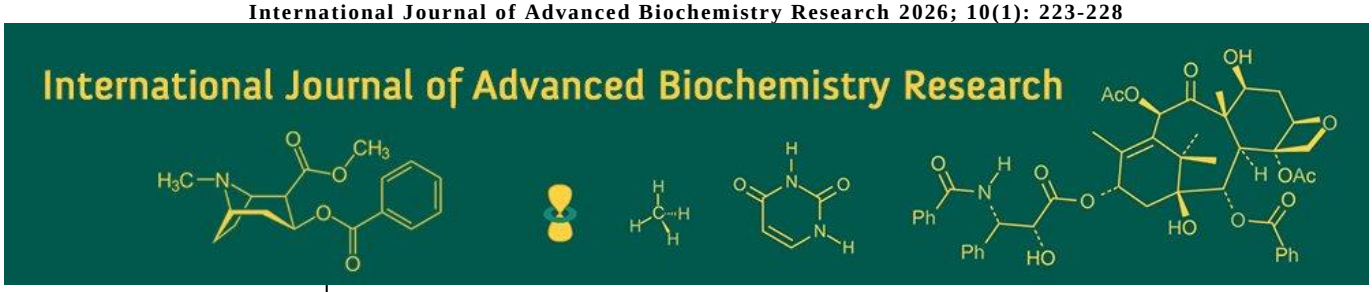

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Histopathological study of tartrazine in Wistar rats (*Rattus norvegicus*)

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Abstract
The present study on toxicopathology of tartrazine was carried out on 80 Wistar rats comprised of 40 male and 40 female divided into four equal groups, *viz.*, Groups I, II, III and IV. Group I served as control and received distilled water, while Groups II, III and IV received tartrazine @ 50, 100, 200 mg/kg body weight orally dissolved in drinking water for 90 days, respectively. All rats were subjected to pathomorphological studies on 91st day of experiment. No appreciable pathomorphological changes were observed in any organs except liver, kidney, stomach and testis. Histopathological changes in liver were characterized by focal necrosis of hepatocyte, micrograniuloma with infiltration of mononuclear cells. Kidney showed congestion, mineralization, infiltration and regenerative tubules whereas infiltration of eosinophils and focal ulceration in mucosal surface of stomach was seen in treatment group rats.

Keywords: Tartrazine, toxicopathology, Wistar rats, histopathology, liver lesions, kidney lesions, stomach lesions

Introduction
Food additives are substances mixed with primary food materials in order to enhance its flavor, taste, appearance, food value and conservation that will fulfill the demand of wholesome and tasty food for rapidly increasing population round the year (Amin *et al.*, 2010). More than 3000 additives and preservatives are available in the markets that are used as antioxidants and antimicrobial agents (Boca Raton and Smoley, 1993) [4]. These ingredients have been employed to increase the taste, shade, constancy, quality and value of foods. These are the result of industrialization, marketing struggle and advances in the technology of food processing and treatment (NRC, 1983).
Dyes are dynamic in food industries in order to make food look more attractive and delicious, providing uniqueness and for imaginative or ornamentation purposes (Elgendy and Al-Zahram, 2015) [8]. From the organoleptic opinion of vision, the visual aspect is a key feature for the selection of the products by the consumer. Food colours are usually classified as natural and synthetic (Harris, 1986) [12] and the synthetic colours are additionally divided into permitted and non-permitted colours. In India, 83.6 per cent of the sample contained permitted colours and 16.4 per cent of sample confined non-permitted colours (Dixit *et al.*, 2011) [5]. Natural food dyes are less stable which has been lost through processing and storage. Natural dyes are comprised of four main types of plant pigments as Carotenoids, Chlorophyllin, Anthocyanins and Betanin (Rodriguez-Amaya and Delia, 2016) [18].
Tartrazine (TAZ) also known as E 102, FD and C, Yellow No. 5 is an azo dye and salt of chemical formula 3-carboxy-5-hydroxy-1-(4'-sulphophenyl)-4-(4'-sulphophenylazo) pyrazoletri-sodium salt (Tanaka *et al.*, 2008) [21]. The Pure Food and Drugs Act of 1906 made illegal any nourishment found to be adulterated which may render the food injurious to health. It was found that the foods manufactured by unorganized private sectors and small merchants did contain colours in much greater concentration than permitted range (Biswas *et al.*, 1994) [3]. The toxicity of Tartrazine has been associated to the reductive biotransformation of the azo bond during their metabolism in the intestine and liver generating reactive amines, aryl amines and free radicals (Umbuzeiro *et al.*, 2005) [22] through disturbance of pro-oxidant and antioxidant equilibrium respectively in rat liver (El-wahab and Moram, 2012) [9] and brain

