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Research Article
Assessment of Sub-chronic Effect of Two Artificial Food Additives on Selected Biochemical Parameters in Wistar Rats

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Abstract

Background and Objective: Amaranth dye and vanillin are food additives used in food, drugs and cosmetics. This study was designed to assess the sub-chronic effect of oral ingestion of these food additives on biochemical parameters. **Materials and Methods:** The animals were divided into 7 groups (5 animals/cage) and fed standard diet with tap water *ad libitum*. The animals were treated with 4.7 mg kg^{-1} b.wt., amaranth dye, 47 mg kg^{-1} b.wt., amaranth dye, 12.5 mg kg^{-1} b.wt., vanillin, 125 mg kg^{-1} b.wt., vanillin, 2.35 mg kg^{-1} b.wt., amaranth+6.25 mg kg^{-1} b.wt., vanillin and then 23.5 mg kg^{-1} b.wt., amaranth+62.5 mg kg^{-1} b.wt., vanillin. Blood samples were collected at the end of the study period for biochemical analyses. Data analysis was carried out with SPSS using one-way analysis of variance (ANOVA). **Result:** This study showed a significant ($p \leq 0.05$) increase in alkaline phosphatase, aspartate aminotransferase and alanine aminotransferase in animals treated with amaranth dye. Serum urea and creatinine were significantly ($p \leq 0.05$) elevated in animals treated with amaranth and vanillin. Significant ($p \leq 0.05$) decrease in total protein and bilirubin was observed in animals treated with 4.7 mg kg^{-1} b.wt., of amaranth dye compared to the control group. This study showed significant ($p \leq 0.05$) increase in glutathione and catalase in animals treated with high dose of the combination of amaranth and vanillin. Haematological assessment showed significant ($p \leq 0.05$) elevation in haemoglobin, red blood cells and platelet count in animals treated with 4.7 mg kg^{-1} b.wt., of amaranth dye and also showed significant ($p \leq 0.05$) increase in total white blood cells in groups administered vanillin and combination of amaranth and vanillin. **Conclusion:** Amaranth and vanillin can adversely affect organs as the liver and kidney and alter biochemical parameters.

Key words: Amaranth dye, hepatotoxicity, nephrotoxicity, oxidative stress, sub-chronic toxicity, vanillin

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The food industry has continually created and improved new agents to manipulate, preserve and enhance food products. The use of these agents has enabled scientists to mimic natural flavors, color foods to make them look more natural and preserve foods for longer periods of time. There are such food products that are made entirely from chemicals. Sweets and sugar substitutes consist of artificial agents. This kind of alteration of food can have a huge effect on the body's biochemical balance[1].

Food additives are substances that are deliberately introduced to food to enhance them as well as introduce desired characteristics. These additives are used for various purposes including preservation, aesthetics, taste masking and sweetening[2]. Food additives are added to food to increase the shelf life of the food by maintaining product consistency, wholesomeness and freshness. Food additives could also be defined as substances used in the production, processing, treatment, packaging, transportation or storage of food[3].

With the great increase in the use of food additives, there also has emerged considerable scientific data linking food additive intolerance with various physical and mental disorders, particularly with childhood hyperactivity[4].

Food dyes are added with a principal aim to give a color to a foodstuff, or to restore its natural color. The natural and synthetic color additives were used extensively to color foods, drugs and cosmetics[5] because of low cost[6].

Foods that are aesthetically pleasing are more likely to be consumed. The visual aspect may be an important factor for the selection of products by final consumers; an important reason why food dyes stand out as one of the essential additive class for the food industry in their quest for larger market shares.

There is a dearth of knowledge regarding the toxic effect of coloring agents or their effects on fetal development. Since amaranth structure shown in Fig. 1, had been banned from the U.S.A since 1976, there is no much recent data from the FDA about the toxicity of this coloring agent, however, Amaranth is still used in many other countries to color food.

Flavorings are food additives with aromatic and/or sapid properties used to confer or enhance the aroma and the taste of food without nutritional purpose. They are classified into natural, artificial, synthetic reaction or conversion flavorings and smoke flavorings[7]. However, due to their chemical composition, flavorings in general are considered a controversial advance in the food industry by many healthcare experts, which suggest that these compounds, along with synthetic food dyes, significantly contribute to the dietary

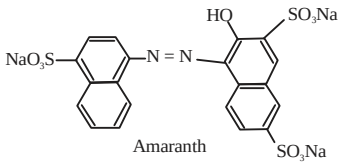

Fig. 1: Chemical structure of amaranth, (FD&C Red No. 2, CAS No. 915-67-3, E123, C.I. Food Red 9, Acid Red 27, Azorubin S, or C.I. 16185

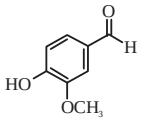

Fig. 2: Chemical structure of vanillin (CAS No: 121-33-5, 4-Hydroxy-3-methoxybenzaldehyde)

impoverishment and also trigger diseases, such as allergies and changes in the functioning of the digestive tract[8]. Vanillin (4-Hydroxy 3-methoxybenzaldehyde), as shown in Fig. 2, is a phenolic aldehyde, which is an organic compound with the molecular formula $C_8H_8O_3$. Its functional groups include aldehyde, hydroxyl and ether. It is the primary component of the extract of the vanilla bean.

