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Adverse Effects of Some Food Additives in Adult Male Albino Rats

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ABSTRACT

This study aims at investigating the toxicological and biochemical effects of different types of food additives (tartrazine, monosodium glutamate, sunset yellow, and sodium benzoate) on adult male albino rats. Thirty five (35) adult male albino rats with body weight (150 – 200 g) were randomly assigned into four treatment groups (n=7), and a control group (n=7). In the 1st treatment group, rats received a daily oral dose of 20 mg/kg b. wt. tartrazine for sixty days. In the 2ndtreatment group, rats received a daily oral dose of 100 mg/kg b. wt. monosodium glutamate for sixty days. In the 3rd treatment group, rats received a daily oral dose of 200 mg/kg b. wt. sunset yellow for sixty days. In the 4th treatment group, rats received a daily oral dose of 100 mg/kg b. wt. sodium benzoate for sixty days. Rats in the control group received 1 ml/100g b. wt. distilled water for sixty days. At the end of the experiment duration, blood samples were collected for determination of different biochemical parameters. Data showed that rats administrated with tartrazine had a highly significant increase in MDA, GPx, and a significant increase in CAT activity. Also, they showed a highly significant increase in ALT, AST, ALP, serum total bilirubin, serum albumin, LDL-cholesterol, HDL-cholesterol, total cholesterol, total lipids, serum urea and creatinine, serum sodium and potassium concentrations, fasting blood glucose level, alpha- fetoprotein, and protein kinase C levels. Monosodium glutamate treated rats showed a highly significant increase in MDA, GPx, CAT., ALT, AST, ALP, serum total bilirubin, serum albumin, LDL-cholesterol, HDL-cholesterol, total cholesterol, total lipids, serum urea and creatinine, serum sodium, potassium, chloride and phosphorous concentrations, fasting blood glucose level, alpha-fetoprotein, and protein kinase C levels. Sunset yellow treated rats showed a highly significant increase in total bilirubin, total cholesterol, total lipids, serum urea and creatinine, serum potassium and chloride concentrations. Sodium benzoate treated rats showed a highly significant increase in MDA, GPx, CAT., ALT, AST, ALP, serum total bilirubin, serum albumin, LDL-cholesterol, HDL-cholesterol, total cholesterol, total lipids, serum urea and creatinine, serum potassium and phosphorous concentration, fasting blood glucose level, alpha-fetoprotein and protein kinase C. They also showed a significant increase in serum sodium and chloride concentrations. We concluded that tartrazine, monosodium glutamate, sunset yellow, and sodium benzoate adversely affect and alter different biochemical parameters, especially those related to vital organs e.g. liver and kidney and that sunset yellow has the least adverse effects in comparison with the other food additives.

Key words: Tartrazine, Monosodium glutamate, Sunset yellow, Sodium benzoate, Male albino rats, Biochemical parameters, Oxidative stress, Alanine aminotransferase (ALT).

Introduction

Food additives are products added to the basic food stuffs with an aim of improving its aspect, flavour, taste, colour, texture, food value and conservation (Imane *et al.*, 2011). It play a vital role in today`s bountiful and nutritious food supply. They allow our growing population to enjoy a variety of wholesome and tasty foods year round (Amin *et al.*, 2010).The use of preservatives in food industry has been increased with the advancement in the production technologies. Food additives are divided into five broad categories according to their function: 1) Taste Enhancers: taste is comprised of the rudimentary sensations of sweet, sour, bitter, and salty, 2) Antioxidants: that protect oily and fatty foods from spoilage by inhibiting lipid peroxidation and preventing the disintegration of lipid-soluble vitamins, 3) Preservatives: enhance food safety and extend shelf life by limiting viral, bacterial, and fungal growth. They are added to a wide variety of foods, especially those with high carbohydrate content, 4) Stabilizers and Emulsifier: these include lecithin, gelatins, corn starch, waxes, gums, propylene glycol, and cation scavengers such as ethylenediamine tetra-acetic acid (EDTA) 5) Coloring agents: they are derived from multiple animal, vegetable and mineral sources (Eman *et al.*, 2014).

Food dyes are the most interesting group of food additives, frequently color of a product determinates its attractiveness for consumer, moreover they are found to have an effect on the food choice by influencing taste, sweetness and pleasantness (Soheila *et al.*, 2012; Visweswaran and Krishnamoorthy, 2012).

Tartrazine is one of the Egyptian famous food additives which are used as a coloring substances (Amin *et al.*, 2010), Moreover, it is used in cooking in many developing countries as a substitute for saffron (Mehedi

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et al., 2009). Tartrazine known as E102 or FD&C Yellow 5 is a synthetic lemon yellow azo dye used as a food coloring. Many products contain tartrazine like foods cotton candy, soft drinks, flavored chips (Doritos, Nachos, etc.), cereals (cornflakes, muesli, etc.), cake mixes, soups, sauces, some rice, ice cream, candy, chewing gum, marzipan, jam and jelly, also, some medical preparations contain tartrazine such as vitamins, medicinal capsules and certain prescription drugs (Amin *et al.*, 2010).

Sunset yellow also known as disodium 6-hydroxy-5-[(4-sulfophenyl)azo]-2-naphthalenesulfonate is a synthetic dye commonly known as sunset yellow, Orange Yellow E110 used in food coloring. This dye gives a reddish-yellow color to foods and drugs, it is found in orange sodas. Also, it is generally used in bakery/biscuits industries (Veena and Geeta, 2013).

Monosodium glutamate (MSG), the sodium salt of amino acid glutamate, is a food additive, popularly used all over the world as "flavor enhancer". It is used to flavor meat, poultry, sauces, and soups. As food additive, MSG is described and listed as flavoring or hydrolyzed vegetable protein (Egbuonu *et al.*, 2009). Through its stimulation of the oro-sensetory receptors and by improving the palatability of meals, MSG influences the appetite positively, and induces weight gain (Manal and Nawal, 2012).