(Gao *et al*., 2011) [10]. Tartrazine is converted into aromatic amine sulfanilic acid after being metabolized by the gastrointestinal microflora (Moutinho *et al*., 2007) [16] and the intended aromatic amines can produce Reactive Oxygen Species (ROS) by interface of these amino groups with nitrite or nitrate-containing foods or in the stomach. The ROS such as superoxide anion, hydroxyl radical, and H_2O_2 could be manufactured in the metabolism of nitrosamines and rise oxidative stress (Bansal *et al*., 2005) [2]. Traditional toxicity studies of azo dyes have been directed on mammalian models principally rats and mice, which commonly have significance on immunological, serological, biochemical and histopathological features (Elbanna *et al*., 2017) [6]. Several safety concerns were described for Tartrazine, but the evidence is limited. The consumption of Tartrazine exceeds their acceptable daily intake and higher exposure may cause health risk in human being. Due to paucity of information available on Tartrazine toxicity in India, the present study has been planned

Material and methods

Location

The present study was carried out at the Department of Veterinary Pathology, College of Veterinary Science and Animal Husbandry, Kamdhenu University, Sardarkrushinagar - 385 506, Gujarat, India.

Experimental animals

A total of 80 Wistar rats were procured from Pavo Research Solutions, Dashrath, Vadodara, Gujarat, India. Before grouping and dosing, the procured rats were retained under acclimatization for 15 days.

Institutional Animal Ethics Committee (IAEC) approval

The protocol was presented before the Institutional Animal Ethics Committee on 13th January 2021 and the protocol was approved as VETCOLL/IAEC/2021/17/PROTOCOL-07 by the IAEC of the College of Veterinary Science and Animal Husbandry, Kamdhenu University, Sardarkrushinagar-385 506, Gujarat, India.

Housing and environmental conditions

Animal management and treatment procedures complied with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India. All the rats were housed in polypropylene cages at the laboratory animal house facility of the college (Figs. 1 and 2) in an environmentally controlled room with 22 ± 3 ºC temperature and 30-70 per cent humidity. Light/dark cycles of 12/12 hours were provided throughout the acclimatization and study period. Corncob was used as a bedding material. Rats were identified by tail markings. All essential managemental procedures were adopted to keep the rats free from stress. Rats had *ad libitum* access to standard pellet diet (VRK Nutritional Solution, Sangli, Maharashtra, India) and RO drinking water throughout the study period.

Fig 1: Laboratory animal facility

Fig 2: Housing of rats in polypropylene cages at the Laboratory animal facility.

Experimental design

The toxicopathology of Tartrazine was studied in 80 Wistar rats comprising of 40 male and 40 female rats. All 80 rats were randomly divided into 4 different groups. Each group consisted of 20 rats. The groups were numbered as groups I to IV. Group I served as control and received only distilled water, while groups II, III and IV were received test

compound tartrazine at the dose of 50, 100 and 200 mg/kg/day respectively in distilled water for 90 days.

Numbering and identification

Identification of animals was done by tail marking with a permanent color marker. Cage labels and body marking were specific to spot the animals. Five animals were kept in one cage. One ring for number one, two rings for number two, three rings for number three, four rings for number four and no marking for number five.

Test compound

The test compound tartrazine was purchased from Sigma-Aldrich, Mumbai, India.

Necropsy and organ weight

All the rats were euthanized, on the 91st day of the study. The rats have been fasted overnight before necropsy. Rats were anesthetized using isoflurane. The organs were collected from all the animals. The organs were cleaned by using filter paper and then weighed by using an analytical balance.

