Assessment of Lipoprotein and Histopathological Changes of Male Rats *Rattus norvegicus* Treated With Formaldehyde

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Abstract

The present study was designed for detection the effect of formaldehyde in some biochemical and physiological parameters which included cholesterol , triglycerides and level of lipid profile and histopathological changes of some organs of the laboratory male rats *Rattus norvegicus* , thirty two of rats were divided into four groups , the first group administrated normal saline as control group while the three others groups administrated formaldehyde orally (10mg/kg body weight/day) for one , two and three months respectively .

The results showed a significant increasing ($P \leq 0.05$) in level of cholesterol , LDL and VLDL in second, third and fourth groups compared with control group, while non-significant increasing in triglycerides and atherogenic index level for treated groups (second, third and fourth groups) compared with control group . Significant decreasing ($P \leq 0.05$) was observed in level of HDL for all treated groups by formaldehyde compared with control group .

Also, the results showed histopathological changes as congestion , destroyed and thickness the walls of alveoli , hemorrhage and infiltration of inflammatory cells in lung . In addition to many damages in liver which included congestion of central vein , necrosis , enlargement of sinusoids , inflammation , hemorrhage and vacuolation of hepatocytes with hypertrophy the nuclei of its

Key Words : Formaldehyde , Lipid profile , Rats , Lung .

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Introduction

Formaldehyde or formalin is an organic compound belongs to aldehyde which characterized as a colorless and flammable gas possess stifling odour and soluble in water , chloroform , diethyl ether and ethanol (1,2) . Formaldehyde was used as a fungicides , a disinfectant especially in veterinary medicine preservative of tissues , embalming fluids , cosmetics , textiles and wood-products (3) . The exposuring for formaldehyde has been by inhalation , ingestion method and by skin contact , some studies reported health hazard effects such as hematological parameters disorders (4) , teratogenic effects , gastrointestinal tract , respiratory system and central nervous system causing mood alterations and losing of concentration that regarded to formaldehyde exposure (5,6) .

The aim of the current study was to examine the effects of formaldehyde on the lipid profile and histological damages of lung and liver in male laboratory rats .

Materials and Methods

Laboratory Animals

Thirty two adult male rats *Rattus norvegicus* , weighting (230-300) grams aged about (2-3) months were obtained from animal house of biology department /college of education for pure sciences / university of Thi-Qar under conditions of controlled temperature and humidity. They were allowed free access to standard diet and tap water .

Experimental Design

Rats were randomly divided into four groups , each group consists of eight animals as follow :

First Group (Control group) : They were given normal physiological saline .

Second Group : They were treated with 10% formaldehyde (10 mg/kg body weight / day) for one month **.**

Third Group : They were treated with 10% formaldehyde (10 mg/kg body weight / day) for two months .

Fourth Group : They were treated with 10% formaldehyde (10 mg/kg body weight / day) for three months **.**

All animals administrated of formaldehyde by orally method .

Serum and Lipid Profile Determination

At the end period of each experiment , blood samples were collected (in tubes without EDTA anti-coagulant) after the animals had been sacrificed . Samples were centrifuged at 3000 rpm . for 15 minutes to obtained serum and was stored at (-20°C) until used for lipid profile measurement . Cholesterol and triglycerides were estimated according to enzymatic methods kits (Biolabo/France) (7,8) respectively .

Lipid profile included high density lipoproteins level (HDL) was measured by

using enzymatic assay kit (Biolabo/France) (9) , low density lipoproteins (LDL) and very low density lipoproteins (VLDL) were determined by (10,11) while index atherogenic level was estimated according to (12) as :

LDL = Total cholesterol – (HDL+VLDL)

VLDL= Triglycerides /5

Atherogenic Index = LDL/HDL

Histological Examination

The animals of all groups were anesthetized by chloroform or ether and killed after one , two and three months of treatment . Lung and liver were kept in formaldehyde (10%) and used for histological examination using paraffin section technique which including washing and dehydration by ascending grades of alcohol . Clearing in xylene then embedding in paraffin wax . Sectioning at 5 microns thickness , staining with hematoxylin and eosin (H&E) then examination microscopically to determination of damages in tissues (13) .

Statistical Analysis

Data were analyzed with analysis of variance (ANOVA) by using SPSS program . Differences were considered statistically significant at ($P \leq 0.05$) .

