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# Evaluation of Reproductive toxicity caused by Indigo carmine on male swiss albino mice

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## Summary

Present investigation was undertaken to evaluate the toxic potential of Indigo carmine, on male reproductive organ : testes, in *Swiss Albino* mice. The animals were fed on diet containing 0.0 (control), 0.017 (Low dose) and 0.039 (High dose) gm per kg body weight of dye for 42 days (6 weeks). The calculated doses of dye was mixed with the standard mice feed and was given daily at a fixed time in the morning during the entire experimental tenure. The body weight of mice was recorded weekly. 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 and sperm motility were found to be reduced significantly. The dye caused a reduction in the sperm density which was found to be non-significant at low dose and significant at high dose. Histologically, the dye caused a profound damage to the complete testis architecture.

Key words: common food dye; Indigo carmine; tubular diameter, sperm density, sperm motility, histopathology, Swiss albino mice

## Introduction

Eatables with bright colouration makes the food more tempting and mouth watery and in response, compells a consumer to purchase and relish it. Thus, food colourants have been added to food for centuries to enhance its appearance. Many researchers studied the toxicological disorders induced by various food colourants in mice and other mammals [1,2,3,4,5,6,7,8,9,10,11,12,13,14,15,16,17,18]. However, no systematic studies have so far been made to evaluate the toxicity of the dye, Indigo carmine.

The present work is a part of the project which concerns to evaluate toxic impacts of various permitted & non-permitted dyes individually and in combinations. The present work is an attempt to evaluate reproductive toxicity of the dye, Indigo carmine 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 Indigo carmine ( E number 132; FD & C Blue # 2 ; C.I. 73015) used in the present study was procured from the local market. It was manufactured and packed by ASES Chemical works laboratory chemical division, Jodhpur (Rajasthan). 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 and III were fed with 0.017 g/kg b.wt and 0.039 g/kg b.wt of Indigo carmine respectively. 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[19].

**Sperm motility**: Sperm motility was assayed by the method given by Prasad *et al*., 1972 [19]. 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 [20] 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 animals fed with the dye showed an increase in their 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*

Oral administration of dye Indigo carmine 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 Indigo carmine caused a marked reduction in the testicular sperm density which was

found to be non-significant at low dose but highly significant at high dose when compared to respective controls. The dye caused a highly significant decrease in the sperm motility at both the doses.(Table 3).

### *Effects on Testes Histopathology*

Oral administration of the dye, Indigo carmine caused severe pathological changes in the testis architecture at both the doses.

At low dose, it caused thickening of the tubular basement membrane, arrest of spermatogenesis at spermatid stage and the tubular lumen showed debris of the broken sperms.

At high dose, the dye caused dissolution of tubular basement membrane, exfoliation of cells in the lumen leading to complete testicular blockage.

Cytoplasmic vacuolation & pycnosis were also prominent.

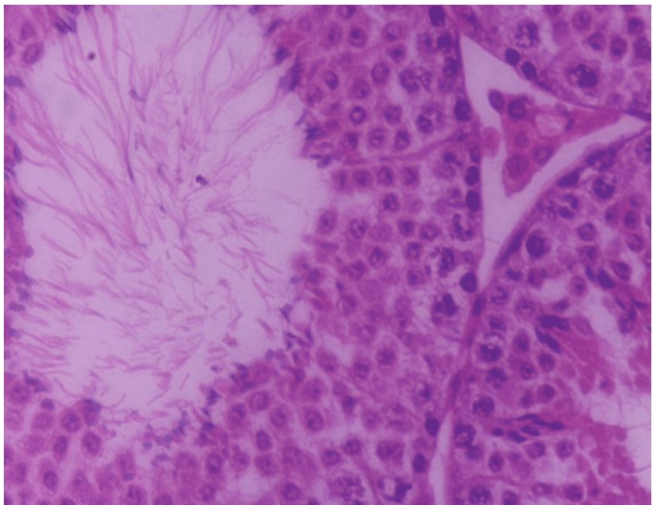

Fig.1. Microphotograph of testis of control mice showing normal testicular architecture (400X).

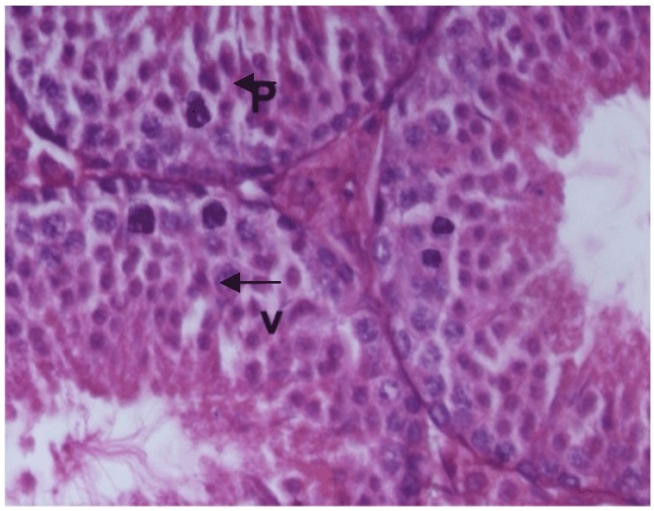


Fig. 2. Microphotograph of testis of Indigo carmine treated (Low Dose) mice, showing thickening of the tubular basement membrane, arrest of spermatogenesis at spermatid stage and tubular lumen showing debris of broken sperms, Cytoplasmic vacuolation(v) and pycnosis(p).(400X).

