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ASSESSMENT OF REPRODUCTIVE TOXICITY CAUSED BY FAST GREEN FCF ON MALE SWISS ALBINO MICE

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Summary

Fast green FCF is one of the commonly used permitted synthetic food colourant which imparts bright green colouration to the food items. The current study investigates the toxic potential of this dye on male reproduction in *Swiss Albino* mice. The experimental animals were fed with 0.017 and 0.041 gm per kg body weight of dye for 42 days (6 weeks). The calculated doses of the dye was mixed with the standard mice feed and was given daily at a fixed time in the morning. The dye at both the doses caused a significant increase in the body weight but a significant decrease was observed in the weight of testes, tubular diameter , sperm density and sperm motility. Histologically, the dye caused a severe damage to the spermatogenic elements.

Key words: common food dye; Fast Green FCF; seminiferous tubular diameter, sperm density, sperm motility, Histopathology, Swiss albino mice.

Introduction

A colour additive, as defined by regulation, is any dye, pigment, or other substance that can impart colour to a food, drug, or cosmetic or to the human body. Colour additives are important components of many products, making them attractive, appealing, appetizing, and informative. Food colours are generally classified as natural and synthetic [1]. The synthetic colours are further divided into permitted and non-permitted.

In India, according to Rule 28 of Prevention of Food Adulteration Act (PFA Act, 1954) [2], eight synthetic colours, which include - tartrazine, brilliant blue, sunset yellow, ponceau-4R, carmoisine, erythrosine, fast green FCF and indigo carmine are permitted to be used in the eatables and that too in a limited quantity. According to Rule 30, maximum limit of the permitted colour shall not exceed 100 or 200 ppm (100 mg per kg of food) of the final food or beverage for consumption.

Many researchers studied the toxicological disorders induced by the food colorant both permitted and non- permitted in mice and other mammals [3,4,5,6,7,8,9,10,11,12,13,14,15,16]. Some of the early studies reported that the Fast Green has immunotoxic properties[17], can induce allergic reponse when ingested orally in food [18], impair hepatic functions[19] and inhibit synaptic activity in rat hippocampal interneurons [20]. However, there is paucity of systematic assessment of toxicity of the dye Fast Green.

The present investigation is a part of the research program which has planned to evaluate in detail the toxic impact of this commonly used food dye on swiss albino mice.

Materials and Methods

Animal's Model

Adult male Swiss albino mice of B-6 strain, 4-5 weeks old, weighing 25+ 3g were selected for the present study. Each animal was housed individually in a polypropylene cage bedded with saw dust and were maintained at standard laboratory conditions (12-h light/dark cycle; 25±3°C temperature; 35–60 relative humidity). Animals were fed on standard mice feed procured from Aashirwad Food Ltd., Chandigarh (India) and water was given *ad libitum*.

Dye Used

The dye Fast Green FCF (E number 143; FD & C Green No. 3; C.I. 42053) used in the present study was procured from the local market. It was manufactured and packed by Research – Lab, Fine Chem Industries, Mumbai (India). The other chemicals used in the experimentation were of analytical grade.

Experimental design

Investigation was carried out for a period of 42 days and the doses of the food dye administered were selected on the basis of LD_{50}. The dose is expressed in terms of the amount of test substance (dye) received by the animal per kg of body weight per day (mg/kg b.wt. /day).

Animals were divided into 3 groups each containing 5 animals and were kept individually. The animals of group I served as control and were fed with the standard diet alone.

The animals of group II were fed with 0.017 g/kg b.wt of Fast Green FCF and the animals of group III were given 0.041 g/kg b.wt of Fast Green FCF. The dye was given orally mixed with the standard food.

see Table 1.

Parameters studied

Body weight and Organ weights

The treated males were weighed and autopsied after 24 hours from the last dose. The animals were sacrificed by cervical dislocation.

The testes and cauda epididymis were carefully dissected out, made free from adherents and

weighed on an electronic top balance.

