Hepatoprotective effect of Peltophorum pterocarpum leaves extracts and pure compound against carbon tetra chloride induced liver injury in rats

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Abstract
Liver has a vital role in the management of physiological processes such as secretions, metabolism, storage and detoxification of various chemicals. Therefore, most of the liver diseases have been documented because of oxidative damages and linked to toxic chemicals. Oxidative stress is one of the mechanism involved in carbon tetra chloride (CCl_4) induced hepatotoxicity which triggers membrane disintegration and loss of membrane associated enzyme and necrosis. To correlate this mechanism, hexane and ethanol leaves extracts of *Peltophorum pterocarpum* at doses of 75, 150 and 250 mg/kg body weight and pure compound bergenin at doses of 25, 75 and 100 mg/kg body weight were studied for hepatoprotective activity in both sexes of albino rats. Liver injury was induced by carbon tetra chloride using 1.5 ml/kg body weight once during experimental period. The protective and therapeutic effects of test samples were compared with the standard drug containing betaine glucuronate + diethanolamine glucuronate + nicotinamide ascorbate. Different biochemical parameters such as ALT (alanine aminotransferase), ALP (alkaline phosphatase), γ-GT (gamma-glutamyltransferase), DB (bilirubin direct), and TB (total bilirubin) were investigated and assessed to match the histopathological examination results. Among various biochemical tests, bergenin demonstrated maximum reduction in AST, ALT, γ-GT, DB and TB serum levels at a dose of 100 mg/kg body weight in prophylactic mode in comparison to toxicant group. The statistical significance ($p<0.01$) were observed against ALT and TB serum levels when compared to toxicant group that were also noted in line with the histopathological examination of rat liver section where bile duct and central vein observed intact, however mild inflammation with absences of fibrosis and negative degeneration of lobular hepatocytes were recorded. Findings of present study demonstrated bergenin as a potent hepatoprotective against CCL4 induced hepatic toxicity.

Key words: *Peltophorum pterocarpum,* hepatoprotective activity, serum enzymes, histopathological examination.

Introduction

Peltophorum pterocarpum (DC.) Backer ex K. Heyne, tree belongs to the family *Fabaceae,* widely cultivated as a roadside tree in Pakistan. The genus (*Peltophorum*) with about 15 species has been reported to be distributed in tropical and subtropical countries including Sri Lanka, Malaysia and North Australia[1]. Review of literature showed that different parts of *Peltophorum pterocarpum* tree are used to treat several diseases and claimed to possess hepatoprotective property as well[2]. In some cases, the traditional healers applied the leaves in the form of decoction for treating skin disorders, while stem infusion and flowers were used in muscular pain[3].

Phytochemical investigations carried out on ethanol leaves extract indicated presence of flavonoids, alkaloids, saponins, sterols and cardiac glycosides, while bergenin was isolated from methanol extract of *Peltophorum pterocarpum* leaves as white crystals[4].

In search of potent hepatoprotective compounds from phytopharmaceuticals, present research work was directed towards evaluation of *Peltophorum pterocarpum* to observe its protective effects using CCl_4 as toxicant in animal models (rats). During metabolic degradation mechanism in the liver, CCl_4 propagates highly reactive trichloromethyl (CCl_3) as a free radical which binds with the lipoprotein of endoplasmic reticulum. The mechanism could initiate with the production of toxins, either generating directly from CCl_4 metabolism or from lipid peroxidation of cytoplasmic membrane. The whole process or chain reaction may lead to many morphological and functional changes in cell membrane associated to the aggregation of lipid derived oxidants causing hepatocellular damage and enhanced production of fibrotic tissue[5, 6].

Under hostile conditions, free radicals are derived from molecular oxygen as a natural intermediate, while as by-product under stress conditions and usually called reactive oxygen species (ROS). Thus, free radicals can originate from both endogenous (metabolism and ageing) and exogenous sources (anxiety and environmental contamination). Excess amount of these free-radicals if not eliminated from endogenous system can lead to cell injury, pathetic immune system, changes in gene expression and may generate atypical proteins, which can cause initiation and propagation of many diseases like liver disorders, immunosuppression, diabetes, neurodegenerative disorders and cancer. Hepatic cells are capable to strike against ROS by means of various antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). These enzymes take part in scavenging the free radicals, thus preventing cell damage. Therefore, it is necessary to overcome this problem and the natural hepatoprotectives with antioxidant effect can be a superior choice over synthetic one due to their poor solubility and moderate antioxidant effect in low concentration[7, 8]. During last two decades, a number of phytochemicals have been identified and reported from different parts of plants, known to exhibit hepatoprotective effect with powerful antioxidant and anti-inflammatory activities and capable of controlling impairment of cells and tissues and thus protect from lipid per oxidation.

