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The Hepato-Renal Protective Potential of Different Fractions of *Celtis occidentalis* against Paracetamol-Intoxicated Hepatotoxicity in Rabbits; In-Vitro Antioxidant Assay

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Abstract

The present study investigated the antioxidant, hepatoprotective, and nephroprotective effects of various fractions (n-hexane, chloroform, aqueous, and ethyl acetate) of *Celtis occidentalis* leaves against paracetamol-induced toxicity in male rabbits. The in vitro antioxidant activity was assessed using the DPPH radical scavenging assay, while in vivo protective effects were evaluated in ninety male rabbits divided into fifteen groups (n=6). Group 1 served as the control, and Group 2 received paracetamol (2 g/kg). Groups 3–14 received different fractions of *C. occidentalis* at doses of 100, 200, and 300 mg/kg along with paracetamol, while Group 15 received silymarin (50 mg/kg) as the standard. After 21 days, hematological, biochemical, and histopathological analyses were performed. Paracetamol administration caused significant alterations in hematological indices, liver and kidney function markers, lipid profile, and body weight. Treatment with *C. occidentalis* fractions significantly ameliorated these changes in a dose-dependent manner, with higher doses showing the most pronounced effects. Histopathological observations supported biochemical findings, confirming tissue recovery. Among all fractions, the chloroform and aqueous extracts exhibited the strongest antioxidant, hepatoprotective, and nephroprotective activities, validating the therapeutic potential of *Celtis occidentalis* leaves.

Keywords: *Celtis occidentalis*, Fractions Antioxidant, Hepato-renal Histopathology

1.0 INTRODUCTION

There are multiple studies which have demonstrated the involvement of free radical in the development of degenerative diseases. The most important free radical is oxygen reactive species which is the derivative of oxygen. Reactive nitrogen species and reactive chlorine species also required oxygen for their production [1-3]. In the reaction with other molecules the free radical capture electron to attain stability which can trigger a chain of reaction leading to lipid peroxidation and results in cell membrane disruption. The over production of reactive oxygen species leading to many pathophysiological conditions like inflammation, genotoxicity, diabetes,

ischemic-reperfusion injury, cirrhosis and cancer etc. [4, 5]. Antioxidant plays a crucial role in preventing and mitigating chronic diseases by reducing the damages to cellular components by reactive oxygen species. Antioxidants of both natural and synthetic origin have a wide range of use in the treatment of different types of human diseases [6]. Liver is the main organ in the biotransformation of drugs and biological toxins [7]. Hepatitis is linked with oxidative stress and redox imbalance [8]. At normal dose the paracetamol metabolism occurs through conjugation with glutathione but in over dose the levels of glutathione are not enough to detoxify it, so the N-acetyl-p-benzoquinone form covalent bond with SH groups in structural proteins which form protein adduct causing hepatocyte cell death, oxidative stress, mitochondrial injury, multi-organ death and may be patient death [9]. The consumption of paracetamol may result in abnormal liver function [10] and may lead to acute liver failure [11]. Paracetamol is reported to have adverse effects on liver and kidney causing hepato and renal necrosis [12]. Paracetamol induce kidney toxicity in different ways including cytochrome P-450 pathway, prostaglandin synthetase and N-deacetylase enzyme. Kidney injury might be occurred due to the inhibition of paracetamol metabolism, acetaminophen-glutathione conjugate or may be one of the metabolites of acetaminophen. An enzyme called prostaglandin endo-peroxidase synthetase found in kidney that convert paracetamol into toxic metabolite which may cause kidney damage. N-deacetylase acts on paracetamol, de-acetylating its substrate to p-aminophenol which than may be converted to free radical and bind to cellular protein and cause injury [13].

Celtis occidentalis commonly known as American Hackberry is a deciduous tree native to North America [14] and its distribution range from Manitoba and Quebec southward to the central eastern United State [15, 16]. Native American used the bark of *Celtis occidentalis* plant as a Phytomedicine for treating sore throat and aid during menstruation while the wood extract of this plant was used for the treatment of jaundice [17-20].

Based on its traditional use different fractions of methanolic extract of *Celtis occidentalis* leaves were prepared using solvents namely: n-hexane, chloroform, ethyl acetate and aqueous with increasing polarity to investigate antioxidant activities using in Vitro DPPH scavenging assay, and hepatoprotective and nephroprotective activities in vivo in paracetamol-intoxicated rabbits.

2.0 Materials and methods

2.1 Plant materials

The leaves of Celtis occidentalis was collected from Lal Qila District Dir Lower, as already used as hepatoprotective and renoprotective agent [21, 19].

2.2 Chemicals and Kits used in the experiment

2,2-diphenyl-1-picrylhydrazyl (DPPH) (Sigma-Aldrich), Ascorbic acid (Abbott), Methanol (Merck), n-hexane (Sigma-Aldrich), Chloroform (Merck), Distilled water, Ethyl acetate (Sigma-Aldrich), Formalin (Merck), Paracetamol (GSK), Ether (Merck), Silymarin (Mendoza), normal saline, Eosin (Merck), Hematoxylin (Merck), Paraffin wax ((Merck), Xylol (Merck), assay kits for liver and kidney chemistry.

2.3 Preparation of plant extract and fractions

The plant materials were dried at room temperature and then cut into small pieces and powders form through electric grinder. Three kilograms of dried powders were soaked in 10 liters of 95% methanol for one month for maximum extractions. After that the extract was filtered

using Whatman filter paper and then shifted to flask for evaporation. The solvent was evaporated using rotary evaporator at reduced pressure at 45°C for concentration of extract. The moist and concentrated extract was then kept in water bath for obtaining crude extract. The crude extract was kept in refrigerator and then successively fractioned using n-hexane, chloroform, distilled water and ethyl acetate. First the crude extract was suspended in 400 ml distilled water and the suspension was shaken in separating funnel by adding 250 ml of n-hexane three time in separating process. The n-hexane fraction was obtained in three separately fractionation process. Second, the aqueous residues were shaken with 250 ml chloroform three times separately and n-hexane fraction was obtained. After obtaining the aqueous fraction the ethyl acetate fraction was obtained by adding 250 ml ethyl acetate to aqueous residues three time in separating process. All the fractions were then concentrated by removal of solvent through Rota-vapor, 55g, 40g, 40g and 35g fractions of n-hexane, chloroform, aqueous and ethyl acetate were obtained respectively. All the fractions were kept in freezer except the aqueous extract which was kept in desiccator until used.

2.4 Animals and ethical committee approval

All the animals were purchased from NIH (National Institute of Health) Islamabad Pakistan having a weight range from 500-1000g and were housed in the animal house of University of Malakand, Pakistan. The rabbits were housed in the separate cages with six rabbits per cage under standard environmental conditions and 12 hours – 12 hours light-dark cycle. The animals were acclimatized for one week before experiments and had free accessed to tap water and fresh vegetable under laboratory conditions. The ethical committee of Zoology, University of Malakand approved the experimental work and research activity according to the law with protocol number No: E-SA-11-2009 according to Bye-Laws 2008 Scientific Procedure Issue-1.

