Combined Effects of Butylated Hydroxyl Anisole and Monosodium Glutamate on Liver Kidney and Blood Profile in Swiss Albino Mice

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Research Article

Abstract

Nowadays the trend of fast food consumption is very common. Butylated hydroxyl anisole is widely used to preserve lipid or fat containing food items and Mono sodium glutamate is popularized for its umami taste and used to enhance flavor in a variety of foods. The present study was conducted to find out the combined effect of Butylated Hydroxyl Anisole (BHA) and Mono Sodium Glutamate (MSG) on marker hepatic and renal parameters, hematological parameters and histology of kidney and liver. Male albino mice were divided into four groups. Group 1 was treated as control and was given normal distilled water. Group 2, 3 and 4 were fed with MSG (150 mg/kg b. wt.), BHA (50 mg/kg b. wt.) and combination of BHA and MSG for 30 days. Mice treated with MSG and combined dose of MSG and BHA revealed a significant increase in the level of urea, creatinine, SGOT and SGPT. Hematological analysis revealed significant reduction in RBC count, WBC count, haematocrit, MCH and MCHC concentration in the group 2 and 4 but no significant effect on blood parameters was noted in mice treated with BHA alone. Based on the results, it can be concluded that MSG alone and in combination with BHA may exert adverse effects on blood parameters, liver and kidney biochemical parameters. However more studies should be conducted at a higher dose level or for a chronic period to confirm the toxicity of BHA.

Keywords: Butylated hydroxyl anisole; Monosodium glutamate; Liver; Kidney; Hematology

Introduction

Food additives are substances that are not normally consumed as food itself but are added to food intentionally to prevent spoiling, improve appearance and maintain the food's nutritional quality. Food additives are classified into many functional classes

such as acidity regulators, antioxidants, colors, emulsifiers, preservatives, stabilizers, sweeteners, and thickeners [1]. These are added to food items like crackers, cereals, breads, snacks, ready to eat meals, cheese, yogurt, meats, sauces and soups etc. [2]. Chemical food preservatives are also widely used in various food industries to provide longer shelf life to food items. According to international institution of health, benzoates (such as sodium benzoate, benzoic acid), nitrites (such as sodium nitrite), sulphites (such as sulphur dioxide), sorbates (such as sodium sorbate, potassium sorbate) are some widely used chemical food preservatives [3]. Antioxidants act as oxygen absorbers/or oxygen scavengers. They nullify the effects of oxygen radicals and retard autoxidation. During autoxidation, food items may combine with oxygen in the air at room temperature and can become rancid. This rancidity of food items can be delayed by retarding autoxidation. β carotene, butylated hydroxyanisole, tocopherols, and ascorbic acid are some of the commonly used antioxidants which can be added to the food items to nullify the effects of oxygen degradation on food items rich in lipids [4].

Monosodium Glutamate (MSG; $C_5H_8NO_4Na$) is one of the most widely used food-additives. It is used as a seasoning or a flavor enhancer in food. It is designated with the E number E621. It was synthesized by Japanese chemist Kikunae Ikeda in 1908. It is a sodium salt of glutamic acid. It dissociates into Sodium cations (Na^+) and glutamate anions ($C_5H_8NO_4$) when dissolved in water. It exists as a crystalline white solid at room temperature. It has an umami taste and it intensifies meaty flavors when added to certain foods. It is used in several canned food products, spice blends, several instant noodle products and also in tobacco to enhance its taste. It is also used in the treatment of hepatic coma [5]. Butyl hydroxyl anisole is one of the most commonly used antioxidants because of its low cost, high performance and wide availability. It is popularized as E320. It is also known as Tert-butyl-4-methoxyphenol ($C_{11}H_{16}O_2$). It consists of a mixture of two isomeric organic compounds 2 tert-butyl-4-hydroxyanisole and 3-tert-butyl-4-hydroxyanisole with a waxy solid appearance [6]. As a food preservative, it is added to edible fats and fat containing food to prevent the rancidification of food which can generate objectionable odor. It is also being used in food packaging, animal food, cosmetics, rubber and petroleum products etc. [7].