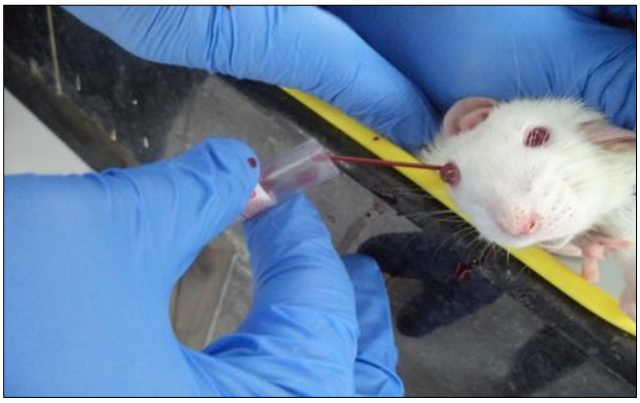

Fig 3: Blood collection from retro orbital plexus of rat.

Clinical pathology

Blood was collected from the retro-orbital plexus (Fig. 3) with the help of a capillary tube for hematology and biochemical estimation on the 91st day of the experiment.

Pathomorphology

After recording the gross lesions, the tissues *viz.*, kidneys, liver, heart, brain, spleen, adrenals, thymus, testes, trachea, lungs, epididymides, ovaries, uterus with a cervix, salivary glands, seminal vesicle, prostate, urinary bladder, stomach, intestines (both), lymph nodes, spinal cord, and skin were collected from sacrificed animals, trimmed for any adherent tissue, as appropriate and subsequently preserved in 10 per cent neutral buffered formalin for at least 48 hours.

Tissue processing and staining

Fixed tissues were trimmed, labeled and washed under running tape water for two hours. Dehydration was done using ascending order (30, 70, 90 and 100%) of isopropyl alcohol. The dehydrated tissues were cleared by 3 changes of xylene and impregnated in melted paraffin. The whole tissue processing was carried out in an automatic tissue processor (Leica TP1020). The paraffin impregnated tissues were embedded using Leica EG1160 paraffin embedding station and cooled on Leica EG1150 C Cold Plate. The 5 microns thick paraffin sections were cut using Leica RM2255 fully automated rotary microtome. The sections were taken on egg albumin-coated slides. The sections were deparaffinized by xylene and rehydrated through descending grades of isopropyl alcohol and water. The hydrated tissue sections were stained with hematoxylin, differentiated in acid alcohol and blueing was carried out by ammonia water. Then tissue sections were stained with eosin, dehydrated with absolute isopropyl alcohol, followed by xylene and mounted with DPX (Luna, 1968; Suvarna *et al*., 2012) [15, 20]. The entire slide staining was done in Gemini AS Automated Slide Stainer, Thermo Scientific.

Results and Discussion

Tissues *viz.*, liver, kidneys, lungs, heart, brain, spleen, adrenal, thymus, testis, epididymides, ovaries, uterus with cervix, seminal vesicle, urinary bladder, stomach and both intestine (Small and Large) were collected for histopathological examination from all the rats during necropsy. No appreciable gross changes were observed in visceral organs *viz.*, liver, kidney, lungs, heart, brain, spleen, adrenal, thymus, ovaries, uterus with cervix, seminal vesicle, prostate, urinary bladder, stomach and both intestine after oral administration of tartrazine daily for 90 days.

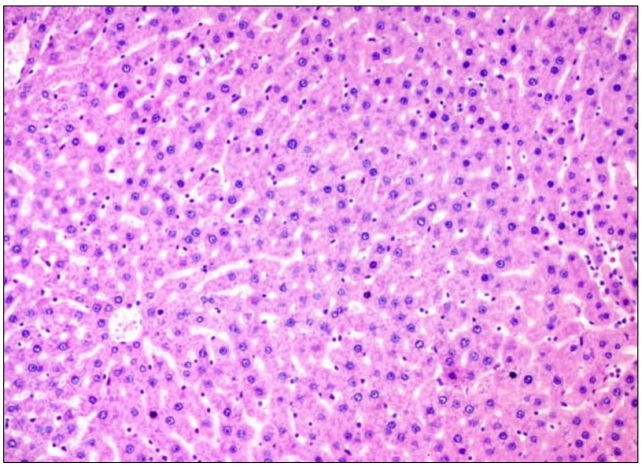

Fig 4: Gorup I Male: Photomicrography of liver with normal histological architecture. H&E X 100

In the present study, lesions were found in the liver of male and female rats belonging to Groups II, III and IV. Normal histological architecture of liver (Fig. 4) was seen in Group I rats. Focal microgranuloma and infiltration of mononuclear cells in portal area of liver (Figs. 5, 6) were observed after the dosing of tartrazine in both sexes of rats. These changes were considered specific to the tartrazine toxicity as all rat groups had lesions of liver microgranuloma, hepatic necrosis with mononuclear cells infiltration. The microscopic changes observed in the present study were in accordance with Ghonimi and Elbaz (2015) [11], Saxena and Sharma (2015) [19] and Khayyat *et al*. (2017) [14].