Liver disorders and symptoms can be characterized as jaundice, tumors, fatty changes, metabolic and degenerative lesions, cirrhosis and hepatitis. The management of liver disorders is still challenging topic

because the drugs available can only provide concise support and symptomatic relief [9]. It is believed that phytopharmaceuticals can fill this gap and may provide certain powerful compounds with better safety profile and thus can be used in the management of some liver disorders. With this aim, present study was designed to evaluate hepatoprotective activity of a phytopharmaceutical - bergenin, along with hexane and ethanol leaves extracts of *Peltophorum pterocarpum* and to compare the efficacy with a drug containing betaine glucuronate + diethanolamine glucuronate + nicotinamide ascorbate, a well-known drug.

Materials and Methods

Plant material:

Leaves of *Peltophorum pterocarpum w*ere collected from University of Karachi and were identified at Department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi, Karachi–Pakistan. The identified sample specimen code *Peltophorum pterocarpum* PR-01-09 is available in Herbarium of the Department of the Pharmacognosy, University of Karachi, Karachi–Pakistan.

Leaves were washed and dried under shade for two weeks and then 3 kg of dried leaves were soaked in hexane for 15 days followed by filtration and evaporation under reduced pressure using rotary evaporator at 45ºC. The residue obtained after filtration was soaked in ethanol for another 15 days. The ethanol extract was then filtered and evaporated under reduced pressure using rotary evaporator at 45ºC and stored in air tight glass vials for further studies.

Isolation of pure compound bergenin:

Dried leaves (5kg) were soaked in methanol, followed by filtration through Whatman filter # 1. Solvent was evaporated under reduced pressure using rotary evaporator at 45^{o}C and the concentrated residue was partitioned between methanol and chloroform. After fractionation of methanol extract through column chromatography, bergenin was extracted out as white crystals at ratio of mobile phase $CHCl_3$: MeOH (83:17) [10].

Chemicals and Standard Drug:

Chemicals used in the study were of analytical grade. Carbon tetrachloride was obtained from Sigma. While the standard drug containing betaine glucuronate + diethanolamine glucuronate + nicotinamide ascorbate was purchased from local pharmacy. Analytical kits used for the estimation of serum ALP, ALT, γ- GT, DB and TB levels were purchased from Ecoline.

Animals:

Swiss Albino rats of both sexes weighing 150 – 200g were used in this study. Animals were kept under laboratory condition of temperature (23±3ºC) with 12/12h light and dark cycles and were allowed free attain of food and water and marked properly.

Experimental protocol

Hepatoprotective assay:

Hepatoprotective activity of pure compound, extracts and standard drug were performed in two models [5].

• Prophylactic study model

• Therapeutic study model

Rats were segregated into six primary groups i.e., A, B, C, D, E and F, each group included five animals (n=5). Three different doses i.e., 25, 75, and 100 mg/kg body weight of pure

compound bergenin were administered to group 1-A, 2-A, 3-A. While hexane and ethanol extracts of *P. pterocarpum* leaves were delivered orally to group 1-B, 2-B, 3-B, and 1-C, 2-C and 3-C at doses of 75, 150, 250 mg/kg body weight accordingly. Group D was served as a standard group and treated with standard drug 5.37, 10.75, 16.13 mg/kg body weight to group 1-D, 2-D and 3-D respectively along with toxicant. Group E was exposed with single oral dose of 1.5 ml/kg body weight CCl_4 in equal volume of olive oil (1:1) and assigned as toxicant group. Group F was given saline presented as control group. The study was performed in duplicate keeping all the parameters same as described above [4].

Prophylactic study model:

This examination pattern was executed via earlier dosing of test samples in experimental animals, to inhibit the liver injury by toxicant. The rats were divided into pre-defined groups. They were given their specific doses for five days constantly and after half an hour of administration of last dose of drug on day five animals were subjected to toxicant by oral administration of CCl_4 except the control group 4.

Therapeutic study model:

The study comprises of creating toxicity first in liver by oral administration of single dose of CCl_4. After half an hour of CCl_4 intoxication, animals of pre-defined groups in study design were given their particular doses for 5 days regularly excluding control group. On day six, rats of both models (prophylactic and therapeutic) were sacrificed using anesthesia and approximately 5 ml blood samples from each animal were drawn by cardiac puncture and were let on to clot at room temperature for half an hour. Blood samples were centrifuged at 2500 rpm for 15 minutes and serum was segregated [4].

