Toxicological Impact of Amaranth, Sunset Yellow and Curcumin as Food Coloring Agents in Albino Rats

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ABSTRACT

BACKGROUND: In this study the possible toxic effects of prolonged administration of three of the widely used food coloring agents are tested.

METHODS: Adult female albino rats were administered amaranth (4.7 and 47 mg/kg body weight), sunset yellow (31.5 and 315 mg/kg bwt.) and curcumin (15.75 and 157.5 mg/kg bwt.) at doses equal to and 10 times the acceptable daily intake (ADI). Following which liver and kidney parameters (glutathione, lipid peroxides, activities of transaminases and alkaline phosphatase and urea and creatinine concentration) were measured. Furthermore, the effects of these coloring agents on blood picture and on the development of rat embryo were also studied.

RESULTS: Our study revealed no effect of amaranth and sunset yellow on liver and kidney glutathione and lipid peroxide levels. Oral administration of curcumin in its low and high doses for 2 months decreased hepatic lipid peroxide concentration. Colorants also did not alter the liver and kidney function when given at the ADI dose but administration of sunset yellow at doses equaling 10 times ADI increased aminotransferases and that of amaranth at 10 times ADI elevated alkaline phosphatase levels. Administration of amaranth at 10 times ADI dose caused skeletal abnormalities in 25% of the examined foeti.

CONCLUSION: It could be concluded that high doses of 47 mg/kg bwt. of amaranth and sunset yellow could impair hepatic function. Moreover, based on the results from this study, amaranth should be avoided during pregnancy.

Key Words: Coloring Agents; Liver; Kidney; Antioxidants; Blood Picture; Teratology

INTRODUCTION

Food additives are substances intentionally added to food. These may be natural or synthetic [1]. Food additives are commonly used in processed foods to improve appearance, flavor, taste, color, texture, nutritive value and conservation. The principal classes of food additives are coloring agents, preservatives, flavors, emulsifiers and stabilizers [2]. Coloring agents, also called colorants, play a significant role in enhancing the aesthetic appeal of food. Foods that are aesthetically pleasing are more likely to be consumed [3,4]. 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 food industry in their quest for larger market shares. Even though all color additives are alike in terms of the Food and Drug Administration's (FDA) regulatory definition, they are regulated in two classes; color additives requiring certification (synthetic), and the color additives exempt from certification (natural). Despite the importance of food colorants, there is an ever growing concern

about the adverse effects of synthetic food colorants on human health [5]. In Egypt, there has been a sharp increase in the use of synthetic food colorants during the past few years and this use of synthetic colorants particularly in food items is not controlled by any regulatory body [6]. There is a dearth of knowledge regarding the toxic effect of coloring agents or their effects on fetal development. Since Amaranth had been banned from the U.S.A. since 1976, there is no recent data from the FDA about toxicity of this coloring agent. However, Amaranth is still used in many countries and is approved for use in Egypt.

Sunset yellow is currently banned in Norway and Finland. In 2008, a European Union EU deal specified that food and drinks containing any of six artificial colorings that may be linked to hyperactive behavior in children, including sunset yellow, will have to carry warnings. Moreover, the European Food Safety Authority EFSA decided in 2009 to lower the acceptable daily intake for sunset yellow from 2.5 mg/kg to 1.0 mg/kg bodyweight per day. Moreover, a study found that mixtures of four synthetic colors plus the preservative sodium benzoate (E211) cause increased hyperactivity in humans. Sensitivity reactions may occur when sunset yellow FCF is mixed with other synthetic colors [7].

