HISTOPATHOLOGICAL AND BIOCHEMICAL STUDYTO EFFECT OF CODEINE-PARACETAMOLIN SPRAGUE DAWLEY RATS

Aseel Kamel Hameed* Adel J. Hussein** S.K.Majeed***

*.Department of Basic Science, College of Dentistry, University of Basrah, Basrah, Iraq.
**.Department of Anatomy, College of Veterinary Medicine,University of Basrah , Basrah, Iraq.
***Department of Pathology, College of Veterinary Medicine, University of Basrah, Basrah, Iraq.

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ABSTRACT

This study performed in twenty four male Sprague Dawley rats for (90) days which divided randomly into four equal groups. Group (1): received normal saline daily. Group (2): received orally codeine-paracetamol(40/2500 mg / kg b.w) daily. Group (3): received orally codeine-paracetamol (80/5000 mg / kg b.w)daily. Group (4): received orally codeine- paracetamol (160 /10000 mg / kg b.w) daily. After end of day (90) of study all animals were sacrificed to do the histopathological and biochemical examinations. The statistical analysis results revealed the body weight effects of codeine-paracetamol toxicity a significant ($P \leq 0.05$) decreases of treated group after (90) days of treatment. The histopathological investigation of liver, kidney and brain of treated groups showed centrolobular necrosis, dilation of sinusoids, vaculation of hepatocytes and septal fibrosis of liver while kidney showed vaculation of mesengial cells of glomeruli, necrosis of proximal convoluted tubules and dilation of renal cortical tubules, also brain of treated group showed vaculation of neurons, these changes are appeared mild in group (2), moderated in group (3) and sever in group (4). The statistical analysis results of biochemical investigations of liver and kidney function tests showed a significant ($P \leq 0.05$) increases of levels of serum AST, ALT, ALP, bilirubin and creatinine respectively in all treated groups which these enzymes increased mildly in group (2), moderately in group (3) and severely in group (4).

INTRODUCTION

Paracetamol (acetaminophen) is a widely used analgesic and antipyretic agent for the relief of fever, headaches, minor pains, it is a major ingredient in numerous cold and flu remedies; in combination with non-steroidal anti-inflammatory drugs and opioid analgesics (1). Paracetamol is used also in the management of severe pain such as post operative pain; its overdose produces hepatic necrosis and renal failure due to increases in lipid peroxide levels and depletion of glutathione (2). Paracetamol was the fourth most common cause of death following self-poisoning in the United Kingdom in 1989 (3). The narrow margin between therapeutic and toxic doses, that reveals when ingestion of (10-15grams) of paracetamol by adults may cause severe hepato-cellular necrosis and less often renal tubular necrosis (4).

Codeine phosphate is predominant alkaloid opium, it is considered as a pro-drug, metabolized to active compounds of morphine and codeine-6-glucoronide (5,6). Codeine is a classic analgesic cause nephropathy is characterized by renal papillary necrosis and papillary calcifications(7).On other hand the relative contribution of the opioid to the hepatotoxicity is unknown; also codeine distributed in the lung, liver, kidney, and spleen, it has pharmacologic effects on the central nervous system (e.g. analgesia, drowsiness, mood changes, respiratory depression, nausea, and dysfunction of the endocrine and autonomic nervous systems) and the gastrointestinal tract like decreased gastrointestinal motility (8,9).

The combinations of codeine with paracetamol produce a significant increase in analgesia compared with Paracetamol alone ; codeine causes less euphoria and sedation than morphine, but CNS depression and coma occur in case of overdose (10,11). The synergistic effect of paracetamol-codeine is possible via a pharmacokinetic interaction between the two classes of drugs, NSAIDs may decrease the renal excretion of the pharmacologically active metabolite of morphine (morphine-6-glucuronide), also codeine, a centrally-acting opioid, can have additive analgesic effects when combined with peripherally acting agents (12,13).

MATERIALS AND METHODS

Twenty fourmale Sprague Dawleyrats were used to do this study, their weights were ranged (150-200) grams. They were taken from animal house of Veterinary Medicine college at University of Kufa, then they were housed in laboratory animal house incollege of Veterinary Medicine at University of Basrah, with (25±2ºC)temperature with lightness system was (10/14) hours darkness/lightness and a mechanical ventilations was used to control of the suitable humidity. They were kept for adaptation in these environments for (14) days before the study. These animals were divided randomly in to four groups and put in specific plastic cages. Each group of those rats were put cages, in each cage, there were six animals per each group to avoid crowding and nutrient a pellet, and water until the end of the study.Also the cages were made from a plastic containing hard-wood chip as bedding. The bedding was changed continuously to ensure a clean environment.Also the animals weighing in day zero, 30^{th} , 60^{th} and lastly in day 90^{th}.

DETERMINATION OF MAXIMUM TOXIC DOSE

In this experiment, we started with normal human tablet contain (500mg of paracetamol / 8 mg of codeine) brought from (Bristol pharmaceutical company / U.K), then gave orally for two rats then started to increase the dose until we reached to a dose of six tablets which gave us a clinical signs of the maximum toxic dose like drowsiness, non active , arching back, piloeraction, sunken of eyes and pin point of the pupil. At this point we decided that will be the maximum toxic dose is (15000 paracetamol / 240 mg of codeine / kg bw).Then we decided that the minimum toxic dose is the high dose (group 4) will be four tablets (10000 mg of paracetamol /160 mg of codeine /kg bw) and the intermediate dose (group 3) is two tablets (5000 mg of paracetamol / 80 mg of codeine /kg bw) while the low dose (group 2) is one tablet (2500mg of paracetamol/ 40mg of codeine /kg bw) (14).