Admissible daily intake of these additives are provided by bodies as food and drug administration[9] and world health organization (WHO) and are recommended. Unfortunately, people consume these additives in amounts way beyond these recommendations over time as a result of their preference for particular food products. There have been several concerns about the safety of food additives and reports are available in literature on the toxicity of some of these additives. Very little literature is available on the sub-chronic effect of the food additives (amaranth and vanillin) on the antioxidant status and biochemical parameters of animals. This study, therefore, was aimed to evaluate the effect of these food additives alone or in combination with each other on antioxidant status (glutathione and glutathione peroxidase) as well as on different body weights and other biochemical parameters in Wistar rats.

MATERIALS AND METHODS

Test substances: Amaranth Extra Pure (Lobachemie Ltd, India), vanillin (Lobachemie Ltd, India), buffer (Randox kit), phosphate bufffer (Randox kit), L-alanine (Randox kit), α-oxoglutarate (Randox kit), 2, 4-Dintrophenyl hydrazine (Randox kit), carbonate buffer pH (Randox kit), xanthine oxide 0.3 μ mL^{-1} (Randox kit), sodium hydroxide solution (Randox kit), 2-Amino, 2-methly-1-propanol pH 11 (7.9 M) (Randox kit),

Na_2PO_4 (80 nM) (Randox kit), ethylene diamine tetraacetate (Drug House, BDH Ltd), Tris-HCl (Drug House, BDH Ltd).

Animals: Thirty-five male albino rats of the Wistar strain aged 10-12 weeks and weighing 120-140 g bred in animal house of the Department of Zoology and Environmental Biology (ZEB), University of Nigeria, Nsukka were used for this study. All animals were housed at controlled room temperature of about 27-30°C with a photoperiod of 12 h light and 12 h dark/day. The animals were acclimatized to their environment and diet for 7 days before experimentation and housed in metal cages in the animal house of Biochemistry Department, Michael Okpara University of Agriculture, Umudike Abia state. This study was carried out in May-July, 2017. Throughout the duration of the study, the test animals were administered the food additives orally alongside normal rat chow and their body weights were measured weekly.

Dose selection: The food additives were administered to the animals daily for 2 months. The doses were calculated for humans and modified for rats using the method of Paget and Barnes[10]. The acceptable daily intake (ADI) of the additives were used according to FDA[11] as well as 10 times of these doses were used to evaluate their possible toxic effects. For the groups that received a combination of amaranth dye and vanillin, half of the ADI dose and half of 10 times the ADI respectively for each of the additives was used.

Experimental design: Thirty-five male Wistar rats were randomly grouped into 7 groups with five animals in each group. The test animals were fed rat chow and distilled water and were treated daily for 60 days with amaranth dye and vanillin as follows:

- **Group 1:** Control rats
- **Group 2:** 4.7 mg kg^{-1} b.wt., amaranth dye
- **Group 3:** 47 mg kg^{-1} b.wt., amaranth dye
- **Group 4:** 12.5mg kg^{-1} b.wt., vanillin
- **Group 5:** 125mg kg^{-1} b.wt., vanillin
- **Group 6:** 2.35 mg kg^{-1} b.wt., amaranth dye+6.25 mg kg^{-1} b.wt., Vanillin
- **Group 7:** 23.5 mg kg^{-1} b.wt., amaranth dye+62.5 mg kg^{-1} b.wt., Vanillin

The animals were observed daily for general clinical conditions. They were weighed on the first day, once every seven days during the administration period and on the last day. The percentage body weight change was determined using the formula:

$$\text{Percentage body weight change} = \frac{\text{Final body weight} - \text{Initial body weight}}{\text{Initial body weight}} \times 100$$

Blood samples were collected for biochemical analyses.

Haematology and clinical chemistry: At the conclusion of the 60 days study periods, blood samples were collected from animals fasted overnight in heparinized tubes. Hematology parameters including total white blood cell (TWBC) count, red blood cell (RBC) count, hemoglobin (Hb), packed cell volume (HCT), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC) and platelet (PLT) count were determined using the BC 2800 Auto Hematology Analyzer (Abbott Laboratories, Illinois). Clinical chemistry analyses including alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine (CREA), total bilirubin (TBIL), urea and total protein (TP) were determined using standard methods (RANDOX kits). Antioxidant and lipid peroxidation parameters analyses including glutathione peroxidase (GPx), catalase, superoxide dismutase (SOD) and malondaldehyde (MDA) were also carried out using standard methods using RANDOX kits.

Statistical analysis: Statistical analysis of the data was carried out with SPSS version 22.0 using one-way analysis of variance (ANOVA). The statistically analysed data were reported as Mean+SEM. Significant difference was accepted at 95% confidence level of probability i.e. if $p \leq 0.05$.

RESULTS

Toxicity assessment: No mortality as a result of administration of the food additives amaranth dye and vanilllin were recorded throughout the duration of this study.