2.5 Grouping and Dosing of Animals

Ninety male rabbits were randomly assigned to fifteen groups [one Control, one toxicant group (Paracetamol), one positive control (PCM + silymarin) and twelve treated or test groups] with six rabbits per group. Control (group 1) has not received paracetamol, no fraction. Toxicant group (group 2) received paracetamol with 2g/kg body weight. Positive control group (group 15) received silymarin 50mg/kg and Paracetamol 2g/kg body weight. Among the treated groups (group 3, 4 and 5) were administered with 100mg/kg, 200mg/kg and 300 mg/kg n-hexane fraction. Treated groups (group 6, 7 and 8) were treated with 100mg/kg, 200mg/kg and 300mg/kg chloroform fractions. Treated groups (group 9, 10 and 11) received aqueous fraction at 100mg/kg, 200mg/kg and 300mg/kg body weight. Treated groups (group 12, 13 and 14) were given ethyl acetate fractions at 100mg/kg, 200mg/kg and 300mg/kg body weight. All the treated groups also received paracetamol at 2g/kg body weight for 20 days. Route of administration for standard drug, toxicant and test sample was orally by using oral gavage.

2.6 In Vitro antioxidant activity

2.6.1 DPPH radical scavenging assay:

DPPH radical scavenging assay was used to measure the antioxidant activities of different fractions of *Celtis occidentalis*. In the DPPH radical scavenging assay, the different fractions were tested at the dose of 62.5, 125, 250 and 500 ug/ml each. Ascorbic acid was used as a standard solution. The EC_{50} values were then calculated for the different fractions of the *Celtis occidentalis* leaves.

2.6.2 Free radical-scavenging activity: The free radical-scavenging activity of different fractions was evaluated by assessing the decrease in absorbance of DPPH at 517 nm according to (White, Oliveira et al. 2014).

2.7 Blood sample and organs collections

At the 21st day of the experiment all the rabbits were anesthetized with mild anesthesia of ether and sacrificed for collections of blood sample, kidneys and livers. The hearts were punctured with 5 ml syringe of 21gauge for blood collections. Each 2 ml of blood were stored in EDTA tube and used for complete blood examination (CBC). For clear serum collection each 3 ml of blood was spun in centrifuge machine at 4000 rpm for 10 minutes.

2.7 Hematological parameters assessment

Two ml of blood was collected from each individual rabbit in EDTA tubes for the analysis of hematological parameters. Automatic Sysmex analyzer XS-1000i (Japan) was used for relevant tests with regular control.

2.8 Biochemical parameters assessment

All rabbits were subjected to fasting for a period of 10 hours and weighed individually and their weight was recorded as initial weight at the beginning of the experiment, at day 14th and at the last day of the experiment. Animals were divided into groups comprising of six animals per group and dosed as described above in the ‘’grouping and dosing of animals’’ section. The clear serum obtained was then used for the liver and kidney functions tests using Cobas c 111 (USA). Analysis of biochemical parameters: cholesterol, LDL (low density lipoprotein), HDL (high density lipoprotein), triglycerides and blood glucose levels were carried out by using automatic analyzer and commercial assay kits.

2.9 Histopathological examinations

The collected liver and kidney from each rabbit were stored and fixed in 10% formalin (with two changes) for qualitative examination. The tissues were then washed with tape water and then dehydrated with 70%, 80% and 90% methanol and cleared in xylol. The cleared tissues were then fixed in paraffin wax. Sections of 3-5 microns thickness were prepared from paraffin embedded blocks and stained with hematoxylin and eosin for histopathological studies. Slides were prepared and studied under microscope. Images were captured using display microscope at an original magnification of 40x.

2.10 Statistical Analysis

Data presented in mean ± standard deviation. For statistical analysis and comparison between the groups all the data were subjected to one way analysis of variance (ANOVA) by using Graph-Pad Prism version 10, followed by Tukey test. Difference between control and treated groups with p- value ≤ 0.05 was considered significant.

3.0 RESULTS

3.1 Antioxidant activity

3.1.1 In vitro DPPH free radical scavenging activity

All the fractions exhibited the strong free radical scavenging activities against DPPH. Among the fractions, chloroform and aqueous fractions showed the strongest free radical scavenging activity against DPPH (84. 12% and 78.06 % at concentration of 500 µg/ml respectively) (Table 1).

3.2 Hematological parameters

Analysis of hematological parameters revealed that RBC count is decreased in paracetamol treated group (Table 2) compared to control and standard group (Silymarin + paracetamol) and this count is increased in some fraction towards control. No significant difference in group 5 [n-hexane (300mg/kg + PCM 2g/kg)], group 8 [Chloroform (300mg/kg + PCM 2g/kg)], group 10 [Aqueous (200mg/kg + PCM 2g/kg)] and group 11 [Aqueous (300mg/kg + PCM 2g/kg)] were observed in the count of RBC compared to group 1 (Control group) and group 15 (Silymarin + paracetamol group) and the RBC was recovered towards

normal. Chloroform fraction showed best result in all of fractions on increasing concentrations of the dose. Paracetamol administration declined the counts of TLC (total leucocytes count), PLC (platelets count); Hb (hemoglobin), MCV (mean corpuscular volume), MCH (mean corpuscular hemoglobin) and MCHC (mean corpuscular hemoglobin concentration). The n-hexane fraction was found to have recovered the count of TLC, PLC and MCHC at dose of 200mg/kg + PCM 2g/kg and on the count of all hematological parameters except MCV at dose of 300mg/kg body weight. Chloroform fraction showed recovery in PLC count at dose of 100mg/kg and in the counts of PLC, MCH and MCHC at dose of 200mg/kg + PCM 2g/kg and all the hematological parameters were recovered and showed no significant difference from the control and silymarin group at dose of 300mg/kg + PCM 2g/kg. Aqueous fraction returned the levels of PLC and MCH at dose of 100mg/kg + PCM 2g/kg and 200mg/kg + PCM 2g/kg and recovered all the given parameters at dose of 300mg/kg+ PCM 2g/kg. The count of TLC and Hb were normalized by ethyl acetate at dose of 200mg/kg+ PCM 2g/kg and all the hematological parameters excluding RBC at dose of 300mg/kg + PCM 2g/kg body weight. All the hematological parameters were normal and no significant differences were noted compared to control.

