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Hepatoprotective Activity of *Plicosepalus acacia* Extract against Carbon Tetrachloride-Induced Hepatic Damage in Wistar Albino Rats

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ABSTRACT

The objective of this study was to investigate the hepatoprotective activity of methanolic extract of *Plicosepalus acacia* flowers against carbon tetrachloride (CCl_4) induced hepatotoxicity. *Plicosepalus acacia* flowers were collected, dried in shade and were ground to get a coarse powder and subjected to soxhlet successive extraction using methanol. The extract obtained was filtered and evaporated in a rotary evaporator to get a brown reddish semisolid residue. Preliminary phytochemical screening of the plant flowers by using preparative thin-layer chromatography (TLC) revealed the presence of flavonoids, Phenol, Tannins, Steroids, Amino acid, and Triterpene glycoside. The methanolic extract of *Plicosepalus acacia* flowers was evaluated for hepatoprotective activity against Carbon tetrachloride (CCl_4) induced hepatotoxicity. Using several groups of Wistar albino rats. The animals were divided randomly into five groups of six animals each. One Control group and other several treated groups animals by measuring the levels of *serum aspartate aminotransferase* (AST), *alanine aminotransferase* (ALT), and *alkaline phosphatase* (ALP). Furthermore, the hepatoprotective activity was confirmed by histopathological examination of liver tissues. The hepatoprotective activity of methanol extract of *Plicosepalus acacia* flowers shows lowered significantly in levels of AST, ALT, and ALP. These biochemical observations were in turn confirmed by histopathological examination of the liver section and are comparable with the standard hepatoprotective drug Silymarin (50mg/kg body weight P.O.) which served as a positive control.

INTRODUCTION

The liver is one of the largest organs in the human body that plays an astonishing array of vital functions in the maintenance, performance and regulating homeostasis of the body and chief site for intense metabolism and excretion **(1, 2).** The major functions of the liver are carbohydrate, protein and fat metabolism, detoxification, secretion of bile and storage of vitamin. Thus, to maintain a healthy liver is a crucial factor for overall health and wellbeing. **(3).** It is continuously and variedly exposed to environmental toxins and abused by poor drug habits, alcohol, prescribed and an over-the-counter drug which can eventually lead to various liver ailments like hepatitis, cirrhosis and alcoholic liver disease. **(2-4)** Liver damage is also associated with cellular necrosis, an increase in tissue lipid peroxidation and depletion in the tissue GSH levels. In addition serum levels of many biochemical markers like SGOT, SGPT, triglycerides, cholesterol, bilirubin, alkaline phosphatase are elevated. **(5-6)** Liver diseases have become worldwide and are associated with significant morbidity and mortality. The principal causative factors for the liver diseases in developed are excessive alcohol consumption and viral-induced chronic liver diseases while in the developing countries the most frequent causes are environmental toxins, parasitic disease, hepatitis B and C viruses, and hepatotoxic drugs (certain antibiotics, chemotherapeutic agents, high doses of paracetamol, carbon tetrachloride (CCL_4) and thioacetamide (tAA)**(7).** Management of liver diseases is still a challenge to modern medicine. The modern medicine offered maximum protection with little side effects. Nearly 150 phytoconstituents from 101 plants have been claimed to possess liver protecting activity. At the same time, surprisingly, we do not have readily available satisfactory plant drugs/formulation to treat severe liver disease. **(7)**

Therefore, many folk remedies from plant origin are tested for it's potential antioxidant and hepatoprotective liver damage in the experimental animal model. Carbon tetrachloride induced hepatotoxicity model is widely used for the study of hepatoprotective effects of drug and plant extracts. **(8, 9)** A large variety of methods are used for the treatment of liver diseases include pharmacotherapy, surgery as well as liver transplantation, all of which have shown limited therapeutic benefits and are associated with serious complications. **(10)**

There is a great demand to figure out an alternative approach for the treatment of liver diseases. The medicinal plants are considered the most recommended approaches for the treatment of liver disorders. **(11)** *Plicosepalus acacia* is one of this medicinal plants. its belong to the family Loranthaceae, which is the largest family that belong to the order Santalales, it is a parasitic plant which is generally known as " Enab Ala'mq – Murad "(in Arabic) and it is found in northeast Africa, Yemen, Jordan, and Saudi Arabia. In Yemen, it is widely distributed in Taiz and Suhban

valley, Ibb city. and It parasiting in different trees which growth in Yemen.

Plicosepalus acacia used in folk medicine to treat various diseases as smallpox, diarrhea and hookworms infections. Also, treatment of tonsillitis and otitis media were reported. **(12, 13)** In addition, it used for the treatment of diabetes mellitus and to enhance wound healing. **(14, 15)**

MATERIALS AND METHODS

Method of the traditional use: The plant's flowers are dried and blended to make a powder, the powder is mixed with honey and taken as one teaspoonful daily.