Sodium benzoate is a widely used food and beverage preservative. It is a common preservative in soft drinks because it suppresses the growth of bacteria and fungi under the acidic conditions found in carbonated beverages. Also, it is used in many foods including salads, carbonated drinks, jams and fruit juices as well as in pharmaceutical industries for preservation of liquid medicines (Nettis *et al.*, 2004). It is usually produced chemically and added as a preservative in foods where it has major antimicrobial function, being most effective against yeast and mould. Sodium benzoate occurs naturally in several fruits like the apples, cranberries, prunes and in spices like cinnamon and cloves. The presence of sodium benzoate in these foods does not make it function as a preservative (Onyemaobi *et al.*, 2012).

Some food additives however, have been prohibited from use because of their toxicity, as excessive use of some of these chemicals in food materials may cause toxicity in human (Muhammad *et al.*, 2012). Food additives are considered of the Xenobiotics that humans are subjected to exposure (Robert *et al.*, 2009). The metabolism of Xenobiotics to large extent takes place in the liver. The byproducts of such metabolism sometimes become more toxic than the initial substance (Oscar *et al.*, 2006). Xenobiotics can produce a variety of biologic effects, including pharmacologic responses, toxicity, immunologic reactions, and cancer (Robert *et al.*, 2009).

The individual response varies not only according to dose, age, gender, nutritional status and genetic factors, but also according to long term exposure to low doses (Amin *et al.*, 2010). Human studies indicated that tartrazine can induce wide range of allergic reactions in sensitive or atopic individuals. Thus, exposure of excessive colorants to those vulnerable in population may pose a health risk (Nabila *et al.*, 2013). A variety of immunologic responses have been attributed to tartrazine including: neurobehavioral toxicity, anxiety, migraines, clinical depression, blurred vision, itching, general weakness, heat waves, feeling of suffocation, purple skin patches and sleep disturbances (Hala *et al.*, 2013). Animal studies also have established the DNA-damage (mutagenic) effect of tartrazine (Visweswaran and Krishnamoorthy, 2012). Certain food colors such as sunset yellow has been examined in bacterial and animal studies and it has been found that its mutagenicity varies widely, depending on the dose consumed, implying that it may also act as mutagenic and/or carcinogenic agent in human (Mohammed *et al.*, 2014). Sunset yellow as a synthetic dye may cause allergy, respiratory problems, thyroid tumors, chromosomal damage, urticaria, hyperactivity and abdominal pain (Soheila *et al.*, 2012).

Monosodium glutamate may have brain damaging potentials, stunted skeletal development, behavioral aberration, neuro-endocrine disorder, possible learning deficits, seizures (epileptic fits), learned taste aversion and hyperglycemia. Added to these, MSG intake has been implicated in the Chinese restaurant syndrome manifested by migraine, diarrhea, weakness, vomiting, stomach ache and tightness of the chest. It was also reported that MSG could produce symptoms such as numbness, flushing, sweating, dizziness, headaches, asthma, atopic dermatitis, ventricular arrhythmia, neuropathy and abdominal discomfort (Bassey *et al.*, 2012). It was found that this food preservative, in human lymphocytes, induced chromosome aberrations and sister chromatid exchanges, and decreased replication and mitotic indices that were dose-dependent (Onyemaobi *et al.*, 2012).

Sodium benzoate is recommended as a preservative for a number of food products consumed by humans at an optimum level of 0.1 %). The recommended limits in foods are 0.1 to 0.5 % for different countries. However, sodium benzoate has already been the subject of concern about cancer because when mixed with another additive, vitamin C, in soft drinks, it forms benzene, a carcinogenic substance. It also may damage mitochondrial DNA. Both short- and long-term studies of the effects of sodium benzoate *in vivo* have investigated various enzymes and suggested adverse effects of both chronic and subchronic intake or the absence of negative effects (Ibekwe *et al.* 2007).

Although many studies have been carried out to look for toxic effects of the food additives used in the present study in laboratory animals, making considerable progress in understanding its *in vivo* effects is still

controversial. The present study, therefore, aims to investigate and compare some of the toxic effects and biochemical changes induced by tartrazine, monosodium glutamate, sunset yellow, and sodium benzoate in adult male albino rats.

Materials and Methods

Experimental animals

Adult males Sprague Dawely white albino rats (*Rattus norvegicus*), weighting about 150 to 200 g obtained from The National Research Centre in Egypt, were housed in well ventilated room with under controlled laboratory conditions of temperature (25^{o}C), 12h light/12h dark cycle, and humid conditions. The animals were housed in plastic cages under standard hygienic conditions, supplied with enough standard rat chow pellets and drinking tap water *ad libitum*. All the animals received human care throughout the duration of the experiment.

Chemicals

Monosodium glutamate (E621), tartrazine (E102), sunset yellow (E110) and sodium benzoate (E211) used in this study were of food-grade packages, while all other chemical reagents and solvents were of analytical grade.

Experimental design

Before the onset of the experiment, the 100 days old thirty five male albino rats were acclimatized for fortnight, in plastic cages with upper steel mesh and were randomly divided into five equal groups (7/cage) according to the following scheme:

Group I: (Normal control group), rats of this group were normal healthy rats and received a daily oral dose of distilled water with a dose volume of 1mL/100 g of body weight. **Group II:** received monosodium glutamate orally in a daily dose of 100 mg/kg body weight for sixty days. **Group III:** received tartrazine by oral gavage in a daily dose of 20 mg/kg body weight. **Group IV:** received sunset yellow in a daily dose of 200 mg/kg body weight for sixty days. **Group V:** received daily dose of 100 mg/kg body weight sodium benzoate. All chemicals were dissolved in distilled water and given to rats in a dose volume of 1 ml/100 g body weight, in order to optimize doses; all animals were fasted for 1 hour prior to doses administration. Experimental duration was two months and rats were observed daily for general conditions.

