EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 10
Issue: 2
233-238
Year: 2023

A STUDY ON SEROBIOCHEMICAL PROFILE IN ORANGE G INDUCED MALE RATS

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Article Received on 24/11/2022 **Article Revised on 14/12/2022** **Article Accepted on 04/01/2023**

ABSTRACT

In the scenario of fast-paced life journey, fast food consumption is increasing rapidly. To make attractive of the fast food to consumers, foods are prepared with additives like color, odor, taste etc. For visual appeal of food, different colorants including synthetic dyes are used nowadays in different commercial preparations. Orange G is one of such synthetic dyes, widely used in different commercial, industrial and domestic purpose. It is also used as food adulterant as colorant in different food preparations. Few reports on general hazards are found till date, but no report on serobiochemical parameters is found at present. In the present study, we tried the same on male rats by administering the Orange G through spontaneous water intake for 14 days at the dose of 3mg / 100gm BW / day. The results showed no change in body weight and relative weights of vital organs like liver, kidney, heart, spleen, adrenal glands and lung in treated rats. The serum biochemical profile in SGOT, SGPT, ALKP and serum protein also showed no significant change. The study of serum lipid profile, showed significant reduction in cholesterol and HDL level but TG, LDL, VLDL showed no significant change. The serum level of LDH showed significant rise in treated groups. The observation expressed no systemic toxicity of Orange G at the selected dose and duration. But rise of serum LDH and fall of serum cholesterol and HDL expressed the apprehension of chronic heart diseases.

KEYWORDS: Orange G, SGOT, SGPT, Cholesterol, LDH, ALKP.

INTRODUCTION

Dyes are used as coloring agent in many industries including textile, leather, cosmetics, paper, printing, plastic, pharmaceuticals, foods etc.[1] It is estimated that more than 100,000 commercially available dyes with over 7×105 tones dye stuff produced annually in the world.[2] Residual dyes are the major contributors to color in wastewaters generated from textile and dye manufacturing industries and other sources.[3] These residual dyes are stable, difficult to degrade, toxic, rendering the water unfit for its intended uses. Further, the color removal is also not adequate by the conventional chemical and biological treatment. Quite apart from the aesthetic desirability of colored streams resulting from dye waste, some dyes in particular can undergo anaerobic degradation to potentially carcinogenic amines.[4] Among these, Orange G is a synthetic azo-dye used in histology in many staining formulations. It is usually available as a disodium salt.[5] Orange G is used in many applied fields as pH indicator, textile and leather dyes, wood stain, coloring inks, different food preparations.[6 - 9] It becomes hazardous when heated to decompose Orange G, which emits toxic fumes of carbon oxides, nitrogen oxides, sulfur oxides and disodium oxides. [10,11] Till date, so many reports on domestic uses, toxicity in different physiological systems including reproduction in male are available on similar azo- dyes like Metanil azo-dyes and other dyes.[12-14] But no systemic toxicity studies on Orange G are yet available. So, in the present study, we aimed to find the primary effect of Orange G regarding its toxicity in a dose and duration dependent manner in adult male albino rats. To study the depth of toxicity we followed up the gravimetric changes in body growth rate and relative weights of vital organs including some serobiochemical parameters. In future our study will ring the alarm of Orange G toxicity to the common people and will find the usage restriction in parallel with the preventing measures.

MATERIALS AND METHODS

Animal selection and maintenance

During the entire course of experimentation, male albino rats of Wister strain having 100-120 days of age and body weights of 100±20gms were purchased from registered breeder and divided by two groups of six animals each which were considered as vehicle control and treated. Three animals were kept in per cage for

maintenance and were used for experimentation. The rats were maintained under standard laboratory conditions. The light: dark ratio of 14:10 hours, ambient temperature $25^0C \pm 2^0C$ and had free access of food and water ad libitum. The composition of standard diet was followed according to Hawk and Oser 1931.[15] The animal treatment was followed by the animal ethical rules of Burdwan University, West Bengal, India.[16]

Preparation of drug, Application and Dose selection

Orange G was purchased from Sigma-Aldrich (USA). Drug was prepared by dissolving with distilled water as homogeneous solution. The drug was prepared as 25 mg % in water and supplied by feeding bottle for spontaneous feeding. [17] But exact consumption of drug was calculated as 3 mg/100 gm BW/day from the water consumed by animals of treated group after 14 days treatment.