3.3 Blood Profile

The results of blood profile in different treated groups as presented in Table 3, showed elevation in the count of lymphocytes and decrease in the counts of neutrophil, monocytes and eosinophil in paracetamol treated group. A dose dependent decrease in the count of lymphocytes and increase in the counts of neutrophil, monocytes and eosinophil were observed in different treated groups towards control and standard group. The n-hexane fraction at high dose (200mg/kg and 300mg/kg) recovered the counts of lymphocytes and eosinophil and neutrophil at dose of (300mg/kg) significantly toward control compared to paracetamol treated group. The count of eosinophil was significantly elevated in chloroform fraction at dose of 100mg/kg compared to paracetamol treated group. The count of lymphocytes was decreased, and that of monocytes and eosinophil were elevated significantly compared to paracetamol treated animals in chloroform fraction at dose of 200mg/kg and all the blood profile were normalized at 300mg/kg body weight. The aqueous fraction showed similar effect on the blood profile (at dose of 200mg/kg and 300mg/kg) similar to chloroform fraction. Eosinophil level was significantly elevated by ethyl acetate fraction at dose of 100mg/kg body weight compared to paracetamol treated rabbits. Decrease in the counts of lymphocyte and increase in the counts of monocytes and eosinophil were elevated towards control and silymarin group at dose of 200mg/kg and 300mg/kg body weight. Silymarin normalized all the blood parameters significantly to that of control (Table 3).

3.4 Liver function tests

Elevated level of ALP, ALT and bilirubin were found in the paracetamol intoxicated animals (Group 2) compared to control and standard (silymarin + PCM treated group). Decrease in the levels of these parameters were observed at high doses (200mg/kg and 300mg/kg) in dose dependent way in all fractions and the significant decrease in the level of bilirubin was also observed at a dose of 100mg/kg in ethyl acetate and chloroform fractions. The chloroform fraction showed strong recovering effect at dose of 300mg/kg in the entire fraction.

3.5 Serum lipids profile and Blood Glucose level

The lipids profile studied includes cholesterol, triglyceride, HDL and LDL (Table 4). The paracetamol administration increased the levels of cholesterol, triglyceride and LDL and decreased the levels of HDL respectively compared to

control group animals. No significant reduction in the level of cholesterol was observed at low dose of 100mg/kg in all fractions and also at 200mg/kg in aqueous fraction compared to control. The cholesterol level decrease at high dose of all fraction towards control and standard with increasing dose. Triglyceride at high doses showed recovery towards control with increasing concentration of dose. At low dose of 100mg/kg the fractions showed no recovery and were comparable to that of paracetamol group. The levels of HDL were increased by n-hexane, chloroform, aqueous and ethyl acetate fractions at dose of 300mg/kg towards control. The fractions at lower doses have no attenuating effect on the levels of HDL and LDL and their levels were comparable to that of control and silymarin + PCM treated group. The levels of LDL were only significantly decreased by chloroform fraction towards control and silymarin treated group. The blood glucose concentration was significantly increased by the treatment of rabbits with paracetamol compared to control and silymarin treated group (13.08±1.8 **). The chloroform fraction at dose of 300mg/kg significantly decreased the blood glucose level toward control and silymarin treated group compared to that paracetamol group.

3.6 Kidney function tests

The levels of serum urea, serum creatinine and serum uric acid were significantly elevated by the administration of paracetamol compared to control and silymarin + PCM administered group. The levels of serum urea were significantly decreased by n-hexane and ethyl acetate fractions at 300mg/kg toward control and silymarin administered group. The chloroform fraction at high doses (200mg/kg and 300 mg/kg) significantly lowered the levels of serum urea towards control and silymarin group. The elevated levels of serum creatinine were declined by chloroform and ethyl acetate fractions at dose of 300mg/kg significantly near to control and silymarin PCM treated animals. The increased level of uric acid was significantly decreased by the chloroform fraction at dose of 300mf/kg. The other fractions have no significant attenuating effect on the levels of uric acid (Table 5).

3.7 Weight change in the body of rabbits during experiment

All the animals were weighted three time during the activity, at the first day, 11th and 21st days of the experiment. The control group animals gained 47 g weight till the last of the activity. The weight of animals in paracetamol group significantly reduced from 1067g to 963 g till the end of the experiment. Notable change in the weight gain of animals treated with different fraction of methanolic extract of *Celtis occidentalis* leaves were observed with increasing dose concentration compared to paracetamol (Fig. 1).

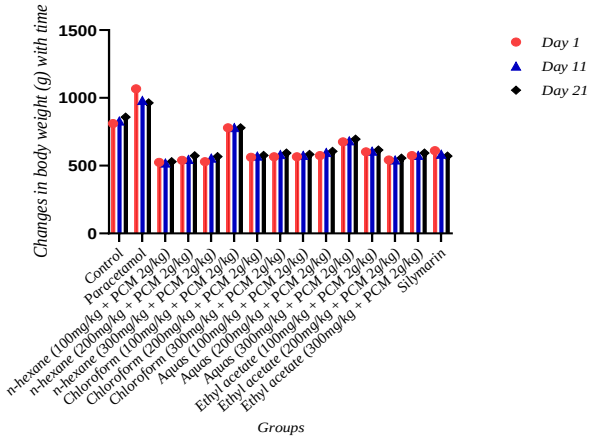

Fig. 1: Effect n-hexane, chloroform, aqueous and ethyl acetate fractions of methanolic extract of *Celtis occidentalis* leaves on the weight (g) changes of rabbits (Figure1) during the experimental period.

3.8 Histopathological analysis

Effects of different fractions of Celtis occidentalis leaves on the liver histopathology and kidney histopathology in paracetamol-intoxicated hepatotoxicity and nephrotoxicity in rabbits are presented in Fig. (2 & 3). It has been observed that the fractions in a dose dependent manner recovered the negative effects on the liver and kidneys of the treated groups. Analysis of serum testosterone and serum cortisol levels male rats.

3.8.1 Histopathological analysis of Liver

3.8.1.1 Descriptions for Figure 2: Microphotograph of liver stained with hematoxylin and eosin (Magnification 40X). (a) represent normal control, (b) PCM, (c) Silymarin, (d) nH100+PCM, (e) nH200+PCM, (f) nH300+PCM, (g) CF100+PCM, (h) CF200+PCM, (i) CF300+PCM, (j) Aq100+PCM, (k) Aq200+PCM, (l) Aq300+PCM, (m) EA100+PCM, (n) EA200+PCM, (o) EA300+PCM. (a): Histological structure of control group showed clear architecture of the liver. Central vein was observed to have normal diameter with normal hepatocytes arrangement. No inflammation and proliferation were noted in Kupffer cells. No vacuolar degeneration was observed and tissues showed normal bile duct and sinusoidal arrangement. (b): Histology of paracetamol group (PCM 2g/kg) showed abnormal architecture of liver. The central vein was dilated and degeneration in hepatocytes was observed. Kupffer cells were proliferated and showed irregular structure and inflammatory cells infiltrated between hepatocytes. Necrosis, bile duct proliferation and cholestasis were observed in PCM treated group. (c): The liver histology silymarin group (silymarin 50 mg/kg) showed normal architecture as that of control group. (d): The histological structure of this group (nH100+PCM) showed severe abnormalities including the dilation of central vein and mucous accumulation in the central vein. Kupffer cells were observed as normal. Inflammation, necrosis and elongated bile duct with accumulated mucus were reported in this group. (e): nH200+PCM fraction showed normal central vein with some deteriorated hepatocytes which showed regeneration. Kupffer cells were proliferated and no inflammatory cells were noted. Little mucus accumulation, mild necrosis and ingested bile duct were in this group. (f): Histology of this group (nH300+PCM) showed normal structure of the liver. No inflammation and necrosis were observed in this group. Normal central vein and Kupffer cells were observed with normal arrangement of hepatocytes. (g): Histological structure liver of CH100+PCM fraction showed severe degeneration of hepatocytes and the cytoplasmic contents were also observed in extracellular spaces. Dilation of central vein with accumulation of mucus was reported. Proliferated Kupffer cells, necrosis and inflammation were observed in this group. (h): This group (CH200+PCM) showed less pathology which reveals some degree of regeneration. Recovery of dilated central vein was observed and no inflammations were reported. (i): The histology of this group (CH300+PCM) showed normal architecture of the liver. Normal arrangement of hepatocyte and normal central vein with no inflammations were observed which revealed greater degree of regeneration. (j): Dilated central vein, degenerated hepatocytes, dispersed cytoplasmic contents in extracellular spaces, swelled Kupffer cells an inflammation were reported in this group (Aq100+PCM). (k): Slight dilated central vein with little accumulated mucus were reported in this group (Aq200). Normal hepatocytes and Kupffer cells were reported with no inflammation. (l): Normal central vein with no accumulation of mucus were observed in this group (Aq300+PCM). Normal hepatocytes and Kupffer cells were noted. (m): Highly degenerated hepatocytes, dilated

