Protective Role of Pomegranate peel extract on the Pituitary gland of adult male rabbits treated with CCL4

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Abstract

This study was carried out to investigate the protective role of pomegranate peel extract for relieving the harmful effects of carbon tetrachloride on the pituitary gland function in adult male rabbits. Twenty four adult male rabbits were used in this study and divided randomly into four equal groups. Rabbits of the 1st group were received 1 ml of orally distal water once a day and olive oil 1/2 ml /kg B.W. intraperitoneal injection [I.P] twice a week as a control group. The second group were treated I.P with 500 mg / kg B.W. of CCL4 mixing with equal volume of olive oil (o.5 ml/kg B.W.) twice a week (group T1). The third group were received orally pomegranate peel extract (100 mg/kg B.W) once a day and olive oil 1/2 ml /kg B.W. I.P twice a week (group T2). The fourth group were received pomegranate peel extract (100 mg/kg B.W) once a day and treated I.P with 500 mg / kg B.W. of CCL4 mixing with equal volume of olive oil (o.5 ml/kg B.W.) twice a week (group T3). Blood samples were collected at on days (0, 14, 28.42 and 56) for measuring LH, FSH concentrations, total serum protein after treatment. Results showed a significant ($P<0.05$) decrease in total serum protein in T1 group compared with control group. While T2 group showed a significant ($P<0.05$) increase in these parameter as compared with the control and T1 group at the end of treatment period. T1 group indicated i a significant ($P<0.05$) increase in serum LH at(zero and 14 day) and FSH at(from zero time to the 28 day) . These levels, however, were significantly decreased in T1 group compared to what we observed in the control ones at the end of the experiment period. T2 group also showed a significant ($P<0.05$) increase in serum FSH at (24 day to the 56day of experiment) and LH concentration (at 56 day) compared to control group. Histological study was showed sever congestion of blood vessel and necrotic area in section of anterior pituitary gland in group treated with CCL4, while demonstrate moderate congestion in T3 group.

In conclusion, Pomegranate peel extract at dose (100 mg/kg B.W.) has positive effects on the pituitary gland function, hence eliminating the deleterious effects or toxic effect of CCL4.

Key words: Pomegranate peel extract, pituitary gland, CCL4.

الدور الوقائي لمستخلص لب الرمان على الغده النخاميه في ذكور ألارانب البالغه المعاملة برابع كلوريد الكاربون

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المستخلص

تهدف الدراسة الحالية الى معرفة الدور الوقائي لمستخلص لب الرمان في وظيفة الغدة النخاميه لذكور الارانب.استعملت اربعه وعشرون من ذكور الارانب البالغه قسمت بصوره عشوائية الى اربع مجاميع بالتساوي وعولجت لمدة 56 يوم كما يلي : أرانب المجموعة الاولى أعطيت 1 مل ماء مقطر يوميا و 2/1 مل/كغم وزن الجسم زيت الزيتون حقنا في البريتون مرتين في الاسبوع واعتبرت مجموعه سيطرة . أرانب المجموعة الثانيه اعطيت مستخلص لب الرمان 100ملغم /كغم وزن الجسم عن طريق الفم يوميا و 2/1 مل/كغم وزن الجسم زيت الزيتون حقنا في البريتون مرتين في الاسبوع (المجموعة الاولى) . أرانب المجموعة الثالثه اعطيت500 ملغم /كغم وزن الجسم من رابع كلوريد الكاربون مخلوطه مع كميه مساويه من زيت الزيتون حقنا في البريتون مرتين في الاسبوع (المجموعه الثانية).أرانب المجموعة الرابعة اعطيت مستخلص لب الرمان 100 ملغم / كغم من وزن الجسم يوميا عن طريق الفم اضافه الى 500ملغم /كغم وزن الجسم من رابع كلوريد الكاربون مخلوطه مع كميه مساويه من زيت الزيتون حقنا في البريتون مرتين في الاسبوع (المجموعة الثالثة). تم سحب الدم في الايام (56,42,28,14,0) لحساب البروتين الكلي في مصل الدم وتركيز الهرمون المحفز للجريبات (FSH) ،تركيزالهرمون اللوتيني (LH) في مصل الدم .