Heamatology and clinical chemistry: ALP, ALT and AST activities were shown to be significantly ($p \leq 0.05$) higher in the Group 3 animals compared to the Group 1 (Fig. 3). Creatinine was significantly ($p \leq 0.05$) reduced in Group 2 and Group 6. TP was significantly ($p \leq 0.05$) reduced in Group 2 but increased significantly in the Group 6 while creatinine also increased significantly in Group 7 (Fig. 4). Serum urea concentration increased in Groups 3, 5 and 6. However, serum urea concentration was significantly ($p \leq 0.05$) reduced in the Group 4 (Fig. 5).

TBIL concentration was significantly ($p \leq 0.05$) reduced in the Groups 2, 4 and 5. However, TBIL concentration was significantly higher in the Groups 3 and 6 (Fig. 6).

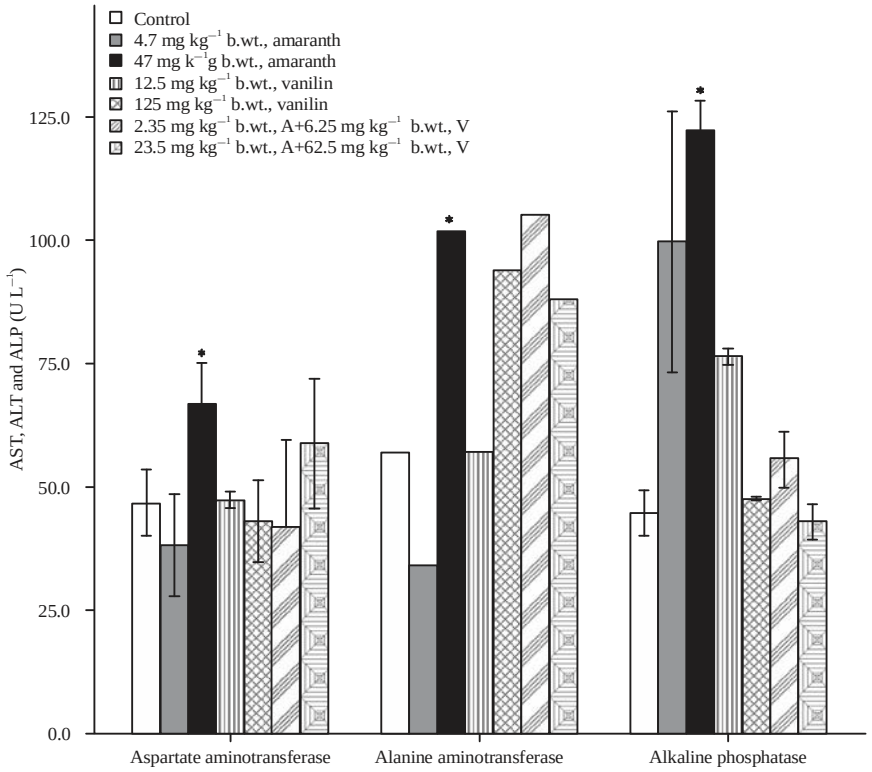

Fig. 3: AST, ALT and ALP activities in different dose groups compared to control group

Data are presented as Means±SEM for each dose group. *Mean difference values compared to control group are significant at the 0.05 level

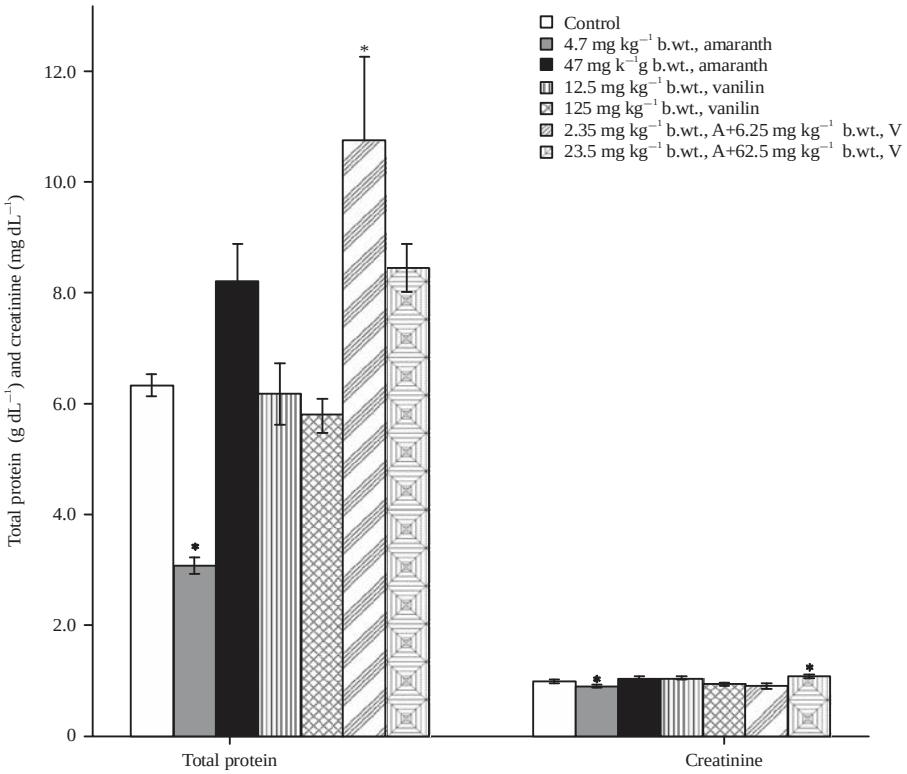

Fig. 4: Total protein and creatinine concentration in different dose groups compared to control group

