Pathological Studies On The Adverse Effect of Food Color Carmine Cochineal on male Albino Rats

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ABSTRACT

Twenty-one mature male albino rats were divided into 3 equal groups to study the adverse effects of the natural food coloring-agent carmine cochineal. Gp. 1 was the control. Gps. 2 & 3 were orally administrated carmine cochineal at a dose 500 mg/kg B wt. (gp.2) and 1000 mg/kg B wt. (gp. 3) dispersed in distilled water by a stomach tube 5 days a week for 45 days. At the end of the experiment, the rats were sacrificed necropsied and tissue specimens were collected from lungs, spleen, liver, kidneys, heart, testes and skin. The specimens were fixed in 10% neutral buffered formalin. Five micron thick paraffin sections were prepared, stained with H&E and examined microscopically.

Macroscopically, the lungs and spleen were enlarged and dark. Gps. 2 & 3 showed a significant decrease in body weight-gain especially gp.3. Microscopically, gps. 2 & 3 showed pulmonary congestion, hemorrhage and perivascular edema. Moreover, the interalveolar septa were thickened. Peribronchial, perivascular and interstitial leukocytic infiltration were observed. The spleen of gp. 3 showed depletion and necrosis of the lymphoid tissues in the white pulp together with leukocytic infiltration in the red pulp. The liver suffered congestion, hemorrhage and degenerative changes. The kidneys displayed perivascular edema and hemorrhage, besides dilated renal tubules and necrosis of the epithelial lining of the renal tubules. Degenerative changes were seen in the cardiac muscle. The testes were congested and presented degeneration and necrosis of the germ cells together with azoespermia in some cases. The skin showed congestion and degenerated hair follicles together with dilatation of the sebaceous glands. The forementioned lesions were more severe in gp. 3.

It could be concluded that, carmine is toxic, particularly at a high dose. It is recommended to limit its use, and to declare its presence to inform the consumers of the product.

INTRODUCTION

Colors are a properties of foodstuff that make them visually more attractive. They facilitate the distinction between ripe and unripe, fresh and old ones. Furthermore they give the flavor to food *(1)*. The coloring food- additives which permitted for use in foods, can be broadly classified into two categories (certified and uncertified coloring agents). The latter could be broadly classified into non synthetic (natural), nature-identical, and inorganic colorants. The natural type, comprise a wide variety of organic and inorganic compounds. They are extracted from animals, plants and minerals *(2,3)*. Carmine cochineal is a natural uncertified coloring agent. Cochineal is a natural red coloring agent obtained from red beetles as an aqueous-alcoholic extract of air-dried bodies of the gravid female insect, Dactylopius coccus costa, which usually parasitic on Cactus, Opuntia Ficus Indica *(4)*. Cochineal extract is the concentrated aqueous solution after removing the alcohol. Cochineal contains approximately 10 percent carminic acid while the remainder (90%) is art insect body fragments. Carmine is the aluminum or calcium-aluminum formed by precipitating carminic acid onto an aluminum hydroxide substrate *(5)*. Cochineal is now widely used as a coloring agent in food, cosmetics, drugs and pharmaceutical products *(6,4)*. Carmine is added to butter, port wine cheese, yogurt, strawberry milk drinks, canned meat products, sausage, fresh and frozen sea food manufacturing, lumpfish eggs and caviar. Cochineal extract is used in fruit drinks, candy and tomato products. Carmine and cochineal extracts are used in drugs and pharmaceutical products such as outside

container or wrapper of the retail package. Carmine has been reported to be used in 814 cosmetic formulation including lipsticks, blushers, make up bases, eye shadows, skin care lotions, bath products and baby products *(5)*. FDA is recorded many cases of hypersensitivity to carmine, carminic acid and cochineal extract. Hypersensitivity reactions included contact dermatitis, urticaria, angioedema, occupational asthma and systemic anaphylaxis *(7- 9)*. More than half of these reports declared the involvement of IgE-mediated diagnostic response to carmine and its derivatives *(10)*. Female patient suffered anaphylaxis after ingestion of Popsicle colored with carmine *(11)*. She experienced nausea within minutes besides pruritus, urticaria and hypotension with tachycardia. On the same context it has been reported that another woman with carmine hypersensitivity within 90 minutes after ingesting of a generic antibiotic azithromycin *(12)*. This allergy was attributed to the carmine dye in the tablet's coating, rather than to the antibiotic.

The aim of the present work was to determine the adverse effects of the oral administration of a food-coloring agent (carmine cochineal) through the induced lesions in the internal organs of male albino rats.

MATERIAL AND METHODS

Cochineal Extract(carmine , carminic acid)

Cochineal extract(color Index No.75470), its derivatives carmine red (E120), cochineal (E124) and indigo carmine (E132) were obtained from Fluka AG, Chem, Fabrik CH-9470 Buchs, Germany by Sigma-Aldrich Laborchemikalien GmbH.

Cochineal extract color varies in shade from orange to red to violet as the pH is increased. It is insoluble in typical solvents, including water, glycerin and propylene glycol but can be dispersed easily in water.

Experimental Design

Twenty-one mature male albino rats weighing from 120-150 gm. B.wt., were randomly divided into three equal groups. They were kept in a metal cages under hygienic conditions, fed on a well balanced ration and provided with water *ad-Libitum* during the experimental period (45 days). Gp.(1) was the control. It was 5 days weekly given distilled water. Gps. 2 & 3 were orally given carmine cochineal dispersed in distilled water by stomach tube 5 days weekly at a dose 500mg/kg.B.wt. for gp.2 and 1000mg/kg.B.wt. for animals were weighted weekly for 6 weeks gp.3 *(13)*. At the end of the experimental period, rats were sacrificed, necropsied and specimens were collected from the internal organs, (lungs, spleen, liver, kidneys, heart, testes and skin), fixed in 10% neutral buffered formalin. Five micron thick paraffin sections were prepared, stained with hematoxylin and eosin, and examined microscopically *(14)*.

Statistical analysis

The obtained data were analyzed statistically using ANOVA test *(15)*.

RESULTS

Rats of gps .2& 3 showed a significantly decreased body weight, particularly gp.(3) when compared with the control (Table 1).