أظهرت نتائج الدراسة الحالية انخفاضا معنويا ($P<0.05$) في وزن الجسم و تركيز بروتين مصل الدم في حيوانات المجموعة (T1) مقارنة مع مجموعة السيطرة ، بينما المجموعة (T2) أظهرت ارتفاعا معنويا ($P<0.05$)في تلك المعايير مقارنة مع مجموعة السيطرة ومجموعه T1 في نهاية فترة المعالجة. كما إن نتائج المجموعةT1 اظهرت ارتفاعاً معنويا ($P<0.05$) في تركيز هرموني (FSH).للايان من (الصفر الى اليوم 14) وال LH من اليوم (الصفر الى اليوم 28) متبوعا بانخفاض معنويا في تلك الهرمونات في المجموعة مقارنةمع مجموعة السيطرة .كذلك اظهرت مجموعة T2 ارتفاعا معنويا ($P<0.05$) في مستوى LH وFSH مقارنة مع T1. في حين اظهرت مجموعة T3 انخفاضا معنويا في هذه الهرمونات في الاسابيع الاولى من المعالجة متبوع بارتفاع معنويا ($P<0.05$) .**كما** أظهرت الفحوصات النسيجيه حدوث احتقان شديد في الأوعية الدموية وتنخر في الغده النخاميه في المجموعه(T1) . هذا وقد شوهد حدوث احتقان خفيف في الغده النخامية(T3)

يستدل من نتائج هذه التجربة أن معاملة ذكور الأرانب البالغة لمدة 56 يوم بجرعة (100 ملغم/كغم من وزن الجسم) من مستخلص لب الرمان أدى إلى تحسين وتعزيز وظيفة الغده النخامية للأرانب ضد التأثيرات المؤذية لرباعي كلوريد الكاربون .

كلمات مفتاحية : مستخلص لب الرمان- الغده النخامية في ذكور الارانب - رباعي كلوريد الكاربون

Introduction:

Herbal medicine is the very important part of modern medicine; it is use plant also, plant extracts (35). Pomegranate peel extract, *Punica granatum. L*, was used as best source of antioxidant compounds like "ellagic acid,ellagitannins , pelargonidin,anthocyanidins,gallic acid, psuedopelletierine and isopelletierine (56)and(36). Pomegranate has become wide separation due to physiological properties, like anticancer, (1) (26) protective of cardiovascular system, (51) hypolipidemic (11). Also, many investigators (37) and (43) had documented that 'pomegranate have free radical scavengers and potent antioxidant effect .

In addition to pharmacological functions as 'hepatoprotective activity (33) and hypoglycemic activity (16) . Although, there is no enough evidence indicate the advantage or negative effect of pomegranate peel extract on male fertility (12) .

tetrachloromethane, also known as Carbon tetrachloride, was consider as colourless; nonflammable; heavy liquid with sweet - aromatic - nonirritating odor (19) and (27).It is used as an 'agricultural fumigant' and as a 'solvent' in the production of semiconductors; in the processing of fats; oil , rubber, and in laboratory applications (21). High concentrations of [ccl4] can affect the hepatocyte, central nervous systemand renal system (47). Chronic exposure to [ccl4] can cause liver, (31) kidney damage and cancer (24). CCl4 also, caused decrement in relative or absolute reproductive organ weights, sperm motility and sperm concentration.(48)

Materials and methods:

Twenty four adult male rabbit (Oryctolagus cuniculus) were obtained from local market of Baghdad, housed in cages and placed in room for two weeks for adaptation. Room temperature was maintained at (21 - 25˚c), air of the room was changed continuously by using ventilation vacuum and with light/dark cycle of 12:12 h/day, and were fed on freshly prepared ration pellet obtain from local market) along the experimental periods.