Curcumin, like many antioxidants, can be a "double-edged sword", where in laboratory settings, anticancer and antioxidant effects may be seen along with pro-oxidant effects. Carcinogenic effects of curcumin are inferred to result from interference with the p53 tumor suppressor pathway, an important factor in many human cancers, including colorectal cancer. Carcinogenic and lethal dose 50% (LD50) tests in mice and rats, however, have failed to establish a relationship between tumorogenesis and administration of curcumin in turmeric oleoresin at >98% concentrations. Other in vitro and in vivo studies suggest that curcumin may cause carcinogenic effects under specific conditions. These conflicting data about curcumin prompted us to study the toxicological effects of curcumin, which is used in Egypt in large quantities. The present investigation was designed (a) to compare the antioxidant effects of two synthetic (amaranth and sunset yellow) and one natural (curcumin) color additives, (b) to study the effects of these agents on peripheral blood, liver, and kidney function tests and (c) effect on fetal development in albino rats [8].

MATERIALS AND METHODS

Animals:

Seventy adult female Sprague Dawley albino rats housed in steel mesh cages for 2 months and provided from the breeding unit of the National Research Center (Giza, Egypt) were used throughout this study. Animals were fed on commercial standard pellets enriched with barley and carrots. Experiments were performed according to the National Regulations on Animal Welfare and Institutional Animal Ethical Committee (IAEC).

Coloring agents

1) Amaranth dye (E123) was obtained from Merck (Darmstadt, Germany). The compound was supplied in a 100g bottle in a powdered form.
2) Sunset Yellow FCF (E110) was obtained from El Gomhouria Co. (Cairo, Egypt). The compound was supplied in a pure powder form.
3) Curcumin: Natural food colorant obtained from Sigma-Aldrich, USA company.

All drugs were freshly dissolved (amaranth and sunset yellow) or suspended with Tween 80 (curcumin) in water and given to rats in a dose of 1ml/100 gram body weight.

Animal grouping

Rats were randomly divided into seven equal groups (10/cage).
Group I: Normal control daily water orally at a dose of 1ml/100g body weight for 2 months.
Group II (Amaranth ADI): Amaranth 4.7 mg/kg body weight.
Group III (Amaranth 10 times ADI): Amaranth 47 mg/kg body weight.
Group IV (Sunset Yellow ADI): Sunset yellow 31.5 mg/kg bwt.
Group V (Sunset Yellow 10 times ADI): Sunset yellow 315 mg/kg bwt.
Group VI (Curcumin ADI): Curcumin 15.75 mg/kg bwt.
Group VII (Curcumin 10 times ADI): Curcumin 157.5 mg/kg bwt.

Drugs were administered daily for 2 months and doses were calculated for humans and modified for rats [9]. The acceptable daily intake was used according to FDA and we used 10 times these doses to see possible toxic effects.

Blood sampling:

Blood samples (3ml) were collected every month from the retro-orbital plexus of each rat into dry centrifuge tubes and left to clot at room temperature. Serum samples were obtained after centrifugation at 1500 rpm for 10 min, and then kept in clean epindorf tubes at -20°C until analysis (AST, ALT, alkaline phosphatase, urea and creatinine). At the end of the experiment, another sample was taken from each animal where the blood was taken in clean dry vials containing 2 drops of 3.8% sodium citrate to be used for red blood cells count [10].

Preparation of tissue homogenate:

At the end of 2 months, a piece of liver and kidney were taken freshly from each animal on ice

and cooling centrifuge and the supernatant was used for determination of reduced glutathione (GSH) and lipid peroxides concentration.

[1]-Determination of antioxidant activity:
Reduced glutathione GSH content in liver and kidney homogenates (20%) was determined by a calorimetric method [11] with some modifications [12], and the lipid peroxide content in the liver and kidney homogenates (20%) was determined by a calorimetric method [13].

[2]-Determination of liver and kidney function:
Hepatic transaminase activity (ALT and AST) [14], alkaline phosphatase activity (ALP) activity [15], serum urea nitrogen level [16] and creatinine concentration [17] were determined.

[3]-Determination of blood picture:
RBC count, hemoglobin (Hb) percentage [18], packed cell volume [19], mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC) were determined.

