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TOXIC EFFECTS OF THE SYNTHETIC FOOD DYE BRILLIANT BLUE ON LIVER, KIDNEY AND TESTES FUNCTIONS IN RATS

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ABSTRACT

Evaluation of the toxic effects of synthetic dyes brilliant blue were tested in rats by measuring their actions on serum activity of glutamate oxaloacetate transaminase (AST), glutamate pyruvate transaminase (ALT), alkaline phosphatase (ALP), acid phosphatase (ACP), serum total bilirubin (SBIL-T), serum creatinine (SCR), serum urea (SUR) and serum testosterone concentrations.

Rats were fed synthetic brilliant blue dye supplemented diet, daily for 15, 30 and 45 days. Brilliant blue dye caused an increase of ALT, AST, ALP, SBIL-T, SUR and SCR. This increase was more pronounced in animals treated with repeated single higher doses than in those receiving the repeated single lower doses. On the contrary, serum ACP and testosterone concentrations were decreased after treatment. Histopathological examinations revealed alterations in kidneys include: congestion and hemorrhage with infiltration, thick walled blood vessels and deformation of the structure of glomeruli. Whereas alterations in liver include: focal necrosis of hepatocytes, infiltration and vacuolation.

Testes showed irregular shape of seminiferous tubules, atrophy of Leydig cells and disturbance in spermatogenesis.

Results indicated that the used doses of the synthetic dye brilliant blue were mostly attributable to hepatocellular damage, renal failure and decrease in spermatogenesis process.

Keywords: Brilliant blue dye – Liver, Kidney, Testes, Histopathology - Biochemistry.

INTRODUCTION

Color is a quality of food that makes it visually accepted and aids in its recognition. Foods containing added colors include candy, confections, bakery goods, soft drinks, cereals, snack food, jam, jelly, dessert powders and dairy products such as butter and ice-cream. Following the passage of the colors Additive Amendment of 1960, 20 natural colors were exempted from certification, whereas all the approved synthetic colors prior to the Amendment were required to be retested if questions regarding their safety dose.

Yet, little work on the toxicity was published before (Borzelleca and Hallogen, 1987) who revealed that the synthetic dye erythrosine had no significant effect on histopathological examination in rats.

It was concluded that feeding of synthetic dye indigocarmine on long term did not exert any carcinogenic effect (Hooson *et al.*, 1975).

Hansen *et al.* (1966) found that brilliant blue had non significant effect on fetuses. Durne *et al.* (1995) concluded that the synthetic dye brilliant blue had non significant effect on chromosomal damage. AbuElzahab *et al.* (1996)

observed that administration of the synthetic dye of indigocarmine induced damage to liver tissue.

This study was employed to elucidate the effect of the synthetic dye brilliant blue on the activity of some organs (liver, kidney and testes) of Albino rats.

MATERIALS AND METHODS

Color used (FAO/WHO, 1974): Brilliant blue FCF, color index no. 42090, E 133 disodium salt of 4-[4N-Ethyl-p-(sulfobenzylaminophenyl)-(2sulfoninophenyl)-Methylene]-[1-(N-Ethyl-N-P-Sulfobenzyl)-Delta 2,5-Cyclohexadienimine] C.1 Food Blue 2, AD1: 0-12.5, color class: triarylmethane

Brilliant blue were provided from P. Robert and Co. (France)

Experimental Animals:

Ninety adult male rats weighting (100-120 g) were used in this experiment and had free access to water. The animals were fed on standard diet (Hegested *et al.*, 1941 and Campbell, 1961). First group consists of thirty rats were left as control. The remaining three groups each consists of twenty rats (ten rats were daily treated with the dye

supplemented diet of oral dose 0.08 g/kg diet (1/10 dose of Ibrahim *et al.*, 1988a), the other ten rats were daily treated with the dye supplemented diet of oral dose 0.4 g/kg diet). The second group was treated for 15 days, the third group was treated for 30 days and the fourth group was treated for 45 days. Ten of the control and treated rats were used for the biochemical and histopathological studies in the two different doses.