Both MSG and BHA are being used at a large scale. A number of studies have been done to evaluate the effect of MSG on different animal models but none study has been performed to assess the combinational effect of MSG and BHA. Therefore present study has been designed to evaluate the combinational adverse effects of MSG and BHA.

Materials and Methods

Animal

Twenty adult male albino mice (*Mus musculus*) 25 g-30 g were used for this study. The animals were housed in plastic cages, with six mice per cage. Floors of cages were covered with soft crushed wood shavings. The animals were provided with tap water ad libitum and fed with the standard commercial chow. The animals were kept in an air conditioned room with an optimum temperature of 25 ℃ ± 2 ℃, humidity (60%-70%) and light/dark condition at animal house facility of IIS deemed to be university, Jaipur. The animal procedures were performed in accordance with guidelines for ethical conduct in care and use of animals [8].

Chemical used

Butylated Hydroxyl Anisole (BHA) and Mono Sodium Glutamate (MSG) were used as test substances and were procured from Merck, Mumbai.

Experimental design

After one week of acclimatization, the animals were divided into four groups (6 albino mice for each) as follow:

Group 1: Was treated as control group and was provided with distilled water for 30 days

Group 2: Animals were treated with MSG orally daily for 30 days (150 mg/kg b. wt.)

Group 3: Animals were treated with BHA orally daily for 30 days (50 mg/kg b. wt.)

Group 4: Animals were treated with combination of BHA and MSG orally by gavage for 30 days (150 mg/kg B. wt. MSG and 50 mg/kg B. Wt. BHA)

The study protocol for study was approved by Institutional Animal Ethics Committee (IAEC) of IIS deemed to be University, Jaipur and Rajasthan, India.

Autopsy schedule

At the end of the experimentation and 24 hours after the last dose, all animals were sacrificed under light ether anesthesia. Blood was collected from cardiac puncture. Half of the blood sample was analyzed for hematological parameters and half sample was centrifuged for serum isolation. Serum was used for biochemical parameters. Liver and kidney were dissected out and were fixed in fixative for histological preparation.

Parameters of the study

Body weight: Body weight of the control and treated mice were recorded on the first day before treatment and just after completion of treatment (day of sacrifice).

Organ weight: Liver and Kidneys were dissected out and cleaned off from adherent fat and blood clot. Each organ was weighed separately on a digital electronic balance.

Biochemical parameters: Biochemical parameters were performed on blood plasma or serum. SGPT (Serum glutamate pyruvate transaminase) and SGOT (Serum glutamate oxaloacetate transaminase) levels, Serum creatinine and blood urea were analyzed [9]. All parameters were studied by CPC stat plus 3000 biochemical analyzer using accurex kits.

Hematological parameters: Total erythrocyte count, WBC count, hemoglobin, haematocrit, Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin Concentration (MCHC) were determined using standard methods.

Histology: At the end of experimentation the kidneys and liver tissues of the albino mice were dissected out and were fixed in Bouin's fluid. Slides were prepared and stained with hematoxylin and eosin for histological examination.

Statistical analysis: Value obtained during the present study was expressed as mean ± SEM (Standard Error Mean). Significance of difference between groups was evaluated by one way ANOVA. The level of significance was considered at $p<0.05$.

Results

Effect on body and relative organ weight

Body weight of mice treated with MSG, BHA and combined dose of MSG and BHA showed no significant increase as compared to the control group [10]. A significant increase was found in the weight of liver in the mice treated with BHA ($p<0.05$) and combined dose of MSG and BHA ($p<0.01$), while no significant change was found in the mice treated with MSG. Weight of the kidney was found to be significantly increased in all the three groups {(group 1 ($p<0.01$), group 2 ($p<0.05$) and group 3 ($p<0.001$)} as compared to control mice (Table 1).

Table 1: Showing changes in body weight and organ weight.