Results and Discussion

Biochemical Parameters

Table (1) demonstrated the serum levels of cholesterol and triglycerides for control and treated groups . Cholesterol of treated groups was significantly increased ($p \leq 0.05$) compared with control group while the triglycerides of all treated groups non-significantly increased compared with control group .

This increasing may be belong to oxidative stress that resulted from formaldehyde treatment by free radical generation which causes lipid peroxidation (LPO) led to attacking the unsaturated fatty acids and high lyses in blood . The triglycerides was elevated as evidence to break down of lipids , Navasumrit *et al*. (14) reported the exposure to formaldehyde generates free radicals and induce LPO for lipids of cellular membranes , this result agree with Yoneda *et al*. (15) who found formaldehyde causes oxygen stress , such as that caused by free radical generation . The other explaining for increasing cholesterol regarded to liver damaged by formaldehyde which affects on lipid metabolism and led to elevated of free fatty acids , the free fatty acids considered as main components of cholesterol and triglycerides . Jaeschke *et al*. (16) showed the hepatocytes damaged affects on metabolism of fats and cause change in level of cholesterol .

Table (1) : Effect of formaldehyde on cholesterol and triglycerides (n=8) (Mean ± Standard deviation)

Group	Cholesterol (mg/dL)	Triglycerides (mg/dL)
First group(Control)	58.11 ± 4.96 [a]	31.80 ± 4.32 [a]
Second group	71.77 ± 8.18 [b]	32.20 ± 4.71 [a]
Third group	79.06 ± 10.23 [b]	32.60 ± 3.97 [a]
Fourth group	84.00 ± 13.50 [b]	37.20 ± 4.86 [a]

Different letters refer to significant difference(P≤ 0.05) compared with control group

Lipid Profile

Lipid profile levels in table (2) detected significantly decreased (P≤ 0.05) in HDL level of treated groups compared with control group while LDL and VLDL of treated groups were significantly increased (P≤0.05) . Also table (2) revealed to non-significantly increased in index atherogenic level of treated groups compared with control group , too .

Decreasing significantly in HDL level due to effect of formaldehyde on induction of LPO by free radicals , as known lipid peroxidation causes increased of cholesterol led to transporting the cholesterol from blood into liver by HDL subsequently decline of it in serum , this agree with Hardell and Sage (17) who noted lipid harmed by ROS .

The current study revealed to a significantly increasing in LDL and VLDL , This may be due to free radicals and LPO that resulted from formaldehyde exposure which led to damage the cellular membranes and affects on receptors . Niu and Evans (18) reported reduction of receptors of VLDL causes prevents enter cells so level of it is rises in blood serum . Also , increasing LDL and VLDL associated with elevated of cholesterol and triglycerides respectively , that agree with results of present study which found rising in both concentrations in treated groups by formaldehyde .

Concerning of the atherogenic index , increasing was observed in treated groups , this result linked to LPO and consumption antioxidants subsequently highly cellular hazards as elevated LDL and reduction of HDL led to rising the atherogenic index .

Table (2) : Effect of formaldehyde on lipid profile

(n=8) (Mean ± Standard deviation)

Group	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)	Atherogenic Index (mg/dL)
First group(Control)	43.00±2.09[a]	8.83±6.69 [a]	7.33±1.74 [a]	0.20±0.15 [a]
Second group	40.33±2.33 [b]	22.70±20.58 [b]	9.30±1.85 [b]	0.56±0.52 [a]
Third group	37.83±4.35 [b]	30.16±9.99 [b]	10.83±1.72[b]	2.85±5.27 [a]
Fourth group	34.33±6.50 [b]	32.66±17.01 [b]	10.23±1.52 [b]	1.00±0.58 [a]

Different letters refer to significant difference(P≤ 0.05) compared with control group

Histological study

The results of the present study revealed to occurrence many histological damages in lungs and livers of all treated groups by formaldehyde that included congestion of blood vessels , destroyed and thickness walls of alveoli with severe pulmonary hemorrhage and infiltration of inflammatory cells in lung , as well as the changes in liver were represented blood congestion , necrosis , enlargement of sinusoids , inflammation , hemorrhage , vacuolation and hypertrophy the nuclei of hepatocytes . The results found tremendous histological effects by increasing periods of formaldehyde treatment (pictures 1-20).