Histopathological Examinations:

At the end of the experiment the control and treated rats were sacrificed. Their livers, kidneys and testes were removed and fixed in Bouin's solution, dehydrated and embedded in wax. Sections of 5 μ thickness were stained with hematoxylin and eosin (Drury *et al.*, 1973)

Biochemical Analysis:

Following decapitation of each animal, blood was collected in clean centrifuge tube placed at 4 °C for 2 to 3 hrs and then centrifuged at 3000 rpm for 15 mins. and serum was separated and kept at -20 °C till biochemical analysis were started. (ALT) and (AST) were determined according to Reitman and Frankel (1957).

Serum creatinine was measured by Husdan and Rupopert (1968). Serum urea concentration was determined by the method of Patton and Crouch (1979).

Serum alkaline phosphatase was determined by Tietz (1976). Serum total bilirubin was measured by Straumfjord and Jan (1973). Serum acid phosphatase was determined using method of Belfied and Goldberg (1971). Serum testosterone concentration was determined by Cumming (1985). The results obtained were statistically analyzed by the method of Kurtz (1983).

RESULTS

1-Histopathological Results:

I- Liver

The normal histological structure of control male rat liver is presented in figures (1&2). Liver sections obtained from rats belonging to the second group showed slight histopathological changes in the hepatic tissue. These changes were manifested in lymphocytic infiltration around the blood vessels, and slight congestion in blood vessels (Figs.3&4). Also Kupffer cells were greatly encountered and they were dilated in different areas of the hepatic tissue. Most of the hepatocytes were approximately eosinophilic cytoplasm. Also some pyknotic nuclei, increased number of Kupffer cells and macrophages were noticed (Figs. 3 and 4).

Inspected liver sections obtained from the third and fourth groups (treated with brilliant blue for 15, 30 and 45 days respectively) displayed necrobiotic changes in the hepatocytes. Pyknotic nuclei, karyolysis, dilated and congested blood vessels and slight areas of haemorrhage (Figs. 5 and 6). In other parts of the liver tissue, hydropic

degeneration and vacuolation in the hepatocytes, complete degenerated hepatocytes and oedematous blood vessels were noticed.

II- Kidney:

Histological structure of the kidney of control group illustrated in figures (7 & 8), showed well developed glomerulus, Bowman's capsule, proximal and distal convoluted tubules. T.S of kidney of rats of the second group treated with brilliant blue for 15 days revealed few disrupted convoluted tubules. Some atrophied and lobulated glomeruli were also detected (Figs. 9 & 10).

The above mentioned pathological lesions are markedly increased with the increasing the period of treatment and doses (third and fourth groups) and represented in figures (11 and 12), respectively.

III- Testes:

Control testis:

The parenchyma of the testis of rat composed of many seminiferous tubules of varying size. Each one is surrounded by an outer thin layer of connective tissue and lined by spermatogenic epithelium, surrounding a central lumen. The epithelium lies on a basement membrane consists of Sertoli cells and spermatogenic cells (Fig. 13).

Sertoli cells are tall pyramidal, non-proliferating population, resting on the basement membrane and reaching the lumen by their apices. They have large oval nucleus, pale cytoplasm and ill-defined boundaries. On the other hand, the spermatogenic cells are, proliferating population, composed of cells of varying phases. The spermatogonia are the initial germ cells arranged in mostly one layer directly on the basement membrane. They are small rounded cells with deeply stained rounded nuclei. The primary spermatocytes are large rounded cells arranged in two or more layers, they have large spherical nuclei.

The secondary spermatocytes are smaller than the primary spermatocytes. The spermatids are located in small groups near the lumen of the tubule and have irregular shape and densely stained nuclei. Spermatozoa appear in the lumen of the tubules and directed with their head toward Sertoli cells. The interstitial cells are large and ovoid with rounded nuclei, they are found mostly in groups between the seminiferous tubules (Fig. 14).