central vein with accumulated mucus, proliferated Kupffer cells and inflammation were observed in this group (EA100+PCM). (n): Liver histology of this group (EA200+PCM) showed recovery in abnormalities to some extent. Mild dilated central vein with little accumulation of mucus, mild bile duct ingestions, scattered inflammatory cells, little degenerated hepatocytes and cholestasis were observed. (o): Normal architecture of the liver, normal central vein, little proliferation in Kupffer cells were observed in this group (EA300+PCm). Hepatocytes showed normal structure with no inflammation and rare coagulative necrosis and ingested bile duct were also observed.

3.8.2 Histopathology of kidney

3.8.1.1 Descriptions for Figure 3: Microphotograph of kidney stained with hematoxylin and eosin (Magnification 40X). (A) Represent normal control, (B) PCM, (C) Silymarin, (D) nH100+PCM, (E) nH200+PCM, (F) nH300+PCM, (G) CF100+PCM, (H) CF200+PCM, (I) CF300+PCM, (J) Aq100+PCM, (K) Aq200+PCM, (L) Aq300+PCM, (M) EA100+PCM, (N) EA200+PCM, (O) EA300+PCM. **(A):** Kidney of control group showed normal architecture with wider distal convoluted tubule lined by cuboidal epithelial cells. Glomeruli and all tubules showed clear histology. **(B):** The histological structure of kidney was severely damaged in this group (PCM 2g/kg). The glomeruli in the cortical regions collapsed and infiltrated by inflammatory cells and dilation in the blood vessels also occurred. Necrosis, inflammation and hyalinization were observed in the epithelial wall of renal tubules. **(C):** Normal histological structure of the kidney was observed in this group (Silymarin 50 mg/kg). **(D):** Inflammation and necrosis in the renal tubules were observed with accumulation of mucus. Wide and dilated blood vessels, collapsed glomeruli and damaged bowman capsule were also observed in this group (nH100+PCM). **(E):** Bowman capsule and blood vessels were found normal in this group (nH200+PCM). A slightly collapsed glomerulus and necrosis in the cuboidal epithelium cells were observed with little accumulation of mucus. **(F):** Kidney histology in this group (nH300+PCM) showed normal architecture with normal epithelial lining. **(G):** Slight increase in the size of glomerulus was observed in the animal group treated with (CF100+PCM). Necrosis and inflammation in the renal tubules and congested Bowman's capsule were also observed. **(H):** Mild degeneration in the glomeruli, congested Bowman's capsules, swelled up epithelial walls and rare inflammations in the cells were observed in the animals treated with CF100+200. **(I):** Normal Bowman's capsule, glomerulus and wider lumen lined by cuboidal epithelium were observed in this group (CF300+300). The chloroform fraction in this group showed high significant recovery and no significant pathology were observed in this group. **(J):** Damage in the architecture of kidney was observed in the group treated with (Aq100+PCM). Collapsed glomeruli, dilated blood vessels, inflammation, mucus accumulation in renal tubules and necrosis were also observed. **(K):** Recovery was observed to some extent in the abnormalities of kidney at the dose of (Aq200+PCM). The glomeruli and Bowman's capsule were found normal and the blood vessels were slightly dilated. Mucus accumulation and necrosis were rare in the renal tubules. **(L):** The histological structure of the kidney of animals treated with Aq300+PCM were like that of control and standard group rabbits. **(M):** The histology of kidney of (EA100+PCM) fraction showed severe necrosis, inflammation, expended glomeruli with mucus accumulation and dilated blood vessels. **(N):** The histological structure in this group (EA200+PCM) showed slight recovery in the abnormalities. A swelled epithelial wall of renal tubules with mucus accumulated

in it, highly elongated glomeruli and congested Bowman's capsule were observed. **(O):** The histology of kidney in this group (EA300+PCM) showed normal architecture with rare inflammation and the renal tubules were lined by cuboidal epithelial cells.

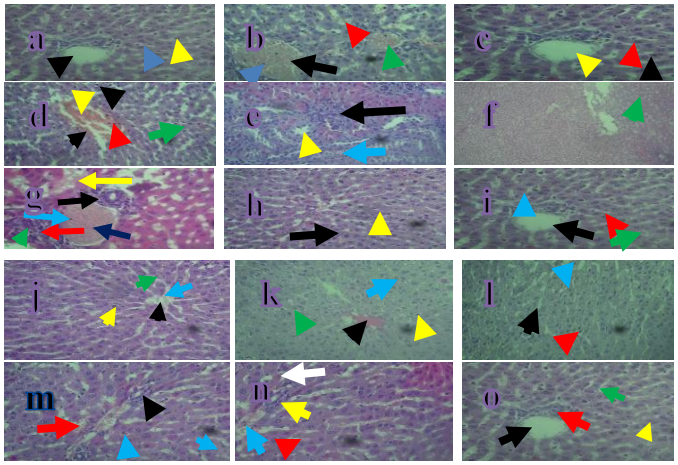

Fig. 2: Microphotograph of liver stained with hematoxylin and eosin (Magnification 40X).

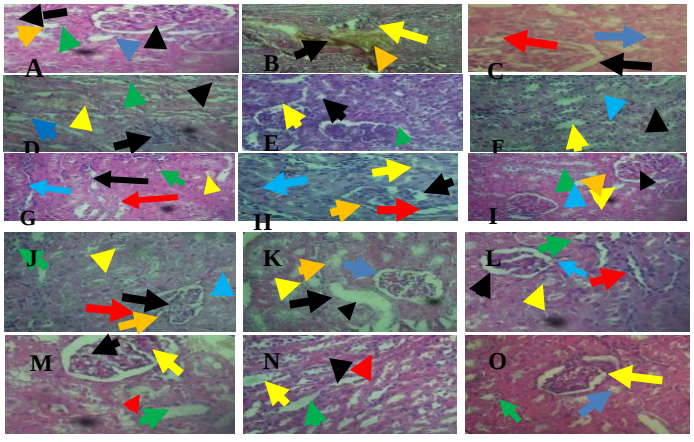

Fig. 3: Microphotograph of kidney stained with hematoxylin and eosin (Magnification 40X).

