



OVARIAN TOXICITY AND OXIDATIVE STRESS INDUCED BY FOOD COLORS IN ALBINO RATS

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ABSTRACT

Food colors have been in use since long to restore the original food appearance. The azo dyes tartrazine, metanil yellow and sunset yellow are used in different food items for coloring purpose. The great increase in the use of these food colors led to cause adverse effects in different body organs. A blend of two or more dyes may produce an altogether different response. So the present study was conducted to evaluate ovarian toxicity of blend of these food colors in female rats. Swiss albino rats were divided into four groups, each group having six animals. Group I served as control, Group II, Group III and Group IV were administered with 25, 50 and 75 mg per kg body weight of food colors for 30 days. The calculated dose of blend was mixed with the rat feed and was given daily at a fix time in morning during the entire experimental tenure. The body weight of albino rats was recorded weekly. The ovary from each animal was weighed, serially sectioned and observed for follicular studies. The activity of superoxide dismutase (SOD), reduced glutathione (GSH) and catalase (CAT) as well as the level of malondialdehyde were accessed in the ovarian tissue. The dye at all the doses caused increase in body weight but a significant decrease in net and relative weight of ovaries. The dye caused increase in malondialdehyde level in ovarian tissue. Significantly lowered levels of SOD, reduced GSH and CAT in ovarian tissue of treated animals were observed when compared with control animals. Histologically the dye caused a profound damage to the complete ovarian architecture. The result indicates that consumption of food color in diet induces reproductive toxicity so indiscriminate use of colors in food products is likely a threat to fecundity rate of experimental animals.

KEY WORDS: Sunset yellow, metanil yellow, tartrazine, Swiss albino rat.

INTRODUCTION

Tartrazine, metanil yellow and sunset yellow belongs to the azo group of dyes. Sunset yellow and tartrazine are permitted food colors and widely used in food products, drugs, cosmetics and pharmaceuticals although the use of metanil yellow as a colorant is not permitted, it is still widely used as a colorant in many food industries. These are generally mixed with food stuff in large amount than acceptable daily intake (ADI) (Dixit *et al.*, 2011). Oral feeding of these food colors in the laboratory animals may increase the production of reactive oxygen species (ROS), which in turn generates oxidative stress in different tissues. Many studies have implicated oxidative damage as the central mechanism of toxicity (Halliwell and Gutteridge, 2002; Celik *et al.*, 2009; Kalender *et al.*, 2010). Oxidative damage occur through production of ROS including hydroxyl radicals and hydrogen peroxide that are generated during the reaction and react with biological molecules eventually damaging membranes and other tissue (Vuillaume, 1987). The food colors may damage membrane by inducing lipid peroxidation (LPO). Malondialdehyde is one of the end products of lipid peroxidation. Thus the aim of this study is to determine the toxicity of food colors in female albino rats by determining oxidative stress parameters such as, SOD, CAT and reduced GSH as well as lipid peroxidation in ovarian tissue.

MATERIALS & METHODS

Animals

Adult virgin female swiss albino rats of 12 weeks old, weighing 135-140g were selected for the present study. Each animal was housed individually in a cage bedded with rice husk and were maintained at standard laboratory conditions (12h light/dark cycle; 25 ± 5°C temperature). Animals were fed on protein rich diet and water was given *ad libitum*.

Dye used

All the chemicals, sunset yellow (C I 15985), tartrazine (C I 19140) and metanil yellow (C I 13065) were purchased from Otto chemical company Pvt. Ltd. Mumbai (India). The blend was formed by mixing them in equal ratio and orally administered to experimental animals.

Experimental design

Investigation was carried out for a period of 30 days. The dose is expressed in terms of the amount of test substance(dye) received by the animals per kg of body weight per day (mg/kg b.wt./day) Animals were divided into four groups, each having six animals and were kept individually. The animals of group I served as control and was fed with normal diet alone. On the basis of average consumption of food commodities and average level of detected colors, animals of group II, III and IV were given blend of food color at a dose of 25, 50 and 75 mg/kg body weight/day respectively. The dye was given orally mixed with food.