Table 1. Body weight of gps.1-3 during the experiment.

Weeks / Group	0	1st	2nd	3rd	4th	5th	6th
	Body weights (gm)						
1	135.71^{a} ±4.70	181.66^{a} ±6.13	207.54^{a} ±4.14	230.55^{a} ±4.25	253.57^{a} ±4.37	263.86^{a} ±5.15	272.00^{a} ±4.59
2	136.57^{a} ±4.63	174.24^{a} ±5.91	194.27^{a} ±5.01	207.71^{b} ±3.92	221.14^{b} ± 2.82	241.14^{b} ±2.69	245.00^{b} ±2.48
3	136.71^{a} ±3.97	143.14^{b} ±5.79	164.36^{b} ±9.00	162.68^{c} ±7.31	161.00^{c} ±5.61	163.14^{c} ±5.11	184.14^{c} ±4.95

Mean with different alphabetical superscripts in the same column are significantly different at P.< 0.05.

Pathological results

Lungs: Macroscopically, the lungs of gp. 3 were red in color and enlarged (Fig.1).

Microscopically, the lungs of gp. 2 showed congestion, thrombosis and perivascular edema (Fig.2). Peribronchial, perivascular and interstitial leukocytic infiltration were observed (Fig.3). The lungs of gp. 3 showed perivascular edema infiltrated with leukocytes with thickened and hyalinized walls of blood vessels (Fig.4). Hemorrhage and proliferation of alveolar cells were seen. Hyperplastic and vacuolated bronchial epithelium was seen with few leukocytes in the lumen (Fig.5). The interalveolar septa were thickened, and leukocytes especially neutrophils were seen in the lumens of some alveoli (Fig.6).

Spleen: Macroscopically, the spleen of gp.3 was dark red and enlarged (Fig.7). Microscopically, the spleen of gp.2 showed hyperplastic white pulp together with thickened splenic trabeculae (Figs. 8 & 9). The red pulp revealed excessive leukocytic infiltration. Depletion and necrosis of the lymphoid tissue were found in the white pulp of gp. 3 (Figs.10 & 11).

Liver: Macroscopically, the liver of gps. 2&3 was dark red. Microscopically, gp.2 showed vacuolar and hydropic degeneration together with periportal fatty change (Fig.12). Mild proliferation of bile ductules and congested portal areas were detected (Fig.13). Similar lesions were seen in gp.3, besides necrotic hepatocytes and hemorrhage (Fig.14). The portal areas showed proliferated bile ductules with leukocytic infiltration and fibroblastic proliferation (Fig.15). Perivascuolar fibroblastic proliferation and congestion were seen (Fig.16). Apoptotic hepatocytes were frequently seen.

Kidneys: Macroscopically, the kidneys of gps. 2 & 3 were dark red. Microscopically, the kidneys of gp.3 showed necrosis and hemorrhage (Fig. 17). Hyaline casts were seen in the lumens of some renal tubules of gp. 2, in addition to vacuolated endothelial lining of the glomerular tuft (Fig.18). Gp. 3 showed similar lesions, besides cystic dilatation of some renal tubules and necrotic epithelial lining of these tubules (Fig.19). Interstitial leukocytic infiltration was associated with dilatated cavities of Bowman's capsules and pressure atrophy of the glomerular tufts, besides lobulation of some glomerular tufts. The medullary collecting renal tubules were focally replaced with leukocytes. The tunica media of some blood vessels were thickened with vacuolated endothelial cells. Perivascular edema and necrotic renal tubules were encountered (Fig.20).

Heart: Macroscopically, the heart of gps. 2 & 3 was apparently normal. Microscopically, gps. 2 & 3 showed congested coronaries, perivascular and interstitial edema (Fig.21). Hyalinized cardiac muscle were noticed, besides interstitial edema (Fig.22).

Testes: Macroscopically, the testes of gps. 2 & 3 were apparently normal. Microscopically, gp. 3 showed congested testicular tissues, besides degeneration, necrosis and disappearance of germ-cells were encountered. Azoespermia was detected in some rats (Fig.23). Thickened tunica albuginea and severe interstitial edema were seen (Fig.24). Some semineferous tubules contained spermatid and spermatozoa. Others revealed degenerated sperms in their lumina (Figs. 25& 26).

Skin: Macroscopically, the skin of gps. 2 & 3 were slightly red especially in gp. 3. Microscopically, congested and degenerated hair follicles were seen together with dilatation of the sebaceous glands. The dermis was congested and edematous. The dermal collagen were disorganized and exhibited bundles hyaline degeneration besides perivascular leukocytic infiltration (Fig.27). Acanthosis with edematous dermis were seen (Fig.28).

