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The Protective Role of CoQ10 and DHEA and Their combination on CCl_4 Induced Liver Injury In Adult Male Rats (*Rattus norvegicus*)

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Abstract

This study was designed to evaluate the protective role of exogenous CoQ10 and DHEA and their combination on CCl4 induced hepatotoxicity in adult male rats. Thirty adult male rats 225-250 grams, 12-14 weeks old were used in this study and randomly divided into five equal groups, 6 animals each as in the following: Control group (G1): 6 male rats received orally DMSO 0.5ml/animal/day, First treated group (T1): 6 male rats received daily CCl4 1ml/kg (1:1 olive oil, IP), Second treated group (T2): 6 male rats received CCl4 1ml/kg and after 1hour injected daily with CoQ10 200 mg/kg IP, Third treated group (T3): 6 male rats received CCl4 1ml/kg and after 1hour injected daily with DHEA 25 mg/kg IP, Fourth treated group (T4): 6 male rats received CCl4 1ml/kg and after 1hour injected daily with a combination of CoQ10 200 mg/kg + DHEA 25 mg/kg IP. The experiment lasted for 28 successive days. The obtained results illustrated that male rats received CCl4 (1ml/kg) caused a significant increase in hepatic function enzymes AST, ALT, and ALP, as well as MDA levels, and caused a significant decrease in antioxidant enzyme activity GPx, SOD, and CAT levels. Also, CCl4 caused various degrees of liver damage such as dilation and congestion of the central vein with hemorrhage, clear fatty degeneration, and infiltration of inflammatory cells compared to the control group. Whereas, the group that treated with CoQ10 200 mg/kg and DHEA 25 mg/kg showed a significant decrease ($P< 0.05$) in serum AST, ALT, and ALP as well as MDA value, and a significant increased in GPx, SOD with declined in CAT levels compared to the group treated with CCl4 intoxication. It is also observed from the results that the combination of CoQ10 and DHEA caused a highly significant ($P < 0.05$) declined in AST, ALT, and ALP as well as MDA levels, and significant elevated in GPx, SOD, and declined in CAT, and almost return to normal level compared to control. As well, the histopathological examination on the liver revealed that rats treated with CoQ10 and

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DHEA and their combination had normal central vein and hepatocytes compared to groups treated with CCl4 due to anti-oxidant, anti-inflammatory, and anti-apoptotic properties. It has been concluded that CoQ10 and DHEA have an evident protective effect against liver damage induced by CCl4 through improving antioxidant enzyme activity in CCl4 treated group leading to a declined MDA level and reduced lipid peroxidation.

Keywords: CoQ10, DHEA, liver injury, CCl4, therapeutic antioxidant

1. Introduction

Coenzyme Q10 (CoQ10) is an endogenous substance act as a vital antioxidant proposed for cellular membrane integrity either by direct reaction with free radicals or by regeneration of other antioxidants (1). It is a lipid-soluble, vitamin-like substance required for the proper functioning of many organs and chemical reactions in the body (2). It has many beneficial effects in human and animal health including cardiovascular disease, age-related disorders, neuromuscular and neurodegenerative disorders, an autoimmune disorder, DNA damage, thyroid disorders, male infertility, cancers, diabetes, fibrosis, apoptosis, and obesity. It is a crucial redox and proton translocations constituent of the mitochondrial respiratory chain, play an essential role in mitochondrial energy production through redox activity in the electron transport chain, transporting electrons between enzymes. Thus, it plays an essential role in cellular bioenergetics and membrane stabilizer and the production of ATP in the oxidative respiration process (3). (4) demonstrated that CoQ10 has anti-inflammatory properties decreasing the production of pro-inflammatory cytokines such as interleukin (IL) and tumor necrosis factor (TNF-α).

Dehydroepiandrosterone (DHEA) is one of the most abundant endogenous circulating steroid hormone with multi-functional properties, it is produced in the adrenal glands, gonads, and brain, where it functions as a metabolic intermediate in the biosynthesis of androgen and estrogen sex steroids (5). It plays a critical endogenous antioxidant and pro-oxidant activity. It can also protect against lipid peroxidation (LPO) of cell membrane induced by oxidative damage (6). DHEA also has anti-inflammatory properties via suppression of pro-inflammatory cytokines secretion like IL and TNF-α and regulation of body immune response (7). DHEA and DHEAS are products of cholesterol metabolism with the first enzymatic reaction occurs in mitochondria and are resulting from the action of cytochrome P450 (8). Cholesterol transport across mitochondrial membranes requires the action of steroidogenic acute regulatory protein (STAR) and converts cholesterol to pregnenolone (9). This study aimed to evaluate the ameliorative effect of CoQ10 and DHEA and their combination on hepatotoxicity induced by CCl4 in adult male rats.

2. Material and methods

2.1. Drugs and chemical reagents

Norfloxacin was obtained Zwijndrecht-Holland, CoQ10 200 mg, and DHEA 25mg was obtained from (Sigma, St. Louis, MO, USA) and administered intraperitoneally. Dimethylsulphoxide (DMSO) were purchased from Merck, Darmstadt, Germany.