A 100 gm of pomegranate peel dry powder was taken and mixed with 50% ethyl alcohol then heated to (60 -70) Ċ for two hours by using Soxhelt extractor, the mixture was separated by centrifuging at 5000 rpm for 20 minutes. The supernatant solution was collected in a sterile container, this process was repeated (three times). After that the solution was collected in a sterile container (32). After the above process done, the ethyl-alcohol was removed from the solution by Rotary Evaporator. The final result of the extracted material was then kept,

The rabbits were divided randomly into four groups (six animals per group) and handled as follows: Control Group: Six adult male rabbits treated orally with distal water daily via Gavage needle and olive oil (1/2ml /Kg B.W. I.P) twice a week for 56 days. Group T1: Six adult male rabbits treated with (1/2ml /Kg B.W. I.P) of CCL4 and mixed with an equal volume of olive oil (1/2 ml / Kg B.W.) twice a week for 56 days (14). Group T2: Six adult male rabbits treated orally with 100 mg /Kg B.W. of pomegranate peel extract[PPE]daily via Gavage needle and with olive oil (1/2 ml /Kg B.W. I.P) twice a week for 56 days (54). Group T3: Six adult male rabbit treated orally with 100 mg /Kg B.W. of [PPE] daily via Gavage needle and (1/2 ml /Kg B.W. I.P of CCL4 mixed with an equal volume of olive oil (1/2 ml / Kg B.W.) twice a week for 56 days.

One ml of blood sample collected in vacuums test tubes were collected at Zero, 14, 28, 42 and 56 days of experiment via cardiac puncture technique. Serum collection separated from coagulated blood sample by centrifugation at 2500 rpm for 15 min. then kept in freezing conditions at -20 C̊ until used (3).

Body weight was measured by balance 'accuracy of the balance is 0.002 grams' before the beginning of experiment and after every 14 day of treatment period for all animals groups. The means of B.W. of each group were recorded in gram

Total serum protein, were measured according to Kit manufacturer's instructions "Bio system, S.A (spain)".

LH and FSH concentrations were measured according to Kit manufacturer's instructions "monobind Inc.(USA)".

After the end of experiment period, animals were killed and pituitary gland was obtained and weighted by sensitive balance after being cleaned from the connective and adipose tissues. The collected pituitary glands o were excised and preserved in 10% formalin buffer solution until preparation of histological section (10).

Results are expressed as mean+ SE. Statistical analysis of data was performed on the basis of Chi square (x2), two-way analysis of variance (ANOVA II), and group differences were determined using least significant difference (LSD) test at $P<0.05$ (9).

Results and Discussion

Table (1) showed a significant decrease ($P<0.05$) in body weight in T1 group when compared with the control and T2 group at day 56 of the experimental period. Also there was a significant elevation ($P<0.05$) in body weight in T2 group when compared with the control and T1 groups at the same experimental period. there were no significant ($P>0.05$) differences showed in T3 group as compared with the control group.

there was a significant (P<0.05) decrease in body weight in T1 group during the experiment as compared with zero time, While T2 group showed a significant (P<0.05) increase in B.W. at day 56 as compared with all other time of treated periods.

Changes in the body weight after CCl4 dosing have been used as a valuable index of CCl4-related organ damage (49) and (35). The exposure to CCL4 has been associated with alternated endocrine function by decrease in secretion of the pituitary gland hormones such as thyroxin stimulated hormones and decrease of T3 and T4 hormone (41). This hormone has a major role in regulation of body weight. CCL4 metabolites affect the permeability of mitochondria, endoplasmic reticulum, and plasma membranes, result in the loss of cellular calcium sequestration and homeostasis, which can contribute heavily to subsequent cell damage (24) and led to decrease in the body weight. The improvement of body weight at protective dose of PPE may be attributed to the biological function of PPE compounds mainly ellagic acid which reduce the oxidative stress and kept the cell function, improving growth and increase of body weight (53).The presence of hydrolysable tannin along with anthocyanins and ellagic acid exhibited good antioxidant capacity and effected scavenger for several reactive oxygen species (32)and (13).