Biochemical analysis:

The level of liver damage was evaluated by measuring hepatic enzymes ALP, ALT, γ-GT, DB and TB[17]. The enzyme activities were measured and read on a Photometric Micro lab [13]. Liver function test was performed at 25ºC, and all the reagents were ready to use.

Histopathological analysis of liver:

Liver samples were removed by incising the abdomen of the test animals and weighed. For histological studies, liver tissues were fixed with 10 % phosphate-buffered neutral formalin, dehydrated in graded (50–100 %) alcohol and embedded in paraffin. Thin sections were cut and stained with hematoxylin and eosin stain for microscopic assessment. The initial examination was qualitative, with the purpose of determining histopathological lesions in liver tissue [14].

Hepatic scoring to evaluate liver pathology:

Laboratory evaluation of liver damages reviewed and scored as hepatic fibrosis, congestion, hepatocellular necrosis and vascular features through biopsy specimen of liver tissues. The detection of degree of fibrosis was based on the observation of various staging, which ultimately reflected a distinctive phase or stages of necrosis or in a broader sense, the damages occurred in liver cells [15, 16].

Criteria used to detect staging of fibrosis in portal tract:

Stage - 0 demonstrated no fibrosis that makes an appearance of normal connective tissues. Stage -1 presented portal fibrosis that introduced fibrous portal expansion. Stage - 2 indicated fibrous portal expansions that

exhibited pericentral and septal fibrosis and no bridging fibrosis. Stage - 3 revealed septal fibrosis that manifested fibrous septa with architectural distortion and no obvious cirrhosis (bridging fibrosis). Stage - 4 identified as cirrhosis with complete nodule formation.

Criteria used to detect grading of inflammation in portal tract:

The inflammatory condition of the hepatic cells was recorded on the basis of lobular damages observed in the portal vein. Grade - 0 demonstrated portal inflammation only that indicted no lobular inflammation and necrosis. Grade - 1 presented minimal inflammation that introduced occasional spotty necrosis. Grade - 2 indicated mild inflammation that exhibited little hepatocellular damage. Grade - 3 reflected moderate inflammation that manifested with noticeable hepatocellular change. While Grade - 4 indicated as severe inflammation that leads to prominent diffuse hepatocellular damage.

Statistical analysis:

Hepatoprotective values were given in mean ± SEM. The biochemical data were analyzed by student's t-test followed by SPSS software version 20. Statistical significance level was set as *P<0.001 and **P<0.01 significant as compared to toxicant.

Results

The preliminary phytochemical screening of ethanol leaves extract of *Peltophorum pterocarpum* indicated the presences of alkaloids, flavonoids, cardiac glycosides, saponins and sterols.

Hepatoprotective effect of pure compound bergenin, hexane and ethanol extracts of *Peltophorum pterocarpum* leaves was examined and presented in Table 1 to 4. Table – 1 shows enzyme analysis results, while Table – 2 indicates histopathological findings in prophylactic model. Similarly, Table -3 reflects enzyme analysis results Table – 4 shows histopathological findings in therapeutic model.

Table 1. Effect of pure compound bergenin, hexane and ethanol leaves extracts of *Peltophorum pterocarpum* on serum ALP, ALT, γ-GT, DB and TB in rats with CCl_4-induced hepatotoxicity (Prophylactic mode)

Compound and Extracts	Dose mg/kg	Liver weight after treatment (g)	ALP (IU/ L)	ALT (IU/ L)	γ-GT (IU/ L)	DB (mg/ dl)	TB (mg/ dl)
Bergenin	25	4.40±0.400	298.60±0.010	50.00±0.001**	1.40±0.052	0.16±0.016	0.56±0.024
	75	4.40±0.400	168.00±0.007**	47.20±0.003**	1.40±0.025	0.06±0.070	0.52±0.001**
	100	4.40±0.400	129.80±0.038	35.20±0.004**	0.80±0.016	0.04±0.178	0.20±0.003**
Peltophorum pterocarpum (Hexane Extract)	75	5.60±0.400	594.80±0.007**	92.80±0.048	2.40±0.024	0.10±0.034	0.58±0.001**
	150	5.20±0.489	569.80±0.009**	71.20±0.001**	2.20±0.151	0.080±0.020	0.48±0.037
	250	5.20±0.489	229.00±0.001**	57.200±0.001**	1.00±0.266	0.080±0.016	0.38±0.009**
Peltophorum pterocarpum (Ethanol Extract)	75	5.60±0.400	598.20±0.008**	110.00±0.032	2.80±0.045	0.08±0.016	0.46±0.002**
	150	8.40±0.400	275.00±0.002**	56.60±0.001**	1.40±0.052	0.06±0.070	0.20±0.034
	250	5.20±0.489	180.80±0.001**	54.40±0.001**	1.40±0.005**	0.14±0.025	0.26±0.073
Standard drug (Jetepar)	5.37	7.50±0.001	279.80±0.001*	59.33±0.002**	4.00±0.099*	0.08±0.016	0.42±0.001*
	10.75	5.00±0.126	199.33±0.025	35.66±0.004**	4.00±0.208	0.08±0.016	0.40±0.002**
	16.13	5.00±0.126	159.00±0.011**	31.00±0.009**	1.66±0.199	0.066±0.03	0.38±0.024
Control	-	5.00±0.003	258..25±0.006	42.00±0.007	1.00±0.034	0.046±0.033	0.13±0.002
Toxicant (CCl_4)	1.5	7.50±0.001	642.30±0.002	141.25±0.017	4.60±0.006	0.98±0.001	1.26±0.043