The histopathological changes in lung and liver of treated groups were associated to administration of formaldehyde , OSHA (19) found occurred many health hazards related to toxic role of formaldehyde after ingestion and inhalation . These histological effects were observed in lung due to formaldehyde application has toxic effects for body , this agree with Fujimaki *et al*. (20) who reported damages in respiratory system , nervous system and gastrointestinal tract by formaldehyde exposure , also , this similar to OSHA (21) which focused formaldehyde concentration can cause death by chemical burns to lungs . Casanova *et al*. (22) noted inhaled formaldehyde appears to be readily absorbed by the upper respiratory tract causing pulmonary damages . The present result was identical with Al-Saeed *et al* . (23) who their study mention highly changes in lung like congestion of blood vessels , thickening of alveolar walls , hemorrhage and distinct inflammation .

Also, the liver showed histological

changes regarded to formaldehyde administration , Corrier (24) demonstrated chronic lesion in liver with damage of hepatocytes and vacuolation . Similarity , congestion in central vein , hemorrhage , necrosis of hepatocytes and inflammation were appeared in liver in study of Al-Saeed *et al*. (23) .

All the results inconsistent with Itami *et al* . (25) who revealed no related signs of toxicity in rats and hamsters after administrated formaldehyde orally or by topical treatment .

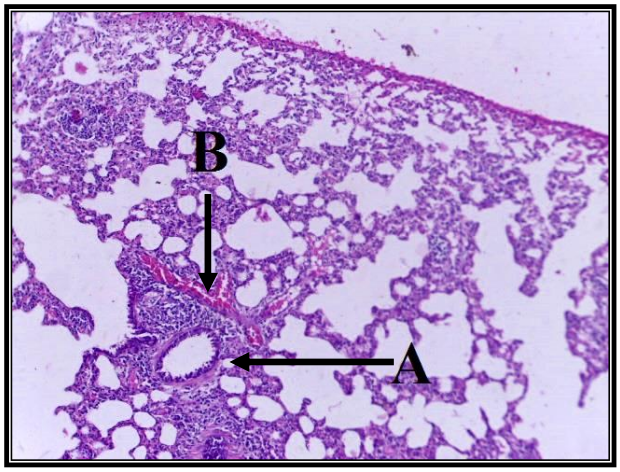


Figure (4) : Section in lung of **third group** showing thickness of the alveoli wall (A) congestion (B) (H&E) (100 X) .

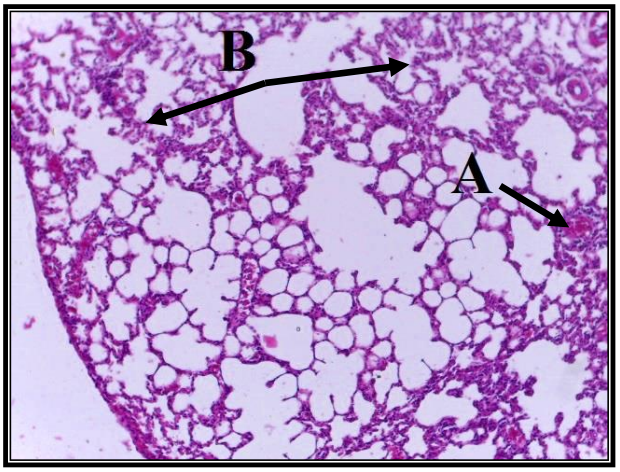


Figure (2) : Section in lung of **second group** showing congestion of blood vessels (A) destroyed walls of alveoli (B) (H&E) (100 X) .

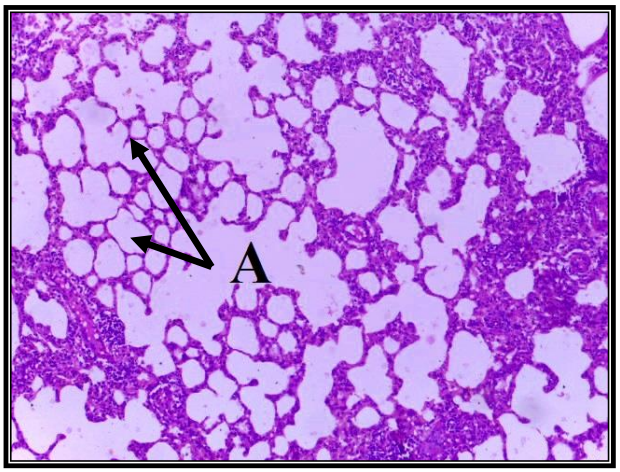


Figure (1) : Section in lung of **control group** showing normal structure of alveoli (A) (H&E) (100 X) .