Table (2) showed a significant (P< 0.05) decrease in the total serum protein values in T1 group as compared with the control group at 42, 56 days of the experimental periods, also showed a significant (P<0.05) decrease of total serum protein value in animals of T1 group as compared with T2 and T3 group during the experiment except at zero time.

On the other hand, the data showed a significant (P<0.05) elevation in the total serum protein value in animals of T2 group (PPE treated) as compared with control group at day 14 and 28 of the experimental periods. Also showed a significant (P<0.05) elevation in the total serum protein value of T2 group when compared with T1 group during the experiment except at zero time.

The results of T3 group showed a significant (P<0.05) elevation of total serum protein as compared with control group at day 14 and 28 of the experiment. Whereas,(T3 group) showed no significant (P>0.05) difference in total serum protein value when compared with T2 group at the experimental period. Within time, the significant (P<0.05) decreases in the total serum protein value showed in CCL4 treated animals (T1 group) when compared with the pretreated periods at day 42 and 56 of the experimental periods.

It is well known that the liver plays a pivotal role in the regulation of various chemicals. Also it’s the major site for protein synthesis (albumin and globulin except

'immuneglobin') (18). According to the above scientific fact, free radicals that may be generated from CCL4 administration may cause toxicity and liver dysfunction (28), which may be in turn, leading to inhibition of protein synthesis and decrement of total serum protein(40)and(23). Treatment of rabbit with pomegranate peel extract ameliorated toxic effect of CCL4 and restore level of total protein toward the control group, this may be due to hepatocyte protective action of polyphenol present in PPE against free radicals damage, protect cell membrane from lipoprotein oxidation and regulation of cell function (20) and(8). Also ellagic acid protects protein from catabolism by free radicals (4) , So that protein synthesis may be increased. The positive effect of ellagic acid on the thyroxin stimulating hormone and thyroid hormone synthesis (2) specially thyroxin hormone may also support the secretion of growth hormone from the pituitary gland , then protein synthesis might be also increased (6) and this cause an increase in body weight as shown in table (1).

Table (3) showed a significant ($P<0.05$) elevation in LH concentration in T1 group as compared with the control, T2 and T3 groups at 14 days of experiment, while showed a significant ($P<0.05$) decrement in LH concentration in T1 group as compared with all groups at days 28, 42 and 56 of experimental periods.

Result also showed no significant ($P> 0.05$) differences in LH concentration in T2 group as compared with the control and T3 group in all periods except day 56. Also showed a significant ($P<0.05$) decrease in LH concentration in T3 group as compared with T1 group at day 14 of experiment , whereas showed significant ($P<0.05$) elevation in LH concentration when compared with T1 group at days 28 ,42 and 56 of experimental periods.

CCL4 caused an increase in the level of LH during the first period perhaps due to its negative effects on testes and caused a reduction of testicular weight, reducing in the number of leydig cells, sertoli cells and diameter of seminiferous tubules, loss of the germinal line, diminutions in cellular proliferation, spermatogenesis and testosterone concentration decrease (30)and(50), So that hypothalamus-pituitary-gonadal axis might be responded to that by increasing secretion of LH to stimulate the testosterone and stimulate spermatogenesis.

In the fourth week until the end of the experimental period LH concentration was observed to be decreased in animals group treated with CCL4. This change may be due to free radical production and decrease anti-oxidative enzymes by CCL4 dosing which led to reduce LH concentration (5) On the other hand, it may be due to destructive effect on the cell of adenohypophesis.