Collection of blood samples

At the end of the experiment period (60 days), rats were deprived of food but not water over night. Blood samples were collected on the day of sacrifice in glass tubes without anticoagulant for the separation of serum. The blood samples were allowed to coagulate at room temperature and centrifuged at 3000 rpm for 10 min, then serum was stored at -80 ^{0}C.

Biochemical assay

Determination of oxidative stress markers

Serum lipid peroxide (malondialdhyde) concentration was determined according to the method described by Satoh (1978). Glutathione peroxidase activity was determined according to the method of Paglia and Alentine (1967). Catalase activity was determined by the method of Aebi (1984) for colorimetric method.

Liver functions

Serum ALT and AST were determined by the method of Reitman and Frankel (1957), for colorimetric method. Serum Alkaline phosphatase (ALP) activity was determined by Belfield and Goldberg (1971) colorimetric method. Serum total bilirubin was estimated by the method described by Walter and Gerade (1970), for colorimetric method. Serum albumin test was performed by the method devised by Doumas et al. (1971), which is a colorimetric method.

Lipid profile

Serum LDL-cholesterol concentration was estimated by the method of Wieland and Seidel (1983), for enzymatic colorimetric method. Serum HDL concentration was estimated by the method of Burstein et al. (1970), for enzymatic colorimetric method. Serum cholesterol concentration was estimated by the method of Richmond (1973), which is an enzymatic colorimetric method. Serum cholesterol concentration was estimated by the method described by Zollner and Kirsch (1962) that is an enzymatic colorimetric method.

Kidney Functions

Serum urea concentration was estimated by the method described by Fawcett and Soctt (1960) which is urease-berthelot method. Serum creatinine concentration was estimated by the method of Schirmeister *et al.* (1964), which is a colorimetric method.

Electrolytes

Serum sodium concentration was determined by the method of Trinder (1951) which is a colorimetric method. Serum potassium concentration was determined according to the method of Sunderman, (1958). Serum chloride concentration was determined by the method of Schales, (1941). Serum phosphorus concentration was determined according of the method of El-Merzabani *et al.* (1977).

Determination of glucose, alpha fetoprotein and protein kinase C

Fasting blood glucose level, alpha fetoprotein, protein kinase C were estimated according to (Baraham and Trinder**,** 1972), (Bates, 1991) and (Wu JC. *et al.*, 1988; Wilkinson and Hallam, 1994**)** methods respectively**.**

Statistical Analysis

Results obtained from this experiment were analyzed by comparing values for different groups with the values for controls. Results were expressed as means ± S.E. The significant differences among values were analyzed by using analysis of variance (one-way ANOVA) with the statistical package for social sciences (SPSS) for windows version 10.0. Differences were considered significant at $P \leq 0.05$ level of significance.

Results

Oxidative stress markers (Lipid Peroxides, Glutathione Peroxidase, and Catalase) activity.

Lipid peroxide (Malondialdehyde)

Oral administration of tartrazine, MSG and sodium benzoate to adult male albino rats caused a highly significant increase in lipid peroxide level. Their values respectively are (2.33±0.14nmol/ml), (2.83±0.18 nmol/ml) and (2.47±0.25 nmol/ml) compared to control (1.23±0.02 nmol/ml). On the other hand, a daily oral dose of sunset yellow for two months did not affect lipid peroxide level (1.50±0.08 nmol/ml) compared to control (Table1).

Glutathione Peroxidase

Oral administration of tartrazine, MSG, and sodium benzoate to adult male albino rats had a highly significant effect on glutathione peroxidase activity (5.31±0.44 mU/ml), (6.98±0.54 mU/ml) and (5.81±0.59 mU/ml), as it increased compared to control (3.36±0.045 mU/ml). On the other hand, sunset yellow produced no effect on glutathione peroxidase activity (Table1).

Catalase Activity

A significant increase in catalase activity resulted from two months of administration of tartrazine, MSG, and sodium benzoate. Their values are (1.30±0.03U/L), (2.46±0.243U/L) and (2.08±0.25 U/L) respectively compared to control. (1.92±0.15U/L), while, administration of sunset yellow has no effect on catalase activity (1.29±0.04U/L) compared to control (Table1).

Table 1: Effect of Tartrazine, monosodium glutamate, sunset yellow, and sodium benzoate in daily doses of 20,100,200,100 mg/kg b.wt., respectively for two months on oxidative stress markers (lipid peroxides, glutathione peroxidase, and catalase) in adult male albino rats (means ± SE).

	Lipid peroxides (nmol/ml)	Glutathione peroxidase (mU/ml)	Catalase (U/L)
Control	1.23±0.02	3.36±0.045	1.30±0.03
Tartrazine	2.33±0.14**	5.31±0.44**	1.92±0.15*
Monosodium glutamate	2.83±0.18**	6.99±0.54**	2.46±0.24**
Sunset yellow	1.51±0.08	3.612±0.29	1.29±0.04
Sodium Benzoate	2.47±0.25**	5.81±0.59**	2.08±0.25**

**significant at p≤ 0.05 compared to control. **Highly significant at p ≤ 0.05 compared to control.*

Liver functions

Oral doses of tartrazine, MSG, or Sodium Benzoate for two months caused a highly significant increase in ALT activity. Their values respectively were (65.68±2.47 U/ml), (47.38±3.15 U/ml) and (64.01±2.58U/ml) compared to control (28.27±1.05 U/ml). Non-significant increase in ALT activity was recorded in the sunset yellow treated rats (37.44±3.02 U/ml) compared to control rats (Table 2).