Data are presented as Means±SEM for each dose group. *Mean difference values compared to control group are significant at the 0.05 level

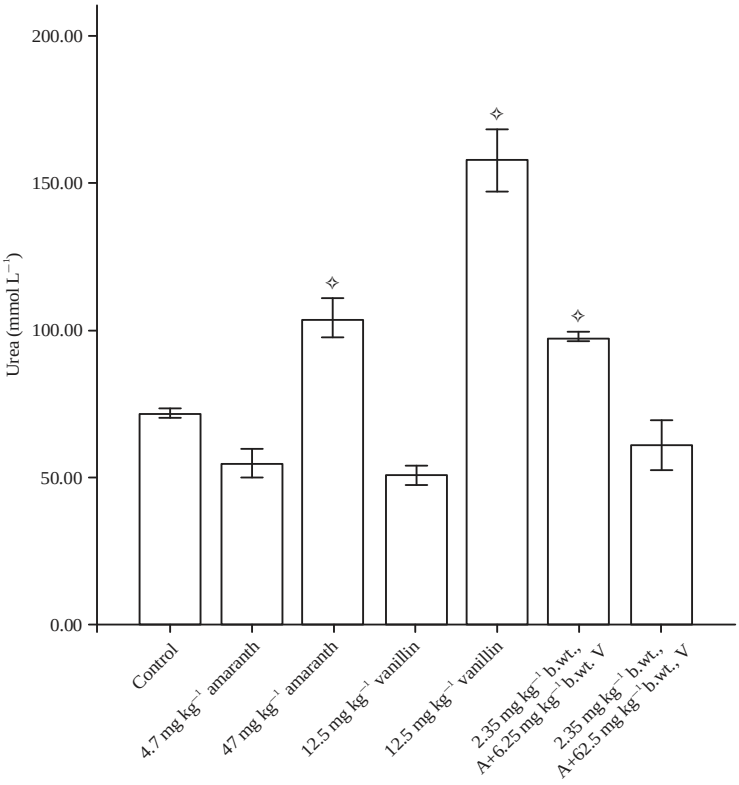

Fig. 5: Serum concentration in different dose groups compared to control group

Data are presented as Means±SEM for each dose group. *Mean difference values compared to control group are significant at the 0.05 level

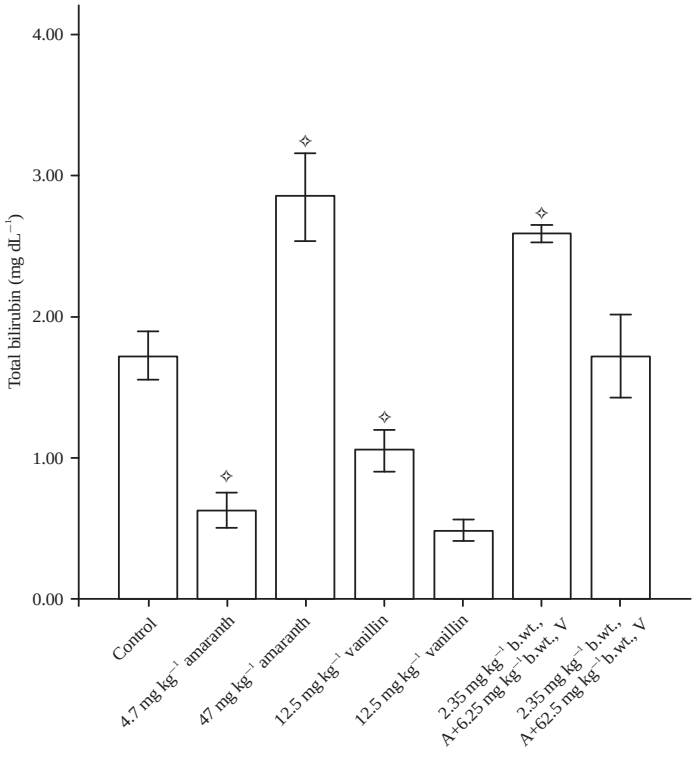

Fig. 6: Total bilirubin concentration in different dose groups compared to control group

Data are presented as Means±SEM for each dose group. *Mean difference values compared to control group are significant at the 0.05 level