There are many studies regarding antioxidant and antimicrobial activities of *Plicosepalus acacia* plant but there is no any study about hepatoprotective of *Plicosepalus acacia* flower.

So the objective of this study was to investigate the hepatoprotective activity *of Plicosepalus acacia* flower against carbon tetrachloride induced liver toxicity.

Equipment:

Cobas c 311 analyzer, (Roche Diagnostics GmbH, D-68298 Mannheim, Germany), Rotafix 32A centrifuge (Andreas Hettich GmbH & Co. KG, D-78532 Tuttlingen,Germany), Light microscope (Carl Zeiss, Ser. Nr. 990746, Axiolab, Switzerland), Apx-100, Denver instruments electric balance(Serial No. 18203624, Germany).

Materials:

Plicosepalus acacia flowers Methanolic extract, Carbon tetrachloride (CCl_4) Conc. 98.9%, Silymarin drug (Mariagon capsules 140 mg, alpha chem. advanced pharmaceutical industries co, Cairo-Egypt), Formalin 10% buffer solution (Sigma Aldrich Co, UK.), Olives oil, Paraffine liquid (Milanero Chemicals Co, USA), Hematoxylin and Eosin dyes (Abbey Color Inc, Philadelphia, USA.

Animals:

The study was carried out using Wistar albino *rats* (100–150 g) of either sex procured from Sana'a University, Faculty of Science, Department of Life Sciences. The animals were kept in the clean barn (Animals house), grouped and housed in cages with not more than six animals per cage and maintained under standard conditions with 12 hrs natural light and dark cycle and were kept at room temperature (25±2°C). They were fed with standard diet and water. The experiment was

carried out in line with the guidelines of Sana'a University, Faculty of Science, Department of Life Sciences, for the use and care of experimental animals.

Methods:

Plant Collection and identification: The plant was collected from Suhban Ibb city, and it was identified by Department of Botany, Faculty of Agriculture, Sana'a University, Yemen.

Preparation of plant extract: The *Plicosepalus acacia* flowers were dried in shade and were ground to get a coarse powder and subjected to soxhlet successive extraction using methanol. The extracts obtained were filtered and evaporated in a rotary evaporator to get a brown reddish semi-solid residue **(16)**.

Selection of the model hepatotoxic agent: CCl_4 was used in this study as a hepatotoxic agent **(7, 16).**

Selection the route of administration of the model CCl_4: Intraperitoneal route of administration was selected for this model as it seems to be the most commonly used method for carbon tetrachloride administration **(17).**

Determination of dose of the extracts: The dose of extracts is 200mg/kg and 400mg/kg body weight. **(7, 18, 19).**

The dose of CCl_4**:** The dose of Carbon tetrachloride (CCl_4) is 1ml/kg body weight. 1 ml/kg CCl_4 dose was found to be optimum or lowest and suitable dose as it caused acute hepatitis**(16, 17, 20, 21).**

The dose of silymarin: The dose of silymarin is 50mg/kg body weight**(18, 22, 23, 24).**

Experimental design:

To assess the hepatoprotective activity of the plant extracts against CCL_4 induced hepatotoxicity the animals were divided randomly into five groups of six animals each as follow.

Group I: Control group, received water and food only for 5 days.

Group II: CCL_4 treated group, received water and food for 5 days followed by a single dose of CCl_4 (1ml/kg i.p.) on the 5th day with olive oil (1:1).

Group III: Standard group, treated with Silymarin (50 mg/kg p.o.) daily for 5days followed by

CCl_4(1ml/kg i. p. with olive oil (1:1) on the 5th day.

Group IV: Treated with *Plicosepalus acacia* extract (200 mg/kg p.o.) daily for 5 days followed by CCl_4(1ml/kg i. p. with olive oil (1:1) on the 5th day.

Group V: Treated with *Plicosepalus acacia* extract (400mg/kg p.o.) daily for 5 days followed by CCl_4(1ml/kg i. p. with olive oil (1:1) on the 5th day **(7, 16).**

Collection and processing of samples:

Animals were sacrificed on the 6th day under mild ether anesthesia. A blood sample from each animal was withdrawn by puncturing retro-orbital plexus and collected in previously labeled centrifuging tubes and allowed to clot for 30 min at room temperature. Serum was separated by centrifugation at 2000 rpm for 15 minutes. The separated serum was transferred into Eppendorf tubes and was analyzed by (Cobas C 311 analyzer, Roche Diagnostics GmbH, D-68298 Mannheim, Germany). for the estimation of various biochemical parameters **(16).**

Biochemical analysis:

The serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) were estimated **(25, 26).**

Histopathological studies:

A portion of the liver was cut into two to three pieces and fixed in 10% formalin. The paraffin sections were prepared and stained with hematoxylin and eosin. The thin sections of liver were made into permanent slides and examined under a high-resolution microscope with photographic facility and photomicrographs were taken **(16, 27)**.