(Ascorbic acid) and different fractions of methanolic extract of *Celtis occidentalis* leaves at different concentration against DPPH.

Fractions + Standard	**DPPH free radical scavenging assay**			
	Concentration in µg/ml			
	62.5 µg/ml	125µg/ml	250 µg/ml	500 µg/ml
Ascorbic acid	68.65%	83.2%	87.3%	90%
n-hexane	60.01%	63.9%	64.35%	67.12%
Chloroform	73.93%	74.42%	76.9%	84. 12%
Aqueous	70.76%	73.63%	74.16%	78.06 %
Ethyl acetate	65.88%	68.65%	70.36%	73.96%

Table 1: Free radical scavenging activities of standard

Table 2: Effect of different fractions of *Celtis occidentalis* on Hematological parameters

Groups	RBC ($\times10^6$/µm)	TLC ($\times10^3$/µm)	PLC ($\times10^3$/µm)	Hb (g/dL)	MCV (fL)	MCH (pg)	MCHC (g/dL)
1.Control	5.2±0.167	9±0.4	439±7	12.28±0.5	61.5±2.4	29.58±1.28	41.7±1.86
2.Paracetamol	3.97±0.22	5.43±0.3	329±11	7.1±0.6	47.58±6.1	17.55±3.27	28.53±3.1
3.n-hexane (100mg/kg + PCM 2g/kg)	4.07±0.307^{ns}	5.15±0.95^{ns}	337±12^{ns}	7.5±1.85^{ns}	47.63±3.25^{ns}	17.48±3.1^{ns}	32.4±3.59^{ns}
4.n-hexane (200mg/kg + PCM 2g/kg)	4.17±0.57^{ns}	7.06±1.3**	359±11***	8.2±2.04^{ns}	53.56±4.6^{ns}	19.83±1.79^{ns}	34.33±4.2*
5.n-hexane (300mg/kg + PCM 2g/kg)	4.9±0.16***	7.04±0.24**	390±8***	9.79±0.51**	53.1±3.3^{ns}	23.5±1.6***	37.1±2.88***
6.Chloroform (100mg/kg + PCM 2g/kg)	3.88±0.29^{ns}	6.03±0.41^{ns}	365±11***	7.65±0.5^{ns}	51.2 ±3.47^{ns}	19.89±0.66^{ns}	33.7±2.1^{ns}
7.Chloroform (200mg/kg + PCM 2g/kg)	4.13±0.18^{ns}	6.68±0.28^{ns}	384±7***	8.87±0.36^{ns}	55.3±2^{ns}	23.11±1.13**	36.61±1.8***
8.Chloroform (300mg/kg + PCM 2g/kg)	5±0.16***	8.2±0.74***	427±15***	11.24±1.15***	58.95±2.7***	27.36±1.67***	39.88±2.45***
9.Aqueous (100mg/kg + PCM 2g/kg)	4.01±0.1^{ns}	6.5±0.6^{ns}	349±8*	7.87±0.45^{ns}	48.08±8.4^{ns}	20.06±2***	32.85±3.5^{ns}
10.Aqueous (200mg/kg + PCM 2g/kg)	4.72±0.55*	6.6±0.56^{ns}	379±8***	8.23±0.94^{ns}	54.31±4.2^{ns}	24.6±1.68***	34.05±2.04^{ns}
11.Aqueous (300mg/kg + PCM 2g/kg)	4.8±0.32**	8.1±0.86***	410±9***	10.93±0.58***	58.16±5.07**	26.78±1.84***	37.4±3.95***
12.Ethyl acetate (100mg/kg + PCM 2g/kg)	3.9±0.12^{ns}	5.89±0.60^{ns}	339±6^{ns}	8.91±0.91^{ns}	47.25±2.4^{ns}	19.11±1.54^{ns}	32.66±1.5^{ns}
13.Ethyl acetate (200mg/kg + PCM 2g/kg)	4.39±0.32^{ns}	6.95±0.36**	346.5±11^{ns}	9.81±0.97**	54.4±3^{ns}	23.25±1.99***	33.9±2.3^{ns}
14.Ethyl acetate (300mg/kg + PCM 2g/kg)	4.48±0.08^{ns}	7.95±0.53***	380.1±6.5***	9.35±0.64*	57.35±1.65**	25.65±1.53***	35.21±2.47**
15.Silymarin (50mg/kg + PCM 2g/kg)	4.98±0.12***	8.42±0.15***	427.6±5.3***	11.5±1.23***	63.8±1.97***	27.15±2.12***	39.23±1.35***

- Significance difference was made between paracetamol and treated group of animals. $P^{ns}\geq0.123$ indicates that there is no significance difference between paracetamol and treated groups. *P**≤0.0332, P**≤0.0021* and *P***≤0.0002* are considered significant compared to that of paracetamol.

Table 3: Effect of different fractions on blood profile of rabbits in different treatment groups

Groups	Lymphocytes %	Neutrophil %	Monocytes %	Eosinophil %
1. Control	54.47±4.4	32.08±3.8	10.65±1.4	2.76±0.08
2. Paracetamol	76.3±3.64	16.2±8.46	5.49±0.8	1±0.04
3. n-hexane (100mg/kg + PCM 2g/kg)	74.2±4.34^{ns}	18.2±5.6^{ns}	6.29±0.68^{ns}	1.04±0.092^{ns}

4. n-hexane (200mg/kg + PCM 2g/kg)	65.32±6.2**	25.1±6.4 ns	7.16±1.2 ns	1.4±0.06***
5. n-hexane (300mg/kg + PCM 2g/kg)	61.34±3.8***	28.33±7.2*	8.9±1.42 ns	1.42±0.07***
6. Chloroform (100mg/kg + PCM 2g/kg)	73.82±4.42 ns	18.1±6.8 ns	6.24±0.98 ns	1.24±0.02***
7. Chloroform (200mg/kg + PCM 2g/kg)	61.18±3.6***	28.2±3.88*	8.4±1.34***	1.92±0.04***
8. Chloroform (300mg/kg + PCM 2g/kg)	56.87±2.9***	30.2±4.8**	9.8±2.02***	2.53±0.086***
9. Aqueous (100mg/kg + PCM 2g/kg)	5.7±4.4 ns	17.8±7.9 ns	5.34±0.89 ns	1.02±0.058 ns
10. Aqueous (200mg/kg + PCM 2g/kg)	62.28±4.6***	24.8±5.7 ns	8.82±1.32**	1.38±0.082***
11. Aqueous (300mg/kg + PCM 2g/kg)	58.69±2.8***	28.24±4.6*	9.4±1.68***	2.66±0.066***
12. Ethyl acetate (100mg/kg + PCM 2g/kg)	74.5±2.58 ns	16.02±4.9ns	6.63±0.86ns	0.84±0.024**
13. Ethyl acetate (200mg/kg + PCM 2g/kg)	66.46±3.74*	23.2±6.3ns	8.82±1.56**	1.26±0.046***
14. Ethyl acetate (300mg/kg + PCM 2g/kg)	62.8±6.43***	25.6±5.64ns	9.48±1.86***	1.8±0.074***
15. Silymarin (50mg/kg + PCM 2g/kg)	60.34±3.8***	27.8±3.86*	9.8±1.86***	1.94±0.084***

- Significance difference was made between paracetamol and treated group of animals. $P^{ns} \geq 0.123$ indicates that there is no significance difference between paracetamol and treated groups. *P*≤0.0332, P**≤0.0021* and *P***≤0.0002* are considered significant compared to that of paracetamol. (Values presented as mean±standard deviation).