Histometry: With the help of occulomicrometer circular appearing seminiferous tubules were traced at x100 and the diameter of each tubule was measured separately. The measurement was expressed in terms of mean of all the traced tubules.

Sperm Density: Total number of sperms were counted using haemocytometer after further diluting the sperm suspension. The sperm density was calculated in million per ml as per dilution[21].

Sperm motility: Sperm motility was assayed by the method given by Prasad, 1972 [21]. The epididymis was removed and known weight of cauda epididymis was gently squeezed in physiological saline (0.09% Nacl) to release the spermatozoa from the tubules.

The sperm suspension was examined within 5 minutes after their isolation from the epididymis. The results were determined by counting both motile and immotile sperms in at least 10 separate and randomly selected fields. The results were finally expressed as percent motility**.**

Histopathological studies: Testes were fixed in Bouin's fixative, paraffin sections were obtained and stained in Ehlrich's hematoxylin and eosin for histopathological studies.

Ethical Aspects

The study was approved by the ethical committee, Center for Advanced Studies, Department of Zoology, University of Rajasthan, Jaipur (India). The guidelines of Indian National Sciences Academy, New Delhi [22] were followed for maintenance and use of the experimental animals.

Statistical analysis

Statistical significance between the control and experimental data were subjected to one way analysis of variance (ANOVA).

Results

Effects on Body and Organ weight

The dye caused a significant increase in the body weight at both the dose levels which was found to be highly significant statistically. However, a highly significant decrease was observed in the average weight of the testes at both the dose levels (Table 2).

see Table 2.

Effects on Seminiferous tubular diameter

The dye Fast green FCF caused a highly significant reduction in the average diameter of the seminiferous tubules at both the dose levels (Table 3).

see Table 3.

Effects on Sperm Dynamics

The dye Fast green FCF when administered orally caused a marked reduction in the testicular sperm density and caudal sperm motility at both the dose levels which was found to be highly significant when compared with the respective controls (Table 3).

see Table 3.

Effects on Testes histopathology

Oral administration of the Fast Green FCF dye caused severe pathological changes in the testis at both the doses. At low dose, it caused arrest of spermatogenesis at spermatocyte stage and the tubular lumen showed debris of the broken sperms. At high dose, the dye caused a total disruption of the spermatogenetic elements. Cytoplasmic vacuolation & pycnosis were also prominent.

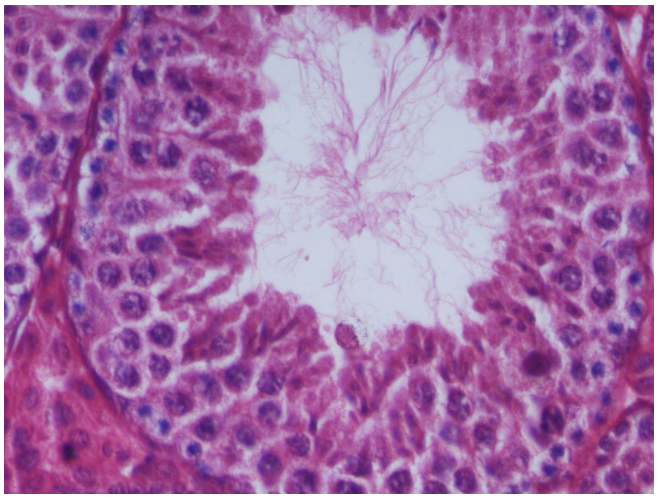

Fig.1. Microphotograph of testis of control mice showing normal testicular architecture (400X).

