{b}	2.05±0.34^{a,b}	8.56±0.22^{b}	3.03±0.19^{a}
Maggi	8.57±0.12^{b}	2.35±0.06^{a}	8.48±0.30^{b,c}	2.84±0.12^{a}

All values are expressed as mean ± SEM. Values with different superscript indicate significant difference at p<0.05.

Effect of monosodium glutamate-containing flavour enhancer on liver lipid profile:

The result of the lipid profile showed there was no significant*(P<0.05)* difference in the level of total cholesterol (TC), triglyceride (TG), HDL-cholesterol (HDL-C), LDL-cholesterol (LDL-C) and VLDL-cholesterol (VLDL-C) of *Vedan* and fermented Locust Bean seed (Iru) when compared with those of the control (Table 3). Also, there was no significant difference in the level of total cholesterol (TC), triglyceride (TG), HDL-cholesterol (HDL-C) and VLDL-cholesterol (VLDL-C) of animals fed with Maggi when compared with those of the control. Maggi significantly *(P<0.05)* increased the level of LDL-cholesterol (LDL-C) when compared with those of the control and Vedan (Table 3).

Table 3 Effect of MSG-containing flavour enhancer on liver lipid profile

Group	TC	TG	HDL-C	LDL-C	VLDL-C
Control	369.67±83.67^{a}	8667.67±98.23^{a}	234.85±34.85^{a}	322.69±78.22^{a}	46.97±6.59^{a}
Vedan	334.00±62.07^{a}	7474.33±1024.83^{a}	229.67±8.63^{a}	288.07±63.64^{a}	45.93±1.73^{a}
Locust Bean (Iru)	474.00±38.32^{ab}	7395.67±1223.60^{a}	265.07±20.83^{a}	420.99±38.38^{a,b}	53.01±4.17^{a}
Maggi	572.00±37.47^{a}	7632.00±1013.00^{a}	220.76±52.96^{a}	527.85±28.69^{b}	44.15±10.59^{a}

All values are expressed as mean ±SEM. Values with different superscript indicate significant difference at p<0.05.

Effect of monosodium glutamate-containing flavour enhancer on kidney lipid profile:

The results of the lipid profile showed there was no significant *(P<0.05)*difference in the level of the kidney total cholesterol (TC), triglyceride (TG), HDL-cholesterol (HDL-C), LDL-cholesterol (LDL-C) and VLDL-cholesterol (VLDL-C) of the animals administered *Vedan*, fermented *Locust Bean (Iru)* and Maggi when compared with those of the control (Table 4). Similarly, there was no significant *(P<0.05)* difference in the level of kidney total cholesterol (TC), triglyceride (TG), HDL-cholesterol (HDL-C), LDL-cholesterol (LDL-C) and VLDL-cholesterol (VLDL-C) of the animals administered *Vedan*, fermented *Locust Bean (Iru)* and Maggi (Table 4).

Table 4 Effect of MSG-containing flavour enhancer on kidney lipid profile

Group	TC	TG	HDL-C	LDL-C	VLDL-C
Control	1103.33±238.58^{a}	3962.67±1079.56^{a}	162.32±25.79^{a}	840.87±424.56^{a}	32.46±8.93^{a}
Vedan	960.00±124.74^{a}	1530.33±157.67^{a}	186.49±13.04^{a}	922.70±124.25^{a}	37.30±2.61^{a}
Locust Bean (Iru)	1565.67±81.46^{a}	1868.50±450.64^{a}	216.72±24.20^{a}	1522.33±83.81^{a}	43.34±4.84^{a}
Maggi	1151.00±120.77^{a}	877.67±169.81^{a}	172.14±24.15^{a}	1116.57±119.35^{a}	34.42±4.83^{a}

All values are expressed as mean ± SEM. Values with different superscript indicate significant difference at p<0.05.

Discussion

Monosodium glutamate is a commonly used food enhancer, which many producers believed, should be used moderately by the consumer.[23] In this study, we investigated the effect of monosodium glutamate-containing flavour enhancer on the enzymes activities, antioxidant/lipid peroxidation and lipid profile of theliver and kidney of rats.

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were found in liver parenchymal cells and are clinically used as markers to evaluate the degree of liver injury.[24] Moderate amount of MSG-containing flavour enhancer did not induce enzymatic changes (changes of liver enzymes activity which are involved in metabolism of chemicals).

Catalase, an enzymatic antioxidant defence of cellular system removes free radicals such as reactive oxygen or nitrogen species (ROS/RNS) continually generated in living system as a result of metabolism, pathological condition or exposure to xenobiotic[25] and stabilizes membrane structure through the removal of acyl peroxides formed during lipid peroxidation reaction.[26] This may have contributed

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to the stabilisation in the concentration of non-protein thiol compounds (NPSHs) observed in MSG treated animals as the compounds play important roles in various aspects of cellular functions which include enzyme activity, signal transduction, cell division, cell protection against reactive oxygen and nitrogen species, and removal of reactive electrophiles.[27]

Lipid peroxidation is a major indicator of oxidative damage initiated by the generation and accumulation of ROS and causes impairment of membrane function.[28] The unaltered lipid peroxidation observed in this study may be attributed to a direct effect of non-generation of reactive oxygen ROS from MSG treatment. MSG-containing flavour enhancer treatment for 32days have noeffect on the livertotal cholesterol (TC), triglyceride (TG), HDL-cholesterol (HDL-C), LDL-cholesterol (LDL-C) and VLDL-cholesterol (VLDL-C) concentration. This perhaps indicates unaltered cholesterol metabolism and no attendant risk of cardiovascular disease in the rats.[29] This may be adduced to moderate intake of MSG-containing flavour enhancer as oppose to the research work of Egbonu and Osakwe[30] where glutamate (15 mg/kg), such as through its inadvertent abuse, may alters lipid status in animals by damaging high metabolic organs, such as the liver, resulting in compromised triacylglycerol and cholesterol metabolism.

Conclusion

Results from this study shows that *Vedan*, fermented *Locust Bean (Iru)* and Maggi cubes consumed at 15ml/Kg (0.6g/Kg) pose no adverse effect on the liver and kidney functions, generated no reactive species implicated in oxidative stress, tissue and organ damage, increased catalase activity and prevented cholesterol deposition in the liver and kidney.

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