PARAMETERS STUDIED

Body and ovary weight

The initial and final body weights of animals were recorded to access percent changes in the body weights of the control and the experimental animals. The ovaries of

both control and the experimental animals were dissected out, cleared off adherent tissues and were weighed on an electronic top pan balance.

The percent change in body weight was calculated as follows

$$\frac{\text{Mean final body weight} - \text{Mean initial body weight}}{\text{Mean initial body weight}} \times 100$$

The relative organ weight was calculated as follows

$$\frac{\text{Absolute organ weight (g)}}{\text{Mean final body weight (g)}} \times 100$$

Preparation of ovarian homogenate

At the end of experimental period, animals were sacrificed by cervical decapitation and the ovaries of different groups was dissected out and rinsed thoroughly with ice cold normal saline. It was smashed in a homogenization buffer and solution was sonicated in an ice bath for 30 second followed by centrifugation at 3000 rpm for 15 min at 4°C. The supernatant was used for determination of the level of malondialdehyde level, the concentration of reduced glutathione and the activities of superoxide dismutase and catalase.

Ovarian oxidative stress

Reduced GSH was determined by the method of Moron *et al.* (1979). The level of GSH is expressed as µg of GSH/mg protein. Lipid peroxidation was evaluated on the basis of MDA level and MDA was determined by method of Jain *et al.* (1989). SOD activity was determined by the method of Marklund and Marklund (1974). The activity of SOD was expressed as units/min/mg protein. Catalase activity was determined by the method of Sinha (1972). The activity was expressed as amount of H₂O₂ utilized/min/mg protein.

Histological preparation

Ovarian tissues were collected from sacrificed animal. Ovaries were washed in normal saline and fixed in 10% neutral formalin and embedded in paraffin wax. 10 µm thick sections were prepared by rotary microtome and stained using H&E as described by Fukuzawa *et al.* (1997).

Statistical analysis

All group values are expressed as mean ± standard error of mean. Statistical analysis was performed by one way analysis of variance followed by Tukey's multiple comparison tests for comparison between different treatment groups. The level of significance was assessed at p<0.05.

RESULTS

Effect on body and ovary weight

The effect of repeated dose of food colors on body weight gain of the female rats was recorded in table 1. A significant increase was observed in the mean final body

weight of the experimental animals in comparison with the mean final body weight of control animals. The weight of the animals treated with food colors at highest dose increased gradually from 137.9 ±0.83 g in the first week to 155.6 ±0.88 g at the end of the fourth week. The weight of the control animals increased from 131.2 ±1.38 g to 146.20 ±1.28 g. So more percent change in body weight was recorded in treated animals than that of control animals. The effect of food color administration for 30 days on ovary weight is shown in table 1. A significant decrease in the relative weight of the ovaries was recorded in the treated animals. The oral ingestion of food colors at all the dose level decreased the relative weight of ovaries by 15.78%, 21.05% and 31.57% with respect to control animals.

Effect on lipid peroxidation and antioxidative parameters

Food color treatment after 30 days of oral administration exhibited a significant increase in the level of MDA (marker of lipid peroxidation) at 25, 50 and 75 mg/kg body weight dose as compared to control group. The mean value of MDA content was recorded 24.07 ±1.55 nmol/mg in control group and it was increased by 14.49%, 32.47% and 74.49% in treated groups II, III and IV respectively. The antioxidative status of female rats was affected by the administration of food color at the three different dose levels in the present study. Significant decrease in the activity of the antioxidant enzymes were observed in the female rats, when administrated at different dose level after 30 consecutive days (Table 2). At highest dose GSH, SOD and CAT level decreased by 50.53%, 29.92% and 62.79% respectively in comparison to control group.

Histological findings

Histology of ovaries is shown in the photomicrograph panel in figure 1. The ovaries of the control group showed normal histological architecture, illustrating a well defined zona granulosa surrounding the oocyte and compact theca (Figure 1A). No significant changes were found in the ovarian histology at 25 mg/kg b.w. dose level. However atrophied ovarian follicle and pyknotic granulosa cells of antral follicle were observed at 50 mg/kg b.w. dose level. At highest dose widespread ovarian follicle atresia, degenerating antral follicle and degenerated oocyte were observed.