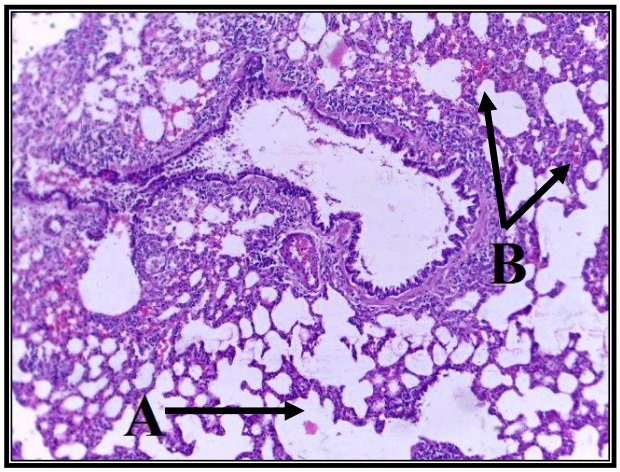


Figure (5) : Section in lung of **third group** showing destroyed walls of alveoli (A) hemorrhage (B) (H&E) (100 X) .

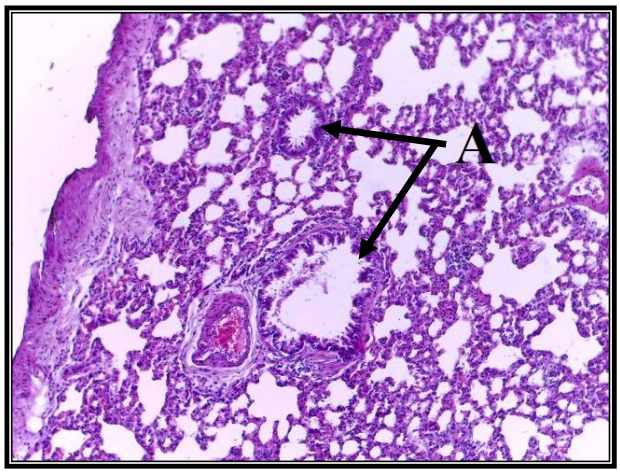


Figure (3) : Section in lung of **second group** showing thickness of the alveoli wall (A) (H&E) (100 X) .

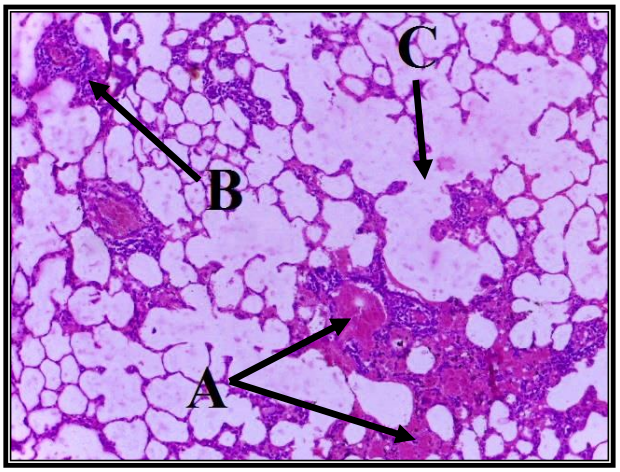


Figure (8): Section in lung of **fourth group** showing severe hemorrhage (A) infiltration of inflammatory cells (B) destroyed of the alveoli wall (C) (H&E) (100 X) .

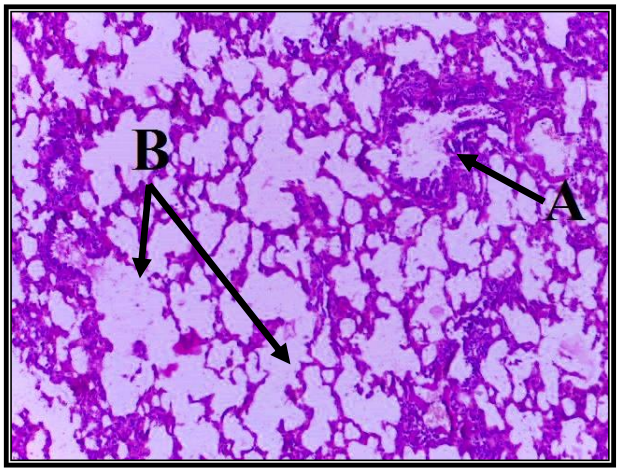


Figure (6) : Section in lung of **fourth group** showing thickness (A) destroyed of the alveoli wall (B) (H&E) (100 X) .