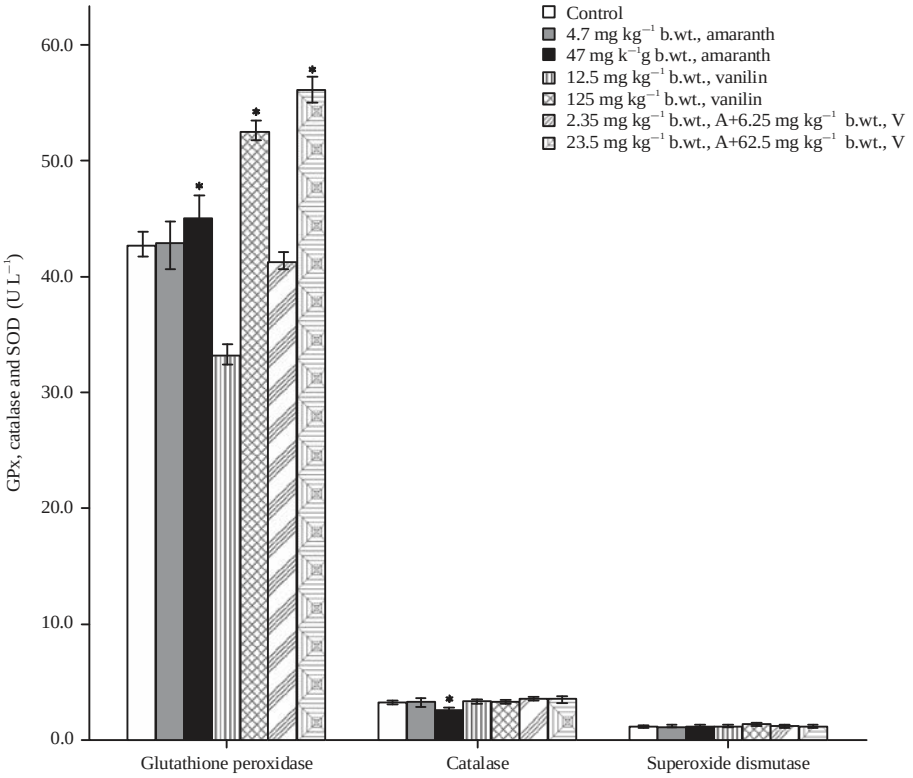

Fig. 7: Glutathione peroxidase, catalase and superoxide dismutase activities in different dose groups compared to control group
Data are presented as Means±SEM for each dose group. *Mean difference values compared to control group are significant at the 0.05 level

Table 1: Effect of two food additives on Hb, RBC and PCV in Wistar rats

Groups	Hb (g dL^{-1})	PCV (%)	RBC($\times 10^6$ mm^{-3})
Control	16.12±0.43	39.60±1.67	7.92 ±2.51
4.7 mg kg^{-1}	19.17±0.67*	47.67±2.52*	10.40±1.00*
47 mg kg^{-1}	14.90±0.88*	40.40±1.67	8.56±1.51
12.5 mg kg^{-1}	16.23±0.56	33.75±2.63*	7.6±1.41
125 mg kg^{-1}	15.70±0.57	41.25±4.72	8.20±1.29
A (2.35 mg kg^{-1})+V(62.5 mg kg^{-1} b.wt.,)	14.87±0.25*	40.00±2.00	8.24±1.52
A (2.35 mg kg^{-1})+V(62.5 mg kg^{-1} b.wt.,)	13.80±0.37*	37.75±4.27	7.48±0.95

*Mean difference values compared to control group are significant at the 0.05 level. Values are represented as Mean±SEM, Hb: Haemoglobin, PCV: Packed cell volume, TWBC: Total white blood cells, RBC: Red blood cells, A: Amaranth, V: Vanillin

GPx and catalase activities were significantly ($p \leq 0.05$) reduced in Group 3. However, GPx activity increased significantly in the Groups 5 and 7. There was no significant difference in SOD activity between the test groups and the Group 1 (Fig. 7).

GSH concentration significantly ($p \leq 0.05$) increased in the Groups 2, 3 and 7 respectively. Groups 3, 5, 6 and 7 showed significantly higher MDA concentration (Fig. 8).

The results for haematological parameters analyses shown in Table 1 and 2. The result showed that Hb significantly ($p \leq 0.05$) increased in Group 2. However, Groups 3, 6 and 7 had significantly lower Hb concentration.

PCV significantly ($p \leq 0.05$) increased in Group 2. However, Group 4 showed a significantly ($p \leq 0.05$) lowered packed cell volume. The Group 2 animals showed a significantly ($p \leq 0.05$) higher RBC count. TWBC count significantly ($p \leq 0.05$) increased in Group 5. Groups 6 and 7 animals, however, showed significantly ($p \leq 0.05$) lower TWBC.

Platelet count significantly ($p \leq 0.05$) increased in Groups 2 and 3. This significant increase was also evident in the Group 4 and 5. The Group 6 animals also showed significantly ($p < 0.05$) increased platelet count. The result showed that all the test groups showed lower MCH values except Group 4. However, these decreased MCH values were