The mean value of LH concentration in T3 group showed restore to the value in the control group. This result might be explained by the biological activity of ellagic

acid, hydrolyzable tannins and other components of pomegranate extract which act as free radical scavenger (7) remove the toxicity of CCL4 and return normal function of organs. As illustrated in histological section of adenohypophesis (figure 1-d). Furthermore [PPE], has positive effect on testicular tissue and elevate testosterone concentration (12).

Table (4) showed a significant (P< 0.05) increased of FSH concentration in T1group as compared with the control, T2 and T3 groups at 14 and 28 days of the experimental periods, while in days 42 and 56 of the experimental period showed a significant decrease (P<0.05) in FSH mean values when compared with control and other treated groups.

Asignificant (P<0.05) increase in mean value of FSH was found in T2 group when compared with the control group at days 28, 42, and 56 of the experimental periods. Result also clarified that there were significant (P<0.05) decrease in the mean value of FSH concentration in T3 group as compared with T1 at days 14 and 28 whereas showed significant (P< 0.05) elevation of FSH mean value in T3 group when compared with T1 group at days 42 and 56 of the experimental period.

A significant (P<0.05) elevation of FSH concentration in T1 group at 14 and 28 days of treated when compared with zero time. Also T1 group showed a significant (P<0.05) decrease in FSH concentration at 42 and 56 days when compared with other period of treated. Also within the time T2 group showed a significant (P<0.05) elevation in FSH mean value concentration during the experiment when compared with pretreated time and day 14, while T3 group showed no significant differences (P>0.05) within group.

The first 4 weeks of treatment with CCL4 (T1 group) showed elevation of FSH concentration result from gonadal dysfunction and testicular atrophy with decrease in testosterone concentration (17). Furthermore, alteration pituitary-gonadal system by CCL4 toxicity (15) leading to elevation of FSH secretion for support spermatogenesis.

While a decrease in FSH concentration in 42 and 56 days of treatment with CCL4 result from effect of CCL4 on the pituitary gland leading to histopathological change in the pituitary gland and decrease of FSH secretion. Also oxidative stress induced by carbon tetrachloride might cause reduction of FSH concentration **(50)and(45**).

Pomegranate peel extract showed an elevation level of FSH when administrated with CCL4 may be due to biological activity of ellagic acid , ellagitanin, gallic acid, punicalgina and anthocyanin are also hydrolysable tannin present in pomegranate peel extract (36) which has anti aromatase activity prevent conversion of testosterone

to estradiol (22) led to increase of testosterone concentration which influence with secretion of FSH from the pituitary gland by feedback mechanism (25).

Table (1): Effect of carbon tetrachloride CCL4 , pomegranate peel extract (PPE) and Pomegranate peel extract plus carbon tetrachloride (PPE+CCL4) on body weight (gm) in adult male rabbits. M ± S.E. (n = 6).

Group / Time	Control group	T1 Group CCL4	T2 Group PPE	T3 Group CCL4+PPE
Zero time	1516.60±33.50 **A a**	1568.30±37.60 **A a**	1475.00±41/20 **A b**	1550.00±48.30 **A a**
14 day	1508.30±35.30 **A a**	1441.60±47.50 **A b**	1516.70±55.90 **A b**	1525.00±48.10 **A a**
28 day	1516.70±38.20 **A a**	1448.70±33.50 **A b**	1508.30±72.60 **A b**	1533.30±44.30 **A a**
42 day	1500.00±38.90 **A a**	1433.30±33.50 **A b**	1500.00±25.90 **A b**	1508.30±43.79 **A a**
56 day	1483.30±42.30 **B a**	1358.30±30.20 **C b**	1625.00± 8.30 **A a**	1525.00±33.70 **AB a**

L SD =118.6

- T1: Animals received CCL4 (500mg/kg B.W). - T2: Animals received PPE (100 mg/kg B.W). - T3 Animal received PPE (100mg/kg) and (CCL4500 mg/kg B.W). - Small letters denote significant differences within group (P<0.05). - Capital letters denote significant differences between groups (P<0.05).LSD = Least significant difference.