Values represent mean ± SEM of five animals in each group.
Significant difference between treated groups*P<0.001 and **P<0.01.

Table 2. Hepatic histopathological finding of pure compound bergenin, hexane and ethanol leaves extracts of *Peltophorum pterocarpum* (prophylactic model)

Compound and Extracts	Dose mg/kg	Portal Tract				Lobules					Loss of central vein
		Fibrosis (Stage)	Inflammation (Grade)	Bile duct	Portal vein	Lobular damage	Hepatocytes		Fatty liver		Finding
							Degeneration	Necrosis	Micro	Macro	
Bergenin	25	2	2	Dam age	Intact	+ve	+ve	-ve	+ve	+ve	Intact
	75	1	2	Intact	Intact	-ve	-ve	-ve	-ve	+ve	Intact
	100	0	1	Intact	Intact	-ve	-ve	-ve	-ve	-ve	Intact
Peltophorum pterocarpum (Hexane Extract)	75	1	2	Intact	Intact	-ve	-ve	-ve	-ve	-ve	Intact
	150	1	2	Intact	Intact	-ve	-ve	-ve	-ve	-ve	Intact
	250	0	1	Intact	Intact	-ve	-ve	-ve	-ve	-ve	Intact
Peltophorum pterocarpum (Ethanol Extract)	75	3	4	Intact	Intact	+ve	+ve	-ve	+ve	+ve	Intact
	150	2	3	Intact	Intact	+ve	+ve	-ve	+ve	+ve	Intact
	250	1	1	Intact	Intact	-ve	+ve	-ve	+ve	+ve	Intact
Standard Drug	5.37	1	1	Intact	Intact	-ve	-ve	-ve	-ve	-ve	Intact
	10.75	1	1	Intact	Intact	-ve	-ve	-ve	-ve	-ve	Intact
	16.13	0	1	Intact	Intact	-ve	-ve	-ve	-ve	-ve	Intact
Control	-	0	0	Intact	Intact	-ve	-ve	-ve	-ve	-ve	Intact
Toxicant (CCl_4)	1.5*	3	4	Intact	Intact	+ve	+ve	+ve	+ve	Mild +ve	Intact

(-ve) Not present, (+ve) Present.

The liver enzymes levels significantly ($p < 0.01$) increased (ALP 642.30 IU/L), (ALT 141.25 IU/L), (γ-GT 4.60 IU/L), (DB 0.98 mg/dl) and (TB 1.26 mg/dl) in CCl_4 treated rats and were considerably reduced in presence of pure compound bergenin at a dose of 100 mg/kg body weight (ALP 129.80 IU/L), (ALT 35.20 IU/L), (γ-GT 0.80 IU/L), (DB 0.04 mg/dl) and (TB 0.20 mg/dl). There was a marked reduction (ALP 168.00 IU/L), (ALT 47.20 IU/L), (γ-GT 1.40 IU/L), (DB 0.06 mg/dl) and (TB 0.52 mg/dl) at a dose of 75 mg/kg body weight. Low dose treatment 25 mg/kg body weight of pure compound bergenin found ineffective in comparison to control group but magnitude of hepatotoxicity was found inferior (ALP 298.60 IU/L), (ALT 50.00 IU/L), (γ-GT 1.40 IU/L), (DB 0.16 mg/dl) and (TB 0.56 mg/dl) to CCl_4 toxicant group. Statistically significant reduction ($p < 0.01$) in liver enzymes levels to ALT and TB were observed by administration of 75 and 100 mg/kg body weight doses. ALP levels were found to decrease significantly only by when bergenin was given at a dose of 75 mg/kg body weight. Hexane extract was suppressed significantly ($p<0.01$) to ALP 594.80 IU/L, TB 0.58 mg/dl and ALP 569.80 IU/L and ALT 71.20 IU/L when compared to toxicant group at doses of 75 and 150 mg/kg body weight respectively, while in comparison with control group it does not reduced increased levels of serum liver enzymes. At high dose of 250 mg/kg body weight hexane extract found significant effective ($p<0.01$) in decreasing of ALP 229.00 IU/L, ALT 57.200 IU/L and TB 0.38 mg/dl levels. Rats treated with high dose 250 mg/kg body weight of ethanol extract showed significant ($p<0.01$) depletion of liver enzymes levels to ALP 180.80 IU/L, ALT 54.40 IU/L and γ-GT 1.40 IU/L when compared to CCl_4 treated group. Administration of low doses 75 and 150 mg/kg body weight showed significant reduction ($p<0.01$) in enzyme level to ALP