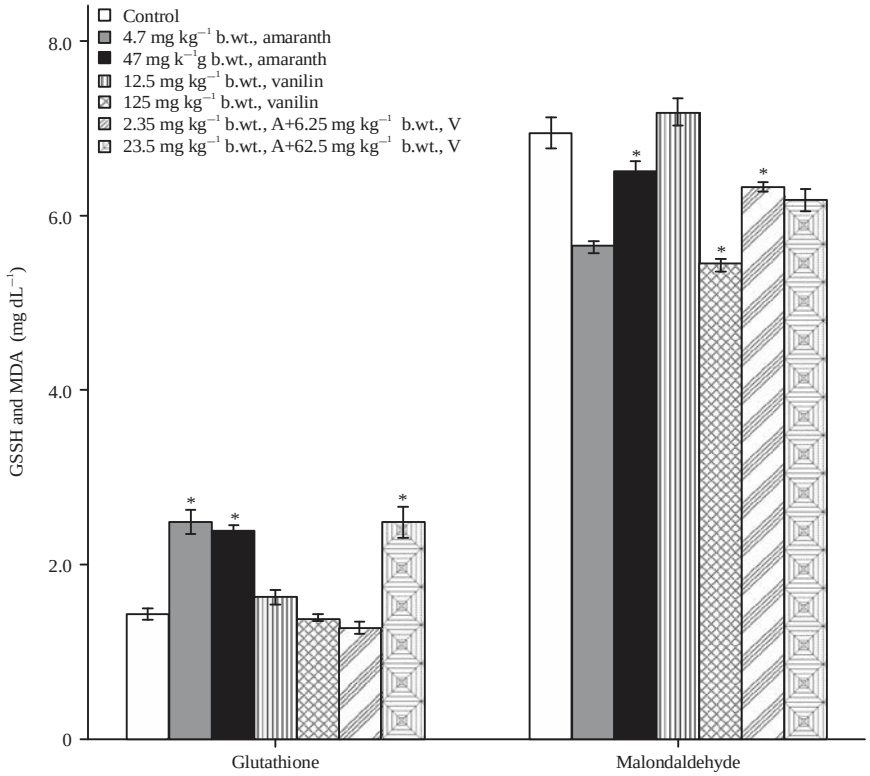

Fig. 8: Glutathione and malondaldehyde concentrations in different dose groups compared to control group

Data are presented as Means±SEM for each dose group. *Mean difference values compared to control group are significant at the 0.05 level

Table 2: Effect of two food additives on TWBC, PLAT, MCH, MCV and MCHC in Wistar rats

Groups	TWBC (mm^{-3})	Platelet (×10^6/L)	MCH (pg)	MCV (fl)	MCHC (g dL^{-1})
Control	4860.00±134.16	85.60±3.85	0.83±0.1	2.05±0.29	40.76±1.97
4.7 mg kg^{-1} Amaranth	4666.67±230.94	167.33±3.06*	0.74±0.02*	1.83±0.06	40.24±0.91
47 mg kg^{-1} Amaranth	4700.00±223.61	142.00±3.74*	0.70±.0.02*	1.90±0.15	36.92±2.45*
12.5 mg kg^{-1} Vanillin	4950.00±191.49	144.00±7.31*	0.86±0.44	1.82±0.19	48.23±2.77*
125 mg kg^{-1} Vanillin	5450.00±191.49*	140.00±1.63*	0.77±0.05	2.02±0.20	38.45±4.72
A (2.35 mg kg^{-1} bw) +V(6.25 mg kg^{-1} b.wt.,)	4066.67±305.51*	105.00±2.65*	0.72±0.04*	1.94±0.10	37.22±1.54
A (23.5 mg kg^{-1} bw) +V(62.5 mg kg^{-1} b.wt.,)	4150.00±191.49*	88.5±4.43	0.74±0.03*	2.00±0.13	36.85±3.56

*Mean difference values compared to control group are significant at the 0.05 level. Values are represented as Mean±SEM, TWBC: Total White Blood Cell count, MCH: Mean corpuscular haemoglobin, MCHC: Mean corpuscular haemoglobin concentration, MCV: Mean corpuscular volume, A: Amaranth, V: Vanillin

Table 3: Percentage body weight change of experimental animals

Groups	Initial Body weight (g)	Final body weight (g)	Body weight change (%)
Control	85.00±3.81	165.00±24.39	93.4740±9.22
4.7 mg kg^{-1} Amaranth	143.40±6.07*	212.20±12.24*	48.0880±3.92*
47 mg kg^{-1} Amaranth	131.00±6.25*	189.00±14.88	44.1960±3.24*
12.5 mg kg^{-1} Vanillin	117.00±2.55*	174.4±22.14	49.2460±9.22*
125 mg kg^{-1} Vanillin	113.60±1.14*	178.60±6.73*	57.1980±2.25*
A (2.35 mg kg^{-1} b.wt.,) +V(6.25 mg kg^{-1} b.wt.,)	106.80±3.49*	188.40±14.17	76.6640±7.34
A (23.5 mg kg^{-1} b.wt.,) +V(62.5 mg kg^{-1} b.wt.,)	93.40±4.56*	159.60±22.46	71.3560±11.75*

*Mean difference values compared to control group are significant at the 0.05 level. Values are represented as Mean±SEM. A: Amaranth dye, V: Vanillin

only significant ($p \leq 0.05$) in Groups 2, 3, 6 and 7. MCV decreased in all the test groups compared to the Group 1. However, none was significant at 0.05 confidence level. MCHC significantly decreased in Group 3 but significantly ($p \leq 0.05$) increased in the Group 4.