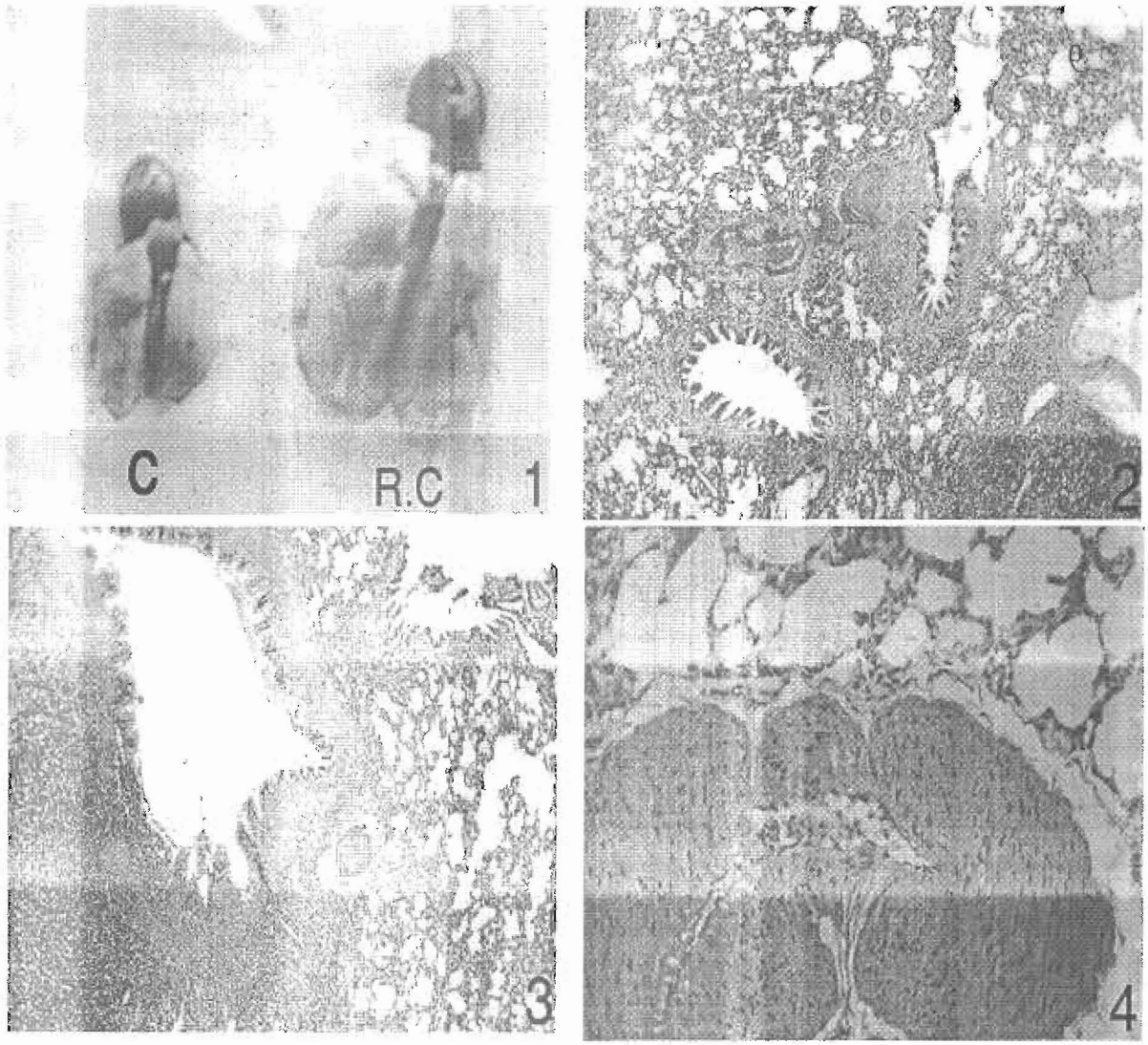
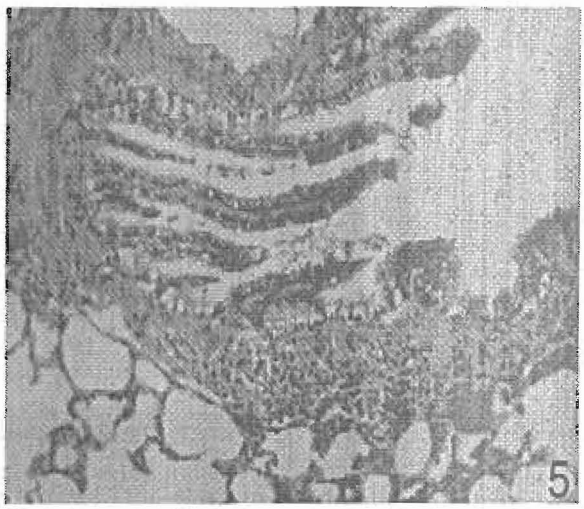
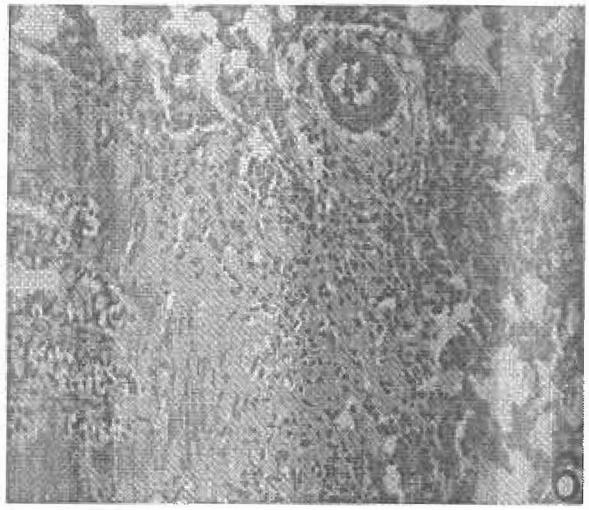
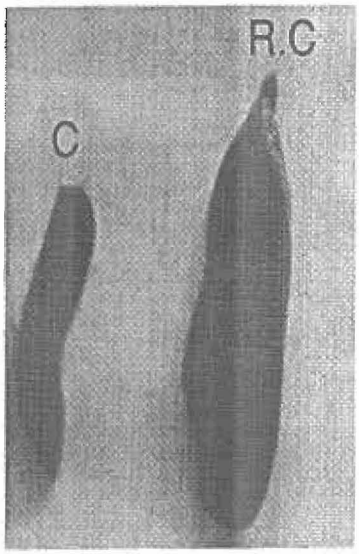


Fig.1. Enlarged lung gp.3 (C: control, R.C. gp.3).
Fig.2. Photomicrograph of the lung gp.2 shows congestion of pulmonary blood vessels, thrombosis and perivascular edema. H & E. X 120.
Fig.3. Photomicrograph of the lung gp.2 shows peribronchial, perivascular and interstitial leukocytic infiltration. H & E.X120.
Fig.4. Photomicrograph of the lung gp.3 shows thickened and hyalinized walls of the blood vessels. H & E. X120.