2.2. Experimental animals

Thirty adult male rats (*Rattus norvegicus*) weighing 225-250 grams, 12-14 weeks old. The rats were housed in the animal house of a college of veterinary medicine/university of Basrah. They were left for 2 weeks for an adaptation previous to the experiment. Each 6 animal was housed in an individual cage measured as15 × 35 × 50 cm and kept under normal temperature 22 - 28 °C and daily light period was 12 hours by use of two fluorescent

lamps, and the humidity rate was about 50 %. Animals were provided with water and diet *ad libitum.*

2.3. Experimental design and study strategy

After the acclimatization period, animals were randomly divided into five equal groups, 6 animals each as in the follows Control group (G1): 6 male rats received orally DMSO 0.5ml/animal/day, First treated group (T1): 6 male rats received daily CCl4 1ml/kg (1:1 olive oil, IP), Second treated group (T2): 6 male rats received CCl4 1ml/kg and after 1hour injected daily with CoQ10 200 mg/kg IP, Third treated group (T3): 6 male rats received CCl4 1ml/kg and after 1hour injected daily with DHEA 25 mg/kg IP, Fourth treated group (T4): 6 male rats received CCl4 1ml/kg and after 1hour injected daily with a combination of CoQ10 200 mg/kg + DHEA 25 mg/kg IP. The experiment lasted for 28 successive days. All animals of the study were sacrificed at end of the experiments. However, the rats before sacrifice were first weighed and then anesthetized by placing them in a closed beaker containing cotton sucked with chloroform for anesthesia. The abdominal cavity was opened up through a midline abdominal incision to take the samples. Blood samples were collected via cardiac puncture according to a method of (10). Then, blood samples were drops directly from the heart by using a 5 ml disposable syringe. The blood was put in-plane tube until it was coagulated, then centrifugated (3000 rpm for 15 minutes) to obtain the serum. The serum samples were separated into many Eppendorf tubes to avoid repeated thawing. All tubes were stored at (-4c) until they were analyzed.

2.4. Histopathological examination

Immediately were removed liver and kidney and separated from surrounding tissues and lipid, then weighed with an electronic balance. Sorted fragments of the liver were collected from all groups and prepared and fixed by using 10% formalin for histological examination according to (11) with aid of the light microscope. The samples were fixated in natural buffered formalin 10 % for 24-48 hours. They were put in the rotary microtome and were sliced by the microtome, steel blade into sections 5 micrometers thick, then sections were floated on a water bath (50-55^{o} C) and then transferred into glass slides coated with Mayers albumin as adhesive substance and left to dry. Histological sections of organs were stained with Hematoxylin-Eosin stain and photomicrographs were taken at 40X magnifications.

2.5. Statistical analysis

In this study, ANOVA Analysis and LSD tests are used according to (IBM SPSS, version 20) program at ($P \leq 0.05$) to find the means for all treatments (IBM SPSS, 2011).

3. Results

3.1. Biochemical assessment

The results in a table (1) demonstrate that male rats received CCl4 (1ml/kg) caused a significant increase ($P < 0.05$) in serum AST, ALT, and ALP levels compared to the control group. Whereas, the groups that treated with CoQ10 200 mg/kg and DHEA 25mg/kg showed a significant decrease ($P < 0.05$) in serum AST, ALT, and ALP level compared to groups treated with CCl4 intoxication, but they were still high significantly ($P < 0.05$) compared to control value. It is also clear from table (1) that the combination of CoQ10 and DHEA caused a highly significant decline in AST, ALT, and ALP levels and almost returns to its normal level compared with the control value. However, results in a table (2) pointed out that male rats who received CCl_4 showed a sharp significantly decrease ($P < 0.05$) in serum GPx and SOD value compared to the control group. Whereas, the groups that treated with CoQ10, DHEA, and a combination of CoQ10 and DHEA showed a significant increase ($P < 0.05$) in serum GPx and SOD levels compared to groups treated with CCl_4 intoxication, and they

seems they reached statistically to GPx and SOD normal value compared to the control value. In contrast, table (2) also showed that male rats who received CCl_4 showed significant elevation (P< 0.05) in serum CAT value compared to the control group. Whereas, the groups that were treated with CoQ10, DHEA, and combination of CoQ10 and DHEA showed a significant sharp decline (P< 0.05) in serum CAT values compared to groups treated with CCl_4, but they return statistically to CAT normal value compared to control value.

3.2. Lipid peroxidation assessment

According to the results obtained in table (2), serum MDA concentration increased significantly (P<0.05) in male rats that received CCl_4 compared to the control group. Whereas, the group that was treated with CoQ10, DHEA, and a combination of CoQ10 and DHEA showed a significant decrease (P< 0.05) in serum MDA value compared to the group treated with CCl_4, but they still high significantly (P< 0.05) compared with the control value.