Tartrazine produced lesions *viz.*, regenerative tubules and mononuclear cells infiltration in group IV male rats (Fig. 8). The lesions were observed in three animals of Group IV male as compared to control rats with normal histologic architecture (Fig. 7) of kidney. Kidney of Group IV female rats showed tubular multifocal mineralization along with presence of tubular cast and congestion (Fig. 10) and regenerative tubules. However, kidney of Group III female rats showed tubular mineralization and peritubular mononuclear cell infiltration. Group II female rats showed

tubular mineralization whereas group I female showed normal histologic architecture (Fig. 9). Elekima *et al*. (2019) [7] also reported similar results. The authors administered tartrazine at the dose rate of 7.5 mg/kg/day for 90 days in rats and reported hyaline cast in proximal tubules, hyper cellularity of mesangial cells and inflammation of the glomerulus in the kidney of the treatment group animals.

Administration of tartrazine produced lesions in stomach only in males when compared to control group. Female rats from all groups showed normal architecture of glandular and non glandular stomach. Rats from Group I showed normal histological architecture (Fig. 11). Male rats from Group IV showed focal extensive area of eosinophilic infiltration extended up to mucosa with focal ulceration in stomach (Fig. 12). This findings of present study were in accordance with Moutinho *et al*. (2007) [16] in which the authors administered tartrazine added mineral water daily at the dose of 7.5 mg/kg for 46 weeks to Wistar rats and observed a significant infiltration of eosinophils and lymphocytes in the gastric antrum mucosa of stomach in treatment group animals.

Ghonimi and Elbaz (2015) [11] had given tartrazine daily at dose rate of 500 mg/kg b. wt. daily for 30 days and revealed degenerative changes in mucosa of stomach. The changes observed in the stomach may be due to functional inability of esterase enzyme which is commonly found in epithelium of stomach to hydrolyze esters with large alkyl groups or to the greater lipophilicity of the higher alkyl esters.

Group I male rats showed normal histological architecture (Fig. 15) whereas loss of round and elongated spermatid was found in multiple tubules. However, few tubules showed formation of multinucleated giant spermatid in testis of high dose treated male rats (Fig. 16). This finding of present study were in accordance with Visweswaran and Krishnamoorthy (2012) [23] in which the authors administered tartrazine at the dose rate of 72 mg/kg/day for 60 days to Wistar rats by oral gavage and observed reduced space between seminiferous tubules, oval elongated tubules and poorly differentiated spermatogenic cells in testis.

Incidental finding were observed in various organs *viz*., Spleen, Heart, Brain, Epididymides and Prostate. Other organs *viz*., both intestine (small and large), ovaries, uterus with cervix, urinary bladder, seminal vesicle, trachea, thymus, salivary gland and adrenals did not reveal any appreciable gross as well as histological lesions were comparable to control group.

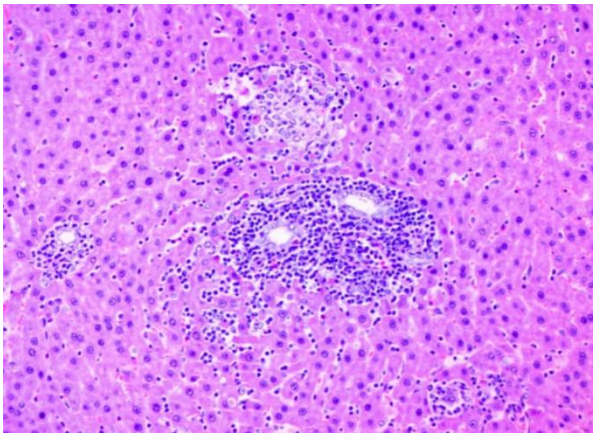

Fig 5: Gorup IV Male: Photomicrography of liver showing focal microgranuloma and portal infiltration of mononuclear cells. H&E X 100