Receiving of tartrazine, MSG, or sodium benzoate showed a highly significant increase in AST activity (62.90±2.47 U/ml), (43.78±3.36 U/ml) and (60.87±2.49 U/ml) respectively as compared to control (24.85±0.76

U/ml), while, administration of sunset yellow for two months showed a significant increase in AST activity (37.37±4.52 U/ml) compared to control (Table 2).

Administration of tartrazine, MSG, or sodium benzoate resulted in a highly significant increase in alkaline phosphatase activity (106.78±3.46 IU/L), (88.41±2.57 IU/L), (107.05±3.44 IU/L) compared to control (73.09±1.39 IU/L). On the other hand, sunset yellow showed a non-significant effect on alkaline phosphatase activity (78.54±2.71 IU/L) compared to control (Table 2).

Tartrazine, MSG, sunset yellow, or sodium benzoate administration increased total bilirubin concentration at a highly significant level, (1.49±0.07 mg/dl), (1.06±0.08 mg/dl), (0.91±0.13 mg/dl), and (1.64±0.08 mg/dl) respectively, compared with control (0.45±0.03 mg/dl) (Table 2).

Table 2: Effect of tartrazine, monosodium glutamate, sunset yellow, and sodium benzoate in daily doses of 20,100,200,100 mg/kg b.wt., respectively for two months on oxidative liver functions (ALT, AST, Total Bilirubin, Alkaline Phosphatase) in albino rats (means ± SE).

	ALT (U/ml)	AST (U/ml)	Alkaline Phosphtase (IU/L)	Total Bilirubin (mg/dl)
Control	28.27±1.05	24.85±0.76	73.09±1.39	0.45±0.03
Tartrazine	65.68±2.47**	62.90±2.48**	106.77±3.46**	1.49±0.07**
Monosodium glutamate	47.38±3.15**	43.78±3.36**	88.40±2.57**	1.06±0.08**
Sunset yellow	37.44±3.01	37.37±4.52*	78.54±2.71	0.91±0.13**
Sodium Benzoate	64.00±2.58**	60.87±2.49**	107.05±3.44**	1.64±0.08**

**significant at p≤ 0.05 compared to control. ** Highly significant at p ≤ 0.005 compared to control.*

Lipid profile

Rats that received tartrazine showed a highly significant increase in LDL concentration (84.43±5.57 mg/dl) compared to control rats (38.54±0.99 mg/dl). Also, LDL concentration highly significantly increased in case of administration of MSG (104.78±10.30 mg/dl) compared to control. Administration of sunset yellow has a non-significant effect on LDL concentration (43.60±3.70 mg/dl) compared to control. Sodium benzoate data showed a highly significant increase in LDL concentration at a highly significant level (101.64±8.38 mg/dl) compared to control (Table 3).

Oral administration of tartrazine resulted in a high significant increase in HDL concentration (51.92±3.39 mg/dl) compared to control (29.56±0.63 mg/dl). Also there was a highly significant increase in HDL concentration level (65.05±6.41mg/dl) upon oral administration of MSG, compared to control. On the contrast, administration of sunset yellow resulted in a non-significant effect on HDL concentration (31.66±2.14 mg/dl) compared to control. Sodium benzoate results recorded a highly significant elevation in HDL concentration (68.70±5.32 mg/dl) compared to control (Table 3).

Oral administration of tartrazine MSG, sunset yellow, or sodium benzoate increased total cholesterol concentration by (154.25±5.93 mg/dl), (175.07±9.99 mg/dl), (106.15±5,75 mg/dl), and (172.16±8.93 mg/dl) respectively, compared to the control (66.35±2.31mg/dl) in adult male albino rats (Table 3).

Total lipids concentration was highly significantly increased after administration of tartrazine MSG, sunset yellow, or sodium benzoate. Concentration levels were (859.39±5.66 mg/dl), (880.16±10.45 mg/dl), (812.89±6.63 mg/dl), and (879.35±9.97 mg/dl) respectively compared to control(139.33±2.48 mg/dl) (Table 3).

Table 3: Effect of tartrazine, monosodium glutamate, sunset yellow, and sodium benzoate in daily doses of 20,100,200,100 mg/kg b.wt. respectively for 2 months on lipid profile (LDL cholesterol, HDL cholesterol, total cholesterol, total lipids) in albino rats (Means ± SE).

	LDL- Colesterol (mg/dL)	HDL-Cholesterol (mg/dL)	Total Cholesterol (mg/dL)	Total lipids (mg/dL)
Control	38.54±0.99	29.56±0.63	66.35±2.33	139.33±2.48
Tartrazine	84.43±5.57**	51.92±3.39**	154.25±5.96**	859.39±5.66**
Monosodium glutamate	104.78±10,30**	65.05±6.41**	175.07±9.99**	880.16±10.45**
Sunset yellow	43.60±3.70	31.66±2.14	106.15±5.75**	812.89±6.63**
Sodium Benzoate	101.64±8.38 **	68.70±5.32**	172.16±8.93**	879.35±9.97**

***Highly significant at p ≤ 0.005 compared to control.*

Kidney Functions

Serum urea concentration was highly significantly elevated in values of (156.69±6.42mg/dl), (185.53±4.35mg/dl), (71.96±4.35 mg/dl), and (126.07±4.23 mg/dl) as a result of administration of tartrazine, MSG, sunset yellow, or sodium benzoate compared to control (44.41±1.61 mg/dl) (Table 4).

Serum creatinine concentration was increased in a highly significant level (0.72±0.07mg/dl), (1.11±0.06 mg/dl), (0.74±0.04 mg/dl) and (0.95±0.03 mg/dl) upon daily oral administration of tartrazine, MSG, sunset yellow, or sodium benzoate respectively compared to control (0.41±0.02 mg/dl) (Table 4).