EXPERMENTAL DESIGN

This experiment was divided into four groups each group contains six male rats as the following:

Group (1): It received normal saline oral doses daily for (90) days which served as control group.

Group (2): It received (40mg of codeine/2500mg of paracetamol/ kg bw) oral doses daily for (90) days which served as low dose group (L.D).

Group (3): It received (80 mg of codeine/5000mg of paracetamol / kg bw) oral doses daily for (90) days which served as intermediate dose group(I.D).

Group (4): It received (160 mg of codeine/10000 mg of paracetamol/ kg bw) oral doses daily for (90) days which served as high dose group(H.D).

COLLECTION OF SPECIMENS

At the end of experiment (90) days, the rats were generally anaesthetized by inhalation of Chloroform and then sacrificed. Blood samples were collected directly from the heart by the use of disposable syringes of (5) ml capacity**,** then was poured into test tubes free from anticoagulant to isolate blood serum and allowed to clot at room temperature and then centrifuged at rate (3000 rotation/minute)for (5) minutes to isolate blood serum and froze at (-20C^{o}) to estimate the biochemical parameters .

After the collection of blood samples from the animals, the gross examination was done for all animals, the macroscopic appearance was recorded to detect any abnormal gross change in the internal organs, including location, color, size, shape, consistency and appearance of cut section, then the organs put in the formalin (10%) for fixation.

PREPERATION OF HISTOLOGICAL SECTIONS

According to (15,16) the preparation of histological sections include:

Tissue samples were obtained from the internal organs like (Brain, liver and kidney), these specimens were fixed at (10%) formalin immediately after removal from the body, then washing the specimens with running water and dehydration in serial alcohol concentrations (70, 80, 90, 100 %).After that cleared in two stages of xylene, and infiltrated with paraffin at (56-57°C), then embedded in paraffin followed block making, and a thin tissue section cut at (6 µm) with rotary microtome, after that the section mounted on the glass slides, attached to the slide it's surface smeared by small drops of Mayer's albumin, then the slides were put on hot plate at

(45C°) for (24) hours, finally the specimens were stained with hematoxylin – eosin, then examined by light microscope to detect any histopathological changes.

BIOCHEMICAL ANALYSIS

Biochemical analysis was measured by using kits which include:

1. Liver function tests like (Aspartate aminotransferase (AST) , Alanine aminotransferase (ALT) , Alkaline phosphatase (ALP), and bilirubin.
2. Kidney function test (creatinine).

STATISTICAL ANALYSIS

The results were analyzed by ANOVA and exposed to independent T-test by using SPSS (special program for statistical system) version 14.0

A value of ($P \leq 0.05$) was accepted as being statistically significant.

RESULTS

The statistical analysis result of effects of codeine-paracetamol toxicity in body weight showedthere was a significant ($P \leq 0.05$) changes between all treated groups when compared with control group in day (90) of treatment which especially there was a significant ($P \leq 0.05$) decrease in body weight of high dose treated group (H.D) as shown in table (1).

The histological examination of liver of control group showed normal liver architecture which consist of normal hepatocytes, central vein and portal areas as in figure (1). The histological study of liver of low dose group (L.D) revealed periportal fibrosis, septal fibrosis, vaculation of hepatocytes, dilation of sinusoids and mild centrilobular enlargement of hepatocytes as in figure (2). The intermediated dose group (I.D) revealed centrolobular enlargement of hepatocytes, septal fibrosis, vaculation of centrolobular region,dilation of sinusoids and mid zonal hepatocytes as shown in figure (3).While the high dose group (H.D) revealed severe centrolobular necrosis, congestion of sinusoids, hemorrhage, early septal fibrosis, diffuse enlargement of hepatocytes as seen in figure (4).

The histological results of kidney of control group revealed normal structures of kidney which contain normal glomeruli, proximal and distal convoluted tubules as shown in figure (5).

The histological study of kidney of low dose group (L.D) and intermediate dose group (I.D) revealed mild to moderate vaculation of mesengial cells and vaculation of proximal convoluted tubules as shown in figure (6,7). The histological study of kidney of high dose group (H.D) revealed necrosis of proximal convoluted tubules, vaculation of mesengial glomerular cells, vaculation of proximal convoluted tubules and dilatation of proximal convoluted tubules as shown in figure (8).

The histological study ofbrain of control revealed normal structures of nervous tissues which contain normal neurons (cell body and axon) , glial cells, astrocytes and oligodendritic cells as shown in figure (9).

The histological examinations of brain of low dose group (L.D) and intermediate dose groups (I.D) showed mild vaculation of neurons as in figure (10) and(11) respectively.While the histological study of brain of high dose group (H.D) revealed vaculation of neurons, vaculation of oligodendritic cells and vaculation of granular layer cells as shown in figure (12).