[4]-Teratogenic effects:
To determine any possible teratogenic effects of amaranth, sunset yellow FCF and curcumin at doses equal to, and ten times ADI, forty female and twenty male albino rats were used where every two females were placed with a male in a separate cage. In the following mornings, a vaginal smear was taken to verify the first day of gestation. Pregnancy was confirmed by the persistence of di-estrus state for 5 days after mating and palpable fetal masses in the abdomen on the 5th day of gestation. Animals were allocated into four groups and given:
Group I (Normal control): Daily water orally at a dose of 1ml/100g bwt. throughout the experimental period.
Group II (Amaranth 10 times ADI): Amaranth at 47 mg/kg bwt.
Group III (Sunset Yellow 10 times ADI): Sunset yellow at 315 mg/kg bwt.
Group IV (Curcumin 10 times ADI): Curcumin at 157.5 mg/kg bwt.
All drugs were given to rats in a dose of 1ml/100 gram body weight from the 6th day of pregnancy through to the 15th day of gestation.
On the 20th day of gestation, the females were anaesthetized by alcohol: chloroform: ether (1:2:3) mixture and caesarean sections were performed to determine the effect of the drugs on fetal development. Morphological examination was performed [20], and the number of implantation and resorption sites was counted [21]. Two thirds of the feti obtained from each female were kept in Bouin's solution for one week to examine visceral abnormalities [20]. The last third were eviscerated and kept in 95% ethanol for 7 days for dehydration, and subsequently placed in 2% potassium hydroxide solution for 24-32 hours. The feti were immersed in Mallsch's solution with alizarin red for 24 hours, and were then washed again by Mallsch's solution. Afterwards, the feti were preserved by serial passages in 20, 50, 80 and 100% glycerin solution for detection of skeletal malformation.

Statisitical methods:
Statistical analysis was carried out by using one way ANOVA and SPSS version 9.0. Data were represented as mean ± SE at $p \leq 0.05$ [22].

RESULTS
Effect on reduced glutathione concentration:
Oral administration of amaranth (4.7; ADI, or 47; 10 x ADI, mg/kg bwt), sunset yellow (31.5; ADI, or 315; 10 x ADI, mg/kg bwt) or curcumin(15.75; ADI, or 157.5; 10 x ADI, mg/kg bwt) for 2 months did not affect liver or renal glutathione levels as compared to control group. (Table 1)

Effect on lipid peroxide (LPO) concentration:
Oral administration of amaranth in doses of 4.7 or 47 mg/kg bwt for 2 months did not affect liver or renal LPO levels as compared to control group, and oral administration of sunset yellow in doses of 31.5 or 315 mg/kg bwt for a longer time period of 3 months also did not affect liver or renal LPO quantity as compared to control group. However, oral administration of curcumin (15.75; ADI, or 157.5; 10 x ADI, mg/kg bwt) for 3 months significantly ($p \leq 0.05$) decreased liver LPO but did not affect renal LPO as compared to control group. (Table 1)

Effect on serum aminotransferases (ALT and AST) activity:
Oral administration of amaranth (4.7; ADI or 47; 10 x ADI mg/kg b.wt.), sunset yellow (31.5 mg/kg bwt; ADI) or curcumin (15.75 mg/kg bwt; ADI) for 2 months did not affect serum ALT or AST activity as compared to control group (Table 1). However, oral administration of sunset yellow 315 mg/kg bwt (10X ADI) or that of curcumin in a dose of 157.5 mg/kg bwt (10X ADI) for 2 months significantly ($p \leq 0.05$) increased serum ALT and AST activity as compared to control group even though, oral administration of curcumin in a high dose of 157.5 mg/kg bwt for only a period of one month did not affect serum ALT or AST activity when compared to control group (Table 2).