Albumin concentration increased in rats treated with tartrazine, MSG, or sodium benzoate at a highly significant levels (5.31±0.17 g/dl), (4.66±0.19 g/dl), and (5.44±0.16 g/dl) respectively compared to the control

(3.67±0.09 g/dl). But, oral administration of sunset yellow did not significantly affect albumin concentration (4.18±0.29 g/dl) compared to control (Table 4).

Table 4: Effect of tartrazine, monosodium glutamate, sunset yellow, and sodium benzoate in daily doses of 20,100,200,100 mg/kg b.wt. respectively for 2 months on kidney functions (urea, creatinine, and serum albumin) in albino rats (Means±SE).

	Urea (mg/dl)	Creatinine (mg/dl)	Albumin (g/dl)
Control	44.41±1.61	0.41±0.02	3.67±0.09
Tartrazine	156.69±6.42**	0.72±0.07**	5.31±0.17**
Monosodium glutamate	185.53±4.35**	1.11±0.06**	4.66±0.19**
Sunset yellow	71.96±4.35**	0.74±0.04**	4.18±0.29
Sodium Benzoate	126.07±4.23**	0.95±0.03**	5.44±0.16**

** *Highly significant at p ≤ 0.05 compared to control.*

Electrolytes

A significant increase in sodium (151.85±0.87nmol/L) resulted from tartrazine administration compared to control (138.75±0.80nmol/L). MSG administration resulted in a highly significant increase in serum sodium concentration (156.77±2.07 nmol/L) compared to control. Oral administration of sunset yellow has no effect on sodium (139.79±1.35 nmol/L) compared to control. Oral administration of sodium benzoate significantly increased sodium (142.45±0.58 nmol/L) compared to control (Table 5).

In case of potassium ions, administration of tartrazine caused highly significant increase in serum potassium concentration (4.90±0.09nmol/ml) compared to control (4.06±0.12 nmol/ml). Administration of MSG resulted in highly significant increase in potassium (5.29±0.05 nmol/ml) on comparing with control group. Also, sunset yellow highly significantly affected potassium level (4.81±0.06 nmol/ml) compared to control. Sodium benzoate administration has a highly significant effect on potassium (5.50±0.08 nmol/ml) as it increased it compared to control (Table 5).

Chloride concentration was highly significantly elevated (100.09±0.93 nmol/L), (106.83±0.52 nmol /L), (106.18±1.43nmol/L) as a result of administration of tartrazine, MSG and sunset yellow respectively compared to control (101.32±0.79 nmol/L). Sodium benzoate administration caused a significant increase in chloride concentration (105.17±0.66nmol/L) as compared to control (Table 5).

Tartrazine results showed no effect on phosphorus concentration (1.01±0.04 nmol/L) compared to control (1.03±0.05 nmol/L), while MSG has a highly significant effect on phosphorus concentration (1.27±0.04 nmol/L) compared to control. Sunset yellow has a non-significant effect on phosphorus concentration (0.95±0.05 nmol/L) compared to control. Sodium benzoate administration resulted in a highly significant increase in phosphorus concentration (1.33±0.06 nmol/L) compared to control (Table 5).

Table 5 : Effect of tartrazine, monosodium glutamate, sunset yellow, and sodium benzoate in daily doses of 20,100,200,100 mg/kg b.wt., respectively for two months on sodium, potassium, chloride, phosphorus levels in albino rats (Means ± SE).

	Sodium (mmol/L)	Potassium (mmol/L)	Chloride (mmol/L)	Phosphorus (mmol/L)
Control	138.75±0.80	4.06±0.12	101.32±0.79	1.03±0.05
Tartrazine	151.85±0.87**	4.90±0.09**	100.09±0.93	1.01±0.04
Monosodium glutamate	156.77±2.07**	5.29±0.05**	106.83±0.52**	1.27±0.04**
Sunset yellow	139.79±1.35	4.81±0.06**	106.18±1.43**	0.95±0.06
Sodium Benzoate	142.45±0.58*	5.50±0.08**	105.17±0.66*	1.33±0.06**

**significant at p≤ 0.05 compared to control.* ** *Highly significant at p ≤ 0.05 compared to control.*

Glucose

Fasting blood glucose concentration in rats treated with tartrazine was highly significantly elevated (133.89±5.67 mg/dl) compared to control (88.03±2.01 mg/dl). MSG resulted in a highly significant increase in glucose concentration (147.18±9.15 mg/dl) compared to control, while, sunset yellow administration did not affect glucose concentration (86.48±7.22 mg/dl) compared to control. Sodium benzoate administration caused a highly significant increase in glucose concentration (140.19±6.49 mg/dl) compared to control (Table 6).

Alpha-fetoprotein (AFP)

Tartrazine administration resulted in a highly significant increase in AFP concentration (103.67±7.42 ng/ml) compared to control (23.34±1.24 ng/ml). There was a highly significant increase in AFP concentration (77.01±6.15 ng/ml) in rats treated with MSG compared to control, on the other hand, sunset yellow has a non-significant effect on AFP concentration (40.51±3.39 ng/ml) compared to control. Sodium benzoate treated rats showed a highly significant increase in AFP concentration (125.09±11.55 ng/ml) compared to control (Table 6).

Protein Kinase C

Protein kinase C concentration in rats treated with tartrazine was highly significantly elevated (4.22±0.35 pmol/L) compared to control (0.97±0.05 pmol/L), Administration of MSG increased protein kinase C concentration (3.20±0.33 pmol/L) compared to control. Rats administrated with sunset yellow showed a non-significant effect on protein kinase C concentration (1.71±0.12 pmol/L). On the other hand, sodium benzoate has a highly significant effect on protein kinase C concentration (5.16±0.51 pmol/L) compared to control (Table 6).

Table 6: Effect of tartrazine, monosodium glutamate, sunset yellow, and sodium benzoate in daily doses of 20,100,200,100 mg/kg b.wt., respectively for two months on fasting blood glucose, α-feto protein, and protein kinase c concentrations in adult male albino rats (means±SE).