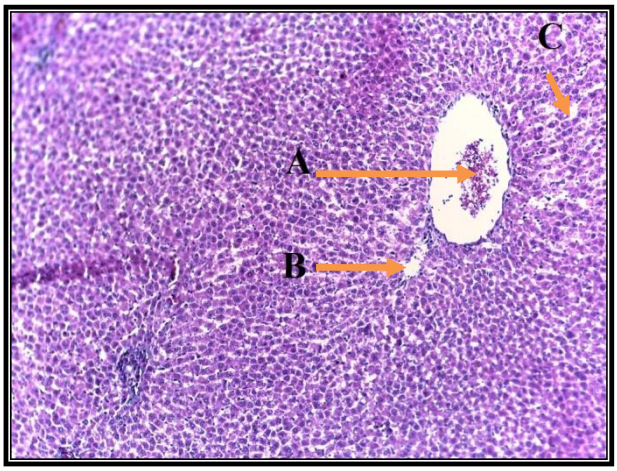


Figure (12): Section in liver of second **group** showing congestion of central vein (A) necrosis (B) enlargement of sinusoids (C) (H&E) (100 X) .

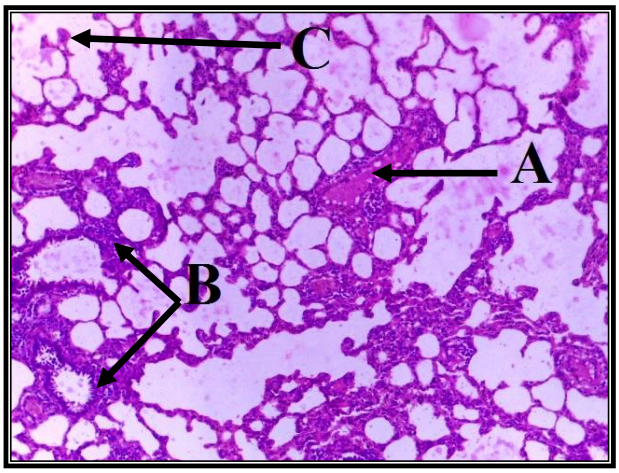


Figure (9): Section in lung of **fourth group** showing congestion (A) thickness of the alveoli wall (B) destroyed of the alveoli (C) (H&E) (100 X) .

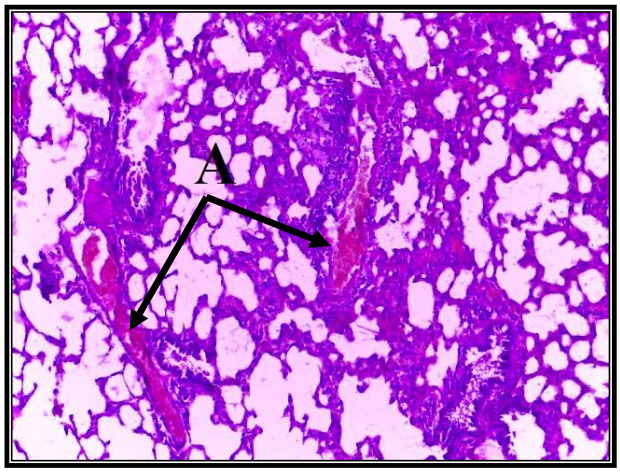


Figure (7): Section in lung of **fourth group** showing large congestion (A) (H&E) (100 X) .

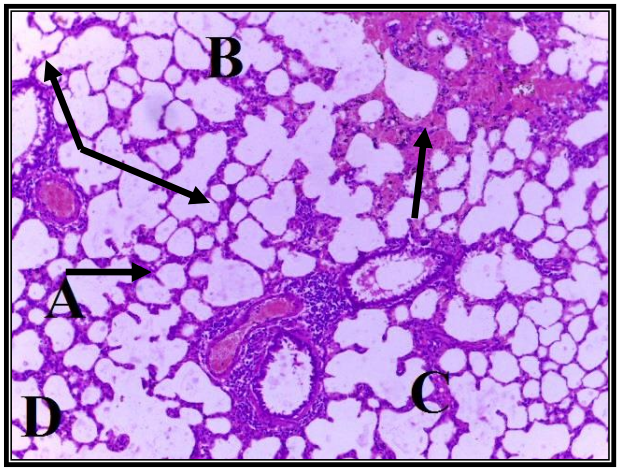


Figure (10): Section lung of **fourth group** showing varying and intensity changes congestion (A) hemorrhage (B) thickness (C) destroyed of the alveoli (D) (H&E) (100 X) .