Statistical analysis:

The data were expressed as mean ± SE. Results were analyzed statistically by one-way analysis of variance (ANOVA) followed by LSD test to compare the groups. p- values < 0.05 was considered significant.

RESULTS

Biochemical findings: As demonstrated in **the table (1)** and illustrated in **figure (1),** the serum level of AST was significantly elevated after treatment with CCl4 alone (839%), Silymarin +

CCl4 (423.3 %) and extract 400 +CCl4 (282.2%), as compared to control. On the other hand, the serum level of AST was significantly decreased in the groups received Silymarin + CCl4 (44.2 %), extract 200 + CCl4 (59.1 %) and extract 400 + CCl4 (47.6%), compared to group received CCl4 alone. But the concentration of AST still elevated compared to control except the group that receives extract 200 + CCl4.

As shown in **the table (2)** and illustrated in **figure (2)** the serum level of **ALT** was significantly elevated after treatment with CCl4 alone (773.5 %), silymarin + CCl4 (594.1%) and extract 400 +CCL4 (587.8 %), compared to control. Administration of extract 200+CCl4 leads to a significant decrease in the level of **ALT** compared to group received CCl4 alone.

As demonstrated in **the table (3)** and illustrated in **figure (3)** there are no changes in the level of ALP in all treated groups compared to control.

Table (1, 2, 3): shows the biochemical parameters AST, ALT and ALP levels and their change after treatment with CCl_4, silymarin + CCl_4, extract 200 + CCl_4 and Extract 400 + CCl_4 which compared to control group.

AST (U/ml)	
Groups	**Conc. ± SE**
A: Control	171.9±23
B: CCL4	1614.2±340*
C: Silymarin + CCL4	899.7±148*+
D: Extract 200 + CCL4	658.8±188+
E: Extract 400 + CCL4	844.7±104*+

Table (1): shows the blood concentration of AST (U/ ml) after treatment with CCL4, silymarin + CCL4, Extract 200 + CCL4 and Extract 400 + CCL4 compared to control.
Data are expressed as mean ± SE
*=Significantly different with respect to control (P<0.05)
+=Significantly different with respect to CCL4 treated group (P<0.05) Statistical analysis was carried out by one-way ANOVA followed by LSD test.

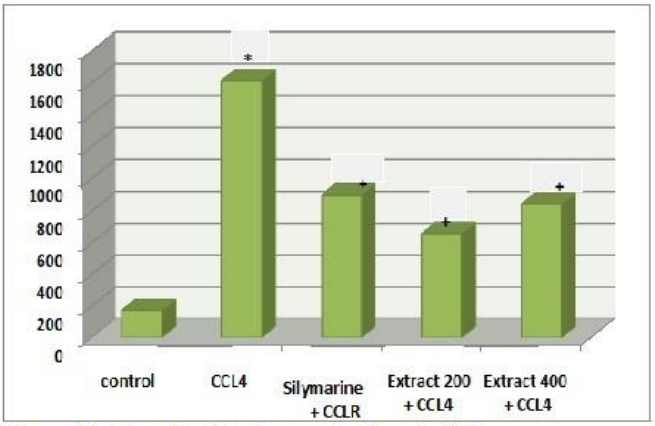

Figure (1): shows the blood concentration of AST (U/ml) after treatment with CCL_4, silymarin + CCL_4, Extract 200 + CCL_4 and extract 400 + CCL_4 compared to control.

*= Significantly different with respect to control (P<0.05)
= Significantly different with respect to CCL4 treated group (P?0.05) Statistical analysis was carried out by one-way ANOVA followed by LSD test.

ALT (U/ml)	
Groups	**Conc.±SE**
A: Control	83.3 ± 16
B: CCL4	727.7 ± 191*
C: Silymarin + CCL4	578.2 ± 123*
D: Extract 200 + CCL4	292.9 ± 60+
E: Extract 400 + CCL4	573 ± 126*

Table (2): Blood concentration of ALT (U/ml) after treatment with CCL4 , silymarin + CCL4 , Extract 200 + CCL4 and Extract 400 +CCL4 compared to control.

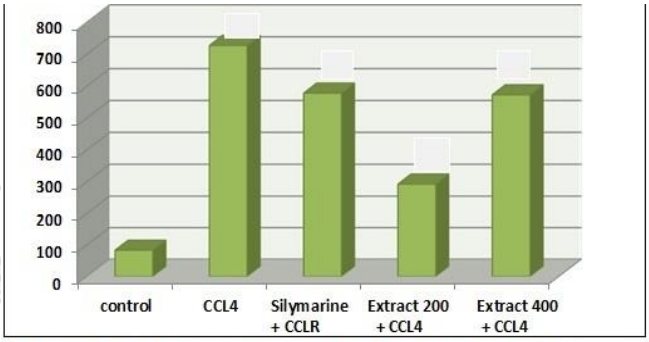

Figure (2): Blood concentration of ALT (U/ml) after treatment with CCL_4, silymarin + CCL_4, extract 200 + CCL_4 and extract 400 + CCL_4 compared to control.