Table 4: Effect of different fractions of Celtis occidentalis leaves, paracetamol and silymarin on enzymes activity, cholesterol, triglycerides, Blood glucose and lipid profile in serum of rabbits treated with paracetamol (Mean±SD, n=6).

Groups	ALT (IU/L)	ALP (IU/L)	Bilirubin (mg/dL)	Cholesterol (mg/dL)	Triglyceride (mg/dL)	Blood Glucose (mg/dL)	HDL (mg/dL)	LDL (mg/dL)
1. Control	31.06±2.4	66.9±3.6	0.37±0.02	46.2±1.8	84.12±4.6	114.2±3.4	29.4±1.34	10.94±1.2
2. Paracetamol	124.38±6.6	123.4±7.4	2.7±O.O7	88.9±6.6	130.4±5.6	137.9±8.46	18.1±2.6	18.84±2.8
3. n-hexane (100mg/kg + PCM 2g/kg)	120.28±5.4ns	119.4±8.23 ns	2.72±0.05 ns	84.36±4.6 ns	126.7±6.42 ns	135.8±3.88 ns	19.26±1.8 ns	18.62±3.34 ns
4. n-hexane (200mg/kg + PCM 2g/kg)	90.6±5.8***	96.4±4.6***	2.03±0.03***	70.4±4.8***	98.2±4.6***	130.6±5.64 ns	24.4±4.2 ns	17.26±2.32 ns
5. n-hexane (300mg/kg + PCM 2g/kg)	69.2±2.4***	80.8±3.4***	1.36±0.02***	63.4±3.9***	93.8±3.4***	128.2±6.43 ns	27.2±3.6*	15.4±1.7 ns
6. Chloroform (100mg/kg + PCM 2g/kg)	120±4.8ns	118.3±4 ns	2.5±0.06***	81.54±4.2 ns	127.6±5.8 ns	134.6±2.8 ns	20.2±3.23 ns	17.2±1.8 ns
7. Chloroform (200mg/kg + PCM 2g/kg)	99.8±5.6***	90.7±4.8***	1.64±0.04****	66.6±3.9***	99.04±6.2***	127±4.9*	25.2±2.68 ns	15.88±3.2 ns
8. Chloroform (300mg/kg + PCM 2g/kg)	50.6±4.4***	76.1±4.8***	0.92±0.03***	52.2±5.4***	90.8±6.4***	120±5.3***	28.22±3.2**	13.08±1.8**
9. Aqueous (100mg/kg + PCM 2g/kg)	125±7.3 ns	118.5±3.8 ns	2.66±0.02 ns	86.6±6.2 ns	134±7.2 ns	132.4±2.88 ns	18.8±5.8 ns	19±2.56 ns

10. Aqueous (200mg/kg + PCM 2g/kg)	114.9±5.4 ns	111.4± 7.8*	1.99±0.04***	81.6±4.6 ns	124.3±3.88 ns	126.2±3.6**	25.4±3.6 ns	18.6±3.2 ns
11. Aqueous (300mg/kg + PCM 2g/kg)	100.4±2.88***	96.1±5.4***	1.64±0.06***	70.3±4.2***	108.9±4.6***	121.7±4.8***	27.16±4.4*	15.2±2.34 ns
12. Ethyl acetate (100mg/kg + PCM 2g/kg)	118.6±4.6 ns	120.6± 6.7ns	2.45±0.04***	83±5.4 ns	128.9±4.6 ns	129.8±6.2 ns	19.21±5.2 ns	20±2.68 ns
13. Ethyl acetate (200mg/kg + PCM 2g/kg)	114.4±4.8*	112.4± 4.8ns	2.12±0.07***	51.3±3.6***	119.8±8.2 ns	126.8±4.2*	24.6±3.22 ns	17.46±1.88 ns
14. Ethyl acetate (300mg/kg + PCM 2g/kg)	98±4.3***	108.4± 6.4**	1.34±0.04***	32.8±2.8***	97.5±6.4***	120.4±3.6***	27.2±6.6*	15.34±2.38 ns
15. Silymarin (50mg/kg + PCM 2g/kg)	28.7±2.4***	74.7±5.4***	0.72±0.02***	34.76±3.4***	90.6±4.6***	113.24±3.22***	28.22±5.6**	12.28±2.24**

- Significance difference was made between paracetamol and treated group of animals. $P^{ns} \geq 0.123$ indicates that there is no significance difference between paracetamol and treated groups. *P*≤0.0332, P**≤0.0021* and *P***≤0.0002* are considered significant compared to that of paracetamol.

Table 5: Effect of fractions of *Celtis occidentalis* leaves on the level of serum kidney biochemical parameters in paracetamol-induced rabbits.

Groups	**Urea (mg/dL)**	**Creatinine (mg/dL)**	**Uric Acid (mg/dL)**
1. Control	28.82±3.6	0.52±0.04	4.8±1.3
2. Paracetamol	60.36±7.8	2.48±0.12	10.14±3.54
3. n-hexane (100mg/kg + PCM 2g/kg)	62.6±6.4ns	2.34±0.14 ns	10.78±2.87 ns
4. n-hexane (200mg/kg + PCM 2g/kg)	54.4±5.91ns	2.32±0.8 ns	9.06±2.38 ns
5. n-hexane (300mg/kg + PCM 2g/kg)	41±3.88***	1.58±0.4 ns	7.16±1.24 ns
6. Chloroform (100mg/kg + PCM 2g/kg)	54.6±8.2 ns	2.06±0.26 ns	8.9±1.22 ns
7. Chloroform (200mg/kg + PCM 2g/kg)	40±3.6***	1.58±0.09 ns	7.28±1.42 ns
8. Chloroform (300mg/kg + PCM 2g/kg)	34.8±4.52***	1.02±0.2***	5.76±0.82*
9. Aqueous (100mg/kg + PCM 2g/kg)	57.8±2.98 ns	2.47±0.88 ns	9.82±1.6 ns
10. Aqueous (200mg/kg + PCM 2g/kg)	54.6±5.24 ns	1.86±0.68 ns	9.68±2.6 ns
11. Aqueous (300mg/kg + PCM 2g/kg)	46.26±6.2**	1.16±0.46**	7.99±1.64 ns
12. Ethyl acetate (100mg/kg + PCM 2g/kg)	56.58±4.78 ns	2.36±0.86 ns	8.94±2.42 ns
13. Ethyl acetate (200mg/kg + PCM 2g/kg)	51.42±7.4 ns	1.96±0.34 ns	8.48±3.22 ns
14. Ethyl acetate (300mg/kg + PCM 2g/kg)	42.38±6.48***	1.34±0.62*	7.67±1.98 ns
15. Silymarin (50mg/kg + PCM 2g/kg)	31.4±4.2***	0.48±0.04***	4.6±0.68**

- Significance difference was made between paracetamol and treated group of animals. $P^{ns} \geq 0.123$ indicates that there is no significance difference between paracetamol and treated groups. *P*≤0.0332, P**≤0.0021* and *P***≤0.0002* are considered

significant compared to that of paracetamol. (Values presented as mean±standard deviation).