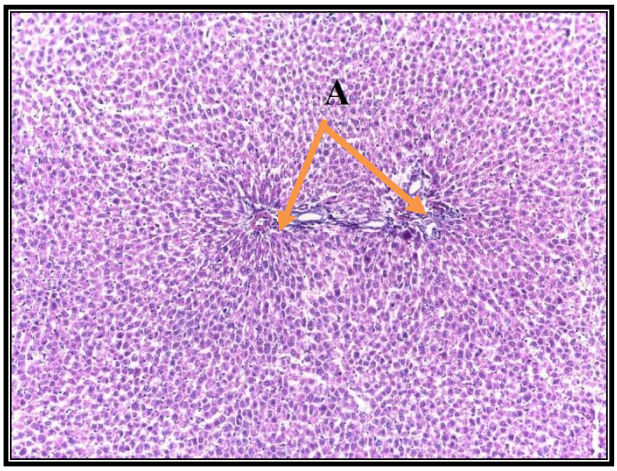


Figure (16): Section in the liver of **third group** showing large infiltration of inflammatory cells (A) (H&E) (100X) .

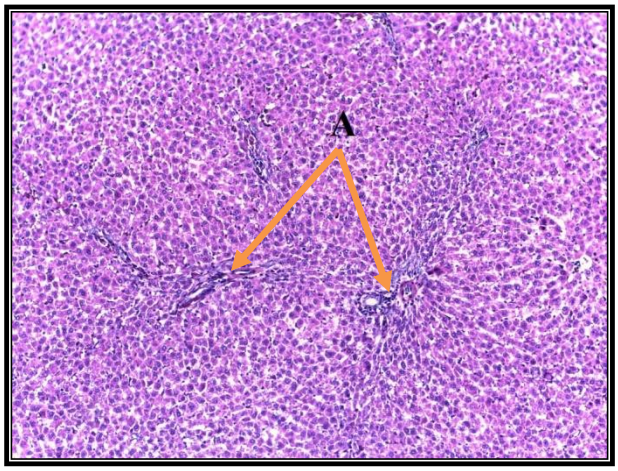


Figure (13): Section in the liver of **second group** showing infiltration of inflammatory cells (A) (C) (H&E) (100X) .

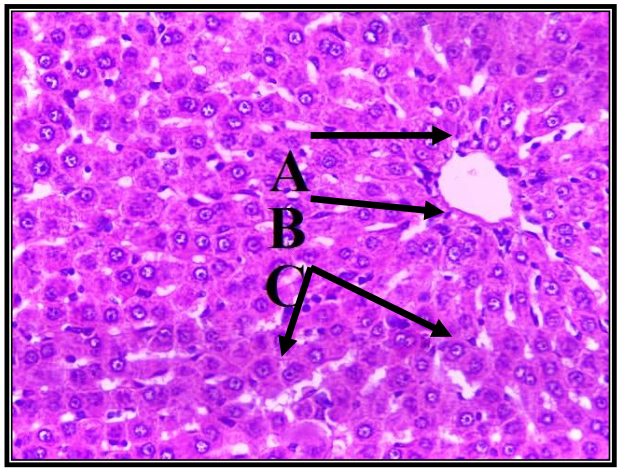


Figure (11): Section in liver of **control group** showing central vein (A) hepatocytes (B) sinusoids (C) (H&E) (100X) .

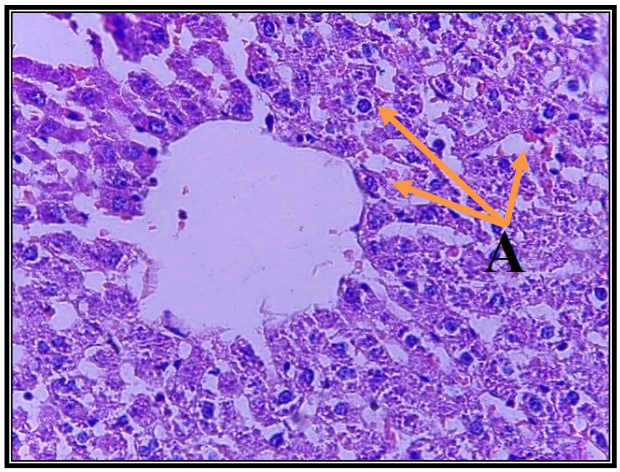


Figure (14): Section in the liver of **second group** showing hemorrhage (A) (H&E) (100X) .