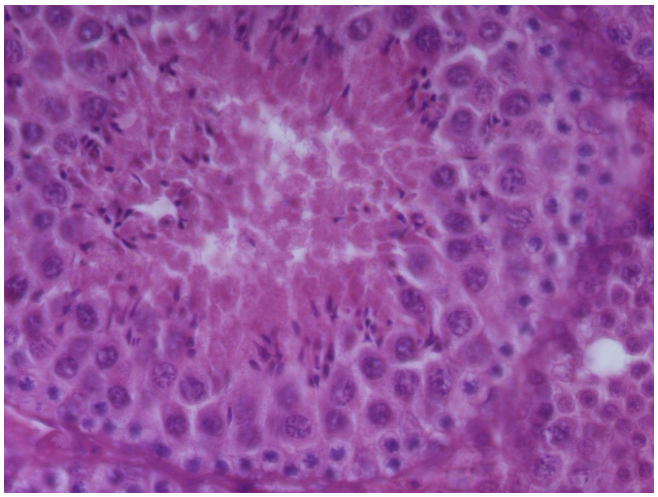

Fig. 2. Microphotograph of testis of Fast Green FCF treated (Low dose) mice, showing arrest of spermatogenesis at spermatocyte stage and blocked lumen with debris of broken sperms. (400X)

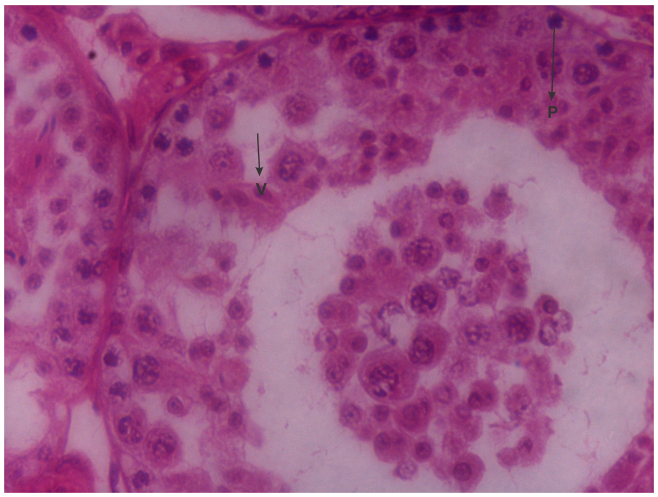

Fig. 3. Microphotograph of testis of Fast Green FCF treated (High dose) mice, showing total disruption of spermatogenetic elements, cytoplasmic vacuolation(v) and pycnosis(p).(400X).

Discussion

The present investigation revealed a marked increase in the body weight of all the experimental animals. Similar increase in the body weight was also reported in mice fed with Ponceau 3R [23]; in mice fed with synthetic food colourants [24]; in rats fed with Metanil yellow [25]; in mice fed with Chocolate brown[3], Orange red [4], malachite green [5], orange G [6], Apple green [7], Tomato red [8,9] & lead chromate[10]; in female mice fed with Tartrazine & Kesari powder [11,12] and in mice fed with Taratrazine [13]. On the contrary, a decrease in the body weight was reported in Sprague-Dawley rats fed with allura red [26]; in rats fed with some synthetic and natural food colourant [27]; and rats fed with sunset yellow and sodium nitrite [28]. Increase in the body weight of the experimental mice can be attributed to the hormonal imbalance caused due to dye toxicity, as there is no evidence of increased food intake by the experimental mice. It has been reported that the reduction in the testosterone levels causes increase in the BMI [29,30,31]. Hence it is possible that this dye somehow caused a reduction in the testosterone level which in turn increased the body weight. Further, it is evident that any type of stress in the body causes excess secretion of stress hormone, cortisol which inturn increases body fat [3]. Hence, the chemical stress caused by the dye might be the another cause of weight gain in the experimental animals.

