Repeated Ingestion of combined Trona and Monosodium Glutamate alters selected Biochemical and Hematological Parameters in Wistar Rats

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ABSTRACT

Background: *Trona and monosodium glutamate (MSG) are cooking ingredients commonly used together in some West African countries including Nigeria and Ghana. Discrepancies regarding the safety of these agents are causes for concern.*

Objective: *To investigate the effects of repeated ingestion of combined trona and MSG on selected biochemical and hematological parameters in Wistar rats.*

Method: *Twenty four male rats weighing 160 ± 20 g were assigned into four groups (n = 6 per group). Rats were treated with distilled water (control), MSG (300 mg/kg bw), trona (500 mg/kg bw), and combined MSG (300 mg/kg bw) and trona (500 mg/kg bw) for 21 days. After the treatment, rats were euthanized and blood samples were collected for hematological and biochemical assays. Data were analyzed by one way analysis of variance (ANOVA) followed by Student's t-test, and $p < 0.05$ was considered significant.*

Results: *Repeated ingestion of monosodium glutamate and trona caused significant increase in platelets and white blood cells compared to control. Total bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and total protein were significantly raised ($p < 0.05$). Catalase and glutathione peroxidase activities were significantly reduced. Serum level of creatinine was also significantly raised ($p < 0.05$) in the treated rats compared to control.*

Conclusion: *Findings from the study indicate that repeated ingestion of combined trona and monosodium glutamate can dangerously alter the levels of endogenous antioxidants, hematological parameters, and biochemical indices of liver and kidney functions.*

Keyword: *Monosodium glutamate, trona, liver, kidney, hematology*

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INTRODUCTION:

Food is one of the necessities of life. Nutritious and balanced diet is needed for energy, growth, and healthy living [1]. Appropriate proportions of carbohydrate, protein, fat and oil, minerals and

vitamins constitute a balanced diet. These classes of food are obtained mainly from plant and animal sources. They are eaten raw or processed by cooking, drying, frying, roasting, and other methods. In processing his food, man has sought ways to preserve and enhance the flavor and palatability of what he consumes [2]. As a result, the use of food additives, food preservatives, and artificial sweeteners or seasonings has become widespread. These additives and preservatives are nutritional aids to the extent that they improve taste or retard spoilage of perishable foods [3]. The use of preservatives reduces wastage and increases the range of foods that can be made available, but the use of these chemical substances has been reported to entail risks. For example, nitrites used in meat to prevent botulism can contribute to methemoglobinemia [4]. They are also metabolized to nitrosamines that are carcinogenic [5]. Monosodium glutamate used as preservative and taste enhancer in many foods can cause angina-like symptoms known as 'Chinese Restaurant Syndrome' [6]. Other preservatives and artificial sweeteners that are used in foods and medicines include saccharin, aspartame, trona, and thaumatin [7]. The risks associated with these agents have been reported to include carcinogenicity, teratogenicity, respiratory and neurological disorders [8]. However, literature also abounds with reports that some of these agents are not that toxic. As a matter of fact, it is generally believed among scientists that monosodium glutamate is safe, even in children, pregnant, and lactating mothers [9]. Today, the use of monosodium glutamate and trona as food additives is still common. Trona is the crude form of sodium carbonate. In Nigeria, it is known as 'Kaun' among the Yoruba tribe, and is widely used to cook vegetables, and to soften meat, beans, and other foods. Its use as tenderizer is also common in other West African countries, including Ghana, Cameroun, Republic of Benin, and Togo [10]. Apart from being used for culinary purposes, it is also used as alternative medicine to treat ailments such as constipation, fever and some forms of respiratory disorders [10]. Monosodium glutamate, marketed in Nigeria as 'Ajinomoto' by West African Seasoning Company, is also widely used as food seasoning to prepare stews, the popular jollof rice, and other foods. Many times, both trona and monosodium glutamate are consumed together in one meal. Considering the discrepancies that surround the safety of these agents and their widespread use, this study was designed to evaluate the effects of co-administration of trona and monosodium glutamate on selected biochemical and hematological parameters in adult Wistar rats.