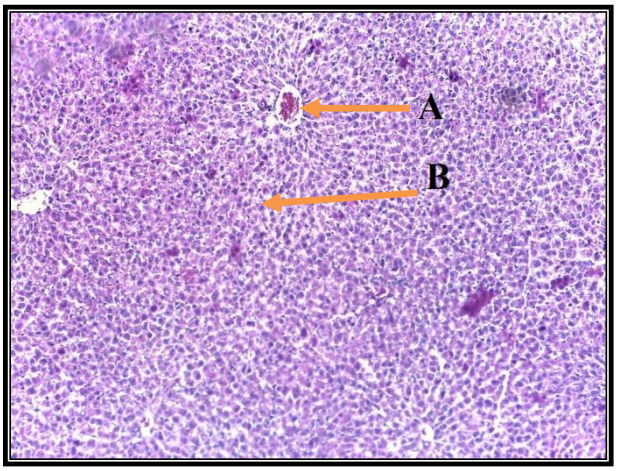


Figure (20): Section in the liver of **fourth group** showing congestion(A) enlargement of sinusoids (B) (H&E) (100X) .

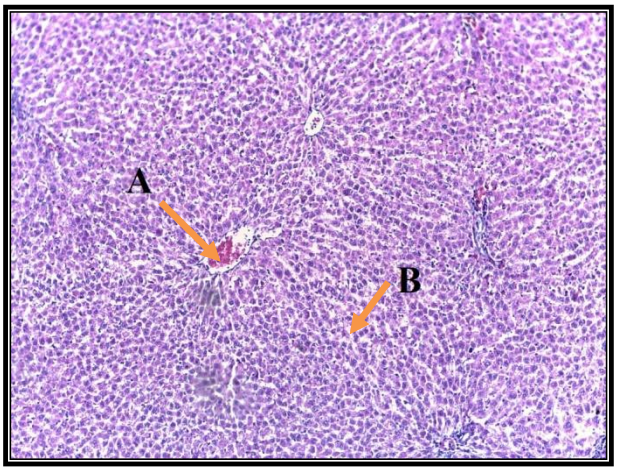


Figure (15): Section in the liver of **third group** showing congestion (A) enlargement of sinusoids (B) (H&E) (100X) .

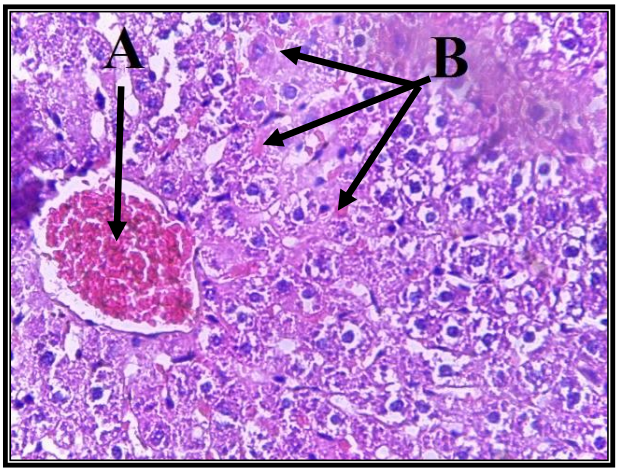


Figure (17): Section in the liver of **third group** showing congestion (A) hemorrhage (B) (H&E) (100X) .