4.0 Discussion

In the current study the methanolic extract of *Celtis occidentalis* was fractionated and evaluated for their antioxidant (Table 1), hepatoprotective and nephroprotective activities. The chloroform (84. 12%) and aqueous fractions (78.06 %) showed most strong antioxidant activities at 500 µg/ml against DPPH in the given fractions. A voilet solution is produced in ethanol by stable free radical DPPH with maximum absorption band at 517 nm [22]. The strong antioxidant potential of all these fractions maybe responsible for their hepatoprotective and nephroprotective activities. DPPH is mostly used to evaluate antioxidant potential of plant extract [5]. One of the most relevant parameter considered in the experiment was body weight of the rabbits. The animals were weighted three times during the current study at day first of the activity, day 11 and at the final day. Animals treated with paracetamol showed significant decrease in the body weight of the animals (Fig. 1) which show the toxic effect of paracetamol [23]. All the fractions and silymarin increase the body weight of animals significantly compared to paracetamol group.

Decrease in the hematological parameters ((RBC, TLC, PLC, Hb, MCV, MCH, and MCHC) were observed in the paracetamol treated animals which is inconsistent with reported study [24]. Decrease in the Hb level was observed in the toxicant group (table 2) which indicates the toxic effect of paracetamol. Decrease in the level of Hb, RBC and the other blood parameters reflect the excessive decomposition of RBC in the liver [25]. Anemia can cause decrease in the above-mentioned parameters which may have occurred due to blood loss, hemorrhage or the deficiency of vitamin B12 [26]. Different fractions of the *Celtis occidentalis* used in the current study at high dose (200mg/kg and 300mg/kg) had increased the counts of all these parameters including Hb significantly compared to paracetamol treated animals. The count of RBC raised significantly compared to toxicant group in the animals treated with fractions likes, n-hexane (300mg/kg + PCM 2g/kg), Chloroform (300mg/kg + PCM 2g/kg), Aqueous (200mg/kg + PCM 2g/kg), Aqueous (300mg/kg + PCM 2g/kg) and Silymarin (50mg/kg + PCM 2g/kg). This increase in the RBC count might be due to the presence of compounds like flavonoids, alkaloids and ascorbic acid which have activated bone marrow and lymphoid organs [27]. The results of blood profile in different treated groups as presented in table 3, showed elevation in the count of lymphocytes and decrease in the counts of neutrophil, monocytes and eosinophil in paracetamol treated animals (group table 3). The different fractions at high doses normalized the aforementioned parameters at high doses 200mg/kg and 300mg/kg combined with paracetamol as shown in the table 3. The findings of the current study showed that chloroform and aqueous fractions has strong therapeutic potential on the blood profile.

Paracetamol overdose increased the levels of AST, ALT and bilirubin. These findings are already being reported [28], which demonstrates that hepatotoxicity is caused by paracetamol overdose indicated by rise in serum enzymes and bilirubin level. For the assessment of liver functions ALT and AST have been used for long time. The damaged hepatocytes released these enzymes and their activity is increased in the plasma. The levels of these enzymes are thus used as marker for the extent and type of hepatocellular damage [29]. Hepatotoxicity results in the defective excretion of ALP by the liver in the bile which is reflected as elevated in the serum. The elevated levels of these enzymes are the conventional indicator of liver injury [30]. Increase in liver enzymes and in the level of bilirubin in the paracetamol treated group showed paracetamol had induced hepatocellular membrane

disturbance leading to the release of the hepatic enzymes from the cell cytoplasm into the blood circulation [31]. The main focus of the current study was comparison between silymarin and different fractions of *Celtis occidentalis.* The co-administrations of all the fractions of *Celtis occidentalis* at high dose of 200mg/kg and 300mg/kg with paracetamol 2g/kg decreased the serum enzymes and bilirubin significantly compared to paracetamol treated group (Table 4). Chloroform fraction at dose of 100mg/kg decreased the level of bilirubin significantly compared to paracetamol group. The effect of different fractions at high doses was like that of silymarin on the serum enzymes and bilirubin. Silymarin is widely and most used standard drug for hepatotoxicity. Silymarin scavenges free radicals and retains membrane permeability, so prevent hepatotoxicity [32].

In our study the levels of cholesterol, triglyceride and LDL in paracetamol treated group were significantly increased and HDL was decreased as compared to control group animals which is previously reported [33-35]. The higher concentration of cholesterol, triglyceride and LDL and low level of HDL indicates the development of cardiovascular diseases, hyperlipidemia, steatosis and necrosis of hepatocytes [21]. Different fractions of *Celtis occidentalis* at different doses has reduced the levels of the above-mentioned parameters significantly compared to paracetamol administered group towards control as mentioned in table 4. The n-hexane fraction at dose of 200mg/kg with paracetamol of 2g/kg significantly reduced the levels of triglyceride and cholesterol compared to paracetamol group towards control and silymarin group and lowered the levels of triglyceride, cholesterol and elevated the level of HDL at dose of 300mg/kg with combination of paracetamol compared to paracetamol group. Chloroform fraction at dose of 200mg/kg in combination with paracetamol lowered the levels of triglyceride, cholesterol and blood glucose and at high dose of 300mg/kg with paracetamol of 2g/kg decreased the levels of triglyceride, cholesterol, blood glucose, HDL and LDL significantly to that of paracetamol group. Aqueous fraction at dose of 200mg/kg reduced the levels of blood glucose and at 300mg/kg decreased the levels of triglyceride, cholesterol, blood glucose levels and HDL to that of paracetamol group animals. Ethyl acetate fraction at dose of 200mg/kg in combinations with paracetamol 2g/kg reduced the levels of cholesterol and blood significantly compared to paracetamol group. At dose of 300mg + PCM 2g/kg the effect of ethyl acetate was like that of aqueous fraction at the same dose. The standard drug silymarin in combinations with paracetamol (Silymarin 50mg/kg + PCM 2g/kg) normalized all the parameters like that of control group animals (table 4).