Duration of the treatment

All the animals were treated for 14 days (equivalent to one spermatogenic cycle) consecutively.

Animal Sacrifice

The animals were sacrificed under light ether anesthesia at 15th day of experiment following the cessation of drug treatment for 24 hours along with corresponding vehicle injected controls.

Blood and Tissue Collection

Blood samples were collected from the hepatic vein of each rat, immediately after light ether anesthesia and the serum was collected properly. Different organs like liver, kidney, spleen, lungs, heart and adrenal glands were dissected out, trimmed, blotted and weighed immediately after sacrifice.

Preparation of serum

Blood collected in non-EDTA vials are kept for at least 30 minutes undisturbed at room temperature. After that it was centrifuged for 15 minutes at 3000 rpm by a Spinwin centrifuge. The transparent fluid was collected from the upper layer of the tube. The collected serum was then preserved at - 20˚C for further use.

Gravimetric method

The body weights of rats were recorded to the nearest 0.5gm, initially on the day of commencement of the experiment and then every alternative day and finally on the day of sacrifice. The tissues of each rat were weighed to the nearest 0.1mg, in a single pan electronic balance. The weights of tissue were expressed after correction for difference in body weight i.e. relative weights (per 100gm body weight).

Serobiochemical studies

All serobiochemical estimations from serum were performed using standard kits, i.e. SGOT and SGPT,[18] ALKP,[19] total Cholesterol, [20] HDL cholesterol,[21-23] LDL cholesterol and VLDL determination, [24] TG, [25-28] HDL and LDL ratio, Total protein,[29-30] and LDH activity.[31-33]

Statistical analysis

Data were analyzed using graph pad InStat version 3 followed by two tail t- test to determine the effects of individual treatments. Differences with $p<0.05$ was regarded as significant. Results were analyzed using the raw data under the significance level.

RESULTS

The change in body growth showed no significant change in between vehicle treated control and Orange G treated groups. Similar pattern was also observed in relative weights of liver, kidneys, spleen, lungs, heart and adrenal glands (Table -1, Fig - 1). Serum enzyme profile i.e. SGOT, SGPT, ALKP and total protein showed no significant change in between the groups, but LDH activity showed significant elevation in treated group (Table -2, Fig - 2). The estimation of serum lipid profile in control and treated groups, the total cholesterol, HDL and HDL:LDL ratio were reduced significantly in treated group. Though, LDL, TG and VLDL showed no significant change when compared between the groups (Table -3, Fig – 3).

Table 1: Body growth rate % and relative weights of liver, kidneys, spleen, lungs, heart and adrenal glands in vehicle treated control and orange G treated groups. Values are expressed as means ± SEM, N=6.

Parameters	Control (gm)	Treated (gm)
% Body growth rate	3.202 ± 0.801	2.707 ± 0.611
Liver	3.577 ± 0.104	3.763 ± 0.193
Kidneys	0.700 ± 0.022	0.725 ± 0.044
Spleen	0.262 ± 0.011	0.235 ± 0.014
Lungs	0.558 ± 0.021	0.721 ± 0.128
Heart	0.330 ± 0.013	0.355 ± 0.029
Adrenal glands *	0.200 ± 0.009	0.230 ± 0.014