ALP (U/ml)	
Groups	**Conc.±SE**
A: Control	648 ± 16
B: CCL4	897.8 ± 191
C: Silymarin + CCL4	883.4 ± 123
D: Extract 200 + CCL4	845.9 ± 60
E: Extract 400 + CCL4	632.3 ± 126

Table (3): Blood concentration of ALP (U/ ml) after treatment with CCL4, silymarine + CCL4, Extract 200 + CCL4 and Extract 400 + CCL4 compared to control.

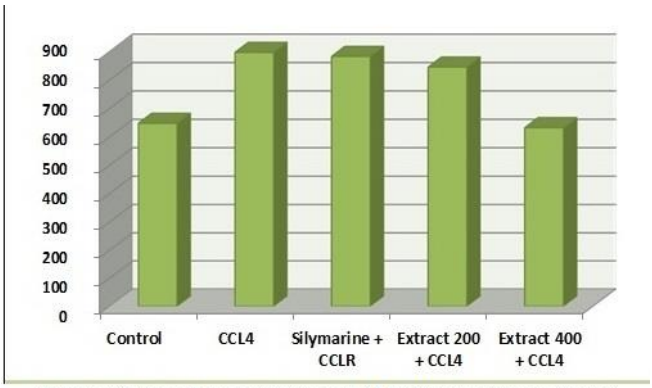

Figure (3): Blood concentration of ALP (U/ ml) after treatment with CCL4, silymarin + CCL4, Extract 200 + CCL4 and Extract 400 +CCL4 compared to control.

Histopathological findings:

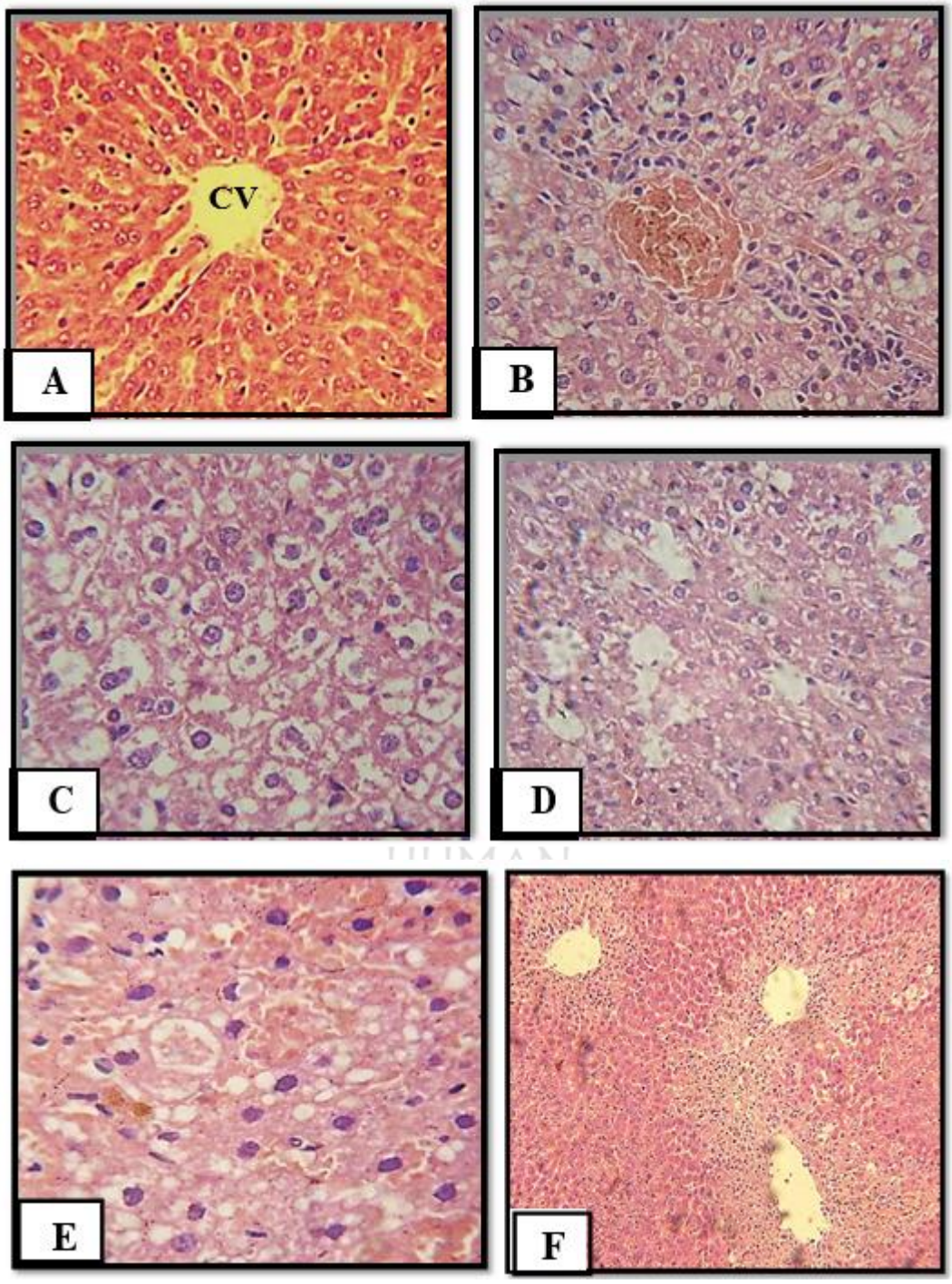

Figure (5): Photomicrograph of the liver section treated with CCL_4 (A): Control rat showing normal hepatocytes architecture with normal central vein (CV). (B): Congested central vein and Infiltration of mononuclear cells. (C): Hydropic swelling. (D): Malty foci. (E): liver hemorrhage. (F): Necrotic area.

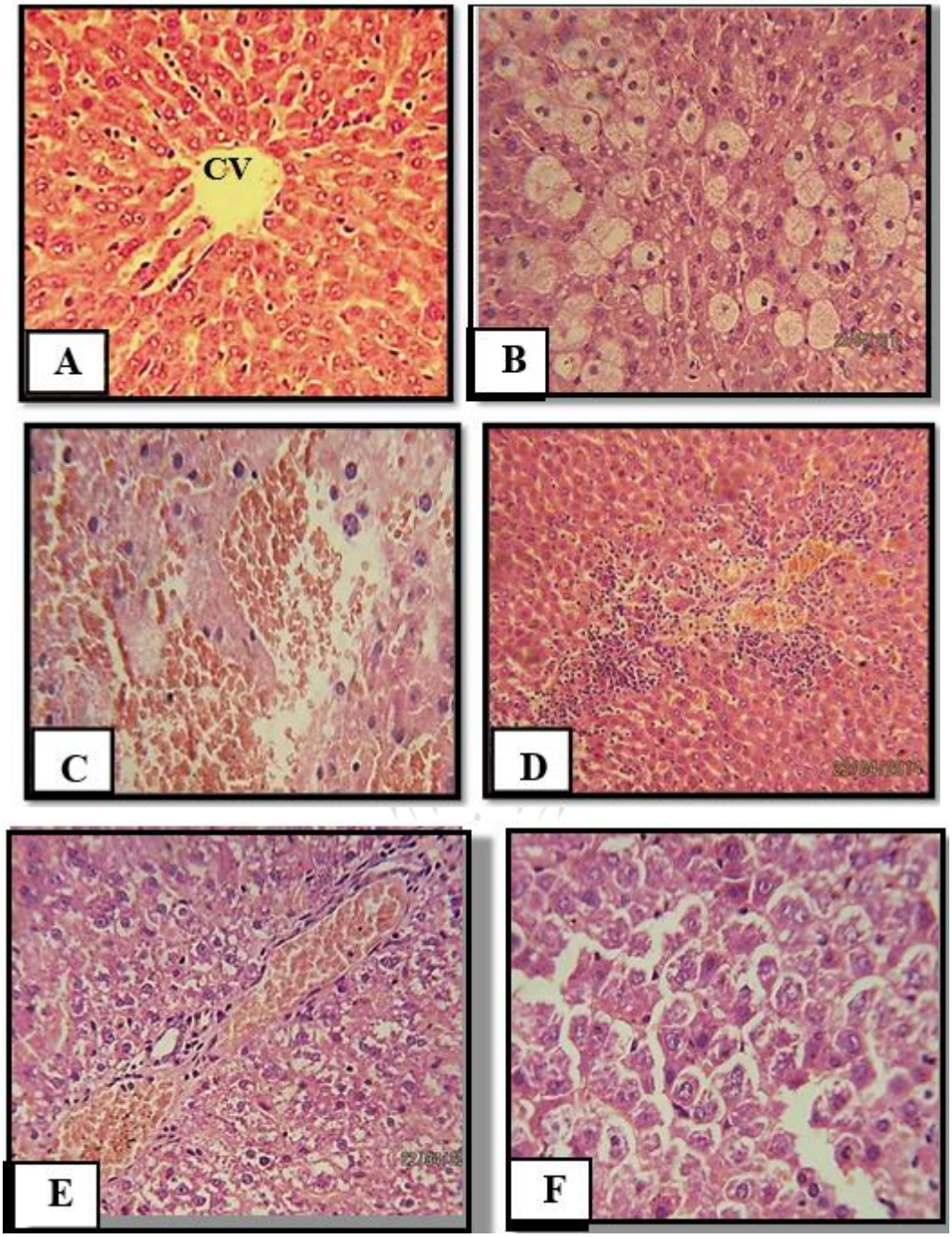

Figure (6): Photomicrograph of the liver section treated with *CCl_4* & Silymarin (A): Control rat showing normal hepatocytes architecture with normal central vein (CV). (B): Hydropic swelling. (C): liver hemorrhage. (D): Infiltration of mononuclear cells and congestion. (E): Congested central vein and Infiltration of mononuclear cells. (F): Hypertrophy.