Table (1): The effect of CCl4 and the protective role of CoQ10, DHEA, and their combination on liver function enzymes of male rats for 28 days of exposure. Mean ± SD

Parameters / Groups	AST (U/L)	ALT (U/L)	ALP (U/L)
Control	**57.21 ± 5.45 c**	**26.10 ± 2.75 c**	**112.4 ± 3.20 d**
CCl_4 1ml/kg	**92.73 ± 3.64 a**	**40.14 ± 3.06 a**	**163.2 ± 3.20 a**
CCl_4 1ml/kg + CoQ10 200 mg/kg	**73.72 ± 5.42 b**	**32.30 ± 3.38 b**	**134.1 ± 1.3 b**
CCl_4 1ml/kg + DHEA 25 mg/kg	**75.74 ± 5.40 b**	**35.43 ± 3.37 b**	**136.1 ± 1.2 b**
CCl_4 1ml/kg + Combination CoQ10 200 mg/kg + DHEA25 mg/kg	**63.76 ± 6.56 c**	**27.58 ± 3.39 c**	**122.1 ± 1.3 c**
LSD	**7.4**	**3.8**	**6.2**

Small letters mean significant differences between treatment at (P≤0.05)

Table (2): The effect of CCl4 and the protective role of CoQ10, DHEA, and their combination on antioxidant enzyme activities of male rats for 28 days of exposure. Mean± SD

Parameters / Groups	MDA (µm / L)	GPx (µm / L)	SOD (µm / L)	CAT (IU/ml)
Control	1. 68 ± 0.30 c	78.4 ± 2.23 b	28.60 ± 2.20 b	2.40 ± 0.50 b
CCl_4 1ml/kg	10.25 ± 1.20 a	39.2 ± 1.30 a	12.34 ± 0.26 a	4.00 ± 0.30 a
CCl_4 1ml/kg + CoQ10 200 mg/kg	6.51 ± 0.20 b	75.2 ± 2.23 b	28.80 ± 1.10 b	2.50 ± 0.60 b
CCl_4 1ml/kg + DHEA 25 mg/kg	6.55 ± 0.30 b	73.2 ± 2.25 b	28.56 ± 1.12 b	2.43 ± 0.52 b
CCl_4 1ml/kg + Combination CoQ10 200 mg/kg + DHEA25 mg/kg	5.43 ± 0.30 b	77.2 ± 2.33 b	30.30 ± 1.40 b	2.37 ± 0.50 b
LSD	2.25	5.23	1.26	0.43

Small letters mean significant differences between treatment at (P≤0.05)

3.3. Histopathological studies

The results in figure (2) pointed out the male rats that received CCl_4 dose (1mg/kg) caused a various degree of liver injury such as dilation and congestion of central vein with hemorrhage, clear fatty degeneration, and infiltration of inflammatory cells after 28 days of exposure compared to the control group (Figure 1). Whereas, the male rats that were treated with CoQ10, DHEA, and a combination of CoQ10 and DHEA showed normal central vein and hepatocytes compared to groups treated with CCl_4 intoxication (Figure 3, 4, and 5) respectively. It is also observed from figure (5) that the combination of CoQ10 and DHEA showed the best results with normal central vein and hepatocytes compared to control and other treated groups.