MATERIALS AND METHODS

Experimental animals:

Twenty four adult male Wistar rats weighing 160 ± 20 g were obtained from Animal House of College of Health Sciences, LAUTECH, Ogbomoso, Nigeria. They were kept in a well-ventilated laboratory with the temperature ranging between 22°C and 25°C. They were fed with animal pellets (Ladokun Feeds Ltd, Ibadan), and allowed free access to clean drinking water. The animals were allowed to acclimatize in the Laboratory for two weeks before commencement of the experiments. The animals were treated in accordance with the instructions from National Research Council Guide for the Care and Use of Laboratory Animals [11].

Treatment and Blood Sample Collection:

After procuring monosodium glutamate (MSG) and trona from Igbona market Osogbo, Nigeria, rats were assigned to four groups (n = 6 per group). Weights of rats were measured before commencement of treatment (Day 0) and after treatment (Day 22) before they were euthanized. They were treated as follows: Group 1 (control) was treated with distilled water (5ml/kg bw). Groups 2, 3, and 4 were treated with MSG (300 mg/kg bw), trona (500 mg/kg bw), and a combination of MSG (300 mg/kg bw) and trona (500 mg/kg bw) respectively. The drugs were administered orally by means of an intragastric cannula for 21 days. Thereafter, rats were made to fast overnight and then euthanized. Whole blood was taken from retro-orbital sinus into EDTA bottles for the analysis of hematological parameters [12]. Serum samples were obtained for biochemical assays from blood samples

centrifuged at 3000 rpm for 10 minutes.

Hematological Assay

Whole blood samples were assayed to determine the levels of red blood cells, white blood cells, platelets, lymphocytes, neutrophils, hemoglobin (Hb), mean cell volume (MCV), mean cell hemoglobin (MCH), and mean cell hemoglobin concentration (MCHC). The assay was carried out by automatic analyzer Sysmex KX-21N (SYSMEX, Co., Ltd., Kobe, Japan).

Biochemical Assay

Serum samples were subjected to analysis of creatinine, aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin, and total protein, using an automatic analyzer, TBA-120FR (Toshiba Medical Systems, Co., Ltd., Japan). Catalase (CAT) was assayed as previously described [13, 14]. Superoxide dismutase (SOD) activity was determined using the method of Kakkar *et al*. [15] and glutathione peroxidase (GPx) was assayed by the method previously described [16, 17],

Statistical Analysis

Data obtained were analyzed and presented as mean ± standard error of mean (SEM). Differences between groups were analyzed by one way analysis of variance (ANOVA) followed by student's *t*-test, and $p < 0.05$ was considered significant. GraphPad Prism version 6.1 for Windows (GraphPad software, San Diego California, USA) was used for the analysis [18].

Results

Co-administration of monosodium glutamate and trona caused significant increase in the platelet counts and white blood cells compared to control. The platelet count increased from 576.33 ± 10.31 to 788.50 ± 12.72, while white blood cell count increased from 7.62 ± 1.05 to 13.48 ± 2.16 after 21-day treatment with combined MSG and trona. The treatment did not cause any significant change in the level of red blood cells as shown in Table 1. Likewise, red blood cell indices were not significantly altered after treatment with trona and MSG. Co-administration of MSG and trona also resulted in significant increase in the levels of circulating lymphocytes and neutrophils.

As shown in Table 2, treatment with MSG induced an increase in the level of total bilirubin. Treatment with combined MSG and trona also significantly raised ($p < 0.05$) the levels of AST, ALT, total bilirubin, and total protein.