	Glucose (mg/dL)	α fetoprotein (ng/ml)	protein kinase C (pmol/L)
Control	88.03±2.01	23.34±1.24	0.97±0.05
Tartrazine	133.89±5.67**	103.67±7.42**	4.22±0.35**
Monosodium glutamate	147.18±9.15**	77.01±6.15**	3.20±0.33**
Sunset yellow	86.48±7.22	40.51±3.39	1.71±0.12
Sodium Benzoate	140.19±6.49**	125.09±11.55**	5.16±0.51**

***Highly significant at $p \leq 0.05$ compared to control.*

Discussion

Assessment of blood constituents of experimental animals as changes from the normal levels due to administration of different products consumed by humans have been continued to play valuable method in studying effects of these products on human health. The wide use of a great number of food additives has caused adverse effects on human health that require continuous evaluation.

Reactive oxygen species (ROS) play an important role in pathological changes in the liver (Poli and Parola, 1997). Biological membranes are particularly prone to theROS effect. The peroxidation of unsaturated fatty acids in biological membranes leads to a decrease of membrane fluidity and disruption of membrane integrity and function, which is implicated in serious pathological changes (Halliwell, 1987). Lipid peroxidation is a major indicator of oxidative damage initiated by ROS and causes impairment of membrane function (Selvakumar *et al.*, 2006), It was explained that MDA level is increased as a product of lipid peroxidation occurred by ROS action on lipids of cellular membrane (Amin *et al.*, 2010). Catalase (CAT) catalyzes H_2O_2 and forms molecular oxygen and water. Glutathione peroxidase (GPx) detoxifies H_2O_2 and organic peroxides at the expense of glutathione (Omca *et al.*, 2012).

This study revealed that rats orally administrated with tartrazine, MSG and sodium benzoate exhibited a highly significant increase in MDA level and the activities of the antioxidant enzymes GPx and CAT. The increased lipid peroxidation observed in this study may be attributed to direct effect of increased generation of ROS resulted from tartrazine, MSG and sodium benzoate administration. The increased activities of GPx and catalase on administration of tartrazine, MSG and sodium benzoate in this study could be attributed to their increased synthesis resulted from the induction, as antioxidant enzymes are induced in response to oxidative stress (Oscar *et al.*, 2006). The role of GPx is a protective one, as it significantly delay irreversible oxidative degradation of lipids and concomitant formation of malondialdehydes (Choudhary *et al.*, 1996).

Because the food dyes (tartrazine) are from the group of azo dye food colorants, they are metabolized into aromatic amine by intestinal flora and the formed aromatic amines can generate ROS as part of their metabolism by interaction of these amino groups with nitrite or nitrate containing foods or in the stomach (Moutinho *et al.*, 2007). The superoxide free radical was produced by tartrazine only after reduction by the intestinal bacteria *Enterococcusfaecalis* (Sweeney *et al.*, 1994). The ROS such as superoxide anion, hydroxyl radical and H_2O_2 could be produced in the metabolism of nitrosamines and increase oxidative stress. As a result of the ROS formation, the MDA level was increased as a product of lipid peroxidation occurred by the ROS action on lipids of cellular membrane (Bansal, 2005). .

Previous studies showed that sodium benzoate can create free radicals and damage cells. It may induce oxidative stress by enhancing lipid peroxidation and by altering the antioxidant enzyme systems. Moreover, previous studies demonstrated that administration of sodium benzoate was associated with lowered antioxidant enzymes; as the activities of CAT and GPx decreased and the MDA level increased. The decrease in CAT and GPx activities was attributed to the inactivation of these enzymes (PiPer, 1999)

The significant increase in MDA and lipid peroxidation could also be due to the increases in the blood glutamate and glutamine which are reported to favor lipogenesis. Glutamate is poorly transported across cell membranes and could accumulate intracellularly, altering the redox state of the cell. In this altered redox state, the cell favors lipid synthesis and tends to shut down lipolysis. The increased level of glutamate increases the concentration of glutamine which may cause toxicity in various organs of body, especially brain. In liver, glutamine degradation yields glutamate which then undergoes oxidative deamination to produce ammonium

ions and α-ketoglutarate. Hence, the increased level of glutamine could also initiate lipid peroxidation by changing the redox potential of the cell (Choudhary *et al.*, 1996).

Oral administration of sunset yellow produced non-significant effect on lipid peroxide (MDA) level and the activities of antioxidant enzymes (GPx) and CAT. This can be explained by that the tested substance did not induce lipid peroxidation, producing no oxidative stress, hence the antioxidant enzymes were not provoked, and the values of their activities were approximately similar to that of the control rats.

It was mentioned that the release of abnormally high levels of specific tissue enzymes into blood stream is dependent on both the degree and the type of damage exerted by the toxic compound administration. The elevation of aminotransferases activities in serum may be due to tissue damage particularly in liver, kidney and heart, and increased permeability of cell membrane. (Amin *et al.* 2010).

The present study revealed that rats consumed tartrazine, MSG and sodium benzoate exhibited a highly significant increase in serum ALT, AST, and ALP activities and significant increase in serum total bilirubin concentrations when compared to control rats. On the other hand, sunset yellow exhibited significant increasing effect on AST activity and total serum bilirubin concentrations only. Previous studies showed that food additives as tartrazine, carmoisine, erythrosine, fast green, indigotine, brilliant blue, sodium benzoate and MSG showed a significant increase in serum AST, ALT, and ALP activities. These results was attributed to hepatocellular damage caused by the toxic effect of these agents which was indicated by vaculation, swelling, necrosis and pyknosis of the liver cells (Nabila *et al.*, 2013). Increase in both serum AST and ALT of rats was attributed to the changes in liver function and hepatocellular impairment which subsequently caused the release of greater than normal levels of intracellular enzymes into the blood (Abdel- Rahim *et al.*, 1989).