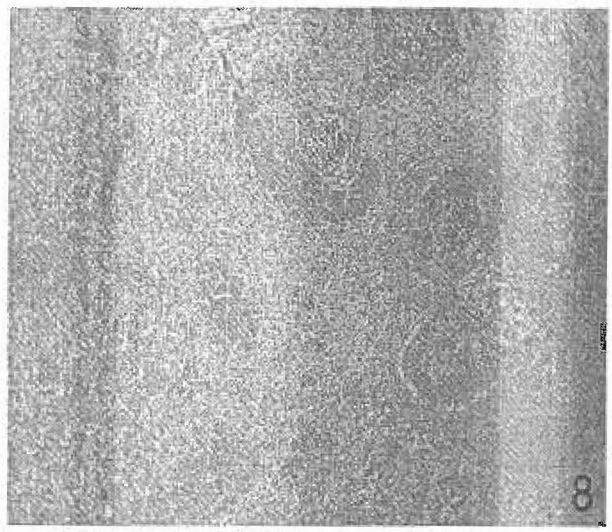


Fig.5. Photomicrograph of the lung gp.3 shows hyperplasia of the bronchial epithelium with vacuolated cytoplasm and few leukocytic infiltration. H&E. X300.

Fig.6. Photomicrograph of the lung gp.3 shows thickened interalveolar septa and some alveoli containing leukocytes especially neutrophils. H&E. X150.

Fig.7. Photomicrograph of the splenomegaly gp.3 with dark red color (C: control, R.C.: gp.3).

Fig.8. Photomicrograph of the spleen gp.2 shows hyperplastic white pulp and thickened splenic trabeculae. H & E. X120.

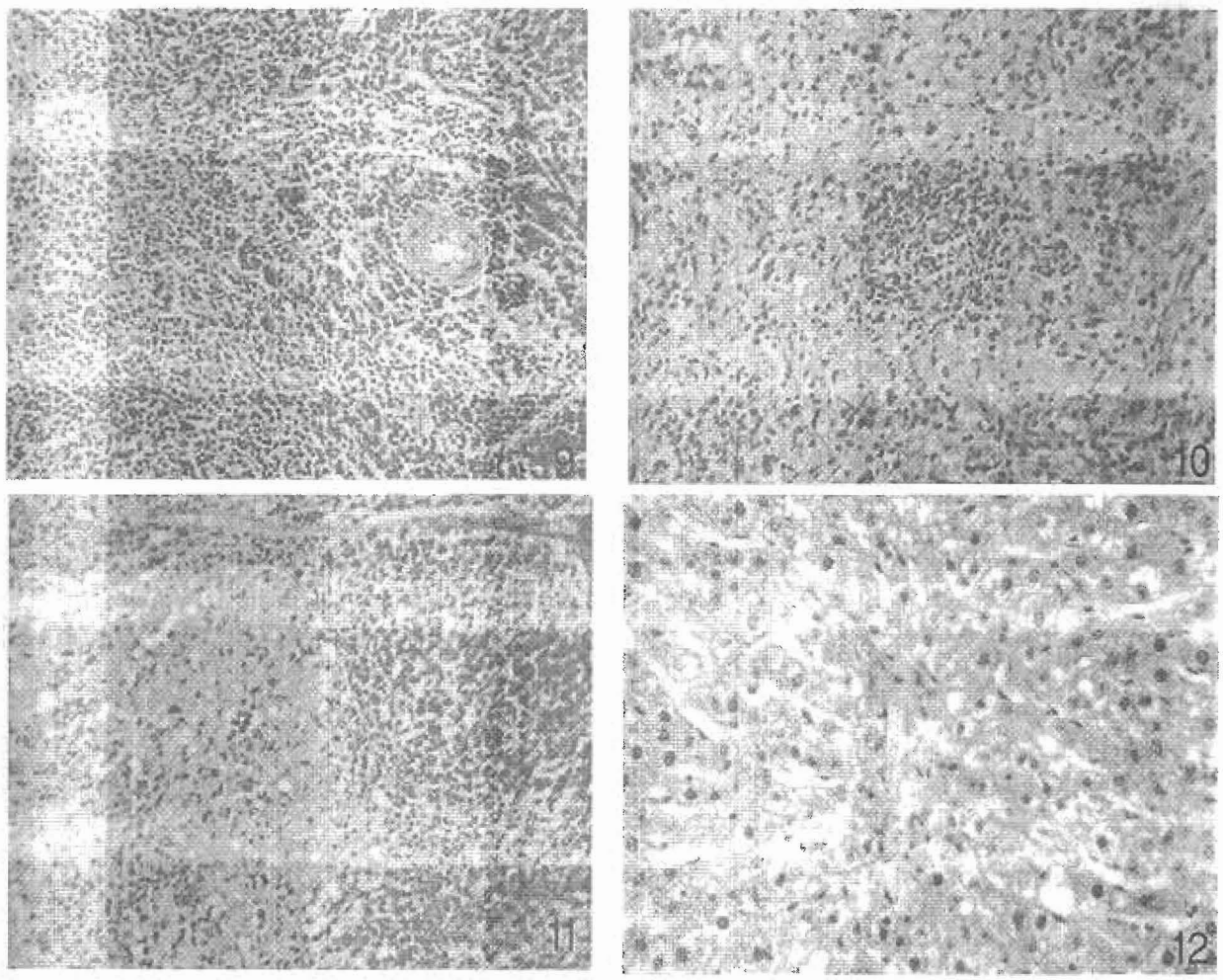


Fig.9. Photomicrograph of the a high power of fig.(8) to shows the hyperplastic white pulp H&E. X300.

Fig.10. Photomicrograph of the spleen GP.3 shows depletion and necrosis of the lymphoid tissue H&E. X150.

Fig.11. Photomicrograph of the high power of the pervious figure to show the depletion and necrosis of the lymphoid tissue. H&E. X300.

Fig.12. Photomicrograph of the liver gp.2 shows vacuolar and hydropic degeneration with periportal fatty changes. H & E. X120.