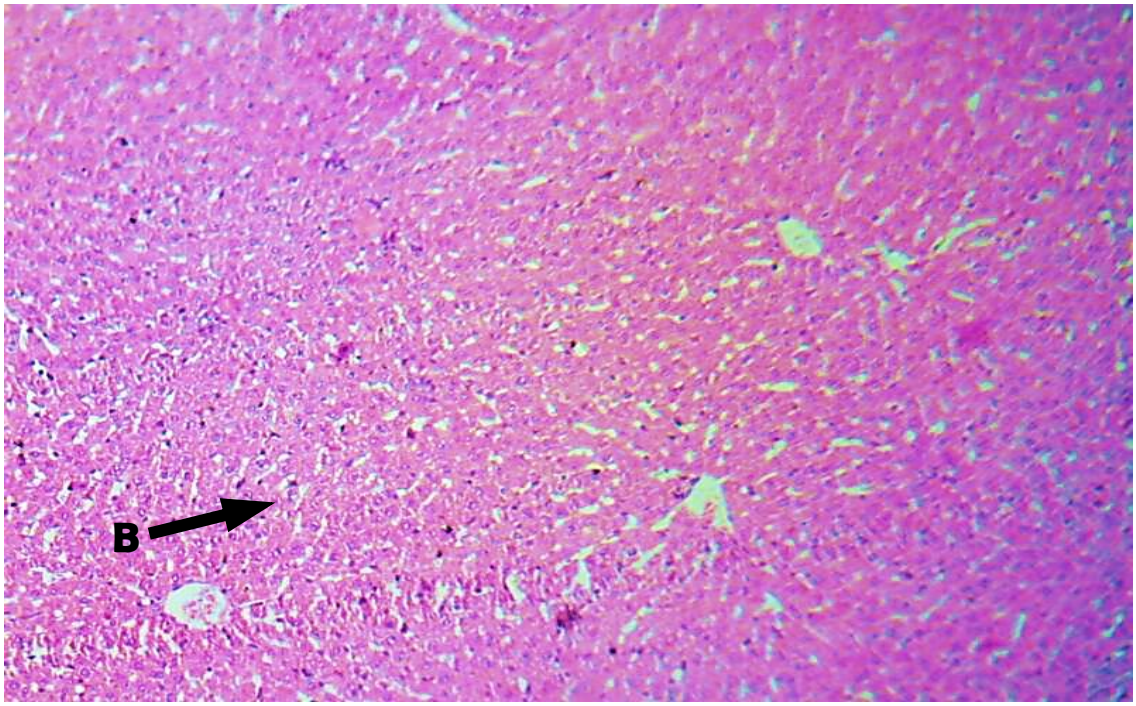

Figure (1). Liver of male rats treated with 0.5 DMSO as a control (H&E Stain, 100 X) observe normal central vein (A), and normal hepatocytes (B).

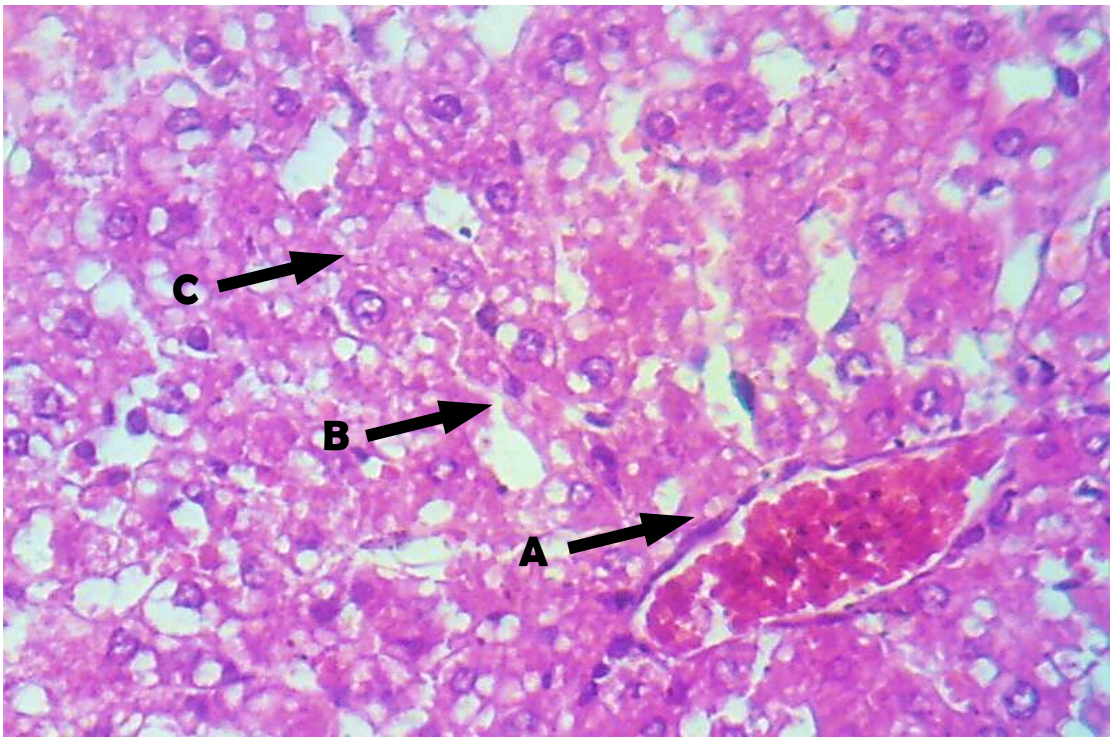

Figure (2). Liver of male rats treated with CCl4 (T1) for 28 days (H&E Stain, 400X) observe dilation and congestion of central vein (A), and clear fatty degeneration (B), infiltration of inflammatory cells (C).