Alkaline phosphatse occurs in the canalicular and sinusoidal membranes of the liver, thus damage to the liver will result in elevated serum ALP activity (Bassey *et al.*, 2012). Cholestatic liver disease is characterized by increased level of ALP coupled with high level of bilirubin. The trend of ALP significantly increase gave an indicator that the hepatic capacity of the liver is grossly affected by tartrazine, sodium benzoate and MSG (Inuwa *et al.*, 2011).

Also, the significant elevation of serum aminotransferases may be attributed to what mentioned that under pathological conditions, the parenchymal cells of hepatic lobules fail to carry out vital functions, which usually results in disturbed or imbalanced intermediatory metabolism. As a result of cellular damage, several enzymes like ALT, AST and ALP beach out into the serum and hence their level indicate the type and extent of damage inflicted (Amin *et al.* 2010). Serum bilirubin concentration may be elevated from acute hepatocellular injury, cholestatic injury, or biliary obstruction (Nabila *et al.*, 2013).

MSG could dissociate easily to release free glutamate. The diminution of glutmte produces ammonium ion (NH_4^+) that could be toxic unless detoxified in the liver via the reactions of the urea cycle. Thus, the possible ammonium ions overload that may occur as a result of an increased level of glutamate following MSG intake could damage the liver, consequently releasing the ALT enzyme. This increase could also be explained by free radical production which reacts with polyunsaturated fatty acids of cell membrane leading to impairment of mitochondrial and plasma membranes resulting in enzyme leakage (Poli *et al.*, 1990).

Sodium benzoate caused derangement of liver function as revealed by significant elevation of serum ALT, AST and ALP. In blood plasma, sodium benzoate has a binding affinity for plasma proteins where it is carried out to different tissues. In the liver, it is metabolized by conjugation with glycine, resulting in the formation of hippuric acid (Kubota & Ishizaki 1991). The observed elevation in the activities of serum enzymes as ALT, AST and ALP in response to sodium benzoate are similar to results from rats treated with N-nitrosodiethylamine (Bansal *et al.* 2005) or N-nitrosoamines (Pevicharova *et al.* 1997). Alkaline phosphatase is present on cell surfaces in most human tissues, especially those of the intestine, liver, bones, spleen and kidneys. The specific location of the enzyme within sinusoidal and bile canalicular membranes could account for its serum elevation in the current study in response to sodium benzoate administration.

The present study revealed that rats consumed sunset yellow exhibited a highly significant increase in serum total bilirubin concentration, and a significant increase in AST activities, but serum ALT, and ALP activities did not significantly affected when compared to control rats. Sunset yellow was administered orally, and later they reach the liver through the portal vein and may cause destructive changes in hepatic cells leading to the release of AST enzyme from the cytoplasm into the blood circulation after rupture of the plasma membrane and cellular damage (Ganong, 2005), but these changes did not affect the antioxidant capacity of the tissue (Eman *et al.*, 2000).

The ALT enzyme is a strong positive indicator of insulin resistance, diabetes mellitus and obesity which are risk factors for coronary heart disease (Manal and Nawal, 2012). The ALT enzyme is also a sensitive marker of liver damage (Al-Mamary *et al.*, 2002). Therefore, the non- significant effect of sunset yellow on the serum ALT activity might be an indication of its safety and that it may exhibits less toxic effect on liver function and tissues than other used agents in the present experiment

Since the liver is the principle organ for various metabolic and detoxification reactions, it is important to continue to study the adverse effects that these food additives may exert on this vital organ.

The total body content of cholesterol depends on the balance between the amount of cholesterol formed in the body plus that absorbed from diet. Intestinal cholesterol absorption represents another major route for the entry of cholesterol into the body, and, thus, this source can influence the plasma LDL-cholesterol concentration. The cholesterol pool in the intestine comes from dietary cholesterol and the majority from biliary excretion. The deviation from normal values of cholesterol, in the blood serum is considered as symptoms of liver diseases (Amin *et al.*, 2010).

Our work revealed that rats consumed tartrazine, MSG, and sodium benzoate exhibited a significant increase in total cholesterol, HDL-cholesterol, LDL cholesterol, and total lipids. It was observed that in the absence of increased exogenous dietary lipid, rats consumed MSG exhibited a significant increase in total cholesterol, HDL-cholesterol, LDL cholesterol, and total lipids. MSG was seen to increase hepatic lipid catabolism via up regulation of oxidative genes. It was specially seen to activate genes involved in bile acid pathway including key regulatory enzyme, cholesterol-7-α hydroxylase (CYP7A1). Lipid mobilization and storage processes were shown to be affected in liver of rats on MSG diets (Bassey *et al.*, 2012). Because blood lipids values were significantly increased in all case groups. These effects of sodium benzoate, tartrazine,and sunset yellow on the lipid profile may due to imbalances between normal rates of fat metabolism and secretion (Glaser & Mayer 1972). The possible explanation of these observed increments may reside in the direct or indirect action of these food additives on lipid metabolism or lipid peroxidation. Our results revealed that rats consumed sunset yellow exhibited a significant increase in total cholesterol, and total lipids but a non-significant effect on HDL-cholesterol, LDL cholesterol. The effect of sodium benzoate and tartrazine on lipid profile and their increasing effect in cholesterol concentration in the present study may be an indication of membrane structure and function disruption, thus influence its fluidity, permeability, activity of associated enzymes and transport system (Mohammed *et al.*, 2014).