Table 1: Effects of trona and monosodium glutamate on hematological parameters

Parameters	Control	MSG	Trona	MSG + Trona
Red Blood Cells (%)	47.15 ± 6.55	41.61 ± 5.50	46.62 ± 6.43	44.75 ± 3.53
White Blood Cells ($x10^3$/L)	7.62 ± 1.05	11.27 ± 1.20	10.53 ± 2.00	13.48 ± 2.16*
Platelets ($x10^3$/L)	576.33 ± 10.31	521.50 ± 12.06	734.81 ± 18.64*	788.50 ± 12.72*
Hb (g/dl)	11.34 ± 2.42	9.76 ± 1.22	10.08 ± 2.14	8.92 ± 1.40
MCV (fl)	61.88 ± 5.52	59.60 ± 5.90	63.03 ± 6.84	64.57 ± 7.51
MCH (pg)	16.38 ± 2.04	18.27 ± 2.07	17.83 ± 1.90	17.81 ± 2.32
MCHC (g/dl)	25.02 ± 3.62	30.85 ± 4.06	28.37 ± 2.80	29.93 ± 3.51
Lymphocytes (%)	67.82 ± 6.40	68.35 ± 8.31	70.98 ± 6.05	86.60 ± 10.62*
Neutrophils (%)	29.18 ± 4.22	34.98 ± 5.83	39.02 ± 4.64	56.40 ± 7.55*

*$p < 0.05$ compared with the control

Table 2: Effects of trona and monosodium glutamate on AST, ALT, total bilirubin, and total protein

Groups	AST (U/L)	ALT (U/L)	Total bilirubin (mg/L)	Total protein (g/L)
Control	32.83 ± 4.41	19.50 ± 2.93	6.57 ± 1.22	6.11 ± 1.74
MSG	49.00 ± 5.63*	16.17 ± 2.80	10.55 ± 2.30*	8.43 ± 1.02
Trona	35.17 ± 4.50	31.83 ± 2.64*	6.84 ± 1.62	6.07 ± 1.25
MSG + Trona	52.72 ± 6.65*	33.33 ± 3.06*	9.02 ± 2.13*	9.99 ± 1.64*

*p < 0.05 compared with the control

Table 3: Effect of trona and monosodium glutamate on enzymatic antioxidants

Group	SOD (units/ml)	CAT (units/ml)	GPx (units/ml)
Control	2.21 ± 0.14	28.93 ± 3.61	3.33 ± 1.00
MSG	1.97 ± 0.17	23.68 ± 2.19	2.25 ± 1.06
Trona	2.61 ± 0.10	31.14 ± 2.21	2.31 ± 1.03
MSG + Trona	2.07 ± 0.12	16.63 ± 3.25*	1.18 ± 1.52*

*p < 0.05 compared with the control

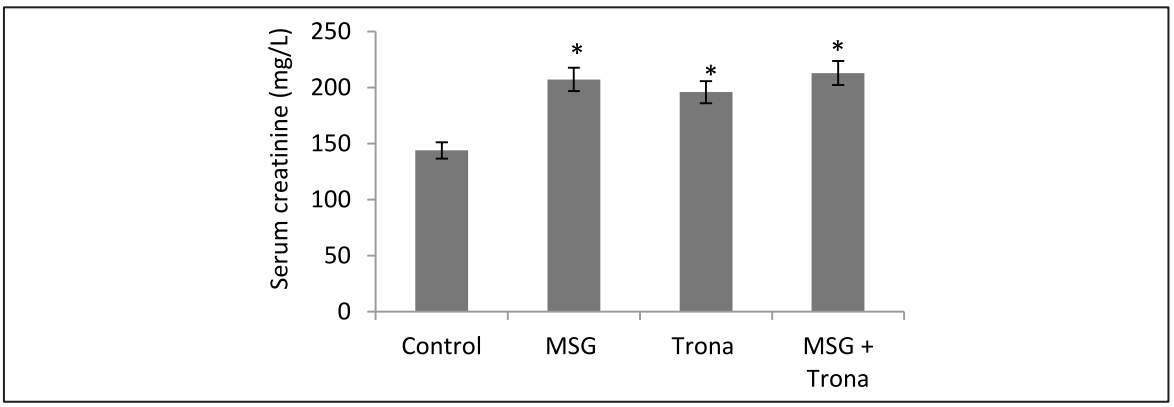

Figure 1: Effect of trona and monosodium glutamate on serum creatinine
*p < 0.05 compared with the control

Table 4: Effect of trona and monosodium glutamate on body weight (g)

Group	Day 0	Day 22	% Change
Control	175.54 ± 6.24	200.45 ± 8.22	+14.3
MSG	176. 73 ± 9.03	184.40 ± 8.07	+4.5
Trona	172.55 ± 8.81	141.07 ± 8.44*	- 18.0
MSG + Trona	182.74 ± 8.51	134.92 ± 7.74*	- 26.4

*p < 0.05 compared with the control

Antioxidant status was significantly affected by the administration of combined MSG and trona. The level of catalase was reduced from 28.93 ± 3.61 to 16.63 ± 3.25, while that of glutathione peroxidase was reduced from 3.33 ± 1.00 to 1.18 ± 1.52, as shown in Table 3.