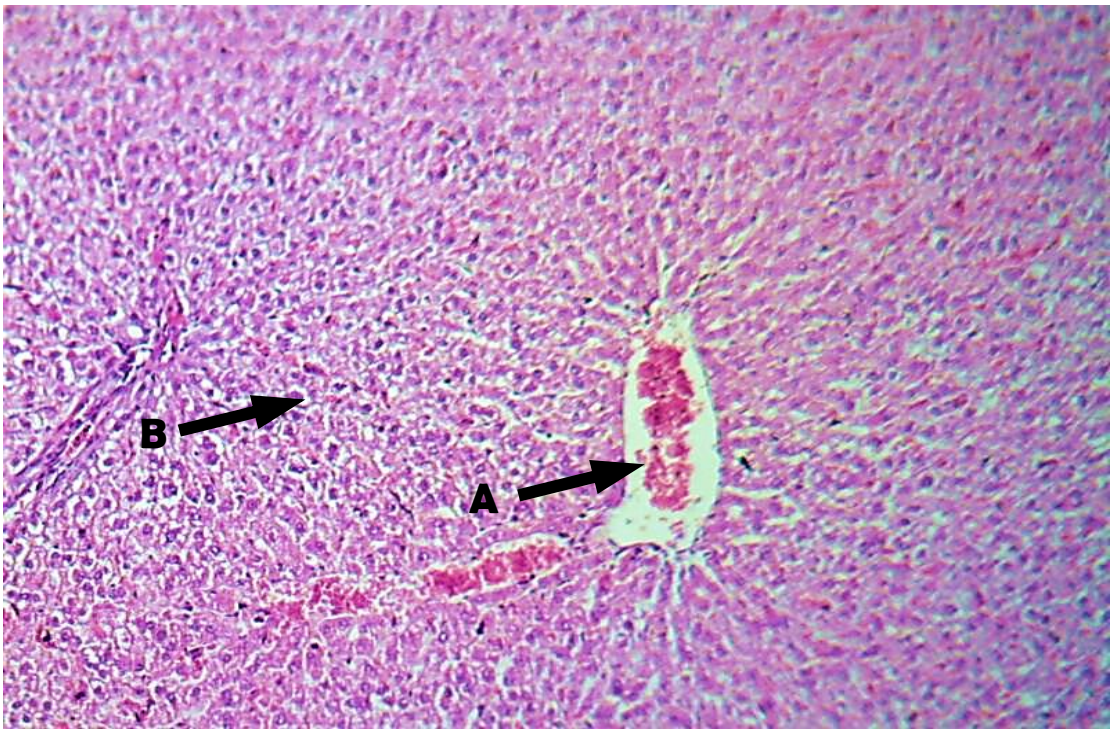

Figure (3): Liver of male rats treated with 1m/kg CCl4 + 200 mg/kg CoQ10 for 28 days (H&E stain, 100X) observe normal central vein (A), and normal hepatocytes (B).

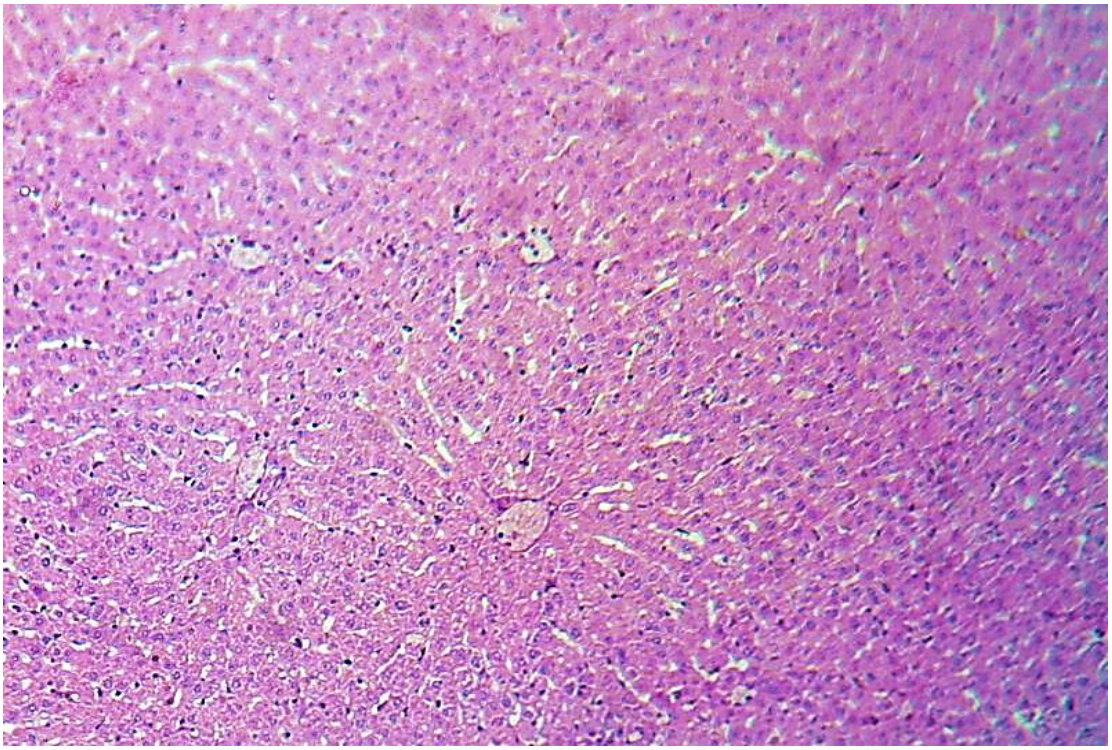

Figure (4): Liver of male rats treated with 1m/kg CCl4 + 25 mg/kg DHEA for 28 days (H&E stain, 100X) observe normal central vein (A), and normal hepatocytes (B).