Group/ parameters	Group 1 (control)	Group 2 (Monosodium Glutamate; MSG)	Group 3 (Butylated Hydroxyanisole; BHA)	Group 4 (BHA + MSG)
Body weight gain (%)	11.46 ±1.37	16.43 ± 3.1	12.44 ± 1.44	16.98 ± 2.70
Liver (g)	5.1 ± 0.2	6.1 ± 0.1	6.2 ± 0.3*	5.6 ± 0.2**
Kidney (g)	1.5 ± 0.009	2.0 ± 0.005**	1.6 ± 0.009*	1.4 ± 0.01***
Level of significance: Mean ± SEM *Represent p<0.05; **Represent p<0.01; ***Represent p<0.001				

Effect on kidney parameters

There was no significant change found in the level of urea in mice treated with MSG and BHA while mice treated with combination of MSG and BHA showed significant increase (p<0.001) in the serum urea content. A significant increase was noted in the level of serum creatinine in mice treated with MSG (p<0.001), BHA (p<0.05) and combined dose of MSG and BHA (p<0.01) (Table 2).

Table 2: Showing changes in urea and creatinine levels in mice of different treated groups.

Group/ Parameters	Group 1 (Control)	Group 2 (Mono Sodium Glutamate; MSG)	Group 3 (Butylated Hydroxyanisole; BHA)	Group 4 (BHA + MSG)
Urea (mg/dL)	34.5 ± 0.64	36.3 ± 0.45	36.3 ± 0.57	40.64 ± 0.58***
Creatinine (mg/dL)	0.54 ± 0.06	1.71.7 ± 0.09***	0.95 ± 0.07*	1.24 ± 0.09**
Level of significance: Mean ± SEM *Represent p<0.05; **Represent p<0.01; ***Represent p<0.001				

Effect on liver parameters

A significant increase was found in the level of both SGPT and SGOT in the mice treated with MSG (p<0.001) and combined dose of MSG and BHA (p<0.01) as compared to the control group.

Effect on blood parameters

RBC count (p<0.01; p<0.01), WBC count (p<0.001; p<0.01), haematocrit (p<0.01; p<0.001), MCH (p<0.01; p<0.01) and MCHC (p<0.01; p<0.01) concentration was significantly reduced in the group 2 and 4 while no significant change was observed in group 2 mice treated with BHA. In group 2 and 3 mice, no significant change was found in the hemoglobin concentration while mice treated with combined dose of MSG and BHA showed significant decline (p<0.01) in hemoglobin content. MCV and MCH revealed significant (p<0.01) increase in both group 2 and 4 while in group 3 after treatment with BHA only, significant (p<0.05) decline was noted in MCV only (Table 3).

Table 3: Showing changes in level of SGOT and SGPT in mice of different groups.

Group/ Parameters	Group 1 (Control)	Group 2 (Mono Sodium Glutamate; MSG)	Group 3 (Butylated Hydroxyanisole; BHA)	Group 4 (BHA + MSG)
SGOT (u/L)	29 ± 1.1	55 ± 1.7***	30 ± 1.8	42 ± 2.1**
SGPT (u/L)	61.3 ± 1.1	84.17 ± 1.9***	66.4 ± 2.3	76.4 ± 1.1**
Level of significance: Mean ± SEM *Represent p<0.05; **Represent p<0.01; ***Represent p<0.001				

Histology

Histological analysis of liver in control mice reveals normal appearance of hepatocytes arranged in cordlike manner around the central vein. Histology of the liver of mice treated with MSG showed necrotic and degenerative changes in hepatocytes and Kupffer cells, hemorrhage, dilation of sinusoids and presence of bi-nucleated cells. Mice treated with BHA showing widened sinusoids, dilation of the

central vein, and cytoplasmic vacoulaization in few hepatocytes. Mice treated with MSG+BHA also showed enlarged Kupffer cells, widened sinusoids and vacuolization.

Kidney histopathology of control mice revealed normal renal cortex and glomerular tufts surrounded by Bowman's capsule. Proximal convoluted tubules were seen with cuboidal cells with round basal nuclei. Distal convoluted tubules were also seen normal with cubical cells having centrally located nuclei. Mice treated with MSG showed distorted glomeruli, widened Bowman's capsule along with degenerative changes in tubules. Treatment of BHA did not exert severe effects on the histology of the kidney. Degeneration in glomeruli and Bowman's capsule along with vacuolization and dilation in tubules was also observed in kidney of mice treated with combination of MSG and BHA.

Discussion

Mono Sodium Glutamate (MSG) and Butylated Hydroxyl Anisole (BHA) are being used as food additives (flavor enhancer) and preservatives respectively at a large scale in food items. Humans are more prone to exposure to both of these as lifestyle and food habits of people have changed extensively now a days [11]. In the present study, the effect of MSG and BHA alone and in combination was assessed on blood profile along with renal and hepatic parameters (Table 4).