Our study demonstrated that the daily intake of MSG, tartrazine and sodium benzoate resulted in a significant increase in serum albumin, urea and creatinine concentration when compared with control rats, while, sunset yellow did not have significant effect on albumin level. Blood urea is the principal end product of protein catabolism and a good indicator for kidney function, while, creatinine appears in the serum in amounts proportional to the body's muscle mass, and is more readily excreted by the kidneys than urea and uric acid.
Also, it was determined that the blood urea can be increased in all forms of kidney diseases such as hydronephrosis congenital cystic, kidney renal tuberculosis, and condition in which deposition of calcium occurs as hypervitaminosis D (Varely, 1987). Also, plasma creatinine increases in renal diseases gave more prognostic significance than those of other nitrogenous substances (Amin *et al.*, 2010).

As it is believed that the significant elevation in urea and creatinine levels is closely related to the impairment of renal function (Nabila *et al.*, 2013 and Timbrell, 2009), this may indicate that tartrazine, MSG, sunset yellow and sodium benzoate could impair kidney function that may be due to the effect of these food additives metabolites on the kidney tissues. Also, serum urea and creatinine increase when the ability of the kidney to filter fluid within the body declines. However, tartrazine, MSG, sunset yellow and sodium benzoate might have either interfered with creatinine metabolism leading to increased synthesis or the tissues might have compromised all or part of its functional capacity of tubular excretion (Manal and Nawal, 2012).

The elevated levels of albumin noticed in the present study may be indicative of the toxic effect of tartrazine, MSG and sodium benzoate on hepatic and renal tissues. Moreover, exposure to tartrazine, sodium benzoate and MSG may cause an adverse effect on the renal function due to oxidative stress induced by these agents on the renal tissue. In a very general term a rising level of creatinine significance an increasing problem with poorly performing kidneys (Inuwa *et al.*, 2011), hence there is a possible link between these food additives and renal impairment. These impairments could also be attributed to the changes in the threshold of tubular reabsorption, renal blood flow and glomerular filtration rate. Also, The impaired levels of sodium, potassium, phosphorus and chloride in this study are indicative of the toxic effect of the food additives used in the present study on renal function or their inhibitory action on electrolytes transport in tissues and suggest that they may interfere with these electrolytes in several metabolic pathway leading to increase in their levels (El-Sheikh and Khalil, 2011)..

Our results showed a highly significant elevation in fasting blood glucose level in rats orally administrated with sodium benzoate, MSG and tartrazine. The elevation of glucose level can be explained by stimulation of glycogenolysis and gluconeogenesis by the liver with temporary loss of endocrine functions of pancreas leading to hyperglycemia (Mohammed and Nabawy, 2013). The increased blood glucose level following MSG administration was attributed to increased gluconeogenesis from glutamate and glutamine. It has been suggested that a possible deterioration of glucose tolerance in rats following MSG administration. The abnormal glucose tolerance could be attributed to decreased cellular insulin sensitivity even under conditions of hyperinsulinemia observed in animals treated with MSG (Macho *et al.*, 2000). Under conditions of hyperinsulinemia, cells could switch to pathways that favor gluconeogenesis to compensate for the increased insulin release (Onyema *et al.*, 2012).

Previous studies demonstrated that sodium benzoate may play a role in enhancing pancreatic secretions, glycogen metabolism or gluconeogenesis, and hence glucose mobilization to the blood (Papadopoulos & Boakou 1991). The elevation in the level of glucose can result in peroxidation of membrane lipids by increasing the events responsible for glucose oxidation which, in turn, promotes formation of thiobarbituric acid reactive substances (TBARS), in the presence of cytochrome P450 (Pfeifer and Mc Cay, 1971, Baynes, 1991). Sunset yellow showed non-significant change in the fasting blood glucose level of rats on comparison to the control ones. That revealed that sunset yellow does not interfere with the metabolism of glucose, as well as, it has no effect on gluconeogenesis.

Our study revealed that alpha-fetoprotein and protein kinase C were significantly increased as a result of tartrazine, monosodium glutamate, or sodium benzoate oral administration to the adult male albino rats. On the other hand, no significant changes were observed on their concentrations after oral administration of sunset yellow.

The increased levels of PKC are indictors of adverse effects of tartrazine, monosodium glutamate and sodium benzoate on different body systems and metabolism because of the following: 1) It has been implicated as a pivotal regulatory element in a variety of cellular functions such as gene expression, growth, differentiation, and exocytosis. 2) Currently, much evidence supports the direct activation of different PKC isoforms by ROS generation. The signaling pathway triggered upon PKC activation by ROS depends on the specific isoform, cell type, and the site of ROS generation. Thus, depending on the particular condition, this mechanism can be involved in either cell protection or death. 3) PKC activation is also involved in cell damage induced by hyperglycemia. Hyperglycemia results in a loss of antioxidant reducing equivalents, which culminates in an overproduction of O2 and Sorbitol, the product resulting from the enzymatic conversion of glucose. 4) PKC also has been shown to promote the production of endogenous ROS to induce a positive feedback loop. It seems that under pathological conditions, a general signaling mechanism is triggered in mitochondrial dysfunction. In this context, an increase in ROS production by mitochondria activates local PKCs, which in turn activate Nox enzymes. Nox enzymes could also activate another group or the same group of PKCs in a feedback mechanism. Consequently, the redox state of the cell becomes imbalanced. 5) Protein kinase C, may phosphorylate potent activators of transcription, and thus lead to increased expression of oncogenes, promoting cancer progression or interferes with other phenomena (Daniela *et al.*, 2012).

Alpha-fetoprotein (AFP) is a type of protein produced in the developing fetus and found in adults. If an individual has high levels of alpha-fetoprotein in the blood, it may be a sign of liver damage, failure or even liver cancer (Blohm *et al.*, 1998). The results of the present study support the safety of sunset yellow and indicate the toxic side effect of the other food additives included in the present study.

We concluded that tartrazine, monosodium glutamate, sunset yellow, and sodium benzoate adversely affect and alter different biochemical parameters, especially those related to vital organs e.g. liver and kidney and that sunset yellow has the least adverse effects in comparison with the other food additives.

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