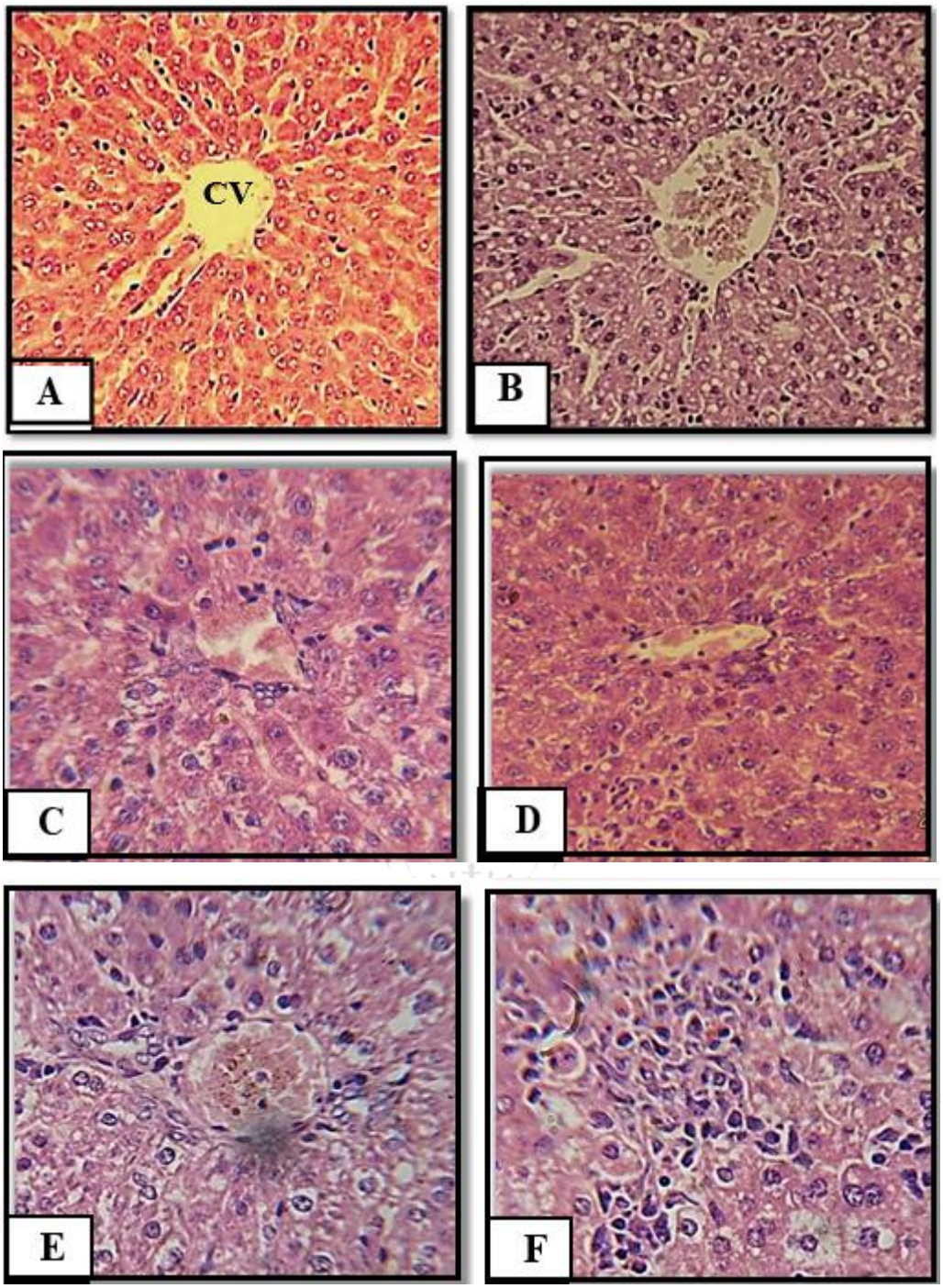

Figure (7): Photomicrograph of the liver section treated with *ccl4 & Plicosepalus acacia* (200mg) (A): Control rat showing normal hepatocytes architecture with normal central vein (CV). (B): Fatty changes. (C): Slight hydropic swelling. (D): Slight infiltration. (E): Congested central vein. (F): Inflammatory region.