Table 4: showing changes in hematological parameters and indices in mice of different groups.

Group/Parameters	Group 1 (Control)	Group 2 (Mono Sodium Glutamate; MSG)	Group 3 (Butylated Hydroxyanisole; BHA)	Group 4 (BHA + MSG)
RBC count ($^{*}10^{6}$)	5.7 ± 0.18	4.2 ± 0.17**	5.0 ±0.22	4.08 ± 0.32**
WBC count ($^{*}10^{3}$)	7.03 ± 0.14	5.28 ± 0.19***	7.36 ± 0.20	6.20 ± 0.32**
Hemoglobin (g/dl)	13.4 ± 0.40	11.9 ± 0.24	13.7 ± 0.41	11.4 ± 0.39**
Hematocrit (%)	43.4 ± 0.89	37.8 ± 1.05**	42.2 ± 0.40	35.2 ± 1.19***
MCH (pg)	22.1 ± 0.56	26.3 ± 0.69**	20.4 ± 0.68	26.7 ± 1.17**
MCV (fl)	65.0 ± 0.78	70.4 ± 0.54**	61.5 ± 0.38*	69.5 ± 1.39**
MCHC (g/dl)	33.98 ± 1.05	30.06 ± 0.47**	31.1 ± 0.63	29.9 ± 0.99**
Level of significance: Mean ± SEM *Represent p<0.05; **Represent p<0.01; ***Represent p<0.001				

Body weight and organ weight measurement after exposure to any toxicant has been considered as a major parameter to reflect the adverse effects of any compound. The body weight of mice treated with MSG and BHA did not represent any significant change in association with the control group [12]. Similar results were observed in mice after treatment with MSG by Celestino, et al., significant increase in the relative weight of liver was observed in the mice after treatment with BHA and combination of both while relative weight of kidney showed significant increase in all the three treated groups (MSG, BHA and combination of both). Similar increases in the weight of liver after BHA treatment and kidney after treatment with MSG and BHA has been recorded. Alterations in the weight of kidney and liver reflect treatment related changes including hepatocellular hypertrophy, renal toxicity, tubular hypertrophy or chronic progressive nephropathy [13].

Biochemical and histo pathological analysis is one of the routinely used procedures for detecting organ specific effects related to chemical exposure (Figure 1). Urea and creatinine are frequently calculated to gain information about renal function [14]. Significant

increased serum urea in group 3 and creatinine level in all the treated groups can be correlated with altered tubular reabsorption and glomerular filtration rate or enhanced level of free radicals [15].

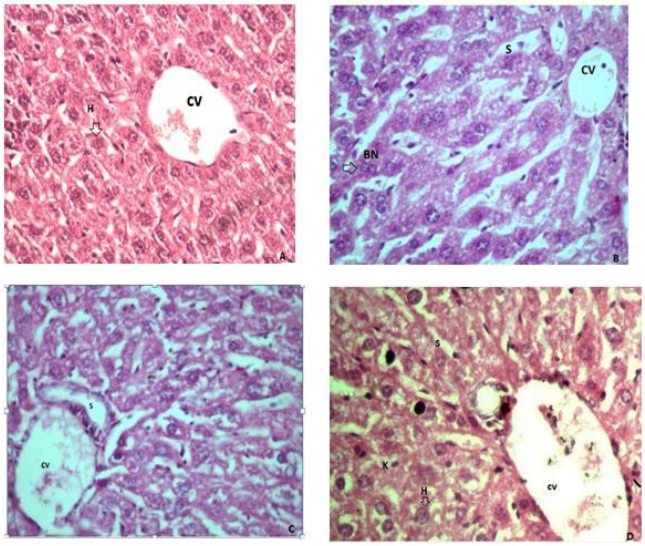

Figure 1: A) Showing normal histo architecture of liver at 400 X in control mice; B) Group 2 treated with MSG showing binucleated cells and widened sinusoids; C) Group 3 treated with BHA showing widened sinusoids; D) Group 4 treated with MSG +BHA showing enlarged kupffer cells, widened sinusoids and vacuolization at 400X (H and E stained).