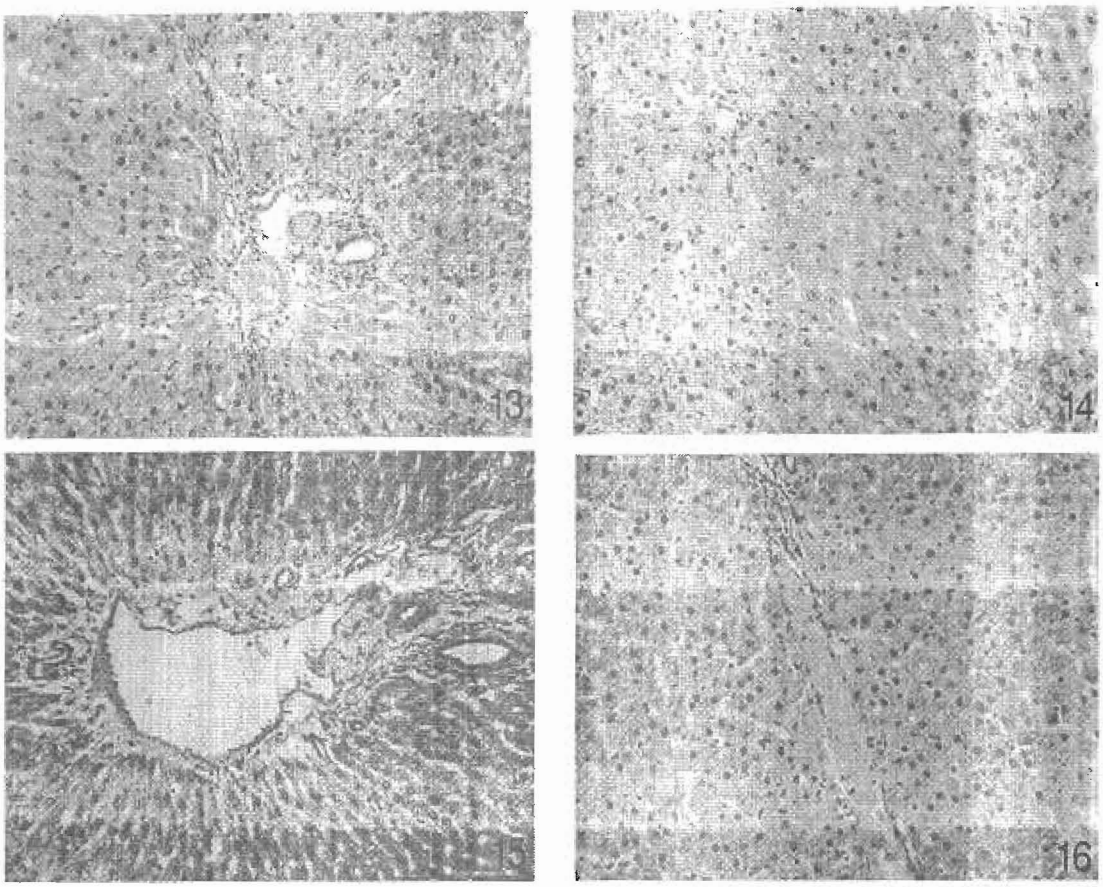


Fig.13. Photomicrograph of the liver gp.2 shows mild proliferation of the bile ductules and congestion of hepatic blood vessels. H&E. X120.

Fig.14. Photomicrograph of the liver gp.3 shows necrosis of the hepatocytes. H&E. X120.

Fig.15. Photomicrograph of the liver gp.3 shows proliferation of the bile ductules, leukocytic infiltration and fibroblastic proliferation. H&E. X120.

Fig.16. Photomicrograph of the liver gp.3 show congestion. H&E. X 120.

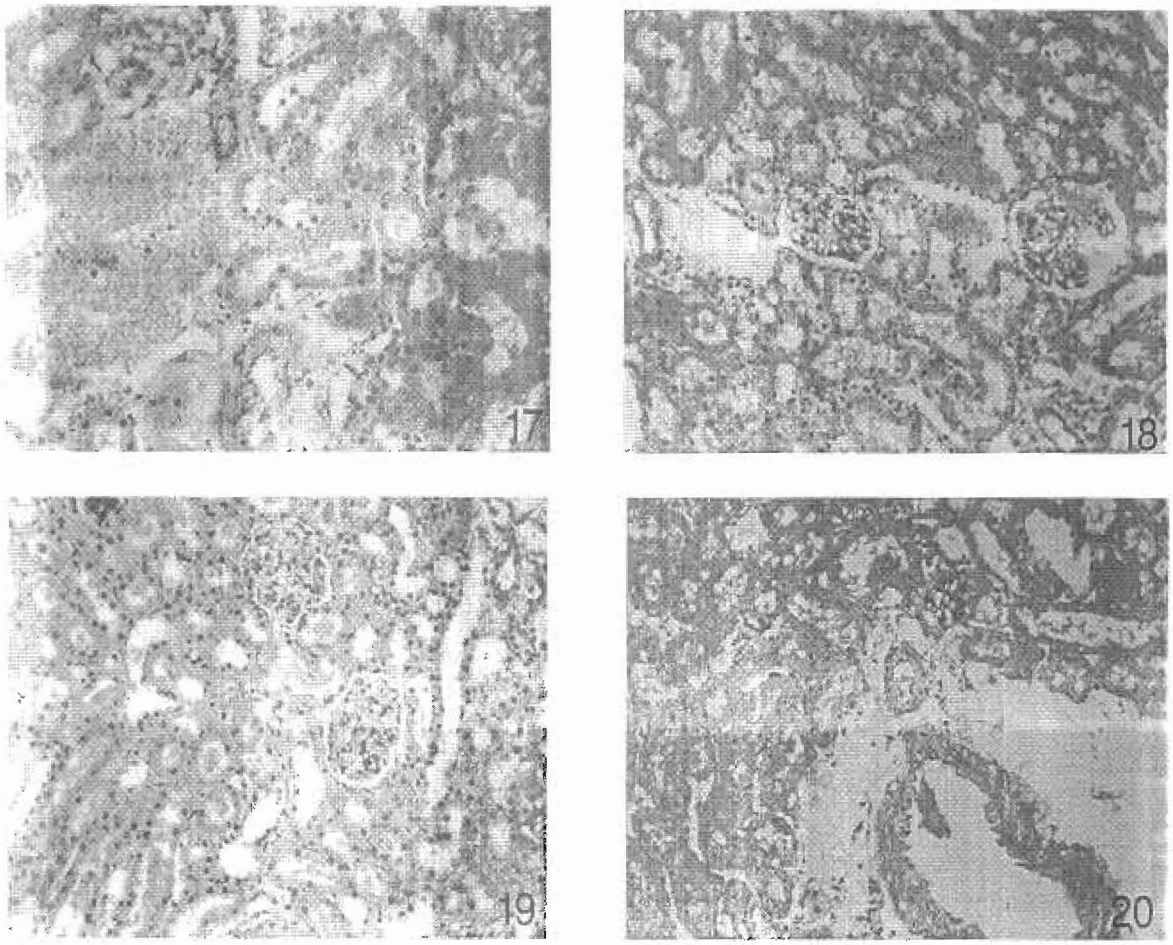


Fig.17. Photomicrograph of the kidney gp.3 shows perivascular edema and hemorrhage. H&E. X300.