**Data has been raised to 10 folds to make contrast in the figure.*

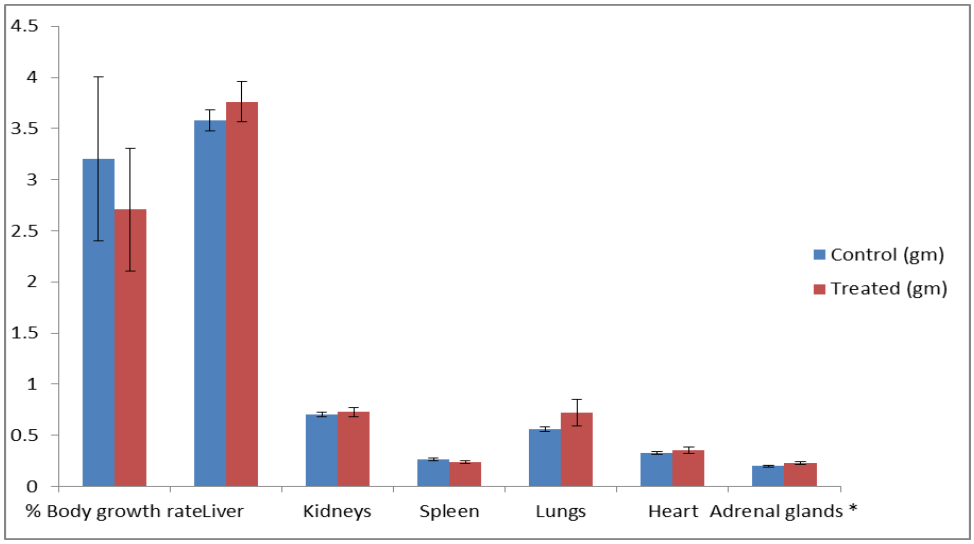

Fig. 1: Bar diagram showing gravimetric changes in Orange G induced (3mg / 100gm BW / day for consecutive 14 days) male rats. Values are expressed as mean ± SEM, N=6.

Table 2: Serum level of SGOT, SGPT, ALKP, LDH and Total protein in vehicle treated control and orange G treated groups. Values are expressed as means ± SEM, N=6.

Parameters	Control	Treated
SGOT (IU/L)	12.466 ± 1.109	10.860 ± 1.104
SGPT (IU/L)	03.478 ± 1.346	05.620 ± 1.440
Total Serum Protein (g/dl)	05.875 ± 0.826	07.375 ± 0.239
ALKP (KA Units)	12.758 ± 2.480	09.208 ± 1.880
LDH (IU/L) *	29.573 ± 3.820	51.597 ± 4.650

***** *Data has been reduced to 10 folds to make contrast in the figure.*

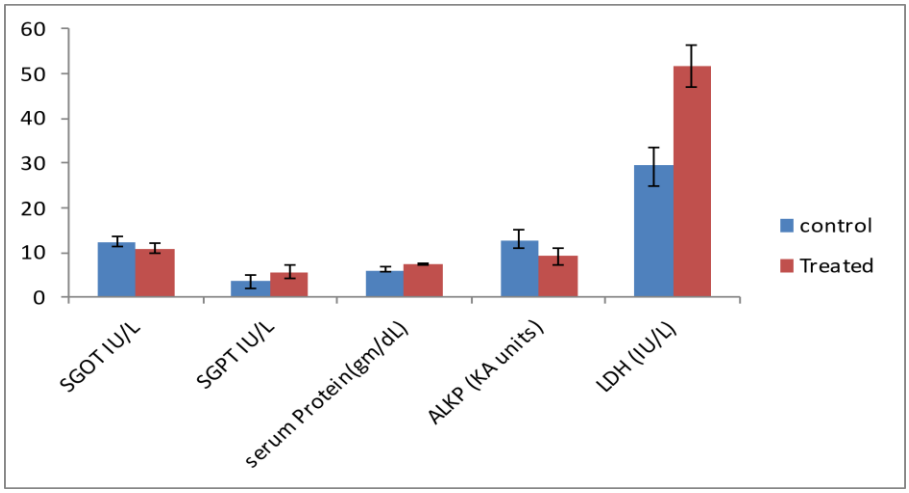

Fig. 2: Bar diagram showing different serobiochemical changes in Orange G induced (3mg / 100gm BW / day for consecutive 14 days) male rats. Values are expressed as mean ± SEM, N=6.

Table 3: Serum lipid profile in vehicle treated control and orange G treated groups. Values are expressed as means ± SEM, N=6.

Parameters	Control	Treated
Total Cholesterol (mg/dl)	92.589 ± 4.782	58.605 ± 1.880
HDL (mg/dl)	51.255 ± 3.261	15.538 ± 3.219
LDL (mg/dl)	20.708 ± 4.179	26.098 ± 5.370
TG (mg/dl)	103.23 ± 13.858	84.098 ± 5.954
VLDL (mg/dl)	20.625 ± 2.722	16.875 ± 1.197
HDL: LDL*	20.887 ± 6.820	10.683 ± 0.790