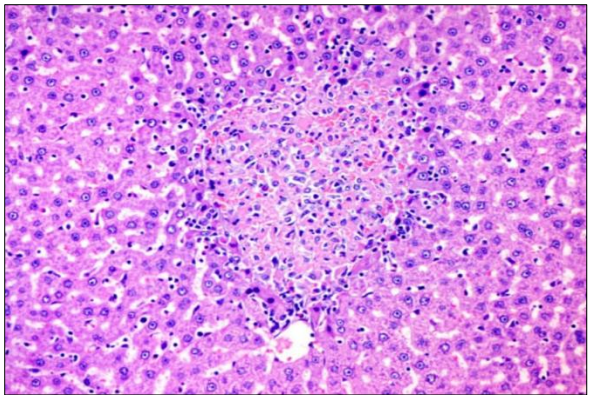

Fig 6: Gorup IV Female: Photomicrography of liver showing focal microgranuloma. H&E X 100

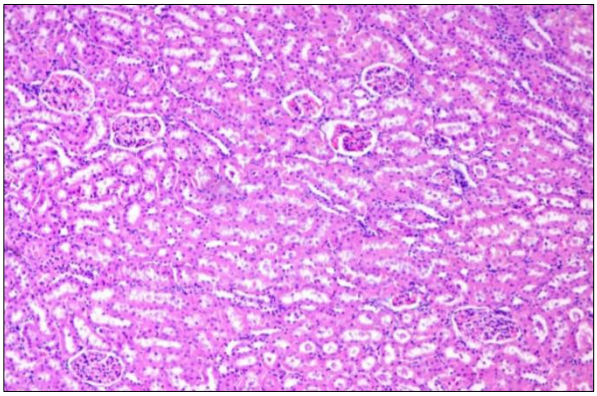

Fig 7: Gorup I Male: Photomicrography of kidney with normal architecture and cellular details. H&E X 100

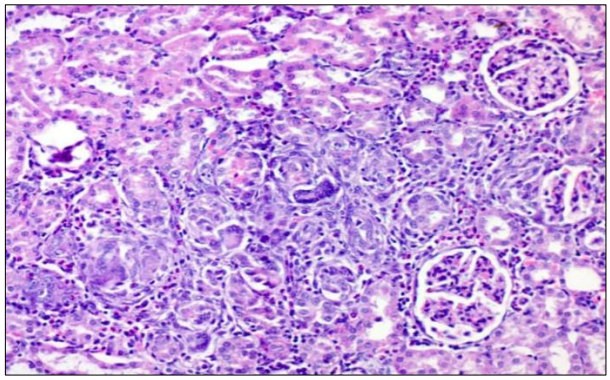

Fig 8: Gorup IV Male: Photomicrography of kidney showing regenerative tubules and infiltration of mononuclear cells. H&E X 100

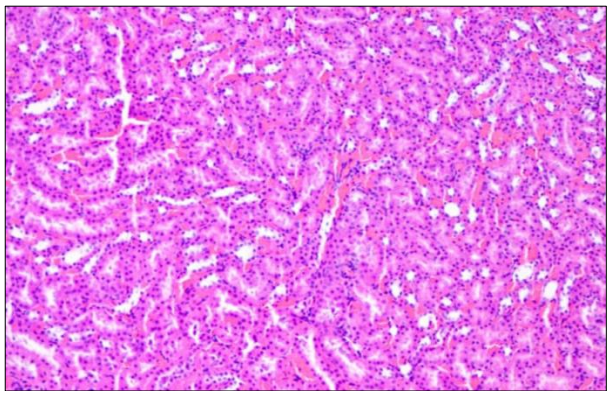

Fig 9: Gorup I Female: Photomicrography of kidney with normal architecture and cellular details. H&E X 100

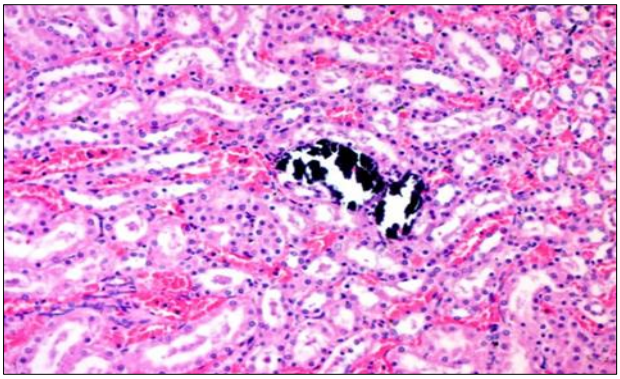

Fig 10: Gorup IV Female: Photomicrography of kidney showing multifocal tubular mineralization along with presence of tubular cast and congestion. H&E X 100