Fig.18. Photomicrograph of the kidney gp. 2 shows hyaline casts in the lumens of some renal tubules and vacuolated endothelial cells of the glomerular tufts. H&E. X120.

Fig.19. Photomicrograph of the kidney gp.3 show cystic dilatation of some renal tubules and necrosis of its epithelial lining. H&E. X120.

Fig.20. Photomicrograph of the kidney gp.3 shows thickened tunica media of the renal blood vessels with vacuolation of its endothelial cells, perivascular edema and necrosis of the renal tubules. H&E. X300.

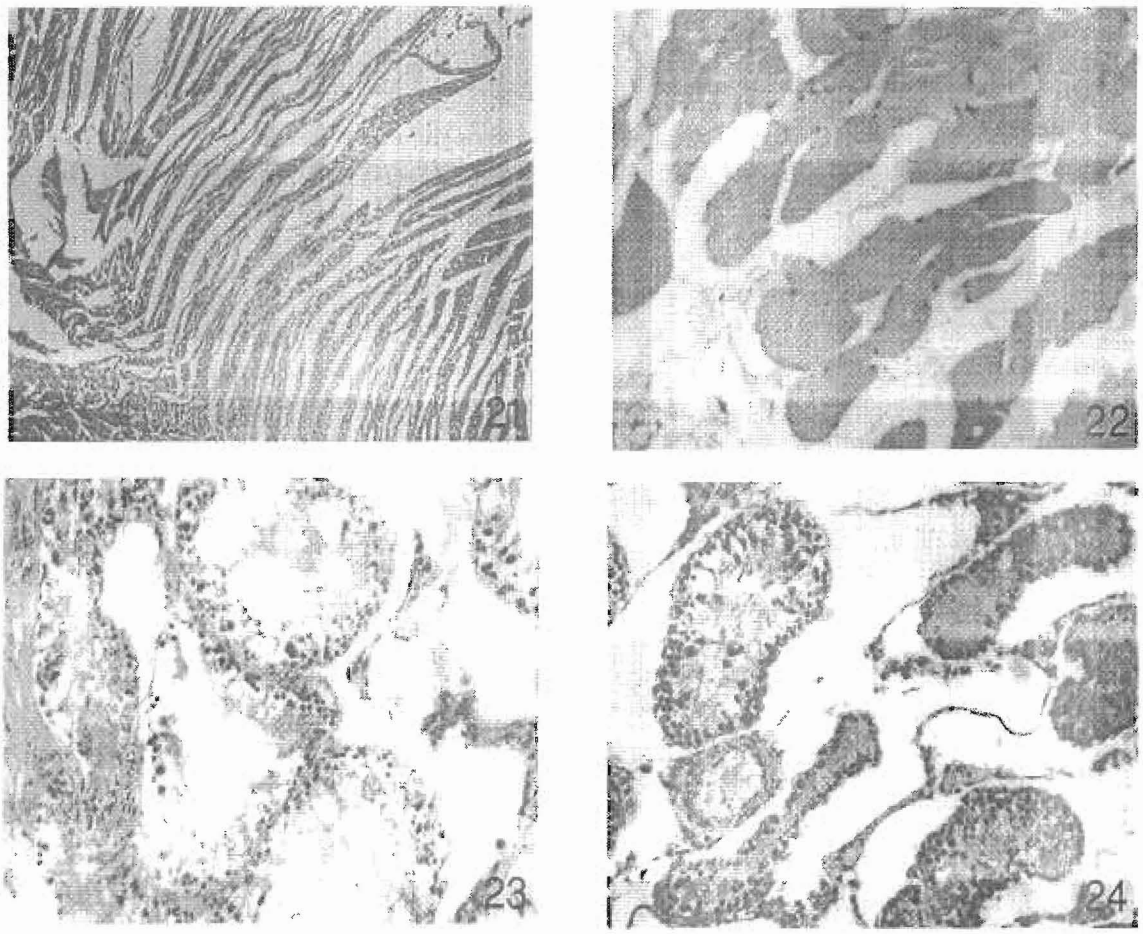


Fig.21. Photomicrograph of the heart gp.3 shows interstitial edema. H & E. X120.
Fig.22.Photomicrograph of the heart gp.3 show hyaline degeneration and interstitial edema. H&E. X300.
Fig.23. Photomicrograph of the testes gp.3 shows degeneration and necrosis of the germ cells and azoespermia. H&E. X300.
Fig.24. Photomicrograph of the testes gp.3 shows severe interstitial edema. H&E. X150.