The percentage change in body weight of the rats after 8 weeks of feeding is shown in Table 3. The result showed significant ($p \leq 0.05$) increase in body weight of the animals when the initial weights of all the animals were compared with their final weights in all groups. The animals in all the test

groups showed significant percentage weight change compared to the control group. However, no significant change was observed in the percentage weight change of the Group 6 animals compared to the control group.

DISCUSSION

In this study, the possible toxic effects of prolonged (8 weeks) oral administration of two widely used food additives (amaranth dye and vanillin) were evaluated in Wistar rats.

Amaranth dye is an azo dye that is absorbed in the gastrointestinal tract and metabolized to products as 1-Amino-4-naphthalene sulfuric acid and 1-Amino-2-hydroxy-3,6-naphthalene disulfuric acid as a result of reductive fission of the azo linkage by a suspension of bacteria obtained from large intestine and cecum of rats[12]. Researchers found that it is reduced by rat liver homogenates as well as by rat intestinal contents. Products of reductive cleavage of amaranth, namely, 1-Amino-4-naphthalene sulfonic acid and 1-Amino-2-hydroxy-3,6-naphthalene disulfonic acid (R-amino salt), are found in the urine of rats fed the dye[12]. Vanillin (4-Hydroxy-3-methoxy-benzaldehyde) is a well-known food additive that can be metabolized primarily to vanillic acid, conjugated vanylyl alcohol and catechol[13].

Cytoplasmic enzymes such as ALT, AST and ALP are employed in the assessment of hepatic disorders and elevation in these enzymes activities reflect liver damage and inflammatory hepatocellular disorders[14]. The result of this study showed that administration of 47 mg kg^{-1} b.wt., of amaranth dye caused liver damage demonstrated by significant ($p \leq 0.05$) elevation of serum ALP, AST and ALT compared to the control group. Due to the cytoplasmic nature of these enzymes, upon liver injury, they enter into the circulatory system as a result of alteration of membrane permeability. Results of this study agree with reports in a previous study by Makni *et al.*[14]. These findings also agree with reports by Mekkawy *et al.*[15], who indicated that two doses of synthetic azo showed a significant increase in serum AST, ALT and ALP activities. The toxic effect of azo dye can be a result of direct action by the compound itself or the formation of free radicals and aryl amine derivatives generated during reductive biotransformation of the azo bond[16]. These free radicals generate oxidative stress that leads to degeneration of liver cells. Halliwell[17] reported that oxidants induce serious pathological changes caused by loss of membrane fluidity and disruption of membrane integrity and function.

Repeated oral exposure of the rats to 12.5 and 125 mg kg^{-1} b.wt., of vanillin did not cause any significant effect on the activity of cytoplasmic enzymes ALT, AST and ALP. This observation is in agreement with the report of Janbaz and Gilani[18].

Proteins are the fundamental components of all living cells and include many substances such as enzymes, hormones and antibodies that are necessary for the proper functioning of an organism[19]. Serum protein has many functions including transport of other substances, immune defense, blood clotting and inflammation defense. Serum protein levels and assays are useful in evaluating nutrition status, infection and various disorders[20] as well as an indicator for assessing the total amount of protein in the blood plasma or serum. This present study found decreased levels of total protein in animals administered amaranth dye at 4.6 mg kg^{-1} b.wt., doses. This suggests that amaranth at that dose may have caused suppression of protein synthesis. The depletion of the serum protein might be attributed to the impaired protein synthesis due to the azo linkage of amaranth dye. This possibly as a result of amino acid deamination by the azo linkage. Studies have reported that azo dyes are involved in many biological reactions such as inhibition of protein synthesis, DNA, RNA, carcinogenesis and nitrogen fixation[21]. The disturbance in liver functions also depresses serum protein and thus results in hypoproteinemia in animals. Moreover, increase in amino acid deamination as a result of some toxic compound also cause hypoproteinemia. However, a significant increase in total serum protein was observed in the test group that received a low dose of the combination of amaranth and vanillin. Increased release of enzymes by the damaged tissues and the antibodies to counteract the dye might be the cause of the increase in observed serum protein[22]. So increased release of liver enzymes caused by the toxic effect of synthetic food dyes amaranth and vanillin can result in increasing of serum protein concentration. The results observed here are in agreement with Mekkawy *et al.*[15], who found a significant increase in serum total protein when two doses of synthetic dyes were administered to rats. Sharma *et al.*[23] reported that total protein was significantly elevated when Tomato Red was consumed by Swiss albino mice. These agree with the findings of Aboel Zahab *et al.*[24], who also reported the same effect on serum total protein with rats whose diets were supplemented with chocolate colors A and B.