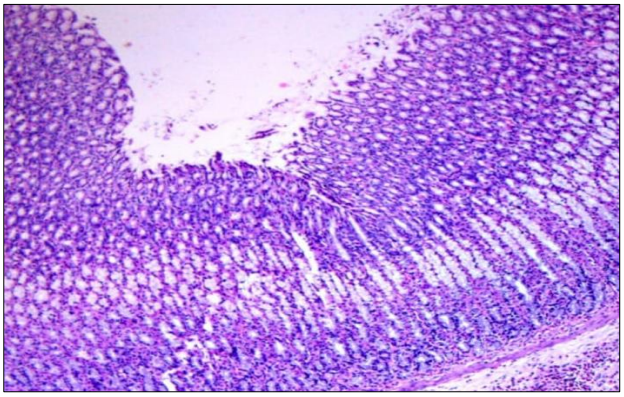

Fig 11: Gorup I Male: Photomicrography of stomach with normal histological architecture. H&E X 100

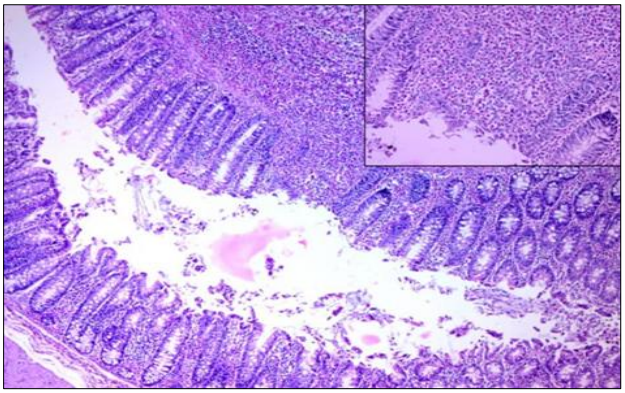

Fig 12: Gorup IV Male: Stomach showing focal extensive area of eosinophilic infiltration extended up to mucosa with focal ulceration. H&E X 100

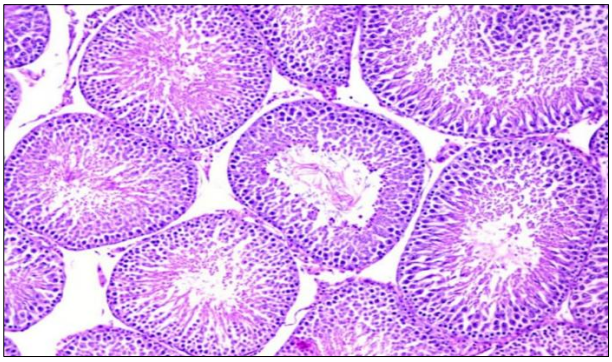

Fig 13: Gorup I Male: Photomicrography of testes with normal histological architecture. H&E X 100

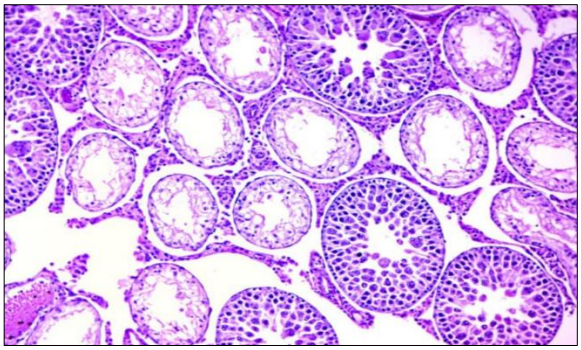

Fig 14: Gorup IV Male: Multiple tubules showing loss of round and elongated spermatid and few tubules showed formation of multi nucleated giant spermatid. H&E X 100

Conclusion

Tartrazine is an aromatic azo group synthetic food dye which is widely used in food industry to give better colouring of foods as easy way and cost effectively than natural colour. Oral administration of tartrazine in Wistar rats @ 50, 100 and 200 mg/kg b. wt. did not produce any noticeable symptoms and clinical as well as behavioural signs throughout the experimental period of 90 days. Tartrazine produced appreciable histopathological changes in liver, kidney, testis and stomach.

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