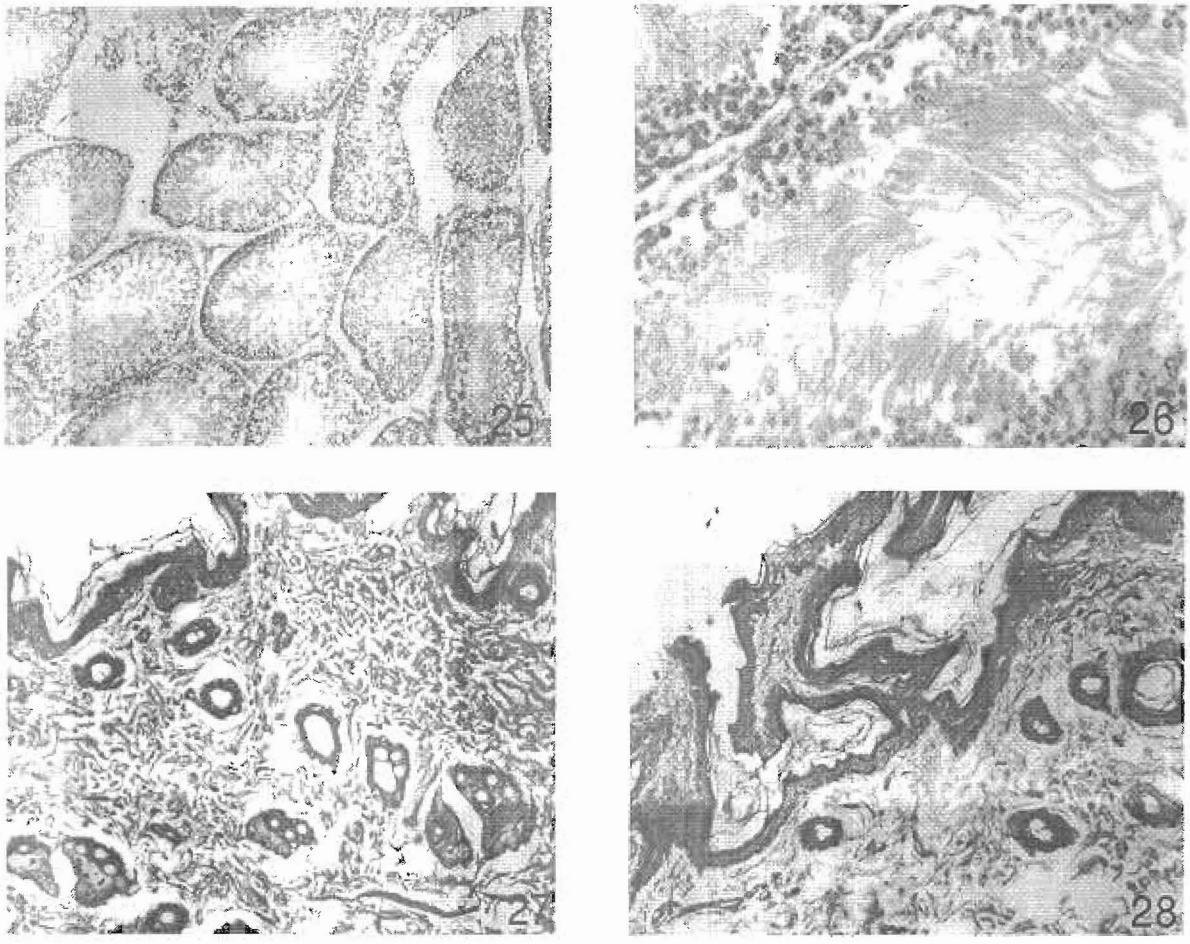


Fig.25. Photomicrograph of the testes gp.3 shows degenerated sperm in the lumen of the seminiferous tubules. H&E. X120.

Fig.26. Photomicrograph of the high power of fig.(25) to show the degenerated sperm in the lumen of seminiferous tubules. H & E. X300.

Fig.27. Photomicrograph of the skin gp.3 shows disorganized fibrous connective tissue bundles and hyaline degeneration of some bundles. H & E. X120.

Fig.28 Photomicrograph of the skin gp.3 shows acanthosis and edematous dermal layer. H&E. X120.

DISCUSSION

Carmine is a widely used pigment derived from gravid cochineal insects. Carminic acid is the source of its color. Allergic contact dermatitis from carmine could be found. The ingredient in carmine causing these delayed hypersensitivity reactions has not been studied. In contrast, there are numerous reports of immediate hypersensitivity reactions from carmine, mostly from its use in foods and beverages, drugs, cosmetics and pharmaceuticals. These are immunoglobulin E-mediated reactions directed against cochineal foreign proteins *(16).*

The carmine cochineal food coloring agent significantly hindered gain in body weight of the male albino rats in our study especially in the gp.3 which received a high dose of carmine. Similar findings were observed in mice *(17)* and rats *(18)* which orally received carmine cochineal at a dose 2.5, 5.0 and 10 g/kg B.wt., 5 days a week for 13 weeks. The retardation in gaining weight was induced by the high dose. Several studies *(19-22)* showed that rats and mice received food colors showed a decrease in body weight. On contrary other investigators *(1)* found that mice given food color tomato red dye (blend of carmoisine and ponceaue 4R) at a dose of 2 and 6g/kg B.wt. for 42 days, significantly increase in the average body weight. This variation may be due to the difference in the species of the experimental animals, difference in the dose administrated and the type of food colors. The retarded body weight gain could be attributed to the disturbing effect of food color additive on the metabolic system *(19)* or to the body immuno-reactions against cochineal foreign protein *(11,16).*

The lungs and spleen, of the current work, were enlarged and dark- red. Such lesions could result from congestion and degenerative changes. Similar findings were previously reported by investigators on other food colors *(1,3,24).* They found a marked increase in the average weight of liver. On the other hand, rats orally given carmine at a dose 2.5,5 and 10 gm/kg B.wt. 5 days a week for 13 weeks, did not induce any remarkable gross lesion *(18).* This deviation could be due to the differences of dose of the coloring agents, species and the duration of the experiment. Our histopathological findings were proportionally correlated with the dose and time dependant. The lungs in gps.2&3 were congested and showed perivascular edema with leukocytic infiltration. Moreover gp.3 presented thickened and hyalinized walls of the blood vessels. Hemorrhage and proliferation of the alveolar cells together with thickening of the interalveolar septa were observed. Many cases of hypersensitivity to carmine, carminic acid and cochineal extract were previously recorded *(7,8 & 25).* Such cases presented angioedema, occupational asthma and systemic anaphylaxis. The induced hypersensitivity may be mediated by IgE response to carmine and its derivatives. The allergic reactions caused by carmine were manifested by nasorespiratory manifestations as nasal congestion, runny nose, itchy nose and throat, wheezing and dyspnea *(5).* Some cases presented a massive release of inflammatory mediators characterized by severe respiratory manifestation of throat swelling and air way closure. Histopathological lesions observed in the spleen of gp.2, showed hyperplastic white pulp and leukocytic infiltration in the red pulp. Other cases (gp.3), showed depletion and necrosis of the lymphoid tissue in the white pulp. Intraperitoneal injections of 1 to 2% aqueous solution of the lithium salts of carminic acid for 60 days, induced a great proliferation of splenic tissue *(17).* Our results may be attributed to the immune response of the rat against the foreign protein of the insect body(carmine cochineal). The liver showed vacuolation and hydropic degeneration together with periportal fatty changes in gps.2&3 besides other lesions, which point out the hepatotoxic effect of carmine. Rats given daily dose of 50, 150, and 500 mg/kg B.wt. of carmine mixed with diet for 6 weeks revealed distended hepatic sinusoids with the high dose *(26).* Food color. Many literatures mentioned that the food coloring-agents accumulate in the liver of the rats especially those given higher dosage levels *(18),* furthermore the degenerative and necrotic lesions observed in the present study indicated the effect of carmine on the hepatocytes. Moreover the liver is an organ of detoxification and bioactivation, as it breaks down toxic