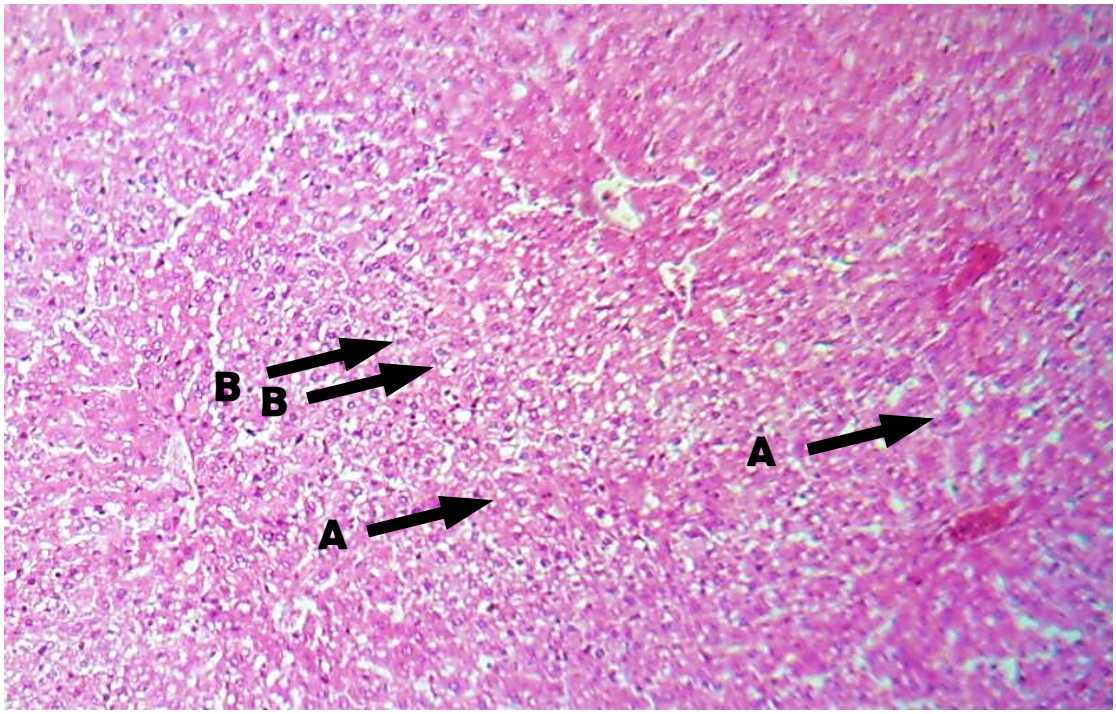

Figure (5). Liver of male rats treated with 1mg/kg CCl4 and combination of 200 mg/kg CoQ10 + 25 mg/kg DHEA for 28 days (H&E Stain, 100X) observe normal central vein (A), and normal hepatocytes (B).

4. Discussion

It is clear from the results that CCl4 intoxicated caused a significant increase in serum levels of liver function enzymes AST, ALT, and ALP activities in male rats may be due to increase serum levels of MDA in this study, where increased lipid peroxidation lead to depression in antioxidant and liver enzymes activity compared to control group. This pointed to detrimental results of CCl4 on the liver is evidenced by an increase of AST, ALT, and ALP, which definite indicator of liver injury. Also, (12) have reported damaged liver after streptozotocin treated, showed a decrease in the activity of serum AST, ALT, and ALP. The CCl4 hepatotoxicity is may be affected in two ways, firstly by the incidence of inflammatory condition, secondly by direct toxic action of CCl4 on liver cell. The utilize of CoQ10 significantly decreased levels of these enzymes compared to control and CCl4 intoxicated groups. Similar results were reported by (13), who established the role of CoQ10 in hepatotoxicity induced by sodium arsenite in male rats, also shows that hepatic enzymes are mainly responsive biomarkers directly concerned with the existence of cellular damage and toxicity due to they are located in the cytoplasm and are released into circulation after cellular damage. It has been known that the increase in AST, ALT, and ALP is a good indicator for impaired liver function. However, the hepatotoxicity induced by CCl4 is may be affected in two ways, firstly by the occurrence of inflammatory condition, secondly by direct toxic action of CCl4 on the liver cell through trichloromethyl- peroxyl free radicals lead to induce lipid peroxidation and destruction of Ca^{2+} homeostasis, finally results in cell death (14). Other authors have shown that CoQ10 can elevate the level of AST, ALT, and ALP after intoxication, this may be attributed to CoQ10 which stabilizes hepatocytes plasma membrane and prevent delivery of AST, ALT, and ALP to extracellular fluid (15). In contrast,