**Data has been raised to 10 folds to make contrast in the figure.*

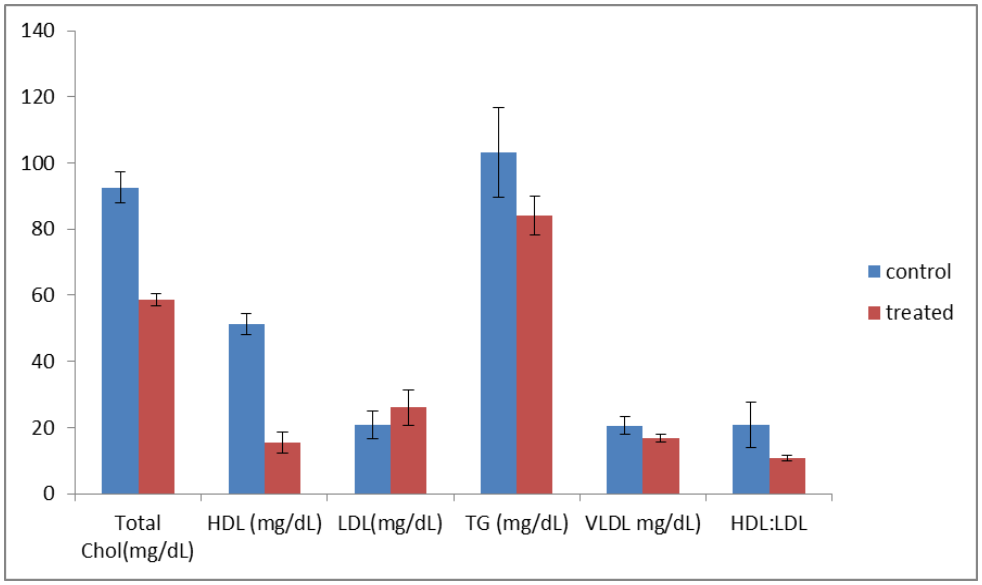

Fig. 3: Bar diagram showing lipid profile changes in Orange G induced (3mg / 100gm BW / day for consecutive 14 days) male rats. Values are expressed as mean ± SEM, N=6.

DISCUSSION

The present study, deals with the treatment of Orange G at the dose of 3 mg/100 gm BW/day for consecutive 14 days, showed no physical sign of toxicity, i.e. death, changes in nature of stool, urine and eye color. Animals did not expressed diarrhea, hematuria, restlessness, uncoordinated muscle movement, respiratory and cardiovascular distress during treatment. Food and water intake were mostly normal only a slightly reduced water intake was noticed in treated group. The change in body weights and relative weights of organs i.e., liver, kidneys, spleen, lungs, heart and adrenal glands of treated group showed no significant difference in respect to control, which suggests the nontoxic nature of Orange G, in this dose and duration of treatment. As, the reduction in body weight gain and changes in internal organ weights serve as the toxicity index which may be due to cellular degeneration and accumulation of cellular debris matter produced by cellular degeneration.[34,35] The nature of nontoxicity in treated group in this dose and duration was also reflected in showing insignificant change in serum SGOT, SGPT and ALKP. Such finding may also be supported by other studies.[36,37] The level of serum total protein also showed no significant change in comparison to treated group showing no possibility of dehydration and proneness of acute infections.[38,39] But in serum lipid profile studies, the total cholesterol, HDL level and HDL: LDL ratio showed significant reduction in Orange G treated group in compare to control, though, LDL, TG and VLDL showed no change at all. The results of lipo-protein status show the good indication against CVD but a mild threat by the significant reduction in HDL level.[40] The body content of cholesterol depends on between amount of cholesterol formed in body plus that absorbed from diet.[41] A deviation from normal values of cholesterol in the blood as symptoms of liver disease and can be attributed to the changes in the activities of specific enzymes responsible for lipid metabolism. In the present study, as no change in liver weight in treated group correlates the non-fatty liver, which is also correlated with the reduction of total cholesterol, indicating the increased cholesterol metabolism. But reduction in HDL in blood of treated group is caused by some unknown mechanism as cholesterol and HDL has inverse relation.[42] In other studies, this finding is supported indicating the nontoxicity of the drug at present dose and duration.[43] The significant lower level of HDL: LDL ratio in Orange G treated group, also supports the same.[40] But the significant rise in serum LDH activity in the treated group, indicates the vermilion cloud of myocardial infarction and chronic heart disease and also may be supported by reduced HDL level.[44,45]

CONCLUSION

From the present study it may be concluded that Orange G exhibits no such major toxic activity at the dose of 3mg/100gm BW/ day for 14 days. But it showed the significant rise of serum LDH activity, reduced level of total cholesterol, HDL and HDL:LDL ratio, which may be the indication of chronic heart disease. In further, the experiment should be repeated in different doses and duration manner vividly.

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