Table (2): Effect of carbon tetrachloride CCL4, pomegranate peel extract (PPE) and pomegranate peel extract plus carbon tetrachloride (PPE+CCL4) on total serum protein (g/l) in adult male rabbits. M ± S.E. (n = 6).

Group / Time	Control group	T1 Group (CCL4)	T2 Group (PPE)	T3 Group (CCL4+PPE)
Zero time	48.66 ± 3.10 A a	50.16 ± 2.94 A a	52.00 ± 1.39 A a	50.83 ± 2.25 A a
14 day	49.83 ± 1.58 B a	47.83 ± 2.80 B a	57.33 ± 0.71 A a	53.50 ± 1.23 A a
28 day	47.82 ± 2.14 B a	47.33 ± 2.10 B ab	53.83 ± 2.50 A a	52.66 ± 0.72 A a
42 day	51.33 ± 2.52 A a	42.66 ± 1.70 B b	56.50 ± 2.30 A a	53.50 ± 2.70 A a
56 day	48.16 ±2.35 A a	38.16 ± 1.56 B b	52.11 ± 5.80 A a	48.50 ± 2.03 A a

L SD =5.8

- T1: Animals received CCL4 (500mg/kg B.W). - T2: Animals received PPE (100 mg/kg B.W). - T3 Animal received PPE (100mg/kg) and (CCL4500 mg/kg B.W). - Small letters denote significant differences within group (P<0.05). - Capital letters denote significant differences between groups (P<0.05). LSD = Least significant difference.

Table (3): Effect of carbon tetrachloride CCL4, pomegranate peel extract (PPE) and Pomegranate peel extract plus carbon tetrachloride (PPE+CCL4) on LH concentration (mIU/ml) in adult male rabbits. M ± S.E. (n = 6).

Group / Time	Control group	T1 Group (CCL4)	T2 Group (PPE)	T3 Group (CCL4+PPE)
Zero time	3.33 ± 0.40 A a	3.75 ±0.25 A b	3.56 ± 0.42 A b	3.45 ± 0.30 A a
14 day	3.36 ± 0.31 B a	4.38 ± 0.21 A a	3.21 ± 0.40 B b	3.81 ± 0.35 B a
28 day	3.53 ± 0.28 A a	2.05 ± 0.41 B c	3.61 ± 0.26 A b	3.81 ± 0.22 A a
42 day	3.66 ± 0.17 A a	1.39 ± 0.15 C c	4.36 ± 0.35 A a	3.83 ± 0.25 A a
56 day	3.90 ± 0.33 B a	1.46 ± 0.18 C c	4.95 ± 0.42 A a	3.71 ± 0.33 B a

L SD =0.86

- T1: Animals received CCL4 (500mg/kg B.W). - T2: Animals received PPE (100 mg/kg B.W). - T3 Animal received PPE (100mg/kg) and (CCL4500 mg/kg B.W). - Small letters denote significant differences within group (P<0.05). - Capital letters denote significant differences between groups (P<0.05). LSD = Least significant difference

Table (4): Effect of carbon tetrachloride CCL4, pomegranate peel extracts (PPE) and pomegranate peel extract plus carbon tetrachloride (PPE+CCL4) on FSH concentration (mIU/ml) in adult male rabbits. M ± S.E. (n = 6).