TABLE 1: Effect of oral administration of food colors on body weight and relative weight of ovary of female albino rat

Group	Body Weight	(gms)	% Change in	Net ovary	Relative weight of
	Initial	Final	body weight	weight (gms)	ovaries (mg/100g bw)
Group I	131.2±1.38	146.2 ±1.28	11.43 ±0.19	0.28±0.006	0.19±0.003
Group II	135.5 ± 1.42	151.8± 1.40*	12.03± 0.49 ^{ns}	0.25±0.006*	0.16±0.004**
		(+3.83)	(+5.25)	(-10.71)	(-15.78)
Group III	135.5±1.57	153.2± 1.75**	13.28± 1.84 ^{ns}	0.23±0.009***	0.15±0.005***
		(+4.99)	(+16.18)	(-17.85)	(-21.05)
Group IV	137.9±0.83	155.6 ± 0.88***	12.85± 0.65 ^{ns}	0.20±0.005***	0.13±0.003***
		(+6.42)	(+12.42)	(-28.57)	(-31.57)

- Values are expressed as Mean±SEM
- Data were analyzed by one way ANOVA followed by Tukey's t test
- Number of animals in each group n=6
- Comparison of all treated group made with control group *p<0.05, **p< 0.01, *** p< 0.001
- a: values in parentheses represents percent change with respect to control

TABLE 2: Effect of oral administration of food color on antioxidant enzyme activity in ovarian tissue of albino rats

Group	MDA	SOD units/min/mg	CAT H ₂ O ₂ utilised/	GSH µg/mg
	nmol/min/mg	protein	min/mg protein	protein
Group I	24.70±0.63	1.37±0.02	0.86±0.02	1.88±0.08
Group II	28.28±0.20**	1.27±0.01*	0.54±0.02***	1.36±0.02***
	(+14.49)	(-7.30)	(-37.21)	(-27.66)
Group III	32.72±0.76***	1.11±0.031***	0.39±0.02***	1.14±0.04***
	(+32.46)	(-18.98)	(-54.65)	(-39.36)
Group IV	43.10±0.83***	0.96±0.013***	0.32±0.01***	0.93±0.02***
	(+74.49)	(-29.92)	(-62.79)	(-50.53)

- Values are expressed as Mean±SEM
- Data were analyzed by one way ANOVA followed by Tukey's t test
- Number of animals in each group n=6
- Comparison of all treated group made with control group *p<0.05, **p< 0.01, *** p< 0.001
- a: values in parentheses represents percent change with respect to control