Thestatistical analysis results of codeine-paracetamoltoxicity in the levels of serum enzymes showed there were non significant ($P \leq 0.05$) differences between intermediated dose group (I.D) and low dose group (L.D) after (90) days of treatment as in table (2).While there was a significant ($P \leq 0.05$) increase in the level of total serum bilirubin (TSB) of the high dose group (H.D) when compared with control group.

There was non significant ($P \leq 0.05$) differences in the level of serum direct bilirubin (D.B) between all treated groups when compared with control group after (90) days of treatment as in table (2).

There were a significant ($P \leq 0.05$) increase in the levels of serum (AST), (ALT), (ALP) and creatinine among treated groups when compared with control group after (90) days of treatment as shown in table (2).

Table (1):Effect of codeine- paracetamol administration in body weight (grams).

Groups	Day zero	Day 30	Day 60	Day 90
G1: Control	185 ± 7.63 a	194.1 ± 6.11 a	203 ± 4.96 a	214.3 ± 4.98 a
G2: L.D	184.1 ± 6.11 a	193.3 ± 6.28 a	200.8 ± 5.54 a	212.1 ± 4.79 c
G3: I.D	183.3 ± 6.66 a	192.5 ± 5.28 a	199.3 ± 5.05 a	204.5 ± 3.6 a
G4: H.D	182.5 ± 7.27 a	187.1 ± 6.37 a	189.0 ± 6.23 a	190.5 ± 5.64 b

- The mean difference is significant at the ($P \leq 0.05$) level.
- The symbol latters (a,b,c) means a significant difference among the groups.

Table(2):Effect of codeine- paracetamol administration in serum liver function test and creatinine levels:

Groups	TSB (mg/dl)	D.B (mg/dl)	AST (U/L)	ALT (U/L)	ALP (U/L)	Creatinine (mg/dl)
(G1) Control	0.13±0.02 a	0.01±0.001 a	89±0.9 a	58.3±0.8 a	25.3±1.02 a	0.15±0.02 a
(G2) (L.D)	0.15±0.02 a	0.02±0.003 a	115±1.5 b	76.3±1.2 d	31.8±0.8 d	0.2±0.03 c
G3 (I.D)	0.13±0.02 a	0.02±0.003 a	133±1.4 b	98.8±2.5 c	42.6±0.8 c	0.3±0.02 b
G4 (H.D)	0.25 ± 0.04 b	0.01 ± 0.002 a	132±11.9 b	163.8 ±1.3 b	57.3±0.4 b	0.2±0.01 b

- The mean difference is significant at the (P
- ≤ 0.05) level.
- The symbol latters means a significant differences among the groups.

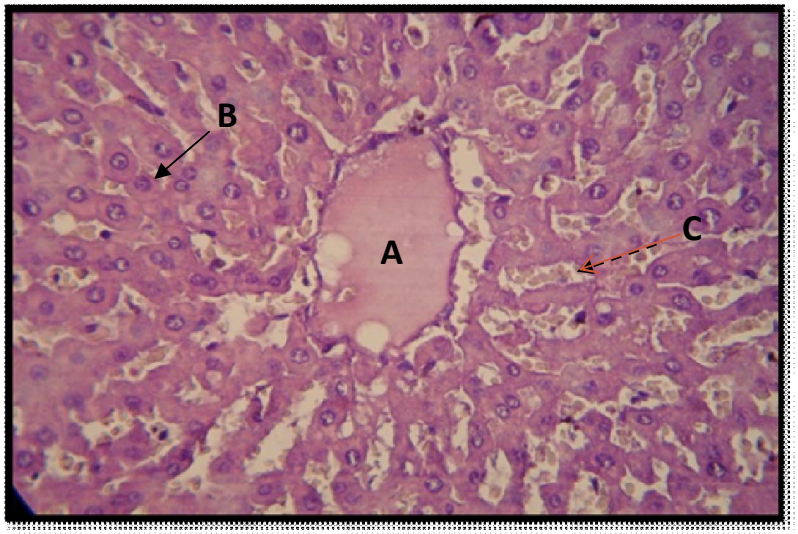

Figure (1):Transverse section through the Liver of control group(H&E stain, 400X). A. Central vein B. Hepatocytes
C. Sinusoids.

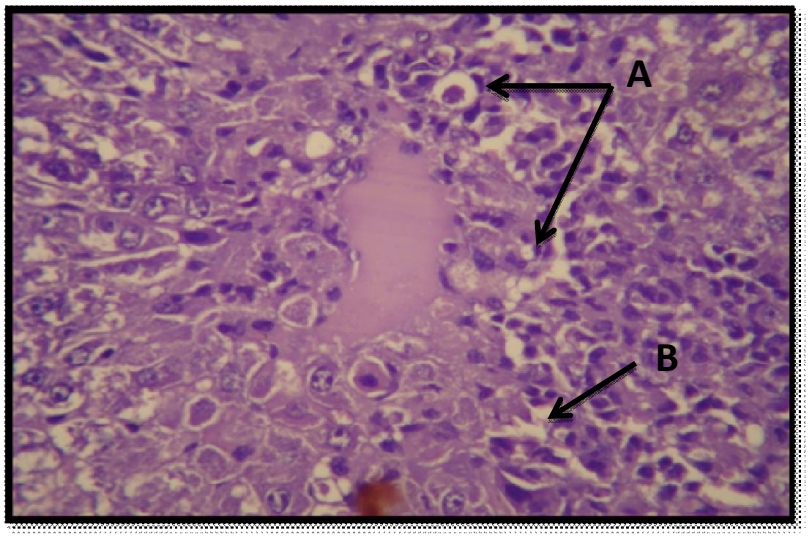

Figure (2):Transverse section through the Liver of (L.D) group(H&E stain ,400X)A. Vaculation of hepatocytes. B. Dilatation of sinusoids.