Group (2):

The administration of brilliant blue for 15 days resulted in different histopathological changes in the testis of rats. The spermatogenic cells were irregularly distributed within the seminiferous tubules and the basement membrane of the tubules was abnormal and splits away from the cells. Moreover, most of the spermatogenic cells appeared necrotic (Fig. 15 and 16).

Group (3):

Testis rat administration of brilliant blue for 30 days induced massive histological changes in the testis of rat (Fig.

15). The spermatogonia and primary spermatocytes showed condensed and strongly basophilic nuclei denoting pyknosis. Also, the primary; secondary spermatocytes and spermatids can not be distinguishable from each other and necrotic cells were perceptible. The blood vessels in interstitial connective tissues were congestion and contained haemorrhage.

Group (4):

Different alterations in the architecture of the seminiferous tubules were displayed 45 days of treatment with brilliant blue. The most prominent alterations were represented in inhibition of the process of spermatogenesis and spermiogenesis, presence of giant multinucleated cells and breakdown of the nuclear material (karyolysis) in most cells (Fig. 18). Also, the intertubular space became abnormally wide and degenerated cells were delineated in the seminiferous tubules. In addition, congestion in the blood vessels and haemorrhage in the interstitial tissue were

noticed. The pathological effect on liver, kidney and testes increased with the high dose.

2-Biochemical Results:

Table (1) reveals ALT, AST and ALP activities and SBIL-T concentration obtained as a mean of 10 rats.

This value was increased significantly after the administration of brilliant blue supplemented diet. In chronic experiments the enzyme activity elevated significantly.

Table (2) showed that SCR and SUR concentrations were significantly elevated following repeated administration of chronic doses of brilliant blue.

Table (3) describes serum acid phosphatase activity and serum testosterone concentration which exhibit a significant decrease as a response of treatment with repeated chronic doses of brilliant blue.

Table (1) Effect of the brilliant blue colour on the liver function in male rats

Parameters	Days	Control	Treated Doses	
			Low dose	High dose
AST (U/L)	15	37.4 ± 0.15	50.2 ± 0.15**	63.3 ± 0.13**
	30	36.1 ± 0.18	52.5 ± 0.18**	68.5 ± 0.16**
	45	38.2 ± 0.16	61.4 ± 0.14**	70.1 ± 0.12**
ALT (U/L)	15	29.3 ± 0.11	58.3 ± 0.14**	60.5 ± 0.28**
	30	28.4 ± 0.14	60.1 ± 0.12**	71.1 ± 0.15**
	45	27.5 ± 0.12	61.2 ± 0.15**	74.5 ± 0.16**
ALP (U/L)	15	20.8 ± 1.7	39.2 ± 2.1**	42.2 ± 3.1**
	30	16.4 ± 1.5	40.4 ± 1.6**	45.8 ± 1.8**
	45	18.5 ± 1.8	41.6 ± 1.8**	48.9 ± 2.6**
Total Bilirubin (mg/dl)	15	0.33 ± 0.02	0.54 ± 0.04**	0.67 ± 0.05**
	30	0.28 ± 0.06	0.56 ± 0.03**	0.71 ± 0.04**
	45	0.35 ± 0.04	0.60 ± 0.06**	0.75 ± 0.05**

Table (2) Effect of the brilliant blue colour on the kidney function in male rats

Parameters	Days	Control	Treated Doses	
			Low dose	High dose
Urea (mg/dl)	15	36.3 ± 0.14	64.3 ± 0.12**	70.1 ± 0.11**
	30	35.8 ± 0.15	66.8 ± 0.15**	72.2 ± 0.14**
	45	37.6 ± 0.17	71.4 ± 0.18**	75.5 ± 0.16**
Creatinine (mg/dl)	15	0.71 ± 0.04	1.41 ± 0.03**	1.60 ± 0.06**
	30	0.81 ± 0.03	1.62 ± 0.05**	1.70 ± 0.04**
	45	0.75 ± 0.04	1.75 ± 0.04**	1.78 ± 0.06**