598.20 IU/L, TB 0.46 mg/dl and ALP 275.00 IU/L and ALT 56.60 IU/L compared in comparison to toxicant group.

Table 3. Effect of pure compound bergenin, hexane and ethanol leaves extracts of *Peltophorum pterocarpum* on serum ALP, ALT, γ-GT, DB and TB in rats with CCl_4-induced hepatotoxicity (Therapeutic mode)

Compound and Extracts	Dose mg/kg	ALP (IU/ L)	ALT (IU/ L)	γ-GT (IU/ L)	DB (mg/ dl)	TB (mg/ dl)
Bergenin	25	216.80±0.001**	63.40±0.005**	1.80±0.009**	0.120±0.070	0.64±0.040
	75	188.00±0.003**	57.80±0.005**	1.00±0.034	0.08±0.016	0.50±0.040
	100	131.00±0.002**	35.80±0.005**	0.40±0.178	0.06±0.070	0.180±0.037
Peltophorum pterocarpum (Hexane Extract)	75	480.00±0.001**	83.30±0.002**	3.33±0.063	0.12±0.178	0.90±0.012
	150	317.60±0.003**	58.40±0.001**	1.60±0.056	0.08±0.016	0.54±0.050
	250	226.00±0.001**	37.66±0.001**	1.00±0.089	0.06±0.184	0.32±0.003**
Peltophorum pterocarpum (Ethanol Extract)	75	300.80±0.013	60.60±0.001**	1.60±0.120	0.08±0.016	0.46±0.002**
	150	268.60±0.025	56.00±0.001**	2.80±0.025	0.08±0.016	0.42±0.005**
	250	249.40±0.003**	51.60±0.005**	1.60±0.227	0.08±0.016	0.26±0.024
Standard drug	5.37	275.200±0.003**	77.000±0.007**	4.000±0.041	0.400±0.041	0.520±0.012
	10.75	263.000±0.008**	63.600±0.001**	3.600±0.029	0.200±0.116	0.360±0.001
	16.13	228.000±0.012	59.400±0.002**	0.600±0.070	0.120±0.004**	0.160±0.056
Control	-	258..25±0.006	42.00±0.007	1.00±0.034	0.046±0.033	0.13±0.002
Toxicant (CCl_4)	1.5	642.30±0.002	141.25±0.017	4.60±0.006	0.98±0.001	1.26±0.043

Values represent mean ± SEM of five animals in each group.
Significant difference between treated groups*P<0.001 and **P<0.01.

Table 4. Hepatic histopathological finding of pure compound bergenin, hexane and ethanol leaves extracts of *Peltophorum pterocarpum* (Therapeutic model)

Compound and Extracts	Dose mg/kg	Portal Tract				Lobules					Loss of central vein
		Fibrosis (Stage)	Inflammation (Grade)	Bile duct	Portal vein	Lobular damage	Hepatocytes		Fatty liver		Finding
							Degeneration	Necrosis	Micro	Macro	
Bergenin	25	2	3	Intact	Intact	-ve	-ve	-ve	+ve	+ve	Intact
	75	2	3	Intact	Intact	-ve	-ve	-ve	+ve	+ve	Intact
	100	0	1	Intact	Intact	-ve	-ve	-ve	-ve	-ve	Intact
Peltophorum pterocarpum (Hexane Extract)	75	1	3	Intact	Intact	-ve	-ve	-ve	-ve	-ve	Intact
	150	0	2	Intact	Intact	-ve	-ve	-ve	-ve	-ve	Intact
	250	0	1	Intact	Intact	-ve	-ve	-ve	-ve	-ve	Intact
Peltophorum pterocarpum (Ethanol Extract)	75	2	3	Intact	Intact	+ve	Focal	-ve	-ve	-ve	Intact
	150	1	2	Intact	Intact	-ve	-ve	-ve	-ve	-ve	Intact
	250	1	2	Intact	Intact	-ve	Focal	-ve	-ve	-ve	Intact
Standard Drug	5.37	4	3	Focal Intact	Intact	-ve	+ve	-ve	Mild +ve	Mild +ve	+ve
	10.75	0	1	Intact	Intact	-ve	-ve	-ve	-ve	-ve	Intact
	16.13	1	1	Intact	Intact	-ve	-ve	-ve	-ve	-ve	Intact
Control	-	0	0	Intact	Intact	-ve	-ve	-ve	-ve	-ve	Intact
Toxicant (CCl_4)	1.5*	2	3	Intact	Intact	+ve	+ve	+ve	+ve	Mild +ve	Intact