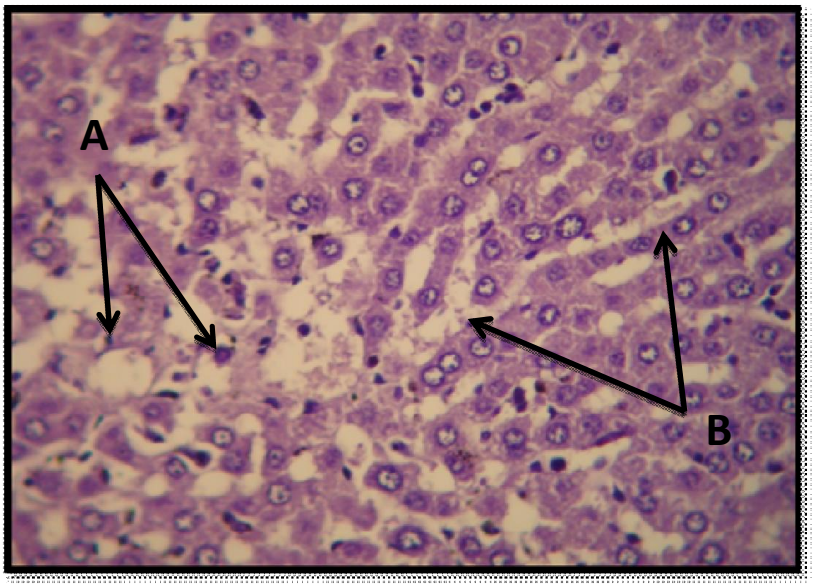

Figure (3): Transverse section through the Liver of (I.D) group (H&E stain, 400X). A. Vaculation of hepatocytes. B. Dilated sinusoids.

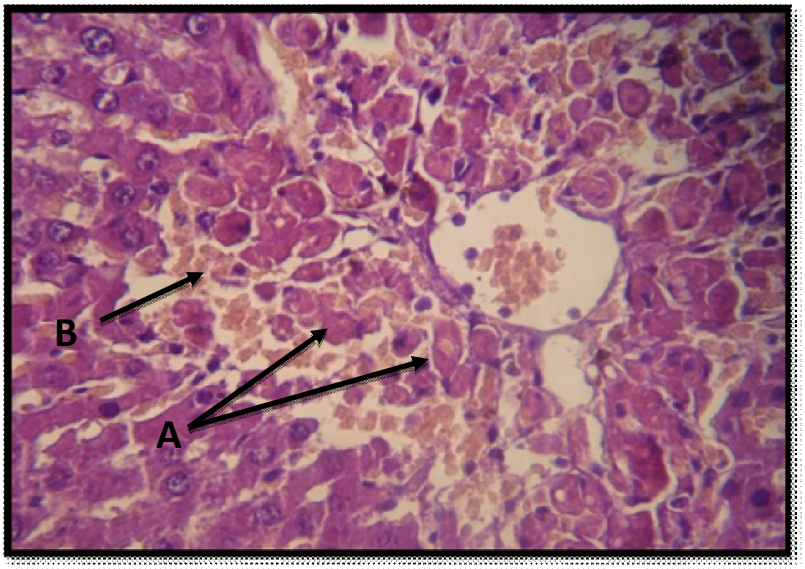

Figure (4): Transverse section through the Liver of (H.D) group (H&E stain, 400X). A. Centrilobular necrosis. B. Hemorrhage.

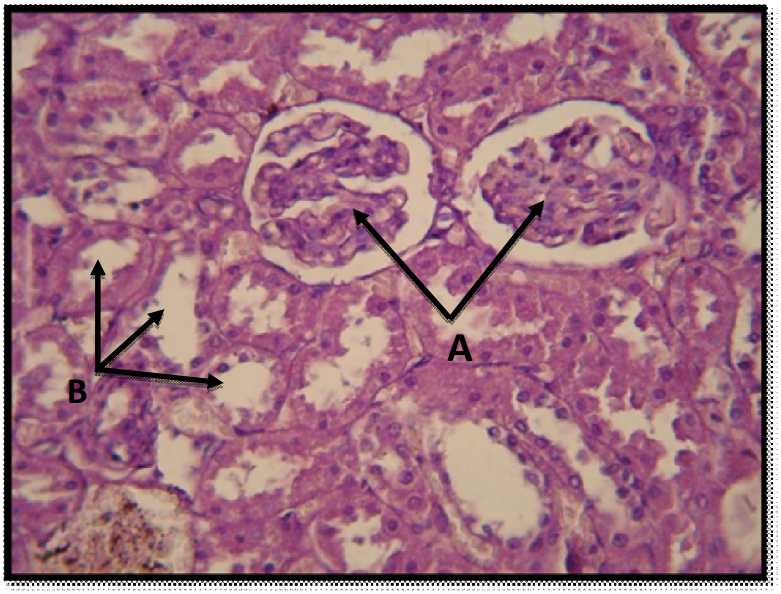

Figure (5): Transverse section through the kidney of control group (H&E stain, 400X).
A. Glomeruli. B. Renal convoluted tubules.