Effect on serum alkaline phosphatase (ALP) activity: Oral administration of amaranth in a dose of 4.7 mg/kg bwt for two months did not affect serum

ALP activity as compared to control group. However, its oral administration in a dose of 47mg/kg bwt for either one or two months significantly ($p \leq 0.05$) increased serum ALP activity as compared to control group. Oral administration of sunset yellow (31.5 or 315 mg/kg b. wt.) or curcumin (15.75 or 157.5 mg/kg bwt) for two months did not affect serum ALP activity as compared to control group. (Table 2)

Effect on serum urea and creatinine:
Oral administration of amaranth in doses of 4.7 and 47 mg/kg bwt, sunset yellow in doses of 31.5 and 315 mg/kg bwt, and curcumin in doses of 15.75 or 157.5 mg/kg bwt for two months did not affect serum urea or creatinine concentration as compared to control group. (Table 2)

Effect on blood picture:
Oral administration of amaranth in doses of 4.7 or 47 mg/kg bwt for 2 months did not affect erythrocyte count, hemoglobin concentration, packed cell volume %, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration or total leucocyte count. Oral administration of sunset yellow at doses of 31.5 or 315 mg/kg bwt for 2months did not affect erythrocyte count, hemoglobin concentration, mean corpuscular volume and mean corpuscular hemoglobin as compared to control group (Table 3), but oral administration of sunset yellow in the higher dose of 315 mg/kg bwt for 2 months significantly ($p \leq 0.05$) decreased PCV% and MCHC as compared to control group. Similarly, even though the oral administration of sunset yellow in doses of 31.5 mg/kg bwt for 2 months did not affect total leucocytes count, its larger dose significantly decreased TLC as compared to control group. Oral administration of curcumin in a dose of 15.75 mg/kg bwt and 157.5mg/kg bwt. for 2 months significantly ($p \leq 0.05$) decreased PCV% as compared to control group.

Effects on pregnant rats and developing feti:
Upon oral administration of amaranth in doses of 47 mg/kg bwt from day 6 up to day 15 of pregnancy into female albino rats, 27.8% of the viable feti were growth retarded (Figure 1), 8.3% showed visceral abnormalities (hypoplasia of heart and lung) (Figure 3) and 25% showed skeletal abnormalities (incomplete ossification of the skull bones, aplasia of metacarpal and metatarsal bones and aplasia of caudal vertebrae) (Figure 4). Oral administration of sunset

Groups	Glutathione		Lipid Peroxide	
	Liver	Kidney	Liver	Kidney
Control	8.6±0.27	5.44±0.16	132 ±9.82	116.45±7.87
Amaranth (ADI)	7.13±0.31	5.51±0.09	141.89±8.98	115.76±3.86
Amaranth (10 xADI)	7.42±0.49	5.84±0.07	115.17±2.99	124.36±3.46
Sunset Yellow ADI	7.48±0.13	5.6±0.07	113.57±2.94	114.18±3.37
Sunset Yellow (10 xADI)	7.63±0.23	5.85±0.18	137±9.81	126.6±4.45
Curcumin ADI	8.5±0.84	5.7±0.17	107±3.66*	118.08±3.52
Curcumin (10xADI)	9.23±0.31	5.84±0.23	73.5±3.61*	104.59±5.88

Table 1: Effect of administration of amaranth, sunset yellow and curcumin in doses of ADI and 10 times ADI daily for 2 months on liver and kidney glutathione (*u*mol/gram tissue) and lipid peroxide (*n*mol/gram tissue) in albino rats (mean ± SE, n=10)

Groups	Hb content g/dl	RBCs count million/mm3	PCV %	MCV (fl=10-15)	MCH (pg=1012)	MCHC %	WBCs count 103/mm3
Control	14.31±0.81	5.68±0.33	30.68±1.5	54.45±2.76	25.53±1.91	46.93±2.54	11.2+0.39
Amaranth ADI	14.24±0.22	5.64±0.27	30.00±0.77	53.71±2.39	25.53±1.17	47.60±1.25	11.83+0.73
Amaranth 10XADI	13.97±0.94	5.04±0.4	28.67±1.98	57.51±3.35	28.01±1.59	48.75±0.7	12.32+0.42
Sunset Yellow ADI	13.58±0.82	5.71±0.28	28.00±1.19	49.16±1.15	23.86±1.33	48.46±1.97	11.7+0.84
Sunset yellow 10XADI	14.14±0.7	5.08±0.24	25.17±1.38*	50.26±4.10	28.21±2.19	37.53±1.05*	9.54+0.54*
Curcumin ADI	12.92±0.69	5.55±0.4	28.00±0.99*	49.34±3.9	24.19±2.65	47.27±1.98	11.22+0.52
Curcumin 10XADI	12.96±0.5	5.31±0.37	28.00±0.78*	54.30±3.54	25.08±1.59	46.63±2.41	9.93+0.58