(-ve) Not present, (+ve) Present

The five days treatment of pure compound bergenin at doses of 25, 75 and100 mg/kg body weight noted to reduce the elevated liver enzymes in a dose dependent manner (25mg / kg body weight = ALP 216.80 IU/L, ALT 63.40 IU/L, γ-GT 1.80 mg/dl, DB 0.12 mg/dl, TB 0.64 mg/dl), (75 mg / kg body weight = ALP 188.00 IU/L, ALT 57.80 IU/L, γ-GT 1.00 mg/dl, DB 0.08 mg/dl, TB 0.50 mg/dl) and (100 mg / kg body weight = ALP 131.00 IU/L, ALT 35.80 IU/L, γ-GT 0.40 mg/dl, DB 0.06 mg/dl, TB 0.18 mg/dl) respectively when compared to toxicant (ALP 642.30 IU/L, ALT 141 25 IU/L, γ-GT 4.60 mg/dl, DB 0.98 mg/dl, TB 1.26 mg/dl) and control group. Statistical significance in reduction ($p<0.01$) of serum enzymes levels was observed in ALP and ALT levels treated with 25, 75 and 100 mg/kg body weight doses as well as the significant reduction of γ-GT level was also observed by 25 mg/kg body weight dose. Treatment of animals with hexane extract at doses of 75, 150 and 250 mg/kg body weight were significantly decreased ($p<0.01$) the levels of ALP, ALT and TB when compared to toxicant but this level of suppression was found to be poor in comparison to control group.

Figure 1: Histopathological micrograph of a normal control rat

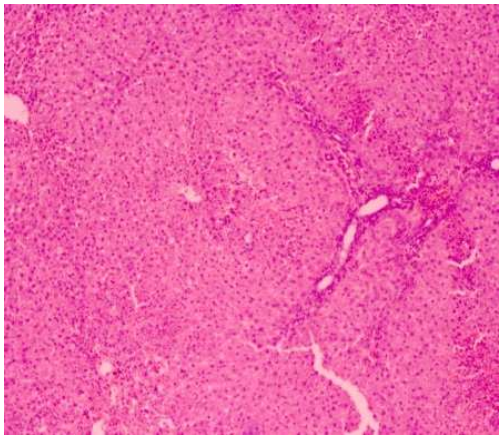

Figure 2: Histopathological micrograph of a rat treated with CCl_4

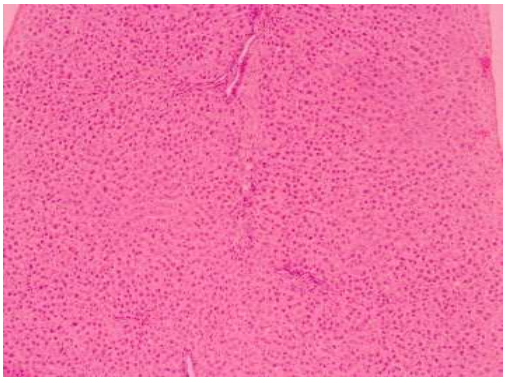

Figure 3: Histopathological micrograph of rat liver section treated with 16.13 mg/kg standard drug and CCl_4 (prophylactic model)

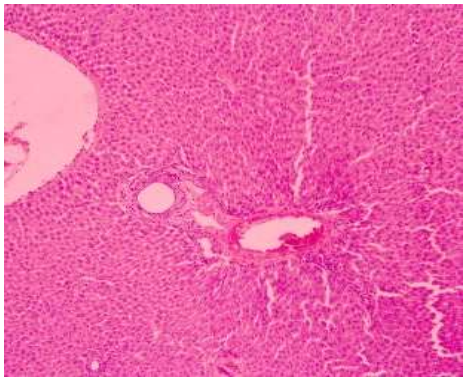

Figure 4: Histopathological micrograph of rat liver section treated with 100 mg/kg pure compound bergenin and CCl_4 (prophylactic model)