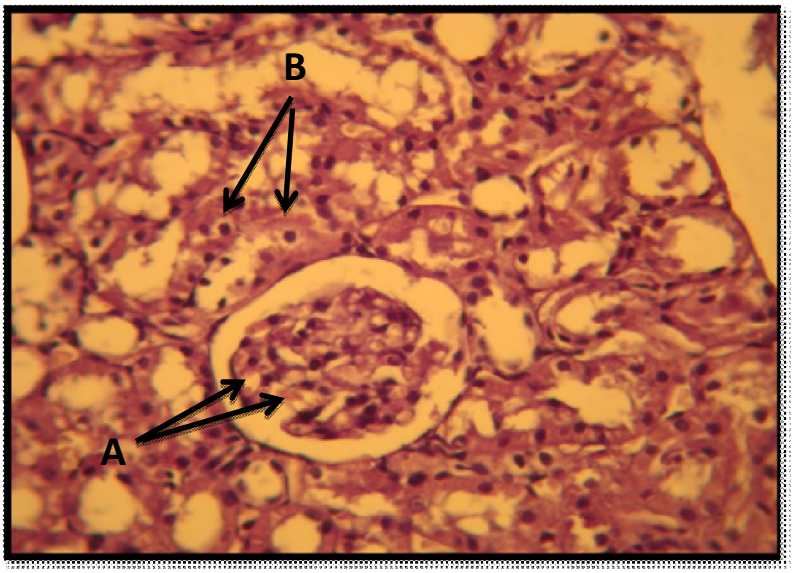

Figure (6): Transverse section through the Kidney of low dose (LD) group (H&E stain, 400X).
A. Vaculation of mesengial glomerular cells.
B. Vaculation of proximal convoluted tubules.

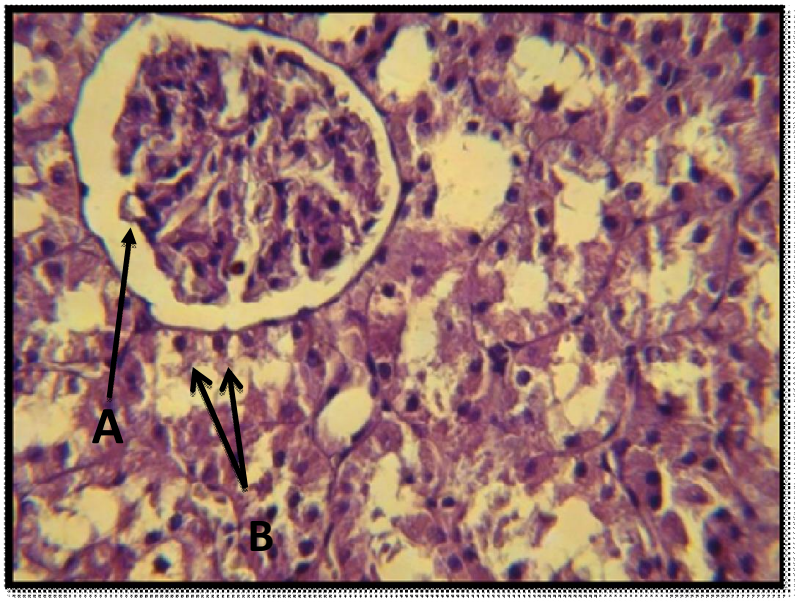

Figure (7): Transverse section through the Kidney of intermediate dose (ID) (H&E stain, 400X).
A. Vaculation of mesengial glomerular cells.
B. Vaculation of proximal convoluted tubules.

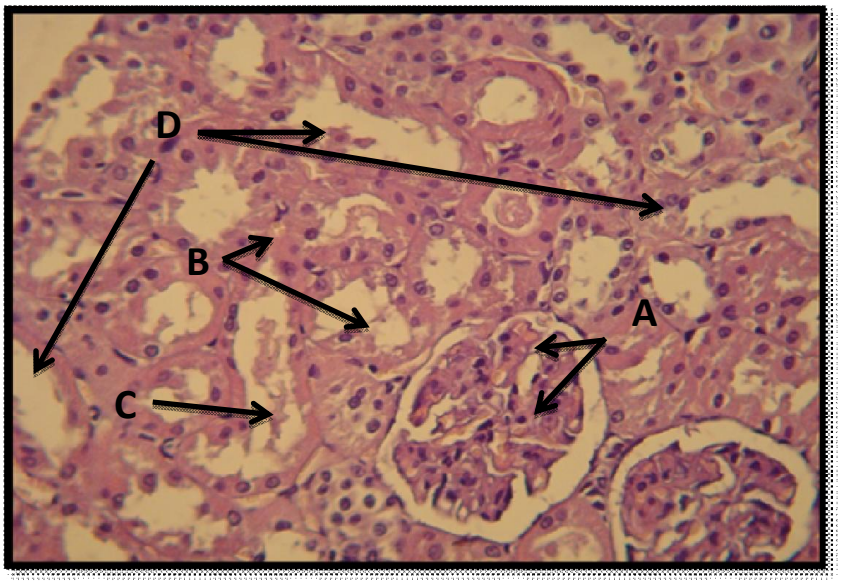

Figure (8): Transverse section through the Kidney of high dose group (HD) (H&E stain, 400X)
A. Vaculation of mesengial glomerular cells.
B. Vaculation of proximal convoluted tubules.
C. Necrosis of proximal convoluted tubules.
D. Dilatation of convoluted tubules.