A significant increase in concentrations of SGOT and SGPT was observed in the mice after being treated with MSG and MSG+BHA. Increased levels of SGOT and SGPT indicate damage in liver cells as these enzymes are released into the bloodstream at greater rate by the damaged hepatocytes [16]. Similar increases in the level of SGOT and SGPT levels have been reported by Emerole, et al., Kolawole, Nnadozie, et al. in MSG treated animals. SGOT and SGPT levels were found to be similar as a control group which indicated no adverse impact of BHA on the liver. Our results are in accordance with the Dassarma, et al. who had reported that BHA showed protective effect on the hepatocytes by reducing the level of free radicals. BHA is a synthetic analogue of Vitamin E and reveals similar activity as vitamin E [17]. It gets converted to its respective radical and halts the chain reaction during lipid peroxidation (Figure 2).

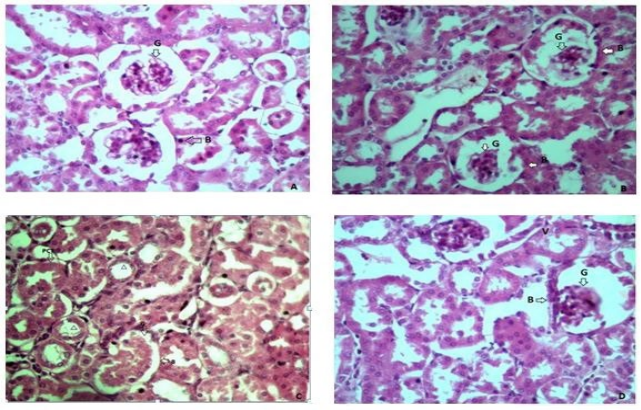

Figure 2: A) Showing normal histoarchitecture of kidney in control mice; B) Showing degenerated glomeruli and vacuolization in mice treated with MSG; C) Showing almost normal histoarchitecture of kidney in mice treated with BHA; D) Showing degeneration in glomeruli and Bowman's capsule along with vacuolization and dilation in tubules at 400X (H and E stained).

Hematological parameters such as Hemoglobin (Hb), Hematocrit (Hct), Red Blood Cell (RBC), White Blood Cell (WBC), and hematological indices such as Mean Cellular Volume (MCV), Mean Cellular Hemoglobin (MCH), and Mean Cellular Hemoglobin Concentration (MCHC) are broadly used in toxicological studies to assess physiological and pathological changes induced by toxicants [18,19]. A significant decrease was noted in the RBC count, hematocrit and MCHC in the mice treated with MSG as compared to the control group. Reduced count of red blood cells might be due to direct toxicity of MSG on RBCs or due to adverse effects on bone marrow stem cells. Increased oxidative stress may also be responsible for reduced RBC count. Reduced erythrocyte numbers and MCHC induced a reduction in the hemoglobin concentration. Mean corpuscular volume and Mean corpuscular hemoglobin act as critical parameters for the diagnosis and morphologic classification of anemia's for over a century. Increased MCV and MCH might result in macrocytic anemia or pernicious anemia respectively.

Distorted histopathology of the liver due to exposure of MSG and combination of BHA and MSG are in accordance with the findings of Abhilash, et al., Dixit, et al., Contini, et al., who reported altered histoarchitecture, glomerular hyper cellularity, tubular swelling and infiltration of inflammatory cells in rat kidneys after treatment with MSG. These histological changes might be due to induced oxidative stress by exposure of MSG. Exposure to BHA didn't reveal any adverse impact on histology of liver and kidney.

Conclusion

Based on observations and results, it can be concluded that consumption of MSG might exert a negative impact on hepatic, renal and hematological parameters. So people should avoid excessive use of MSG in their daily diet. BHA exposure has also shown some changes in biochemical and histological profile but to ensure, more studies on animal models should be conducted to confirm the effects of BHA at higher dose levels and for long duration.

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Conflict of Interest

There is no conflict of interest.

Author Contributions

Ms Prachi Chauhan performed the experiments and analyzed the data. Dr Geeta Pandey supervised, critically reviewed and finally approved the manuscript. Dr Keerti Sharma assisted in analyzing the data and critically reviewing of the manuscript.

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