Co-administration of CoQ10 with CCl4 re-establish the AST, ALT, and ALP activity in male rats as a sign of protecting the effect of CoQ10 against liver injury induced by CCl4 intoxication. (16) reported that CoQ10 characterized the liver as a target organ through the distribution of CoQ10 into different rat tissues. This effect may be related to powerful CoQ10 effectiveness in suppressing increased lipid peroxidation and destruction of the hepatocyte membrane in regenerating liver cells. Furthermore, CoQ10 shows signs of defense against hepatotoxicity by lower levels of TBARs and alanine release leading to a decrease in hepatic enzyme activities (17). This is agreed with (18) who mentioned that CoQ10 has a prophylactic effect against aflatoxin-B1induced hepatotoxicity. Prophylactic effects of CoQ10 on metabolic stress by inhibition of apoptosis in hepatocytes (19). However, (20), (21), and (22) mentioned that CoQ10 protects against metabolic stress-induced hepatotoxicity induced by acute acetaminophen, mainly probably through its antioxidant, anti-inflammatory, anti-apoptosis effects. Moreover, CoQ10 shows the anti-inflammatory and antigenotoxic effect on 1,2-dimethylhydrazine (DMH) induced leukocytic DNA damage in blood cells by direct inhibition and modulating gene expression of cyclooxygenase-2 activity (COX-2) and inducible nitric oxide synthase (iNOS) in the colonic mucosa of male rats (23). In contrast, present results at a similar table show that supplementation of DHEA with CCl4 reduced serum levels of hepatic enzymes ALT, AST, and ALP activities compared to control and CCl4 intoxicated groups. This pointed to a potential protective effect of DHEA against liver injury in adult male rats. While it is stated previously the supplementation with DHEA protects against hepatotoxicity in rats, maybe through its antioxidant, anti-inflammatory, and anti-apoptosis effects (24). Similar results were given by (25). According to (26), the antioxidant role of DHEA may be associated with its active metabolites (DHEA-S), exposing cell membranes to more resistance to be attacked by ROS. Moreover, liver tissue characterized target organ for DHEA on considers circulation of DHEA into various rat tissues as reported previously by (27) after demonstrated of copper-induced oxidative lipid peroxidation. These results may be associated with the efficiency of DHEA in suppressing the elevated lipid peroxidation and damage of cell membrane in a restored liver cell of rats. The administration of CoQ10 by itself did not lead to a significant decrease in liver damage induced by CCl4 intoxication. It seems from the above table that the ameliorating effect of DHEA or may be attributed to their antioxidant properties that would reduce lipid peroxidation through protecting cellular GSH content.

It is also clear from the results that CCl4 caused a significant decrease in the antioxidant enzymes GPx, SOD, and CAT activity in male rats compared to the control group. These results showed that CCl4 causes clear changes of enzymatic GPx, SOD, and CAT constituent of antioxidant due to increased lipid peroxidation which led to depression in antioxidant enzyme activity. These results are agreed with (28) stated that SOD and CAT antioxidants enzymes act as protection agents from oxidative damage by scavenging of ROS. (29) who showed that the effect of CCl4 on the cellular antioxidant defense system is the second mechanism for CCl4 induced oxidative stress by changes in antioxidant activities by inhibiting function SH groups in the SOD, CAT, and GPx enzymes which normally protect against free radical toxicity. However, SOD is the first line of defense against the oxygen free radicals, catalyzes superoxide anion radical ($O2^{-\cdot}$) into less toxic H_2O_2 and O2, whereas CAT reduces

H_2O_2 to non-toxic H_2O and O_2. The CCl4 causes widespread oxidation leading to depletion of GSH and decreased GSH and GPx activity is led to elevate oxidative damage to DNA, lipids, and proteins. Generally, the decline of endogenous antioxidant by exposure to CCl4 could be because CCl4 has a very high affinity for glutathione therefore, exposure to CCl4 decreases GSH level due to either increased use of GSH by the cell to act as a scavenger of free radicals caused by a toxic chemical agent or enhanced utilization of GSH by GPX under oxidative stress and results in increase lipid peroxidation. Also, exposure to CCl4 decreases the generation of nitric oxide (NO), and inhibition of NO synthesis leads to a clear decrease in GSH synthesis through downregulation of the rate-limiting enzyme (30). On the other hand, CoQ10 supplementation significantly increases endogenous antioxidant enzymes GPX, SOD, and CAT which may be due to its direct free radical scavenging activity and decrease in lipid peroxidation as compared to CCl4 intoxicated groups. These results were in agreement with results obtained by (31), who stated that pretreatment with CoQ10 is related to preserving against isoproterenol-induced cardiac hypertrophy in rats heart through decrease myocardial injury by protecting endogenous antioxidant and decrease lipid peroxidation. Whereas, (32) concluded that CoQ10 is a powerful antioxidant either directly or indirectly against CCl4 toxicity through suppresses of oxidative stress and scavenges oxygen radicals, and inhibit lipid peroxidation and thus, increasing glutathione, GPX, SOD, and CAT activities. The current study results agreed with results obtained by many researchers such as (33) in their study on CoQ10 in an adult male with fructose-induced metabolic syndrome, (34) in adult male albino rats fed on a high cholesterol diet in the cerebellar cortex. Ameliorating effect of CoQ10 on an antioxidant enzyme in the present study may be attributed mainly to antioxidant action, which is known to supplemented GSH and antioxidant enzyme levels and scavenge lipid peroxides.