Group / Time	Control group	T1 Group (CCL4)	T2 Group (PPE)	T3 Group (CCL4+PPE)
Zero time	4.61 ± 0.41 A a	4.80 ± 0.34 A c	4.70 ± 0.75 A c	5.00 ± 0.29 A a
14 day	5.10 ± 1/25 B a	11.00 ± 0.31 A a	5.58 ± 0.45 B c	5.70 ± 0.27 B a
28 day	4.88 ± 0.26 C a	9.76 ± 0.49 A b	7.00 ± 1/29 B b	6.25 ± 1/26 B a
42 day	5.71 ± 1/27 B a	2.63 ± 0.46 C d	11.76 ± 0.93 A a	5.61 ± 0.29 B a
56 day	5.63 ± 0.73 B a	2.06 ± 0.35 C d	11.93±0.97 A a	5.30 ± 1/24 B a

L SD =1.45

- T1: Animals received CCL4 (500mg/kg B.W). - T2: Animals received PPE (100 mg/kg B.W). - T3 Animal received PPE (100mg/kg) and (CCL4500 mg/kg B.W). - Small letters denote significant differences within group (P<0.05). - Capital letters denote significant differences between groups (P<0.05). LSD = Least significant difference

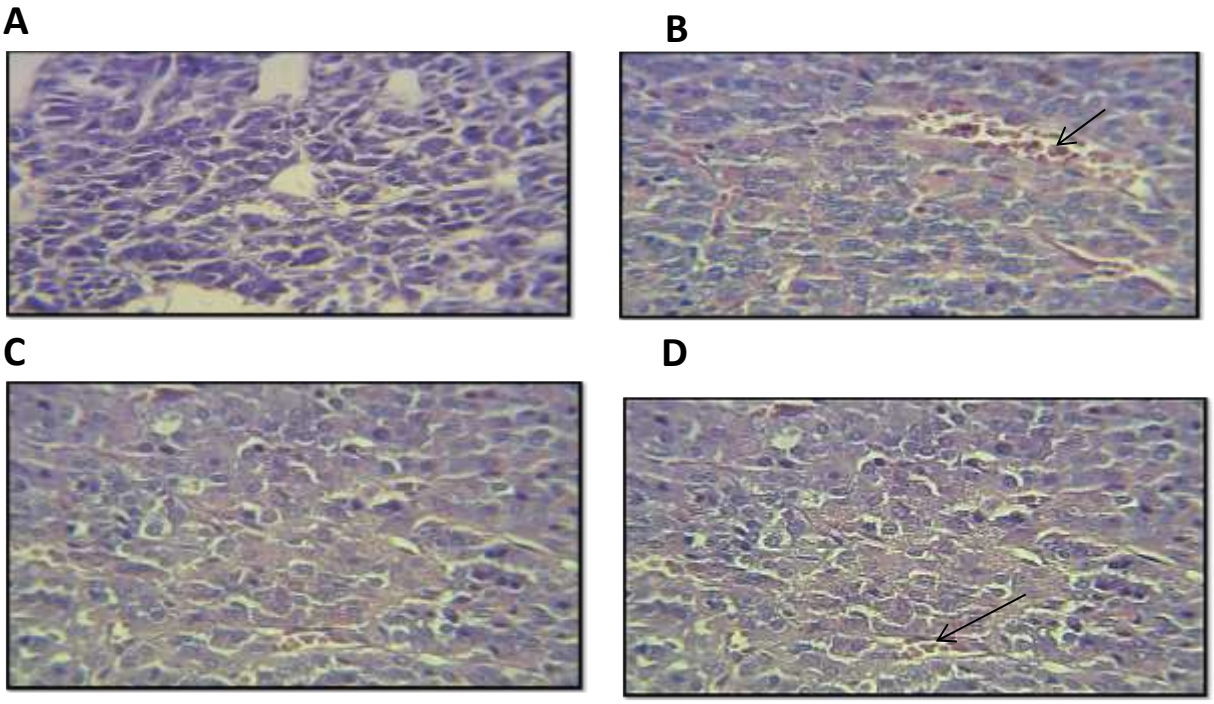

Figure 1: Histological examination of rabbit pituitary gland (A) control group. (B) T1 group treated with 500mg/kg.ccl4 mixed with 1/2 ml of 'olive oil'. (C)T2 group treated with 100mg/kg PPE daily with 1/2 ml of olive oil. (D) T3 group treated with 100mg/kg PPE plus 500mg/kg ccl4 mixed with 1/2 ml of olive oil. After 56 day of treated.