Table 3: Effect of amaranth, sunset yellow and curcumin in doses of ADI and 10 times ADI daily for 2 months on blood picture of albino rats (mean ± SE, n=10)

Drug	No of Pregnant Dams	Total No. of foeti	Growth retardation		Skeletal abnormalities		Visceral abnormalities		Total abnormalities	
			No. of foeti	%	No. of foeti	%	No. of foeti	%	No. of foeti	%
Control 1 ml Distilled Water/rat	6	55	0	0	0	0	0	0	0	0
Amaranth 47 mg/kg bwt	6	36	10	27.8	9	25	3	8.3	22	61.1
Sunset Yellow 315 mg/kg bwt	6	58	6	10.3	0	0	3	5.17	9	15.5
Curcumin 157.5 mg/kg bwt	6	63	2	3.2	0	0	0	0	2	3.1

Table 4: Effect of amaranth, sunset yellow and curcumin in doses of 47, 315 and 157.5 mg/kg bwt respectively (10X ADI) from day 6 to day 15 on foetal abnormalities in albino rats (n=6)

Groups	ALT	AST	Alkaline Phosphatase	Urea	Creatinine
Control	33.24±1.07	59.9±1.2	110.8±2.33	30.39±1.79	0.61±0.01
Amaranth (ADI)	36.51±2.14	60.98±1.41	116.82±3.03	32.51±2.22	0.62±0.01
Amaranth (10 xADI)	37.65±2.16	64.48±7.03	126.73±2.74*	33.74±1.23	0.63±0.01
Sunset Yellow ADI	36.86±2.59	59.56±1.64	117.31±1.58	32.18±2.06	0.63±0.00
Sunset Yellow (10 xADI)	40.74±1.64*	65.52±1.64*	117.05±2.33	33.77±1.57	0.62±0.01
Curcumin ADI	38.25±1.62	59.73±0.69	110.84±2.13	31.66±2.13	0.61±0.01
Curcumin (10 xADI)	37.78±2.33	61.74±2.52	111.88±2.82	35.04±1.39	0.62±0.01

Table 2: Effect of amaranth, sunset yellow and curcumin in doses of ADI and 10 times ADI daily for 2 months on liver and kidney function tests in albino rats (mean ± SE, n=10).

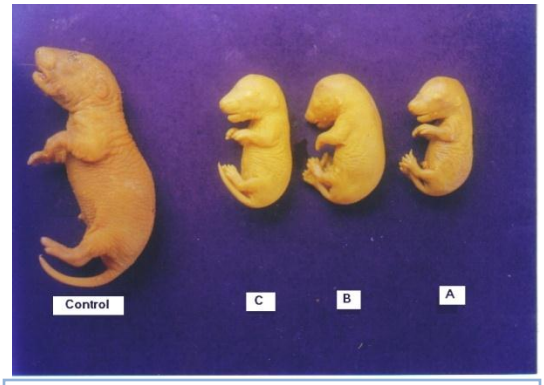

Figure 1: Feti of albino rats treated with (C) curcumin, (B) sunset yellow and (A) amaranth in doses of 157.5, 315 and 47 mg/kg bwt, respectively, from day 6 to day 15 of gestation showing growth

Figure 3: Feti of albino rats treated with amaranth in a dose of 47mg/kg bwt from day 6 to day 15 of gestation showing hypoplasia of heart and lung compared to control