The results of CCl_4 caused a significant increase in serum malondialdehyde (MDA) levels. The study results came in agreement with the results of other articles done by others such as (35), (36), and (37) who studied intraperitoneally administration of CCl_4 and toxicity effects on lipid peroxidation of male rats. The CCl_4 metabolism in the liver results in the generation of reactive metabolites like trichloromethyl (CCl3·) and trichloromethyl peroxy (CCl3OO·) radicals which initiate membrane peroxidation of unsaturated fatty acids and cause fatty liver, fibrosis, and cell necrosis. (38) reported that the increase in oxidative stress and lipid peroxidation may be attributed to the depletion and reduction in hepatic GSH content which has an essential and protective role against oxidative stress. Therefore, CCl_4 begins the production of free radicals and induce lipid peroxidation in subcellular membrane structures and accumulation of ROS to inhibit the electron transfer respiratory chain in the mitochondria (39). However, (40) concluded that CCl4 induced oxidative stress may be attributed to CCl4 induce inflammation in tissue with a production of inflammatory mediators which in turn stimulates the generation of free radicals in tissue. It arises when hydroxyl radicals like oxygen react with membranes unsaturated lipids of resulting in lipid peroxide radicals (ROO•), lipid hydroperoxide (ROOH) generation, and disintegration generate such as malondialdehyde (41). Thus, increased levels of MDA in our study are a good indicator for oxidative stress and lipid peroxidation which is caused by CCl_4 intoxication. Co-administration of CoQ10 with CCl_4 resulted in a significant reduction in the malondialdehyde level. These results indicate

that CoQ10 has a powerful antioxidant activity as obviously by malondialdehyde reduction. The protective role of CoQ10 against CCl_4 induced oxidative stress may be attributed to its potent free radical scavenger activity and inhibit lipid peroxidation or may be attributed to regulation of oxidative phosphorylation and prevention of lipid peroxidation. Also, its ability to normalize endothelial function by combination both oxidative phosphorylation in mitochondria and endothelial nitric oxide synthesis activity (42). Furthermore, (43) and (44) demonstrate that CoQ10 decreased the production of ROS which changes redox balance in cells toward oxidative stress under inflammatory condition due to its anti-inflammatory and immunomodulatory action through suppression release and generation of pro-inflammatory cytokine from the cell and increase of anti-inflammatory cytokine mediators such as TNF-α and IL-10. Alternatively, the results also showed that DHEA supplementation reduced the oxidative stress and lipid peroxidation induced by CCl4 toxicity. This was detected by a potent antioxidant action by decreased MDA level. (45) demonstrate that reduction in the MDA concentration may be related to the antioxidant activity of DHEA to inhibit definite enzymes occupied in free radicals formation. Moreover, ameliorating effect of DHEA against oxidative stress may be attributed to antioxidant properties that would reduce lipid peroxidation through protecting cellular GSH content (46). The antioxidant role of DHEA may be associated with its active metabolites (DHEA-S), exposing cell membranes to more resistance to be attacked by ROS. However, DHEA inhibits NADPH level, which is a substrate essential for NADPH oxidase reaction to generate O•- from O• by inhibiting glucose-6-phosphate dehydrogenase (G-6-PDH). (47) demonstrate that DHEA exerted antioxidant and anti-inflammatory role by reducing tissue susceptibility to the oxidation of both lipid and protein and improving endothelial function which changes redox balance in cells toward oxidative stress under inflammatory condition due to it has anti-inflammatory and immune modulation properties throughout suppression release and generation of pro-inflammatory cytokine from cell and increase of anti-inflammatory cytokine mediators such as TNF-α and IL- 6 and IL-10.

It is clear from the obtained results that CCl4 toxicity caused various degrees of liver injury in treated male rats such as dilation and congestion of central vein, clear fatty degeneration of hepatocytes, and infiltration of inflammatory cells after 28 days of exposure compared to the control group. These results were following those obtained by many researchers such as (48); (49); (50); (51); (52), (53), (54), who studied intraperitoneally administration of CCl_4 induced liver damage and hepatotoxicity. The metabolism of CCl_4 in the liver results in the generation of reactive metabolites like trichloromethyl ($CCl3\cdot$) and trichloromethyl peroxy ($CCl3OO\cdot$) radicals which initiate membrane peroxidation of unsaturated fatty acids and cause fatty liver, fibrosis, and cell necrosis (55). Conversely, supplementation of CoQ10, DHEA, and combination of CoQ10 and DHEA caused reduced changes and restructure reliability with the normal architecture of the liver which may be attributed to anti-oxidant, anti-inflammatory, and anti-apoptotic activities compared to CCl4 intoxicated groups. The pretreatment with CoQ10 and DHEA improved of degeneration effect with inhibited inflammatory infiltration and nearly restored them to normal architecture, possibly due to prevented CCl4 conversion into its reactive metabolites like trichloromethyl ($CCl3\cdot$) and trichloromethyl peroxy ($CCl3OO\cdot$) radicals, reduced oxidative stress with scavenging its free radical, and protected hepatic antioxidant enzymes activities (56).

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