Table (3) Effect of the brilliant blue colour on the testes function in male rats

Parameters	Days	Control	Treated Doses	
			Low dose	High dose
Acid Phosphatase (u/l)	15	17.36 ± 0.59	12.82 ± 1.1**	12.41 ± 1.20**
	30	16.81 ± 0.32	11.85 ± 0.8**	9.60 ± 0.90**
	45	18.26 ± 0.43	10.35 ± 0.70**	9.40 ± 0.85**
Testosterone (ng/ml)	15	1.41 ± 0.28	0.64 ± 0.13**	0.42 ± 0.11**
	30	1.51 ± 0.36	0.53 ± 0.12**	0.41 ± 0.10**
	45	1.45 ± 0.15	0.40 ± 0.14**	0.39 ± 0.13**

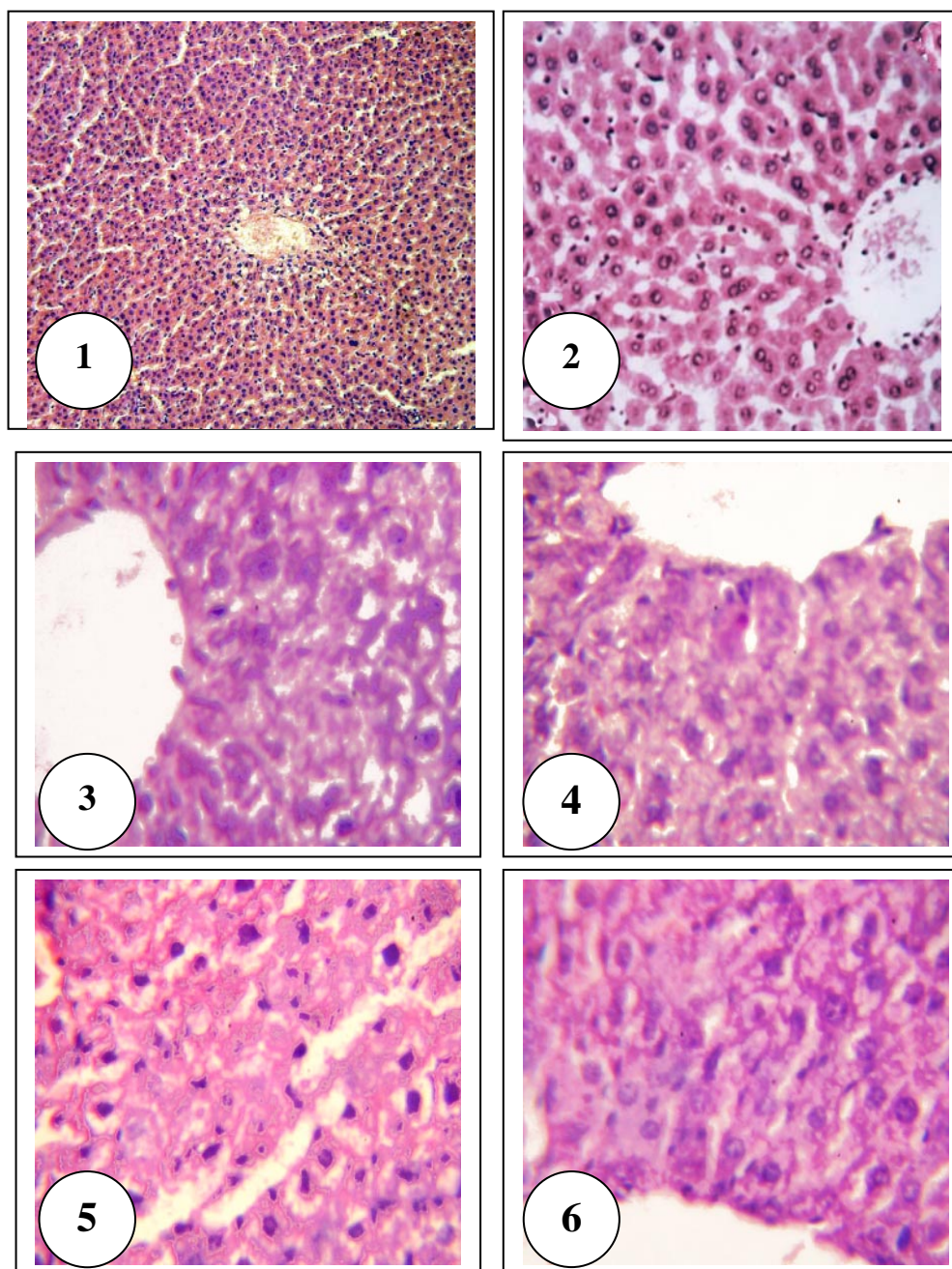


Fig. (1): Liver section of control rat showing normal structures (Hx & E, X 250).

Fig. (2): Magnification of figure (1) showing central vein with endothelial lining and hepatic cords (Hx. & E, X 400).

Fig. (3): Sections in liver of rat treated of the second group illustrating eosinophilic cytoplasm. and some pyknotic nuclei. (Hx. & E, X 400).

Fig. (4): Section of liver of rat of the second group showing increased number of Kupffer cells, dilated and congested hepatic portal vein. (Hx. & E, X 400).

Fig. (5): Sections of rat liver of the third group showing pyknotic nuclei and some vacules in hypatocytes (Hx. & E, X 400).

Fig. (6): Sections of liver of rat of the fourth group shows hydropic degeneration and vacuolation in the hepatocytes, and some complete degenerated hepatocytes (Hx. & E, X 400).