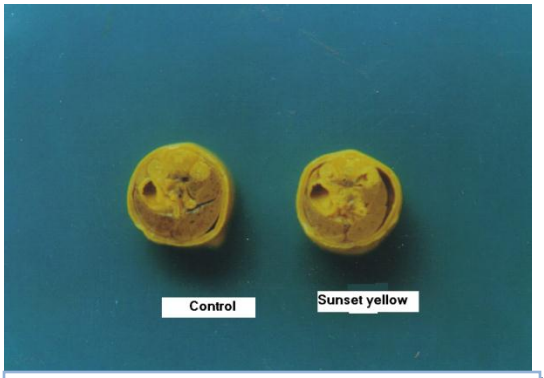

Figure 2: Feti of albino rats treated with sunset yellow in a dose of 315mg/kg bwt from day 6 to day 15 of gestation showing dilatation of the renal pelvis compared to control

Figure 4: Feti of albino rats treated with amaranth in a dose of 47mg/kg bwt, respectively, from day 6 to day 15 showing incomplete ossification of the skull bones, aplasia of metacarpal and metatarsal bones and that of caudal vertebrae

yellow in a dose of 315 mg/kg bwt from day 6 up to day 15 of pregnancy into female albino rats, resulted in 10.3% of the feti showing growth retardation (Figure 1) and 8.3% of the feti showing visceral abnormalities (dilatation of the renal pelvis) (Figure 2). Upon oral administration of curcumin in doses of 157.5 mg/kg bwt from day 6 up to day 15 of pregnancy into female albino rats, 3% of the feti showed growth retardation (Figure 1) with no skeletal or visceral abnormalities (Table 4)

DISCUSSION

In this study, the possible toxic effects of prolonged administration of three of the widely used food coloring agents are tested. Oral administration of amaranth daily for 2 months in doses of 4.7 and 47 mg/kg bwt did not affect either hepatic or renal glutathione as compared to the control group. However, a previous study has reported an increase in liver glutathione content after prolonged oral administration of amaranth daily in a dose of 500 mg/kg bwt [23]. This difference could be attributed to the difference in the dosage regimen. Oral administration of sunset yellow (31.5 and 315 mg/kg bwt) or curcumin (15.7 and 157 mg/kg bwt) daily for 2 months did not affect either hepatic or renal glutathione as compared to the control group, which is consistent with similar observation previously reported for curcumin [24]. Oral administration of curcumin (15.7 or 157 mg/kg bwt) daily for 2 months decreased hepatic lipid peroxide concentration as compared to the control group, which is consistent with a previous study where lipid peroxide was reported to be significantly decreased by administration of curcumin [25]. In the present and previous studies [26] curcumin was found to have a strong antioxidant effect.