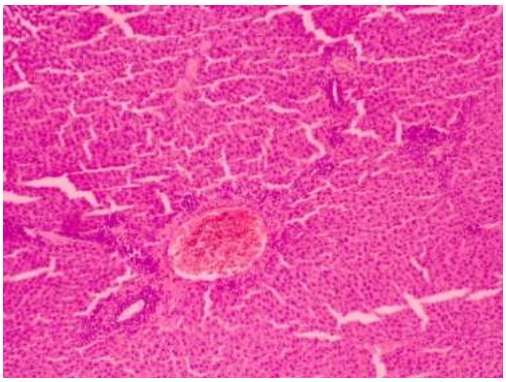

Figure 5: Histopathological micrograph of rat liver section treated with 250 mg/kg hexane extract of *Peltophorum pterocarpum* leaves and CCl_4 (prophylactic model)

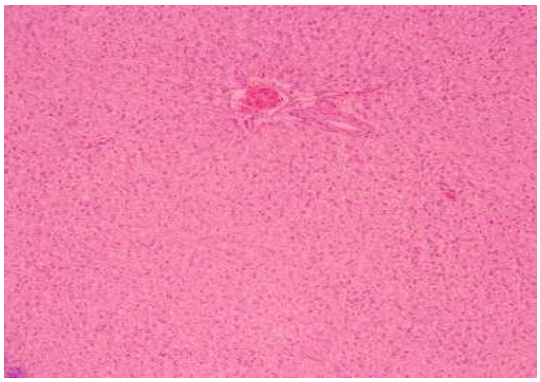

Figure 6: Histopathological micrograph of rat liver section treated with 250 mg/kg ethanol extract of *Peltophorum pterocarpum* leaves and CCl_4 (prophylactic model)

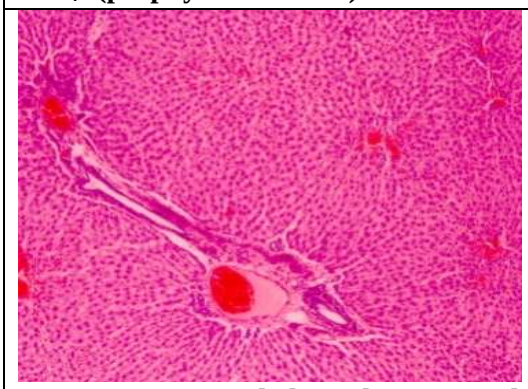

Figure 7: Histopathological micrograph of rat liver section treated with 16.13 mg/kg standard drug and CCl_4 (therapeutic model)

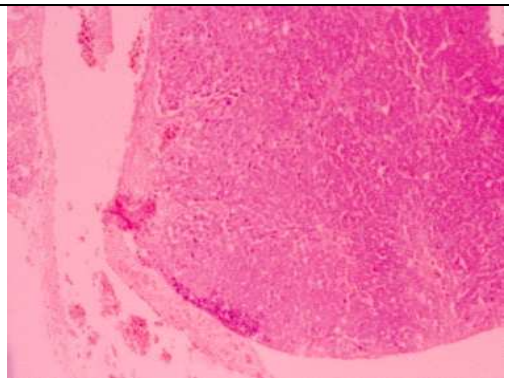

Figure 8: Histopathological micrograph of rat liver section treated with 100 mg/kg pure compound bergenin and CCl_4 (therapeutic model)

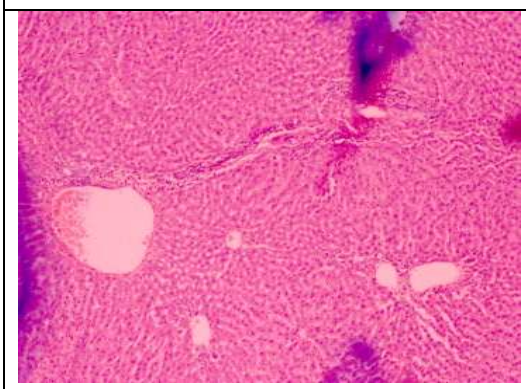

Figure 9: Histopathological micrograph of rat liver section treated with 250 mg/kg hexane extract of *Peltophorum pterocarpum* leaves and CCl_4 (therapeutic model)