The reduction in the testicular weight & tubular diameter indicates inhibitory effect of the dye Fast green FCF on the reproductive organ. Maintenance of structural and functional integrity of the male reproductive organs require a continuous presence of androgen in the blood. Therefore, the finding confirms the anti-androgenic nature of the dye. Histopathological observation revealed that the dye caused a marked reduction in the spermatogenic stages. Hence, it might be the another cause of reduced testis weight in the experimental animals. This observation finds support from the findings of Sharma et al.,2008 [9], Mathur et al.,2005b [25], Sherins et al.,1978[33], Khanna et al., 1978 [34],

Prasad and Rastogi,1983 [35] Takihara et al.,1987 [36], Huang et al.,1997[37], Abdel-Aziz et al.,1997[38], Mathur et al., 2001[39], Mathur et al., 2003[40], Mathur et al.,2005a[41].

Histopathologically, in the microsections of the treated testes apical degeneration and confluence of tubules, denudation of germinal epithelial cells,some tubules with obliterated lumen, hampered spermatogenesis or devoid of sperms were observed. It is known that the differentiation of primordial germ cells into spermatogonia and the consequent appearance of spermatogenic cycles are under the control of gonadotropins and testosterone [42]. These are mediated possibly by sertoli cells [43,44] which regulate cell cycle kinetics and influence both spermatogonia and preleptotene spermatocyte [45,46]. The arrest of spermatogenesis at early stages observed in the experimental animals might be due to direct effect of dye Fast green FCF on the sertoli cells which control spermiation. It is in accordance to the finding of Bardin et al.,1988 [44], Choudhary et al.,2005 [47], Karanth et al., 2004[48]. The production of rounded and elongated spermatids were under the influence of androgens so the alteration in androgen level in testes caused less number of spermatids in the seminiferous tubules [49]. A testosterone deficit is expected to interfere with the completion of meiosis by a direct action on the germ cells [50]. Thus, it seems possible that the dye might have affected the Leydig cells which in turn reduced the production of the testosterone as a result the spermatogenesis gets inhibited. Similarly, giant cells were also observed denuded off from the spermatogenic epithelium into the tubular lumen. These giant cells could be the result of faulty or failed chromosomal replication during cell division due to dye toxicity.

The low sperm density observed in the experimental animals might be attributed to the altered androgen metabolism due to dye toxicity [47,51,52,53,54]. The reduced sperm motility due to treatment might be the result of indirect action of Fast Green FCF on pituitary gonadal epididymal axis because epididymis provides suitable environment for development of spermatozoa under the influence of androgen.

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Groups	No. of mice in a group(kept individually)	Amount of food/mice/day	Dye given/mice/day (gm/kg/b.wt.)	Food intake/mice/day
Group I (Control)	5	10 gm standard mice feed	nil	all food consumed
Group II (Low dose)	5	5gm dye mixed food + 5gm standard food	0.017	all dye mixed food consumed
Group III (High dose)	5	5gm dye mixed food + 5gm standard food	0.041	all dye mixed food consumed

Table 1. Showing consumption of food in both control and experimental mice

Groups	No. of mice	Bodyweight (gm)		Testes weight (gm/100gm b.wt.)
		Initial	Final	
Group I (Control)	5	24.4±0.50	26.0±0.44 (p<0.04)	0.17±0.00
Group II (Low dose)	5	24.6±0.60	30.2±0.80*** (p<0.00)	0.12±0.00*** (p<0.00)
Group III (High dose)	5	23.6±0.24	30.4±0.50*** (p<0.00)	0.10±0.00*** (p<0.00)

Table 2: Showing changes in body weight and testes weight of mice
***= highly significant

Groups	Seminiferous tubular diameter (µm)	Sperm density (million/ml)	Sperm motility (%)
Group I (Control)	171.93±7.6	1.93±0.17	73.8±2.43
Group II (Low dose)	133.27±1.82*** (p<0.00)	1.2±0.13*** (p<0.00)	56±3.16 *** (p<0.00)
Group III (High dose)	101.62±6.8*** (p<0.00)	0.62±0.07 *** (p<0.00)	37.4±1.40*** (p<0.00)

Table 3: Showing changes in seminiferous tubular diameter and sperm dynamics of mice.
***= highly significant