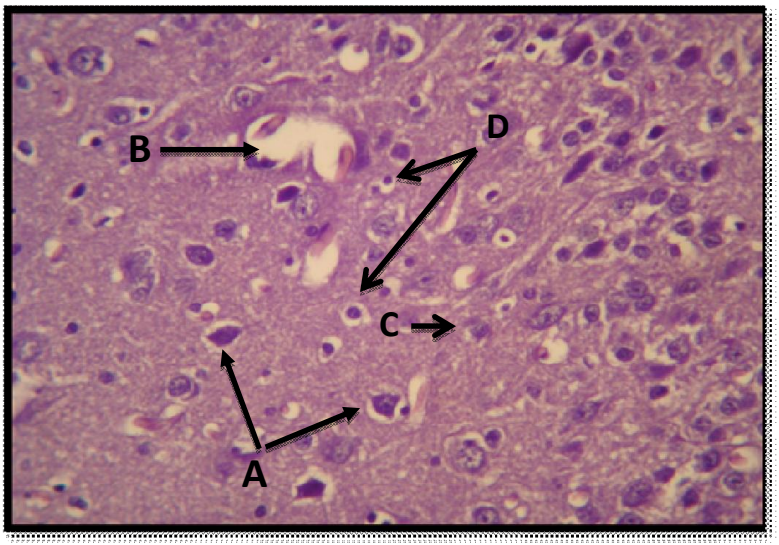

Figure (9):Transverse section through the brain of control group (H&E stain, 400X) A. Neurons. B. Axons. C. Astrocytes. D. Oligodendrocytes.

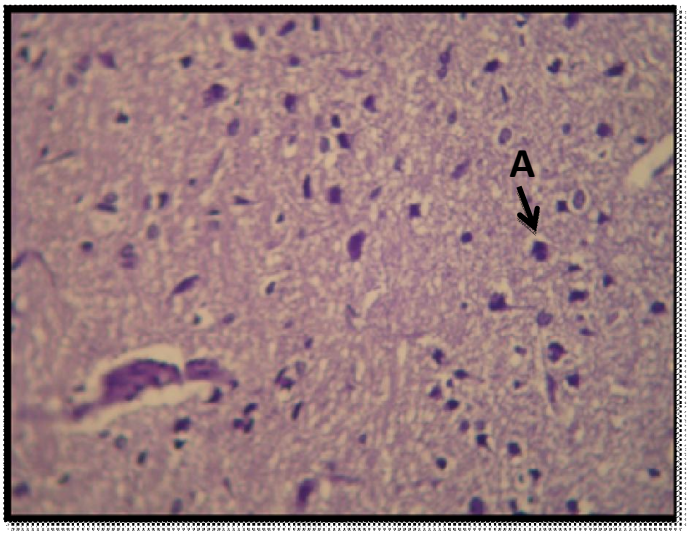

Figure (10): Transverse section through the Brain of low dose (L.D) group (H&E stain, 400X).

A. Vaculation of neurons.

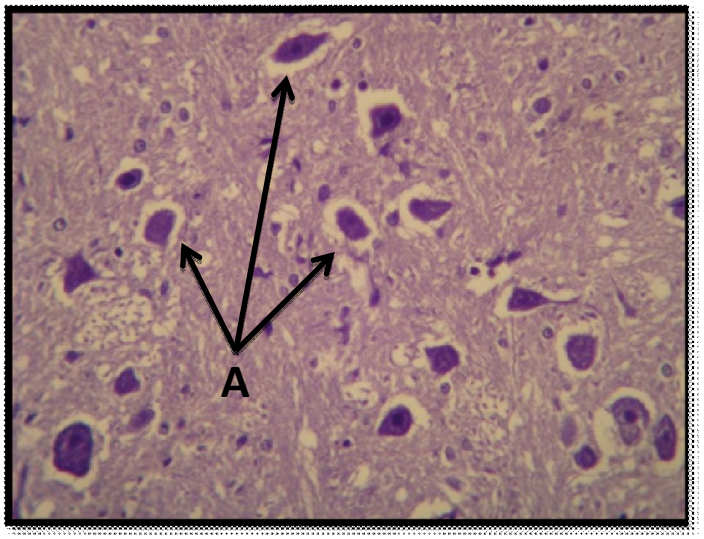

Figure (11): Transverse section through the Brain of Intermediate dose (I.D) group (H&E stain, 400X). A. Vaculation of neurons.