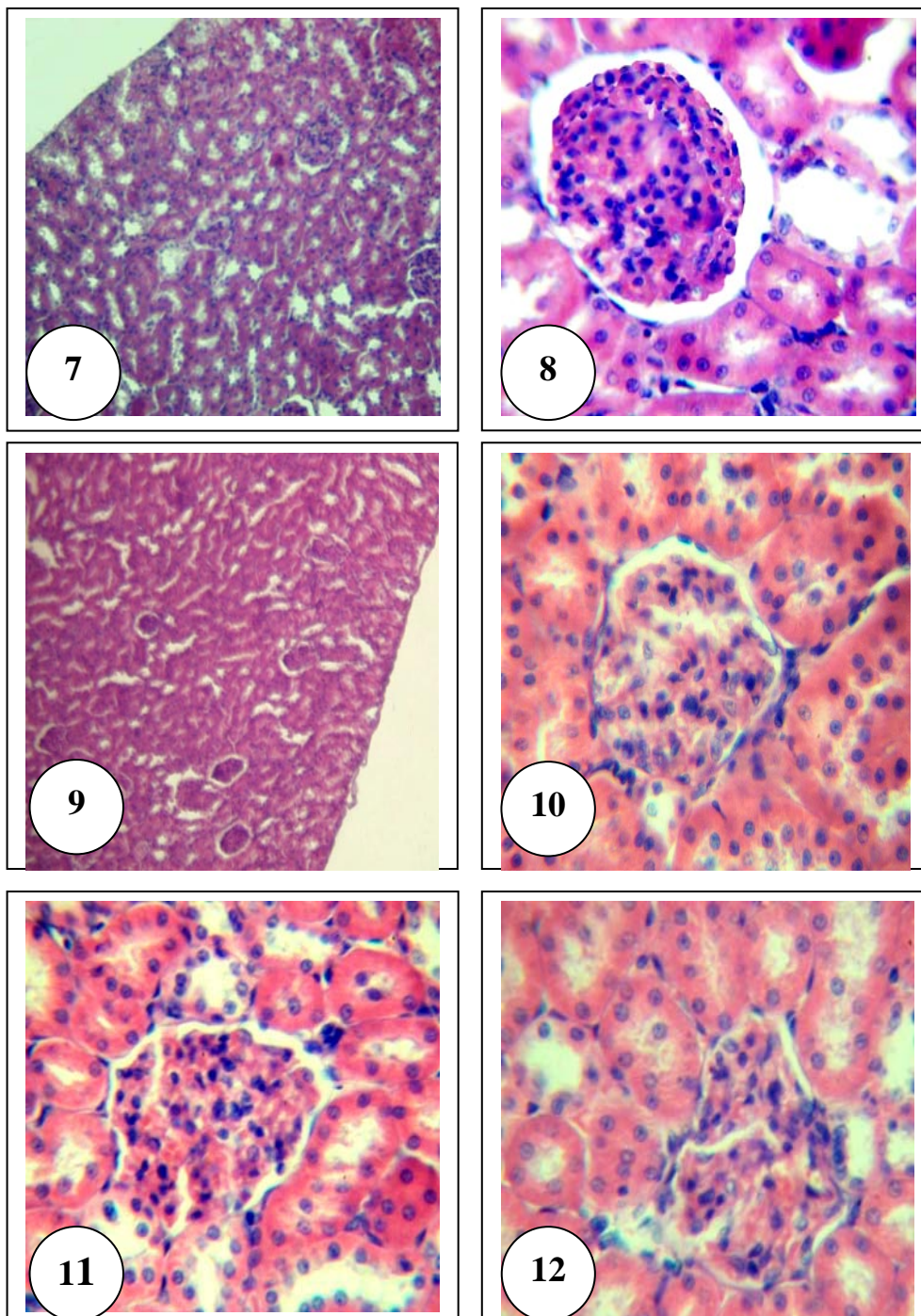


Fig. (7): Section in kidney of control rat showing normal structure of kidney (Hx.& E, X 100).

Fig. (8): Enlarged portion of figures (7) to illustrating normal structure of Malpighian corpuscle . (Hx. & E, X 400).

Fig. (9): Section of treated kidney group 2 showing hypertrophied of glomeruli (Hx & E, X 100)

Fig. (10): Enlarged portion of figure (9) showing hypertrophied and segmented glomerulus .Convoluted

tubules appeared vacuolated with debris of granulated chromatin (Hx & E, X 400).

Fig. (11): Section in rat kidney group 3 showing destruction of most of convoluted tubules and endothelial of glomerulus (Hx.& E, X 400).

Fig. (12): Section in rat kidney of treated group 3 showing atrophied glomerulus with narrow lumen. (Hx. & E, X 400).