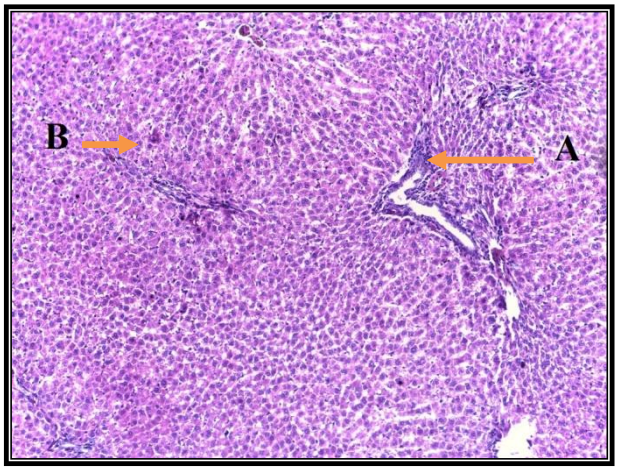
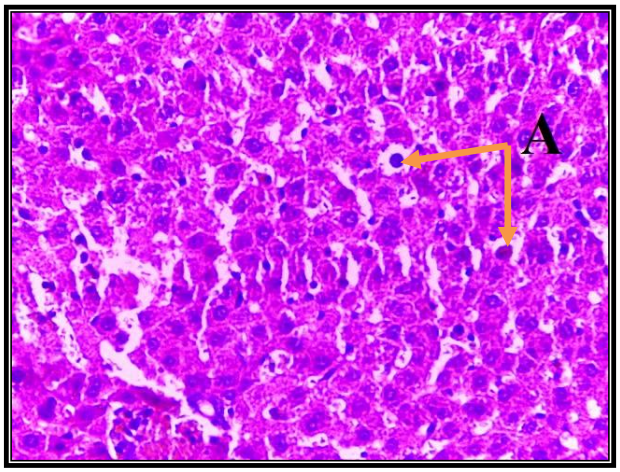


Figure (18): Section in the liver of **fourth group** showing large infiltration of inflammatory cells (A) hypertrophy of nuclei of hepatocytes (B) (H&E) (100X) .

Figure (19): Section in the liver of **fourth group** showing vacuolation of hepatocytes (A) (H&E) (100X) .



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تقييم التغيرات في مستوى البروتينات الدهنية والتغيرات النسجية المرضية لذكور الجرذان المختبرية *Rattus norvegicus* المعاملة بالفورمالديهايد

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الخلاصة

صممت الدراسة الحالية للكشف عن تأثير الفورمالديهايد في بعض المعايير الكيموحيوية التي شملت (تركيز الكولسترول والكليسيريدات الثلاثية ومستوى البروتينات الدهنية) والتغيرات النسجية المرضية لبعض أعضاء ذكور الجرذان المختبرية *Rattus norvegicus* ، إذ أستخدم إثنان وثلاثون من الجرذان قسمت عشوائيا إلى أربع مجاميع : جرعت المجموعة الأولى بالمحلول الملحي الفسلجي وأعتبرت كمجموعة سيطرة فيما عوملت المجاميع الثلاثة الباقية بالفورمالديهايد بمقدار (10ملغم/ كغم وزن الجسم/ يوم) ولفترات زمنية مختلفة (شهر ، شهرين وثلاث أشهر) على التوالي .

إتضح من نتائج الدراسة الحالية إرتفاع معنوي ($p\leq0.05$) في مستوى الكولسترول والبروتينات الدهنية واطئة الكثافةLDL والبروتينات الدهنية واطئة الكثافة جدا VLDL للمجاميع الثانية والثالثة والرابعة مقارنة مع مجموعة السيطرة ، في حين لم يصل الإرتفاع إلى مستوى المعنوية بالنسبة لتركيز الكليسيريدات الثلاثية ودليل التصلب في المجاميع الثانية والثالثة والرابعة المعرضة للفورمالديهايد مقارنة مع مجموعة السيطرة ، بينما قد لوحظ حصول إنخفاض معنوي ($p\leq0.05$) في مستوى البروتينات الدهنية عالية الكثافةHDL لكل المجاميع المعاملة بالفورمالديهايد (الثانية والثالثة والرابعة) مقارنة بمجموعة السيطرة .

أظهرت النتائج أيضا تغيرات نسجية مرضية للأعضاء التي شملتها الدراسة تمثلت بإحتقان الأوعية الدموية وتحطم وتثخن جدران الأسناخ الرئوية مع نزف رئوي شديد وإرتشاح الخلايا الإلتهابية في الرئة ، بالإضافة إلى إحتقان الوريد الكبدي والتنخر وتوسع الجيبانيات الدموية وإلتهاب الكبد ونزف دموي وتفجي الخلايا الكبدية وتضخم أنويتها .

الكلمات المفتاحية : الفورمالديهايد ، البروتينات الدهنية ، الجرذان ، الرئة .