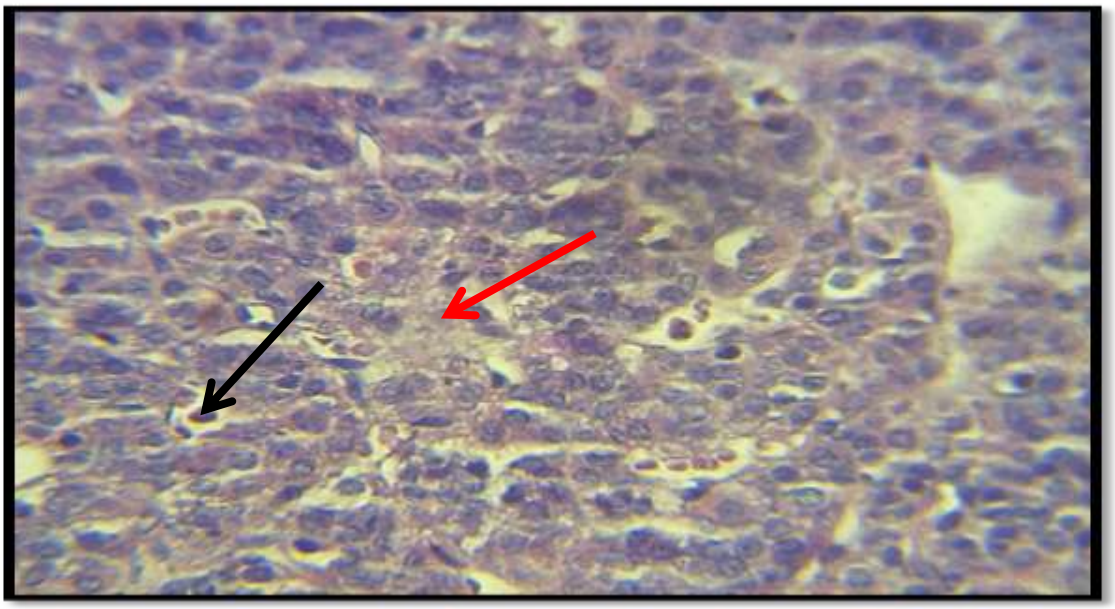

Figure 2: Histological examination of rabbit pituitary gland (T1 group) treated with 500mg/kg.ccl4 mixed with 1/2 ml of 'olive oil'. (⟶) refer to necrosis and (⟶) refer to neotrophile.

Microscopical examination of rabbits pituitary gland which treated with 500 mg /kg CCL4 showed sever congestion of blood vessel (Figure 1-B) with present of neutrophils in the lumen in addition to necrotic area (Figure 2), which characterized by disappear of nuclei . That lesion may result from free radical generated from CCL4 (44) and (39). Free radicals can bind with polyunsaturated fatty acid to produce alkoxy and proxy radicals, that in turn, generate lipid peroxides, that are highly reactive, change enzyme activity and finally induce injury or necrosis (24).

Whereas,microscopically examination of rabbit pituitary gland treated with 100 mg/kg of PPE showed normal structure of gland and no clear lesion in animals of this group (Figure 1-C). PPE possess bioactive ingredients which play important role in reduction of oxidative stress, therefore, the histological examination of rabbit pituitary gland treated with PPE + CCL4 showed moderate congestion of blood vessel without inflammatory cell. (Figure 1-D). Furthermore, Ellagic acid and other components which present in pomegranate peel extract have potent anti-inflammatory effect (34), also pomegranate peel extract inhibits irregular cell growth and induces apoptosis via modulation of proteins regulating apoptosis(46) and (29).

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