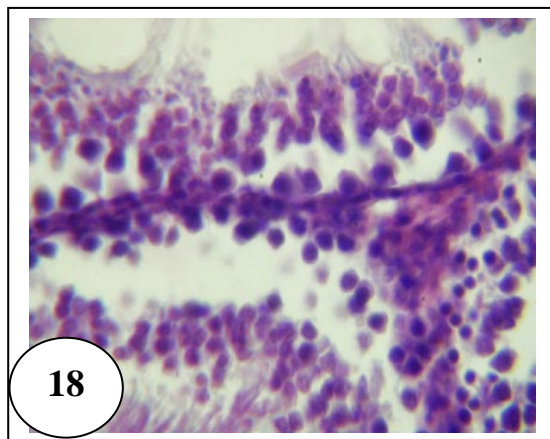
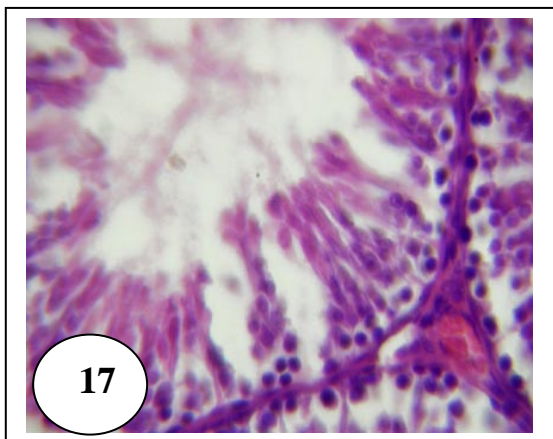
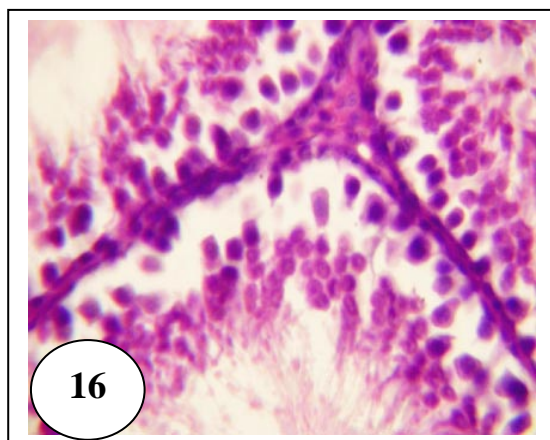
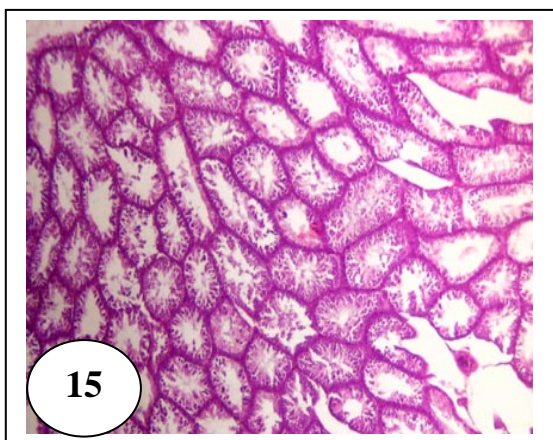
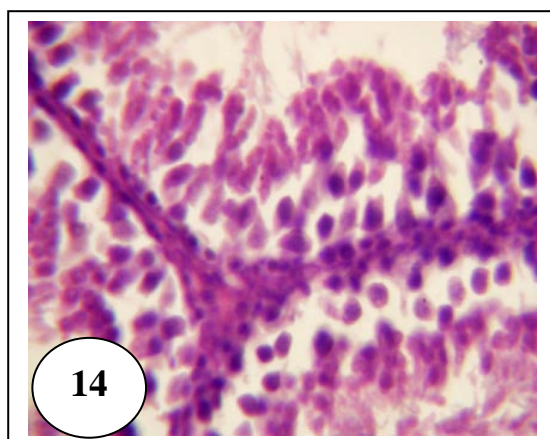
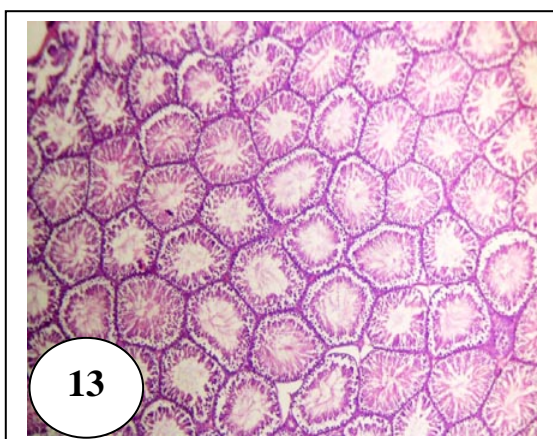


Fig. (13): Section in testes of control rat showing normal structure of seminiferous tubules (Hx. & E, X 100).

Fig. (14): Enlarged portion of figure (13) showing the structure of seminiferous tubules and interstitial cells (Hx. & E, X 200).

Fig. (15): Section of treated testes rat group 3 shows the abnormal architectures of seminiferous tubules (Hx. & E, X 100).