The kidney function parameters revealed alterations in serum creatinine in the test animals that received a combination of amaranth and vanillin. These were observed to be dose dependent as a lower dose of the combination caused a decrease in creatinine while the higher dose group

showed an increase in creatinine level compared to the control group. The results for serum urea concentration showed a significant ($p \leq 0.05$) elevation in the rats that received 47 mg kg^{-1} b.wt., of amaranth and 125 mg kg^{-1} b.wt., of vanillin. This result coincides with Helal *et al.*[25] who observed a significant increase in serum creatinine and urea in rats that were fed synthetic and natural food colorants after 30 days. This study is also in agreement with data reported by Ashour and Abdelaziz[26], who observed a significant increase in serum creatinine and urea level of rats dosed with organic azo dye orally for 35 days. Amin *et al.*[19] also observed a significant elevation in serum creatinine and urea level when rats were administered high dose (500 mg kg^{-1} b.wt.,) and low doses (15 mg kg^{-1} b.wt.,) of tartrazine. The significant ($p \leq 0.05$) elevation in urea and creatinine levels observed in this study might suggest renal function impairment caused by toxicity of amaranth and vanillin at high doses. The blood urea can be increased in all types of kidney impairments such as hydronephrosis, congenital cystic, kidney renal tuberculosis, a condition in which deposition of calcium occurs as hypervitaminosis D. Plasma creatinine increase in renal diseases gave prognostic significance than those of other nitrogenous substances[19].

Bilirubin has been shown to be a natural antioxidant.[27] As an antioxidant, bilirubin demonstrated antiatherogenic function through inhibition of low-density lipoprotein oxidation[28] or through inhibition of vascular endothelial activation, which may mediate the antiatherogenic properties of heme oxygenase-1[29]. This present study demonstrated that administration of amaranth dye at 4.7 mg kg^{-1} b.wt., dose to the test animals for 8 weeks exhibited a significant decrease in total bilirubin compared to the control group. Administration of vanillin at 12.5 and 125 mg kg^{-1} b.wt., dose also caused a significant decrease in total bilirubin compared to the control group. This finding could be as a result of the formation of free radicals by the additives as circulating bilirubin is considered to protect human tissues from peroxidation of organic compounds[30] and diseases associated with oxidative stress[31]. The results showed a significant increase in total bilirubin at the high dose of amaranth which suggests that at high dose, amaranth dye causes liver damage leading to leakage of bilirubin into circulation.

The present study revealed that rats administered with 47 mg kg^{-1} b.wt., of amaranth and those that consumed a combination of amaranth and vanillin showed a significant ($p \leq 0.05$) increase in liver glutathione (GSH) level and catalase activity. When GSH is depleted due to oxidative stress, inflammation, or exposure to xenobiotics, *de novo* synthesis of GSS is upregulated[32] to compensate for the depletion and this could explain the increased GSH levels observed in this study. Catalase is an enzyme that scavenges H_2O_2 and prevents peroxidation of cell wall lipids and lipoproteins. The increase in its activity has been observed in several diseases associated with oxidative stress as a compensatory reaction. These findings are in line with reports of Amin *et al.*[19] aside for the increase in GSH which was a contrary finding. Glutathione peroxidase (GPx) was found to be significantly decreased in the rats that received a low dose of vanillin whereas its activity was significantly elevated in the groups that received high doses of vanillin and combination of amaranth and vanillin. It is possible that vanillin, in combination with amaranth, disrupts electron transport chain (ETC) in the mitochondria causing electrons to leak from sites of the ETC. These electrons combine with molecular oxygen to form superoxides that cause lipid peroxidation which in turn leads to elevation of GPx to reduce lipid hydroperoxides.

The results for hematological parameters in the present study shows a significant elevation in haemoglobin (Hb) and red blood cell count (RBC) in animals that were fed low dose of amaranth compared to the control group. This suggests a compensatory effort of the system in response to low oxygen supply to tissues by the red blood cells as a result of the possible disruption of the electron transport chain by the food additives. Mean corpuscular haemoglobin (MCH) was observed to be significantly reduced in the group that was fed high and low dose of amaranth. This finding suggests a possible inhibitory effect of amaranth on heme synthesis which is indicative of symptomatic anaemic condition. The results showed significant elevation of total white blood cell (TWBC) in the animals that received low dose of vanillin and those fed combinations of amaranth and vanillin. Platelet count was shown to be significantly elevated in all the groups except the animals that received a high dose of the combination of the two additives. These observations suggest physiological inflammatory response as a result of tissue damage and inflammation causing acceleration of the immune system to increase production of white blood cells[33].

CONCLUSION

This 8 weeks sub-chronic study has shown that synthetic food additives such as the azo dye amaranth (E 123) and the flavoring agent vanillin as well as their metabolites can significantly induce adverse changes in renal, heamatological and hepatic parameters. These adverse effects are not only induced at high doses but also at low doses which are primarily as a result of oxidative stress caused by the generation of free radicals. From the findings of this study, it

can be concluded possibly for the first time that combining the food additives did not promote the toxicity of the individual additives at the doses administered in this study. However, cases where the combination of the two additives downplayed the toxicity induced on the animals compared to the individual additives, were observed.

SIGNIFICANCE STATEMENTS

This study discovers the possible effect of the consumption of the food additives amaranth dye and vanillin alone and combined that can be beneficial for consumers of these food additives in commercially available food products at and beyond the recommended admissible daily intake (ADI). This study will help the researcher to uncover the critical area of sub-chronic exposure of these food additives on antioxidant status, hepatotoxicity and nephrotoxicity that many researchers were not able to explore. Thus a new theory on the consumption of these additives alone and combined may be arrived at.

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