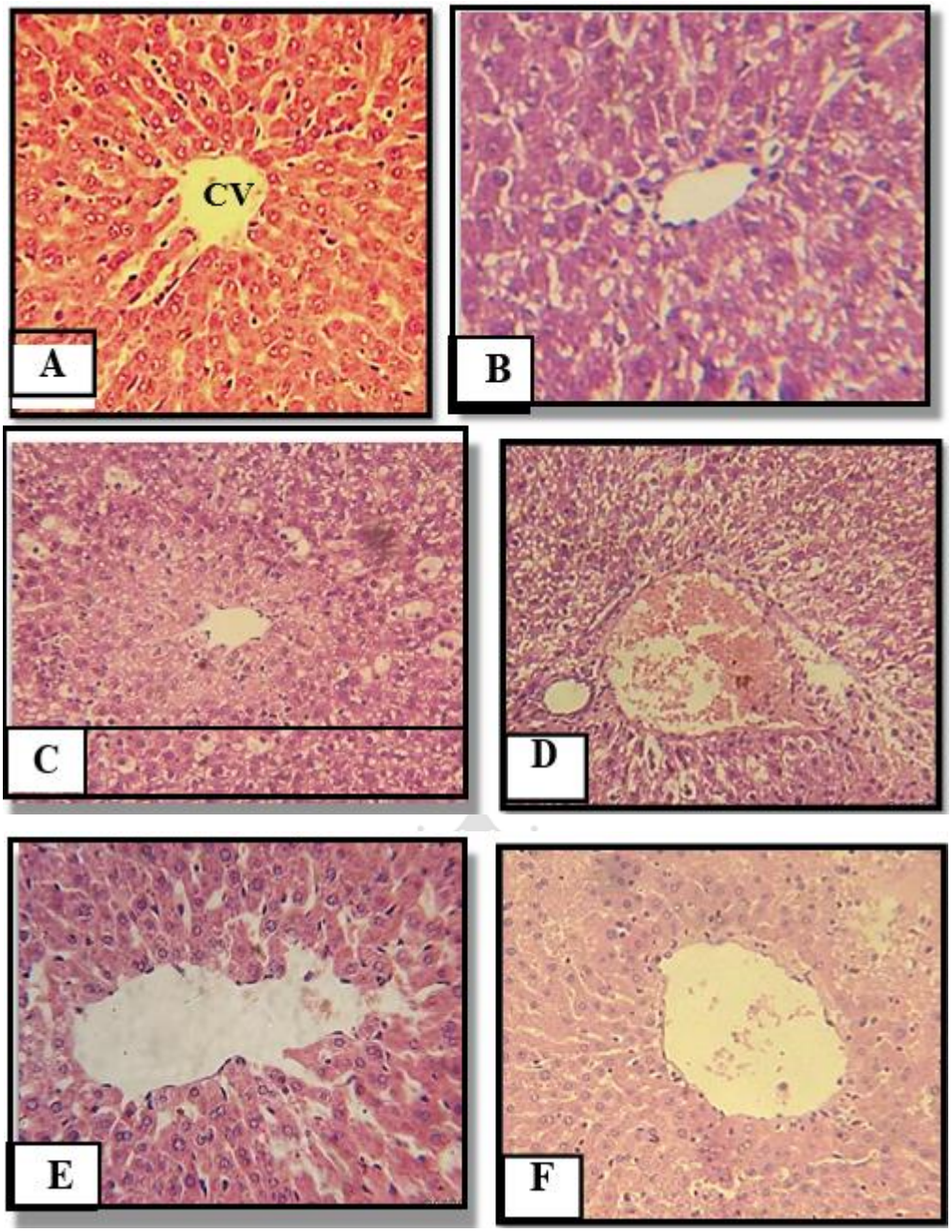

Figure (8): Photomicrograph of the liver section treated with ccl_4 & *Plicosepalus acacia* (400mg). (A): Control rat showing normal hepatocytes architecture with normal central vein (CV). (B): Recovering from Infiltration. (C): Recovering from Hydropic swelling and necrosis. (D): Slight congestion. (E): Recovering from fatty changes. (F): Recovering from hypertrophy.

DISCUSSION

The CCl_4 -induced hepatic injury is an experimental model widely used for hepatoprotective drugs screening.CCl_4 undergoes a biotransformation by hepatic microsomal cytochrome p450, to produce trichloromethyl free radicals (CCl_3), a free radical that binds to lipoprotein and leads to peroxidation of lipids of the endoplasmic reticulum which causes changes in the physical and chemical properties of cellular membranes or organelles leading to necrosis of hepatocytes **(28).**

It induces liver cell necrosis and apoptosis and can be used to induce hepatic fibrosis or cirrhosis by repetitive administration **(29).**

Most experiments involving the induction of liver injury by CCl_4 is usually accompanied by the elevation in the levels of liver enzyme markers (AST, ALT, and ALP). The elevated levels of these biochemical parameters are a direct reflection of alterations in the hepatic structural integrity **(30).**

As a result of hepatic injury, the altered permeability of the membrane causes the enzymes from the cells to be released into the circulation **(31).** The magnitude of hepatic damage is usually assessed by measuring the level of released cytosolic transaminases including ALT and AST in the circulation **(32).**

ALT is a cytoplasmic enzyme found in very high concentration in the liver and an increase of this specific enzyme indicates hepatocellular damage, while AST is less specific than ALT as an indicator of liver function **(33, 34).**

The rise in the serum levels of ALP, AST and ALT as observed in the present study could be attributed to the damaged structural integrity of the liver because these are cytoplasmic in location and are released into circulation after cellular damage **(35)**.