Paracetamol treated group showed significant increase in serum urea, serum creatinine and serum uric acid levels compared to control. These findings are inconsistent with reported study in which paracetamol administration caused the same effect [19]. The increase in the levels of serum urea, creatinine and uric acid might be due to the development of oxidative stress and renal tissues damage [20]. Paracetamol administration causes renal dysfunction by damaging its tubules which leads to necrosis and disturb renal parameters [19]. The n-hexane fraction at dose of (300mg/kg + PCM 2g/kg) decline the level of serum urea significantly compared to paracetamol group. The n-hexane fraction at other doses did not show any significant effect. Chloroform fraction at dose of (200mg/kg + PCM 2g/kg) lowered serum urea level and at dose of (300mg/kg + PCM 2g/kg) reduced the levels of serum urea, serum creatinine and serum creatinine significantly to that of paracetamol toward control group animals. Aqueous fraction at dose of (300mg/kg + PCM 2g/kg) decline the levels of serum urea and serum

creatinine significantly to that of paracetamol group. Ethyl acetate fraction at dose of (300mg/kg + PCM 2g/kg) showed similar effect like that of aqueous fraction at the same dosage. In silymarin all the parameters were comparable to that of control group animals (Table 5). These results showed that among the fractions chloroform is the most potent in therapeutic nature.

4.1 Histopathological Findings

The liver histology of the control group showed cleared architecture of the liver with normal diameter of central vein and normal hepatocytes arrangements (Figure 2, section **a)**. The silymarin group animals were observed to have normal architecture of liver histology (Figure 5, section **c**) like that of control group. The livers' histology of the animals administered with paracetamol showed abnormal liver histology, dilated central vein, necrosis and inflammation. Kupffer cells were proliferated and showed irregular structure and inflammatory cells infiltrated between hepatocytes. Bile duct proliferation and cholestasis were observed in PCM treated group (Figure 2, section **b**). The current findings of paracetamol group are in accordance with reported literature [16]. All the fractions at low dose (100mg/kg + PCM 2g/kg) did not show any curative effect on degeneration and damage caused by paracetamol (Figure 2, sections **d**, **g**, **j** and **m**). The n-hexane fraction at dose of 200mg with combination of paracetamol 2g/kg showed regeneration and little hepatocytes were observed compared to that of paracetamol. Mild necrosis, and little mucus were noted and the Kupffer cells were found proliferated (Figure 2, section **e**). Liver histology of n-hexane fraction at dose of 300mg showed normal structure of the liver. No inflammation and necrosis were observed in this group and the hepatocytes were normally arranged compared to toxicant group. The histology livers of this group were like that of control and silymarin group (Figure 2, section **f**). Chloroform fraction (CH200+PCM) showed some degree of regeneration compared to paracetamol group and the animals treated with low doses of the fraction. Recovering dilated vein was noted and no inflammation in the tissues of livers seen (Figure 2, section **h**). The histology of livers of animals treated with (CH300+PCM) showed normal architecture of livers with no inflammation and normal hepatocytes arrangements and was similar to that observed in control and silymarin group. This group revealed great degree of regeneration compared to other group animals (Figure 2, section **i**). Livers' histology of animals treated (Aqueous fraction 200mg/kg + PCM 2g/kg) showed histological structure similar to that of chloroform fraction at the same dose and showed regeneration to some extent (Figure 2, section **k**) to that of paracetamol group. Normal hepatocytes, Kupffer cells and central vein with no accumulation of mucus, were observed in the livers of animals treated with aqueous fraction at dose of 300mg/kg + PCM 2g/kg (Figure 2, section **l**) compared to that of toxicant group. Liver histology of animals treated with (EA200+PCM) showed recovery in abnormalities to some range compared to animals treated with low doses and paracetamol. Little inflammatory cells and cholestasis were also observed in this group (Figure 2, section **n**). The histology of livers of animals treated with aqueous fraction at dose of 300mg/kg + PCM 2g/kg showed normal architecture with rare coagulative necrosis compared to animals treated with paracetamol (Figure 2, section **o**).

Kidney of control group showed normal architecture and clear histology with wider distal convoluted tubule lined by cuboidal epithelial cells (Figure 3, section **A**). The histological structure of kidney was severely damaged in the animals treated with paracetamol at dose of 2g/kg. The blood vessels were observed dilated and the glomeruli were found collapsed in the cortical regions. Inflammation, hyalinization and necrosis were observed in

the epithelial wall (Figure 3, section **B**) compared to control. These findings are discussed in previous study and comparable to that [19]. Kidneys' histology of silymarin group showed normal architecture (Figure 3, section **C**) compared to toxicant group. Different fractions of *Celtis occidentalis* at low doses not showed any curative effect (Figure 3, sections **D, G, J** and **M**) compared to silymarin group. Kidneys' histology of n-hexane fraction at dose of 200mg/kg + PCM 2g/kg showed normal blood vessels compared to paracetamol administered group. Little accumulation of mucus, slightly collapsed glomerulus and necrosis in the cuboidal epithelium cells were observed in this group (Figure 3, sections **E**) compared to control and silymarin group animals. The n-hexane fraction at dose of 300mg/kg + PCM 2g/kg showed curative effect and reversed the changes caused by paracetamol. The kidney histology in this group showed normal architecture (Figure 3, sections **F**) compared to toxicant group. The current findings are comparable to previous study in which plant extract reversed the histopathological changes caused by paracetamol [35]. Chloroform fraction was found most potent in their therapeutic properties. Animals treated with CF200+PCM showed rare inflammation, mild degeneration in the glomeruli and congested Bowman's capsules (Figure 3, sections **H**) compared to that of control group. The kidney histology in the animals treated with CF300+PCM showed normal architecture of the kidney (Figure 3, sections **I**) compared to paracetamol group. These results are previously described in reported study in which the plant extract used recovered the histological changes caused by paracetamol [19]. The Aqueous fraction at dose of Aq200+PCM showed recovery to some extent to that of paracetamol and low dose treated animals. A slight dilated normal tubules and rare mucus accumulation and necrosis were observed (Figure 3, sections **K**) compared to control animals (Figure 3, sections **A**). The Aqueous fraction showed best therapeutic potential at high dose. At high dose of (Aq300+PCM) the histology of kidneys was founded normal (Figure 3, sections **L**) compared to toxicant group (Figure 3, sections **B**). Slight recovery in abnormalities was observed in animals treated with EA200+PCM. The Bowman's capsule was congested, mild inflammation in epithelial wall and renal tubules and elongated tubules were observed in this group (Figure 3, sections **N**) compared to control and standard groups. The histology of the kidneys of animals treated with EA300+PCM showed normal architecture (Figure 3, sections **O**) similar to that of control and standard groups animals. These findings suggest that different fractions of *Celtis occidentalis* possessed strong hepatoprotective and nephroprotective potential against paracetamol induced hepatotoxicity and nephrotoxicity.

5.0 Conclusion

The current study demonstrates that different fractions of *Celtis occidentalis* have strong antioxidant potential and hepatoprotective and nephroprotective effects against paracetamol administration in over dose. Hematological, Biochemicals and histological analysis confirmed these findings in paracetamol intoxicated-rabbits.

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