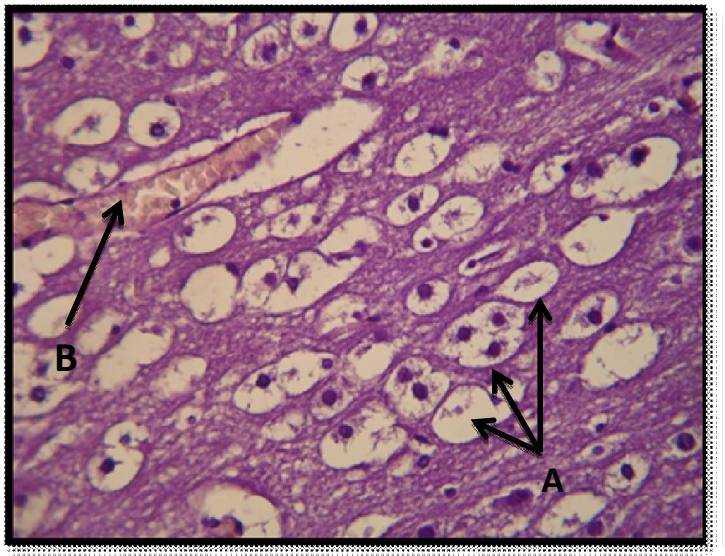

Figure (12): Transverse section through the Brain of high dose (H.D) group (H&E stain, 400X). A. Severe vaculation of neurons. B. Congested blood vessels (vein).

DISCUSSION

The statistical analysis study of codeine-paracetamol in animals body weight showed there was a significant ($P \leq 0.05$) decrease in body weight of high dose treated group (H.D) after (90) days of treatment as in table (1).These could be due to a compromised nutritional status of the rats consequent on gastrointestinal tract derangement, also the loss of appetite combined with drowsiness due to insufficient intake of opium could lead to weight lossas same with (17,18).

This present study is in agreement with (19,20) whomentioned that paracetamol treated rats had a significantbody weight loses ($P<0.05$) which had a greater weight loss of rats in (7.41%) when compared to the control animals, also the body weights decreased in rats exposed to (3.125) or (6.25) ppm of codeine and in mice exposed to (6.25) ppm of codeine were lower than those of the controls.

While this study was in disagreement with(21) who reported that there was no significant change in the mean body weight of all the paracetamol treated groups as compared with control group for (30 days).

The present histological study of liver of the low dose treated group (L.D) and intermediate dose treated group (I.D) revealed centrolobular enlargement of hepatocytes, septal fibrosis, vaculation of centrolobular and mid zonal hepatocytes as shown in figure (2) and (3), while the high dose treated group (H.D) revealed severecentrolobular necrosis, congestion and dilatation of sinusoids, early septal fibrosis, diffuse enlargement of hepatocytes as seen in figure (4).

These resultswere in agreement with (22) who reported that when performed a histopathological studies of rats administered paracetamol showed severe necrosis and disappearance of nuclei of liver,this could be due to the formation of highly reactive metabolites (e.g.N acetyl -p-benzoquinone imine-NAPQI), because of excessiveadministration of paracetamol, also when paracetamol administered at high doses cause liver damage characterized by nuclear pygnosis, leucocytic infiltration and disarrangement of portal vessels.

As a result, hepatocellular supplies of glutathione become exhausted and (NAPQI) is free to react with cellular membrane molecules, resulting in widespread hepatocytes damage and death, leading to acute hepatic necrosis, however, when large quantities of paracetamol are taken, there is an increase in the production of (NAPQI) that may cause severe liver and kidney damage(23). Also other experimental studies done by (24)have also supported toxic effects of chronic use ofopioids on liver and kidneys which showed sinusoidal dilatation and congestion of liver was observed in all rats treated with morphine, and hydropic degeneration(ballooning) was also observed in most of the rats in perivenular region (centrilobular region) as well as degeneration had proceeded to midzonal region in some rats, In addition to perivenular necrosis and hemorrhage and focal microvesicularsteatosis in liver**,** these results in agreement with our result.

The present study disagreed with (21) who reported there were no significant histopathologicalchanges observed in organs of the treated groups of rats and mice as compared tocontrol group.

The histological study of kidney of low dose (L.D) and (I.D) treated groups showedvaculation of proximal convoluted tubules and mesengial glomerular cells as shown in figures (6) and (7)respectively, while the high dose treated group (H.D) revealed necrosis of proximal convoluted tubules, vaculation of mesengial glomerular cells, vaculation and dilatation of proximal convoluted tubules as shown in figure (8).

Paracetamol induced acute renal damage is induced by the elevations in blood urea, uric acid and, urea and creatinine levels and occurrence of tubular necrosis histologically, these results as the same of (2) results.

The results of the present study is agreed with(20) which showed marked reduction in size of the glomeruli (hypoplasia) and apparently wide capsular spaces some of which lacked glomeruli in paracetamol treated animals; also our results agreed with (24) who documented the main histopathologicalchanges was vacuolization in tubular cells in the morphine treated rats, in addition to interstitial mononuclear cell infiltration was found in other rats with focal necrosis and hemorrhage.

The toxicityassociated with paracetamol also resulted in glomerularatrophy and necrosis of the tubules, similar to thoseobserved in acute tubular necrosis in both proximal anddistal parts of the tubules including damage to the glomeruli(25).

The histological study of brain of all treated group (L.D, I.D and H.D) revealed vaculation of neurons, vaculation of oligodendritic cells and vaculation of granular layer cells as shown in figure (10), (11) and (12).