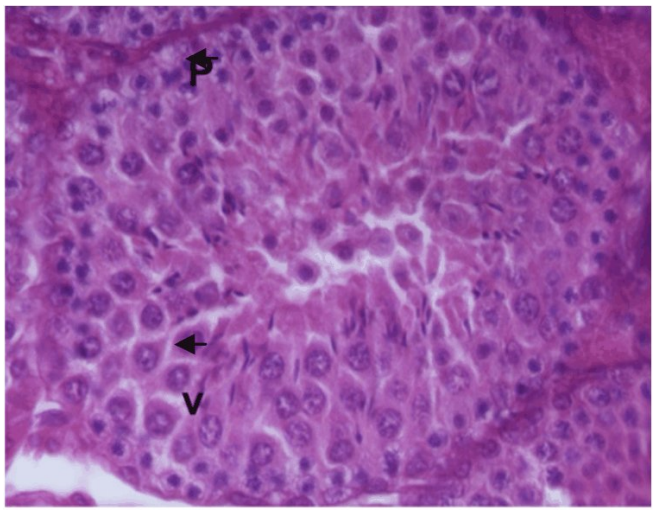


Fig. 3. Microphotograph of testis of Indigo carmine treated (High Dose) mice, showing dissolution of tubular basement membrane, exfoliation of cells in the lumen leading to complete testicular blockage ,Cytoplasmic vacuolation(v) and pycnosis(p). (400X).

## Discussion

Oral administration of the dye, Indigo carmine resulted in exponential increase in the body weight of all the experimental animals. Similar results were recorded in mice fed with Ponceau 3R [21]; in mice fed with synthetic food colourants [22]; in rats fed with Metanil yellow [23]; in male mice fed with Chocolate brown[1], Orange red [2], malachite green [3], orange G [4], Apple green [5], Tomato red [6,7 ] , lead chromate[8] , Tartrazine & Kesari powder [9,10] and Taratrazine [ 11] . On the contrary, a decrease in the body weight was reported in Sprague-Dawley rats fed with allura red [24]; in rats fed with some synthetic and natural food colourant [25]; and in rats fed with sunset yellow and sodium nitrite [26]. The increase in the body weight of experimental mice can be attributed to the hormonal imbalance caused due to dye toxicity. It has been reported, that the low levels of testosterone proportionate causes increase in the BMI [ 27,28,29]. Hence, it is possible that this dye somehow caused a reduction in the testosterone level which in turn increased the body weight. In addition to this, it is also evident that any type of stress in the body causes excess secretion of stress hormone, cortisol which inturn increases body fat [30]. So, it can be inferred that chemical stress caused by the dye may be the another cause of weight gain in the experimental animals.

Significant decrease in the testicular weight & tubular diameter clearly reflects the inhibitory effect of the dye Indigo carmine on the reproductive organ. This indicates the anti-androgenic nature of the dye, as maintainence of structural and functional integrity of the male reproductive organs requires continuous presence of androgen in the blood. Histopathological Observations revealed that the dye caused a marked reduction in the spermatogenic elements.

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 [7], Mathur et al.,2005b [23], Sherins et al.,1978 [31], Khanna et al., 1978 [32], Prasad and Rastogi ,1983 [33] Takihara et al., 1987 [34], Huang et al., 1997 [35] , Abdel-Aziz et al.,1997[36], Mathur et al., 2001[37], Mathur et al., 2003[38] and Mathur et al., 2005a[39].

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 [40]. These are mediated possibly by sertoli cells [41,42] which regulate cell cycle kinetics and influence both spermatogonia and preleptotene spermatocyte [43,44]. The arrest of spermatogenesis at early stages observed in the experimental animals might be due to direct effect of dye Indigo carmine on the sertoli cells which control spermiation. It is in accordance to the finding of Bardin et al.,1988 [42], Choudhary et al.,2005 [45] and Karanth et al., 2004[ 46].

Reduction in the number of spermatids in tubules can be attributed to alteration in androgen level in testes [47].Low levels of testosterone interferes with the functioning of germ cells [48]. Thus, it seems possible that the dye might have altered the functioning of 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 reduction in the sperm density observed in the experimental animals might be attributed to the altered androgen metabolism due to dye toxicity [45,49,50,51,52]. The low sperm motility due to oral administration of dye Indigo carmine clearly indicates its indirect action 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 | 0.039 | 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 | 24.2±0.37 | 25.8.0±0.37 (p<0.01) | 0.18±0.00 |
| Group II (Low dose) | 5 | 24.0±0.44 | 29.4±0.74*** (p<0.00) | 0.13±0.00*** (p<0.00) |
| Group III (High dose) | 5 | 24.0±0.31 | 31.0±0.44*** (p<0.00) | 0.1±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) | 172.52±4.30 | 1.56±0.19 | 70.4±2.35 |
| Group II (Low dose) | 131.29±2.98*** (p<0.00) | 1.24±0.08$^{ns}$ (p<0.1) | 51.4±2.24*** (p<0.00) |
| Group III (High dose) | 99.63±7.62*** (p<0.00) | 0.42±0.07 *** (p<0.00) | 33.6±1.50*** (p<0.00) |

Table 3: Showing changes in seminiferous tubular diameter and sperm dynamics of mice.
***= highly significant, $^{ns}$= non- significant.