The normal concentrations of ALT and AST after administration of curcumin could be attributed to the membrane stabilization effect of curcumin. However, oral administration of sunset yellow in a dose 10 times the ADI for 2 months significantly ($p \leq 0.05$) increases both ALT and AST levels in the serum. This effect may be due to increased cell permeability of hepatocytes. Similar observation was previously reported but these changes did not affect the antioxidant capacity of the tissue [27]. The lack of effect of the tested compounds on the ALP activity in this study may be due to a membrane stabilizing effect of these compounds. However, amaranth in doses of 47mg/kg bwt (10X ADI) for two months significantly increased serum ALP as compared to control group. The enzyme ALP is widely distributed in the body notably in the intestinal mucosa, kidney tubules, osteoblasts of bone, liver, placenta and lactating mammary glands. It is mainly localized at the cell membranes, where it is associated with transport mechanism [28]. Elevation of serum ALP activity is seen in case of cholestasis or hepatic carcinoma [29]. Any of these or other, yet to to be determined, causes may be responsible for this increase in ALP. Urea is a waste product found in blood and formed by the normal break down of protein in the liver. Urea is normally removed from the blood by kidneys and then excreted in the urine. It however, accumulates in the body with renal failure. In this study, no effect of the tested coloring agents when given in doses up to 10 times the ADI for 2 months on serum urea concentration was seen, indicating no effect on protein catabolism, or renal function. Similar findings were reported in a previous study, where no differences were detected between test and control animals in serum urea nitrogen concentration after administration of sunset yellow to pigs at levels of 0 (control), 250, 500 and 1000 mg/kg/day for 98 days [31]. In another study however, after administration of curcumin in a dose of 2.5 and 5% for F344 rats for 13 weeks, the urea concentration was lowered as compared to the control group [32]. This difference may be due to the different animal species, difference in doses, or period of administration. Oral administration of amaranth, sunset yellow and curcumin in both low and high doses for two months did not affect serum creatinine indicating that the three compounds did not affect kidney function at the used doses. Our results agreed with previous results which reported no significant change in serum creatinine concentration after oral administration of curcumin and sunset yellow in a dose of 2.5 and 5 mg/kg bwt respectively (calculated as acceptable daily intake for man) to rats for 1 month [26]. Oral administration of amaranth (4.7 and 47 mg/kg) or curcumin (15.75 and 157.5 mg/kg) for two months did not significantly alter the blood picture (RBCs, Hb concentration, PCV%, MCV, MCH, MCHC and TLC) as compared to control group. This is consistent with findings from another study, which reported no changes in blood picture after giving female rats diets containing 0.03%, 0.3% and 1.5% amaranth for 64 weeks [33]. Oral administration of sunset yellow in a dose of or 315 mg/kg bwt for two months significantly decreased PCV%, MCHC and total leucocyte count as compared to control group. The decrease in MCHC may be attributed to reduction in PCV%, as in a previous study [34]. Oral administration of amaranth at doses equaling 10 times its acceptable daily intake to 6 female albino rats from day 6 up to day 15 of gestation, resulted in growth retardation in 27.8%, visceral abnormalities (hypoplasia of heart and lung) in 8.3%, and skeletal abnormalities (incomplete ossification of the skull bones, aplasia of metacarpal and metatarsal bones and aplasia of caudal vertebrae) in 25% of the 36 viable feti. These results are similar to those of another study which stated that the toxic effect of amaranth could be attributable to two metabolites, sodium naphthionate, which causes sternebral

abnormality in fetuses, and the R-amino salt, which causes skeletal abnormality [35].

Oral administration of sunset yellow at a dose 10 times the acceptable daily intake from day 6 up to day 15 during gestation period did not significantly result in fetal abnormalities and only 5.17% showed dilatation of the renal pelvis. This is in agreement with the results from another study, which stated that oral administration of sunset yellow to rats in doses of

1, 10, 30 and 100 times the ADI caused no reproductive abnormalities [36]. Oral administration of curcumin at a dose 10 times the acceptable daily intake from day 6 up to day 15 during gestation period did not significantly result in fetal abnormalities, except 3.2% of feti showed growth retardation. It was reported in a previous study that curcumin diet at the concentrations of 1500, 3000 and 10,000 ppm for two successive generations caused no reproductive abnormalities, however a small reduction in pre-weaning body weight gain of the second generation (F2) pups at the highest dose level was observed [37].

Strengths and limitations of the study:

This study added new information on these coloring agents. We also examined the teratogenic effect for administering the acceptable daily intake (ADI) which was unlike previous studies that used very high doses of these colorants which were far away from commonly used doses. This study further provides evidence that perhaps, ADI and 10 times this dose for both sunset yellow and curcumin can be used safely in our food but for both amaranth even the ADI is harmful as it caused fetal abnormalities. Further studies need to be performed to confirm our findings and find a better estimate of an acceptable upper limit of these agents.

CONCLUSION

The present study showed that synthetic coloring agents up to 10 times the acceptable daily intake did not cause any oxidative stress. The ADI of the tested compounds did not alter either hepatic or renal function tests. Increasing the dose of synthetic coloring agents up to 10 times the acceptable daily intake caused increase in aminotransferases, ALP and urea concentration in serum. Moreover, amaranth at 10 times ADI leads to fetal abnormalities.

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