(26)showed that morphine increased the number of apoptotic microglia and neurons ; also other work done by (27)showed that prolonged morphine administration increased rat spinal neuronal apoptosis, those results agreed with our result.

Our result which disagreed with(28) mentioned in his work in mice that treated with paracetamol reported no evidence of neurotoxicity of brain ; and in addition study worked by (29) mentioned that neurohistopathology assessment of brain demonstrated normal neuronal cells with no vascular or inflammatory changes after administration of (500 mg /kgbw) of paracetamol intraperitoneal to mice.

The statistical analysisof biochemicalstudy showed a significant ($P \leq 0.05$) increase in the levels of serum (TSB, AST, ALT, ALP and creatinine) of the treated group when compared with control group after (90) days of treatment as shown in table (2).

This is due to the fact that hepatic cells possess a variety of metabolic activities & contain a host of enzymes, when liver plasma membrane gets damaged, a variety of enzymes normally located in the cytosol are released into the circulation, these results also mentioned by(30).

Also, bilirubin is well known metabolic breakdown product of blood heme with great biological and diagnostic values (31).

Increased (ALT, AST and creatinine) could be attributed to nephrotoxicity and hepatotoxicity of morphine as showed in rat (32).

The present study is agreed with (33) who study the damage of liver due to paracetamol over dosage was confirmed by elevated levels of biochemical parameters

like (AST, ALT, ALP, Serum bilirubin) and agreed with (34) who reported that paracetamol treated rats showed significant (P≤0.05) an increase in the level of bilirubin when compared with control rats.

Also agreed with (35)who revealed AST, ALT were increased significantly in addicted patient of opioid at the end of study but we disagreed with their study when mentioned that ALP was significant lower in compare to control group.

Creatinine is an important biochemical parameter for diagnosis of renal impairment (36).

Our study agreed with (37)who observed that administration of paracetamol caused a pronounced increase in serum creatinine level when compared with the control.

(21)documented that no significant changes were seen in (AST, ALT, ALP activities, creatinine and bilirubin) in all the paracetamol treated rats as compared to control group, those results were disagreed with our results.

Increase in creatinin levels in rats receiving morphine can be considered as evidence of renal damage,morever, creatinin levels were found to be significantly higher in the morphine treated group compared to the control group(24), those results agreed with our results.

CONCLUSIONS

we concluded that the codeine-paracetamol toxicity lead a multiple changes in liver, kidney and brain of treated group like necrosis, vacuolation and congestion. While the statistical analysis of serum levels of liver and kidney enzymes showed a significant (p≤0.05) elevation

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دراسة نسجيه مرضية وكيموحيوية لتاثيرعقار الكودائين - باراسيتامول في ذكور الجرذان المختبرية

أسيل كامل حميد * ، عادل جبار حسين ** ، صالح كاظم مجيد ***

* فرع العلوم الاساسية ، كلية طب الاسنان، جامعة البصرة،البصره ،العراق.

** فرع الانسجة والتشريح، كلية الطب البيطري، جامعة البصرة ،البصره ،العراق.

*** فرع الامراض وامراض الدواجن ، كلية الطب البيطري ، جامعة البصرة،البصره ،العراق.

الخلاصة

أجريت هذه الدراسة على اربع وعشرون جرذا ذكر مختبري لمدة ٩٠ يوما حيث قسمت عشوائيا وبشكل متساوي الى اربع مجاميع، المجموعة الاولى جرعت بمحلول الفسلجي يوميا، المجموعة الثانية جرعت بعقار الكودائين – باراسيتامول بجرعة ٤٠ \ ٢٥٠٠ ملغم لكل كيلوغرام من وزن الجسم يوميا ، المجموعة الثالثة جرعت بعقار كودائين – باراسيتامول بجرعة ٨٠ \ ٥٠٠٠ ملغم لكل كيلوغرام من وزن الجسم يوميا ، المجموعة الرابعة جرعت بعقار كودائين – باراسيتامول بجرعة ١٦٠\ ١٠٠٠٠ ملغم لكل كيلو غرام يوميا. وبعد ٩٠ يوم من التجريع جميع الحيوانات تم التضحية بها لعمل الفحص النسيجي المرضي والفحص الكيموحيوي ، حيث اظهرت نتائج الدراسة الاحصائية ان التأثير السمي لعقار الكودائين – باراسيتامول هناك انخفاضا معنويا بمستوى وزن الجسم في المجاميع المعاملة.

اما الفحص النسيجي المرضي لكل من الكبد ، الكلية والدماغ اظهر تنخر مركزي في فصيص الكبد، توسع الجيبانيات ، تفجي للخلايا الكبدية و تليف حويجزي في الكبد؛ بينما الكلية اظهرت تفجي في الخلايا الميزنكية للكبيبة ، تفجي في النبيبات الكلوية الدانية و توسع في النبيبات الكلوية القشرية؛ اما الدماغ فأظهرت النتائج تفجي في الخلايا العصبية.

بينما اظهرت نتائج التحليل الاحصائي لأنزيمات وظائف الكبد والكلية وجود زيادة معنوية في

AST ,ALT , ALP , البيليروبين و الكرياتنين.

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