Fig. (16): Enlarged portion of treated rat testis group 2 showing the decreased number of primary spermatocytes (Hx. & E, X 400).

Fig. (17): Section of rat testis showing the decreased number of spermatogonia and primary spermatocytes and bleeding in blood vessels (Hx. & E, X 200).

Fig. (18): Section of treated testis rats showing bleeding and less number of spermatids group 4 (Hx. & E, X 200).

DISCUSSION

Recent researches of Organic Consumers Association (2005) reported that the toxic effect of combined additives (aspartame, monosodium glutamate (MSG), the artificial colorings brilliant blue and quinoline yellow) stopped the nerve cells growing and interfered with proper signaling system. Haward (2005) and Murray (2005) supported these findings and revealed that this combination developing neuroblastoma in rat cells.

Results obtained in the present work indicated that treating animals with the synthetic dye brilliant blue caused significant increase in the serum levels of ALT, AST and ALP. Damaged organs showed increase in enzyme activity (Jennings *et al.*, 1957). Chronic intoxication was accompanied by continuous increase in serum levels in both ALT and AST activities (Delfavero *et al.*, 1964).

It seems that the changes in serum ALT and AST activities are due to cellular degradation by brilliant blue dye, perhaps on the liver or heart muscle.

The studies following myocardial infarctions (Carrol, 1966) and hepatic dye function (Harper *et al.*, 1979) indicated that these enzymes were liberated in the blood stream following injury to the cells. In this concern, Martin *et al.*, (1983) found that liver tissues which are known of their high contents of transaminases (AST, ALT) lose their enzymes in case of liver cells damage. The effect of the brilliant blue dye on the liver is in accordance with Gaunt *et al.*, (1972), Abdel-Rahim *et al.* (1987 & 1989) and Ibrahim *et al.* (1988a). Abou-Elzahab *et al.* (1997) who recorded a pronounced increase of serum and liver transaminases activity in rats ingested synthetic dyes. AST is considered to be more specific for heart function tests (Ganong, 1991) which indicates that brilliant blue has a retard damage effect on heart function.

Results obtained in the present data indicated that treating animals with the brilliant blue induced significant increase in the SBIL-T. This may be due to defect in liver function produced from hepato cellular damage which agreed with the results of Ibrahim *et al.* (1988b).

ALP has several physiological functions in bone cells, it splits inorganic phosphates from organic phosphate which is a potent inhibitor of mineralization (Charles *et al.*, 1992). The results of the present study showed significant increase in ALP activity for brilliant blue dye may be attributed to the defect in liver function (Hayes, 1994). The influence of the synthetic dye brilliant blue toxicity on hepatocytes was less marked than that on the renal tubules as the necrotic lesions were small, focal and restricted to the central zone despite the large accumulation of brilliant blue dye in the liver tissue homogenates. Gaunt *et al.* (1972) and Abdel-Rahim *et al.* (1989) reported pronounced increase in serum and liver transaminases activity.

Results of the present study revealed that brilliant blue dye treatment induced significant decrease in serum acid phosphatase and testosterone levels. It is well known

that the atrophy of the Leydig cell decrease the testosterone level which promotes the conversion of spermatogonium to spermatocytes (AbdelAziz and AlAshnawy, 1993 and Ahmed, 2000).

Acid phosphatase promotes the increase of the spermatogenesis process (Niemi and Kormano, 1965). The present findings are in accordance with AbdelAziz *et al.* (1989), AbdelAziz and Al-Ashnawy (1993) and Ahmed (2000) who found that the most significant adverse effects of the brilliant blue dye on the testes tissue is probably a direct inhibition of acid phosphatase activity and testosterone levels. These conclusions are in agreement with the present results of the brilliant blue effect on the testes rat tissue.

Mackenzie *et al.* (1992) found that the significant elevation in urea and creatinine levels related to impairment of renal function. These results are in agreement with the present data.

Through this work, the previous data confirmed the destructive effect of the synthetic dye brilliant blue on liver, kidney and testes functions.

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