Compared with the control, serum level of creatinine was significantly increased ($p < 0.05$) after treating rats with trona and MSG as shown in Figure 1. The weights of the animals were significantly reduced ($p < 0.05$) after treatment with trona and combined MSG and trona for 21 days. Trona caused 18% decrease in weight, and combined administration of trona and MSG caused 26.4% loss in weight, as shown in Table 4.

Discussion

Impairment of kidney and liver functions always results in serious health challenges. The liver is the major organ of metabolism while the kidney removes waste products and excess fluid from the body. Any damage to the liver and kidney leads to impaired biotransformation and accumulation of waste products in the blood stream [19]. Some of the food and drug products we consume are potential toxicants to the liver and the kidney [20]. Monosodium glutamate and trona are two food additives widely consumed together in Nigeria. This study examined the effects of co-administration of monosodium glutamate (MSG) and Trona on some indices of hepatic and renal functions. Creatinine is a nitrogenous product produced from the metabolism of creatine in the skeletal muscle and filtered by the kidney and is one of the important indices of renal function [21]. In renal damage, less of creatinine is excreted by the kidney and its serum level consequently increases [22]. This study showed that administration of MSG and Trona in rats, whether given separately or combined, results in significant increase in serum level of creatinine compared to the control. This indicates that co-administration of MSG and Trona could be deleterious to kidney function. Although some researchers have reported that MSG is safe for consumption [23, 24], other studies have shown that it induces significant decrease in liver transaminases, indicating hepatic damage [25]. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are two cytoplasmic enzymes released into the circulation only after structural damage. Therefore plasma levels of these transaminases are used as indicators of liver damage [26]. Other biochemical parameters that are indicators of tissue damage include total protein and total bilirubin. In this study, the significant increase in serum levels of these biochemical parameters after repeated administration of MSG and trona in the rats suggests liver damage. Significant increase in the level of circulating white blood cells, lymphocytes and neutrophils in rats treated with combined MSG and trona suggests that the agents elicited inflammatory processes which could also be secondary to tissue damage. This agrees with the report of Roman-Ramos *et al*. [27]. The increase in level of platelet observed following co-administration of MSG and trona also indicates bone marrow toxicity which may lead to thrombocytosis [28].

Oxidative stress is one of the mechanisms through which organs are damaged. When there is increased generation of reactive oxygen species, most cellular contents are oxidized and structural integrity and functionality of organs are adversely affected [29]. Processes of oxidative stress are mitigated by enzymatic and non-enzymatic antioxidants which are molecules that inhibit the production of free radicals and mop up those that are already generated [29]. Although the doses of MSG and trona administered in this study did not cause any significant change in the levels of superoxide dismutase, catalase and gluthathione peroxidase when given separately, combined administration of these agents significantly reduced the level of these antioxidants in rats. This suggests that repeated co-administration of these agents enhances the process of oxidative stress, which results in suppression of the activity of antioxidant system and downregulation of superoxide dismutase, catalase and glutathione peroxidase [30]. As oxidative stress progressed with little or no hindrance, the liver and kidney are damaged. Previous studies have also reported deleterious effect of MSG on the kidney [31, 32].

Ajiboye *et al*. [33] also reported depleting effect of trona on the antioxidant system. Since this is *in vivo* study, little explanation can be given regarding the mode of action of MSG and trona. Further investigations are needed to explain how the drugs interact with living cells at the molecular level.

Conclusion

Findings from the study suggest that repeated ingestion of combined trona and monosodium glutamate could deplete and suppress the antioxidant system and expose cells to oxidative stress. Hematological and biochemical parameters could also be dangerously altered. Caution should be exercised in the consumption of these culinary agents.

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