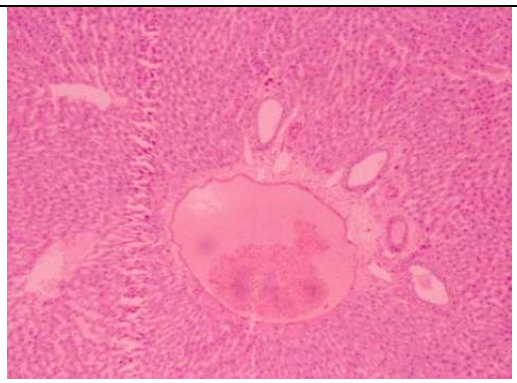

Figure 10: Histopathological micrograph of rat liver section treated with 250 mg/kg ethanol extract of *Peltophorum pterocarpum* leaves and CCl_4 (therapeutic model)

Serum enzymes estimation of test samples has been further ascertained by histopathological observations. The section of liver was focused in portal and central vein. The liver tissue of CCl_4 treated (prophylactic mode) rats viewed under optical microscope appeared with severe and prominent diffuse hepatocellular damage (grade - 4 inflammation) along with prominent bridging fibrosis (stage - 3 fibrosis) and architectural distortion, gross necrosis, interlobular damaged and scattered fatty degeneration were observed in **Figure 2**.

Histopathological observations of the liver of rats pretreated with high doses 100 mg/kg body weight **Figure 4, 5 and 6** of pure compound bergenin, hexane and ethanol extracts (250 mg/kg body weight) of *Peltophorum pterocarpum* leaves and subsequently given CCl_4 showed a more or less normal architecture of liver there was a stage 1 portal fibrosis with mild inflammation of hepatic cells were observed. No hepatic damage was seen in the central vein and showed normal appearance of portal vein almost comparable to the normal group **Figure 1** and the high dose treated 16.13 mg/kg body weight group of standard drug **Figure 3**. Pretreatment of test samples with low doses of pure compound bergenin (25 and 75 mg/kg body weight), hexane and ethanol extracts (75 and 150 mg /kg body weight) showed stage 1 and 2 fibrosis and mild to severe portal inflammation and fatty degeneration. These results are close to the standard drug treatment with low doses 5.37 and 10.75 mg/ kg body weight showing minimal inflammation and periportal fibrosis **(Table 2)**.

Histopathological observations of the liver of rats in therapeutic mode at high doses **Figure 8** treated with pure compound bergenin (100 mg/kg body weight), hexane and ethanol extracts (250 mg/kg body weight) of *Peltophorum pterocarpum* leaves inhibited the indication of fibrosis and inflammation in portal vein **Figure 9, 10**. These histopathological findings of liver section are comparable with liver of rats treated with standard drug at high dose of 16.13 mg/ kg body weight **Figure 7**.

Liver tissue had normal morphology of bile duct, portal central vein, but septal fibrosis with micro and macro vascular fatty liver and moderate grade of inflammation were detected, at low doses treatment of pure compound bergenin (25 and 75 mg/kg body weight), hexane and ethanol extracts (75 and150 mg /kg body weight) of *Peltophorum pterocarpum* leaves. These results are near to treatment of standard drug with low doses 5.37 and 10.75 mg/kg body weight showed moderate grade of inflammation of portal tract and mild fatty liver with lobular degeneration **(Table 4)**.

Discussion

Hepatoprotective experimental study revealed that pure compound bergenin, hexane and ethanol leaves extracts of *Peltophorum pterocarpum* had potential to act both in prophylactic and therapeutic model in a dose dependent manner against liver injury induced by CCl_4. This was an effective investigation test with high negative prognostic value of liver diseases that were supported with histopathological examination of rat liver section. Tested samples might have protected both plasma membrane and liver cells against damage thereby decreasing the leakage of serum enzymes into blood circulation, while the anti-oxidant activity of phytochemicals, may have helped in controlling complication during degenerative liver diseases. This may

be further linked to enzyme suppression results (ALP 129.80 IU/L, ALT 31.00 IU/L, γ-GT 0.80 IU/L, DB 0.04 mg/dl and TB 0.20 mg/dl) as seen in prophylactic model by bergenin at a dose of 100 mg/kg body weight. Further, the reported anti-oxidant activity[4] of phytoconstituents, such as alkaloids, flavonoids and polyphenolic compounds can counteract with free radicals to avoid their destructive effect by increasing capability of anti-oxidant enzymes CAT, SOD and glutathione peroxidase. Results of the present study indicated promising hepatoprotective activity of bergenin against CCl_4 intoxicated animals and may be linked to its capability of enhancing the activity of anti-oxidant enzymes or suppressing the free radical activity.

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