substances or their metabolites, which severely damaged the hepatic cells. The kidneys were severely congested with perivascular edema and hemorrhage. Cystic dilatation of some renal tubules together with necrosis of the epithelial lining of these tubules were seen gp.3. Multiple leukocytic aggregations focally replaced the medullary collecting renal tubules. No body knows about the long term effects of small doses of carmine cochineal and its derivatives that accumulate in the human body during one's whole life *(27)*. The degenerative changes, observed in the kidneys of this work may be attributed to the toxic irritant substances brought to the kidneys for excretion *(1,19,22)* or induced by the accumulation of the food coloring-agents in the renal tissue *(28)*. The myocardium of gps.2&3 in our study, showed congestion and perivascular edema. Moreover vacuolar and hyaline degeneration was seen in the cardiac muscle. No available literature about the histopathological changes in the myocardium of rats given carmine could be found. However, others *(5)* described the allergic reactions caused by carmine. Such allergic reaction was manifested by headache, chest pain and low blood pressure. Some cases of carmine allergy caused a massive release of inflammatory mediators which lead to cardiovascular collapse and shock due to the severity of IgE mediated allergic reactions *(29)*. The testes of the rats given carmine in the present study showed congestion besides necrosis of the germ cells. Some seminiferous tubules revealed necrotic sperms in their lumina in addition to interstitial edema. Similar lesions were previously described *(1,22)*. Such damage could result from the deficit of testosterone which is expected to interfere with the completion of meiosis by direct action on the germ cells, thus it seems possible that the food coloing-agents affect the Leydig cells, leading to reduced the production of testosterone *(1)*. The skin of gps.2&3 was congested and showed degenerated hair follicle together with dilatation of the sebaceous glands. The dermis was congested and edematous. The obtained results could be attributed to allergic reactions which is manifested by an abnormal exaggerated body's immune system to a reaction provoking substance(allergen), usually a protein *(29)*. The majority of such responses are immediate hypersensitivity reactions mediated by IgE antibodies on the surface of mast cells and basophils. The specific allergens from food, binds with IgE antibodies to liberate histamine and other inflammatory mediators involved the allergic response signs and symptoms include skin flushing, urticaria, eczema and angioedema *(5,9,30)*.

Conclusion

We could be concluded that the carmine cochineal coloring agents is toxic, particularly at high dose. It is recommend to limit its use and declare its presence and the source (insect) of its origin in the product. Also require scientific reviews to determine the specific allergen of carmine cochineal and whether it could be eliminated from the coloring agents.

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الملخص العربى

دراسات باثولوجيه على التأثير الضار للمادة الملونة كارمن كوشينيل المضافة للاطعمه على ذكور الجرذان البيضاء

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استهدف هذا البحث دراسة التأثير الضار للمادة الملونة الطبيعية كارمن كوشينيل التي تضاف للاطعمه والادويه و مستحضرات التجميل على ذكور الجرذان البيضاء البالغة بواسطة استخدام جرعتان جرعه صغيره و أخرى كبيرة.

تم استخدام ٢١ من ذكور الجرذان البيضاء البالغة, قسمت بالتساوي إلى ثلاث مجموعات . المجموعة الأولى تركت ضابطة للتجربة, المجموعة الثانية جرعت بجرعة تساوى ٥٠٠مج/كج من وزن الجسم أما المجموعة الثالثة جرعت بجرعة تساوى ١٠٠٠مج/كج من وزن الجسم. و قد تم التجريع بالأنبوبة المعدية خمس مرات في الأسبوع و لمدة ٤٥ يوم.

بعد انتهاء فترة التجربة (٤٥) تم ذبح الجرذان من المجموعات الثلاث لأجراء الصفة التشريحية وتسجيل الأفات الباثولوجية العينية للأعضاء المختلفة و اللتى كانت تتمثل في زيادة حجم بعض الأعضاء وتحول لونها الى اللون الأحمر الداكن. وتم اخذ عينات من الرئة و الطحال و الكبد و الكلية و القلب و الخصيه و الجلد لفحصها مجهريا .

و قد أظهر الفحص المجهرى للرئة وجود احتقان و نزف للأوعية الدموية مع وجود ارتشاح وزيادة فى سمك الحواجر التى بين الحويصلات الهوائيه. و قد اظهر الفحص المجهرى للطحال اضمحلال و تنكرز فى عدد الخلايا الليمفاويه. كما أظهر الفحص وجود احتقان ونزف للأوعية الدموية مع وجود تنكسات مختلفة في كل من الكبد والكلية والقلب و الخصية. كما وجد في الكبد أيضا زيادة فى عدد القنوات المرارية و كذلك فرط فى خلايا كرات الدم البيضاء و النسيج الليفى. كما وجد اتساع فى الأنبيبات الكلوية مما أدى الى ظهور تنكرز فى الخلايا الطلائية المبطنة لهذه الأنبيبات. وقد أظهر الفحص المجهرى للجلد وجود احتقان و تنكسات مختلفة مع اتساع فى الغدد الدهنيه . و قد كانت التغيرات الباثولوجيه اكثر شدة و انتشار فى الجرذان اللتى تجرعت بالجرعة العالية للمادة الملونة.

مما سبق نستنتج ان استخدام المواد الملونة كاضافات للأغذية و الأدوية و مستحضرات التجميل لها اثرها السلبى حتى و لو كانت من مصادر طبيعية.