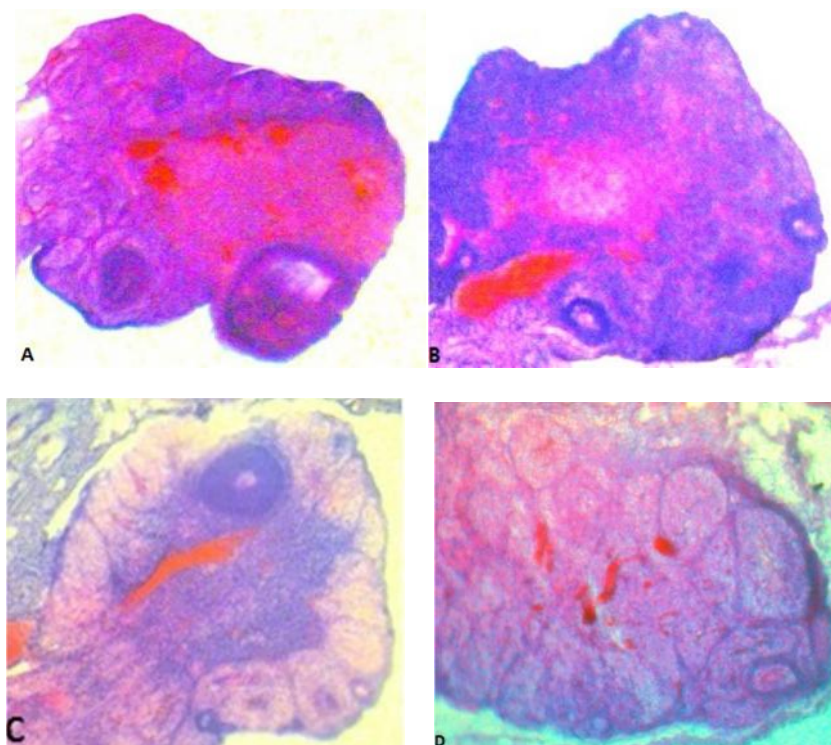


FIGURE 1: Histological observations: (A) ovary of control rat showed normal stroma with developing follicles and mature Graffian follicle. (B) Ovarian section of treated rat at dose 25mg/kg b.w. showed developing follicle. (C) Ovarian section of treated rat at dose 50mg/kg b.w. showed degenerative granulosa cells. (D) Ovarian section of treated rat at 75 mg/kg b. w. showed atretic follicle and degenerative granulosa cells.

DISCUSSION

The present study was planned to evaluate the adverse effect of food colors on fertility, various and ovarian oxidative stress of albino rats. In the present study, sub chronic exposure to food colors resulted in increase body weight of the treated female rats which was significant as compared with control. A similar finding was also reported by Gautam *et al.* (2010). He studied the toxic impact of tartrazine on Swiss albino mice and also found an increase in body weight in both experimental groups *i.e.* for low dose (0.2 g/kg body weight) and high dose (0.4 g/kg body weight) groups. Hasan (1910) also showed weight gain in experimental animals treated with 7.5 mg/kg body weight tartrazine and with 0.15 and 0.3 mg/kg chocolate brown. Chatterjee and Shinde (2002) also reported an increase in the body weight over 20% above the mean body weight. Similar results have also been reported by Osman *et al.* (1995) in mice fed with synthetic food colorant; Sharma *et al.* (2005) in mice fed with orange red; Sharma *et al.* (2006) in mice fed with apple green and Chakravarty *et al.* (2007) in mice fed with lead chromate. Significant decrease in the weight of the ovaries in treated rats was observed in this study. Similar decrease in the weight of the ovaries was also reported by Lorden and Caudle (1986) in rat fed with monosodium glutamate. Sharma (2011) showed decrease in the average weight of the ovaries in the animals fed with kesari powder. According to Simons *et al.* (1997) and Nwanjo *et al.* (2007) the increase or decrease in either absolute or relative weight of an organ after administering a chemical or drug is an indicator of toxic effects of that chemical. In

organophosphates treated female rats, a significant decrease in the ovarian weight was recorded Kaur and Dhanju (2005). The observed lipid peroxidation in the ovary and reduced oxidative enzyme by food colors affirms its ability to induce tissue damage. The lipid peroxidation in treated rats was accompanied by depletion of antioxidant enzymes SOD, CAT and reduced GSH in ovary of female rat. The role of antioxidants is to neutralize the excess of free radicals and to protect the cells from toxic effects (Douglas *et al.*, 2007). Roopha and Padmalatha (2011) observed a significant decrease in SOD and CAT in the ovaries of the Cadmium exposed animals. Cd induced oxidative damage in ovarian rat cells was also observed by Tribowo *et al.* (2014). Similar findings were observed in tissues of female rats treated with nicotine (Iranloye and Oludare, 2011). The food color toxicity caused the degenerative changes in the ovary which includes reduction in number of ovarian follicles and atrophic changes in the oocyte and granulosa cells and marked appearance of vacuolization in the granulosa cells. Sharma (2015) showed degenerative changes in the ovaries in albino mice fed with kesari powder. Similar degenerative changes have been reported by Johari *et al.* (2010) in rat feed with dizonon. In the present study, the ovary of treated animals showed atresia in the follicles. Dutta and Dalal (2008) stated that any disturbance in metabolic factors may initiate the atresia of oocytes. Reduction in number of ovarian follicles has also been observed in mice with organochlorine administration (Rafia and Garieb, 2001). Cypermethrin might have been

exerted some deleterious effects on ovaries in terms of increased atresia (Bretvard *et al.*, 2006).

CONCLUSION

Present study revealed that intake of food colors has deleterious effect on body weight, ovary weight, oxidative stress in ovarian tissue and ovarian histology. Hence, consumption of these food colors would cause adverse effect on health of humans. So it is necessary to create consumer awareness regarding ill effects due to excessive intake of food colors in diet.

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