The flavonoids compound which is acting as an antioxidant is responsible for the hepatoprotective effect of the extract, as also approved by the earlier researchers **(36).**

The compounds that present in the total extract of *Plicosepalus acacia* have a high antioxidant activity such as flavonoids quercetin, ethyl gallate, gallic acid, loranthin, and rutin. Suggesting that the antioxidant capacity of the plant extract is due to a great extent to

its flavonoids content. Hence this plant can be considered as potential sources of bioactive compounds acting as a natural antioxidant.

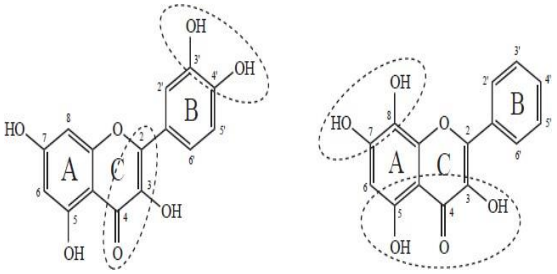

Figure (4). Structural features of flavonoids with high antioxidant activity

Mechanisms of the antioxidant action of flavonoids can include direct scavenging of reactive free radicals such as superoxide and hydroxyl, effectively "mop up" these damaging oxidizing species, chelating of trace metal ions involved in the free radical formation, inhibition of enzymes involved in free radical production, and regeneration of membrane-bound antioxidants such as tocopherol . Leading to hepatoprotective activity **(37).**

The antioxidant activity of these compounds is due to its structure which showing features important in defining the classical antioxidant potential of flavonoids **figure (4)**.

Quercetin, the structure of the flavonol quercetin Showing important features. The most important of these is the catechol or dihydroxylated B-ring.Other important features include the presence of unsaturation in the C- ring and the presence of a 4-oxo function in the C-ring. The catechol group and other functions may also ascribe an ability to chelate transition metal ions such as copper and iron (38).

Rutin, O-dihydroxy groups in the B-ring, the presence of a C 2-3 double bond in conjunction with 4-oxo in the C-ring, and 3- and 5- hydroxyl groups and the 4-oxo function in the A and C- ring are associated with antioxidant activity. **(39)**

Loratnthin, the structural feature responsible for the antioxidative and free radical scavenging activity of location is the hydroxyl groups**(39).**

Gallic acid and ethyl gallate the structural feature responsible for the antioxidative and free radical scavenging activity of gallic acid is the three hydroxyl groups **(40).**

Catching, the structural feature responsible for the antioxidative and free radical scavenging activity of catchine is the hydroxyl groups in B ring **(41).**

In the present study, the activities of enzymes AST, ALT, ALP as in **Table (1, 2, 3)** were found to increase in the hepatotoxic animals and were significantly reduced in groups of Methanolic extract of *Plicosepalus acacia* and silymarin administered rats as compared to that of toxicant *rats*.

Restoration of the levels of these enzymes to/towards near normal values. This confirms the protective effect of a Methanolic extract of *Plicosepalus acacia* against Carbon tetrachloride that induced hepatic damage.

The results of this study were confirmed by Histopathological observation. In contrast to the control group, **Group B** (CCl_4- intoxicant) showed Congested central vein and Infiltration of mononuclear cells, Hydropic swelling, Malty foci, liver hemorrhage and Necrotic area **figure (5**). But a Methanolic extract of *Plicosepalus acacia* (**Group D** and **E**) and Silymarin (**Group C**) administration for 5 days attenuated this Histopathological changes.

Liver showed fatty change and Slight hydropic change and infiltration in **Group C** and **D**, in **figure (6, 7)** and Recovering from Hydropic swelling, necrosis, hypertrophy and Recovering from fatty changes. in **Group E in figure (8).**

So the *rats* treated with Silymarin and extract along with toxicant showed sign of protection against these toxicants. The studied plant extracts contain antioxidants and hepatoprotective activity through regulator action on cellular permeability, stability and suppressing oxidative stress. Hence, it can be concluded that the possible mechanism of hepatoprotective activity of *Plicosepalus acacia* may be due to the reduction of oxidative stress and its ability to reduce elevated levels of serum marker enzymes or inhibition of Cytochrome P450 and to increase the activity of hepatic cells regeneration.

This might be due to the higher contents of flavonoids present in the extract which could have reduced the accumulation of toxic CCl_3 derived metabolites.

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