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Effect of *Adenanthera pavonina* leaves extracts and β-sito sterol glucoside in CCl_4 induced hepatocellular injury in Wistar rats

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ABSTRACT

The aim of this research work was to investigate the less toxic and more specific hepatoprotective and anti-oxidant effects of hexane, ethanol extracts along with pure compound β-sitosterol glucoside isolated from leaves of *Adenanthera pavonina* in carbon tetra chloride (CCl_4) induced liver damage in rat. The anti-oxidant effect was assessed in different concentrations of 100, 250, 500 and 1000 µg/ml by *in-vitro* model system of ferric cyanide reducing power assay using, ascorbic acid as a standard drug. Ethanol and hexane extracts of *A. pavonina* 75, 150 and 250 mg/kg body weight along with pure compound β-sitosterol glucoside 25, 75 and 100 mg/kg body weight were administered orally for six days to CCl_4 (1.5 ml/kg body weight in equal volume of olive oil (1:1)) treated rats and the hepatoprotective activity was assessed in prophylactic and therapeutic model using biochemical parameters aspartate aminotransferase (AST), alanine biochemical parameters like aminotransferase (ALT), alkaline phosphate (ALP), total bilirubin (TB) and direct bilirubin (DB) along with histopathological studies of liver tissues. The results were compared with standard hepatoprotective drug containing (BetaineGlucuronate + Diethanol amine Glucuronate + Nicotinamide Ascorbate). Five-days treatment of tested samples showed dose dependent reduction of CCl_4 induced elevated activity of serum enzymes and total and direct bilirubin indicating the recovery of hepatic enzymes both in prophylactic and therapeutic model. However oral administration of β-sitosterol glucoside can significantly reduce the activity of ALP 183.40 IU/L, ɣ-GT 0.60 IU/L, DB 0.04 mg/dl and ALT 30.20 IU/L, TB 0.14 mg/dl at a dose of 100 mg/kg body weight in prophylactic and therapeutic model respectively. This hepatoprotective effect was comparable to that of the standard drug. Histopathological study of animals showed intact bile duct and portal vein without any cellular necrosis and fatty infiltration. The ferric cyanide reducing power assay showed that *A. pavonina* possessed magnificent anti-oxidant potential in concentration dependent manner but less than standard drug ascorbic acid and among all tested samples β-sitosterol glucoside indicated highest reducing power 0.279. Results of the present study indicated presence of anti-oxidant phytochemicals in *A. pavonina* leaves and exhibited substantial reducing power of free radicals, responsible for promising hepatoprotective effect.

Keywords: *Adenanthera pavonina*, *in-vivo* hepatoprotective, histopathology, CCl_4, *in-vitro* anti-oxidant.

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INTRODUCTION

The liver is the second largest organ in the body and comprises 2.5% of the body weight (Robin et al., 2012).

Major functions associated with the liver such as cardiovascular system, digestives system, excretory system and metabolism. It has vast capacity to detoxify toxic substances and synthesize useful components. Due to unique metabolism and close relationship with gastrointestinal tract the liver is susceptible to injury from expended therapy with potent drugs, infectious diseases, uncontrolled environmental pollution, poor sanitary conditions and serum inflammatory mediators which may lead to diverse range of liver disorders and loss of liver function capacity. Most of the available modern system of hepatoprotective agents only brings symptomatic relief and in most of the cases have no influence effect on the disorders (Handa et al., 1986). Further their use is associated with wide range of side effects. Natural products are playing vital role in health care for decades and are potential sources of more likely to yield pharmacologically active compounds, which may serve as ingredients in formulations containing synthetic drugs. That stimulates liver function, offer protection to the liver damage or help in regeneration of hepatic cell. Ara et al. (2010) reported that the different parts of *A. pavonina* are used in traditional medicine to treat inflammation and rheumatism. The pharmacologic effects of *A. pavonina* as, anti-oxidant and anti-inflammatory have been reported by Mujahid et al. (2013) and hepatoprotective activity was reported by Godoi et al. (2014). Previous chemical investigation reported various secondary metabolites mainly alkaloids, carbohydrates, glucosides of β-sitosterol, glycosides, flavonoids, saponins, steroids, stigma sterol, tanins and terpenoids are present in *A. pavonina* (Khare, 2007; Partha and Rehaman, 2015). In view of numerous traditional uses and documented pharmacological activities of *A. pavonina*, this study was designed and attempt have been made to investigate the effect of hexane and ethanol extracts along with pure compound β-sitosterol glucoside as a protective and therapeutic agent on CCl_4 induced hepatocellular injury in rats in the light of liver function test and histological changes with respect to the phytochemicals linked with anti-oxidant and free radical defense system. The aim of the study is to discover naturally derived therapeutic agent with hepatoprotective effect; since anti-inflammatory activities are crucial factor in management of liver disorder. Therefore, *A. pavonina* suggested being an efficient hepatoprotective agent.

MATERIALS AND METHODS

Plant materials

The leaves of *Adenanthera pavonina 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 of *A. pavonina* is available in the Herbarium of Department of Pharmacognosy, University of Karachi, Karachi, Pakistan.

Preparation of crude extracts

Washed leaves of *A. pavonina* were dried under shade for two weeks and then 3 kg of dried leaves soaked in hexane for 15 days followed by filtration and evaporation under reduced pressure by using rotary evaporator at 45°C. The residue obtained after filtration was soaked in ethanol for next 15 days. The ethanol leaves extract was filtered and evaporated under reduced pressure using rotary evaporator at 45°C and stored in air tight glass vials for biological studies with proper identification (Tasleem, 2015).

Isolation of β-sitosterol glucoside from *Adenanthera pavonina*

β-sitosterol glucoside was isolated from the leaves of *A. pavonina* and identified using High performance liquid chromatography method (Saeidnia et al., 2014; Tasleem, 2015). Dissolved the ethanol extract (20 g) in minimum quantity of petroleum ether and allowed to adsorbed on 80 g of silica gel of 100 to 200 mesh particle size. The slurry was allowed to dry. A dried and neat column (95 cm × 6 cm) was plugged with a cotton bung at the base of column and then petroleum ether with silica gel was slowly added to the slurry. The absorbed extract was charged into the column. The column was first washed out by petroleum ether and then with solvent by constantly increasing the percentage of ethyl acetate in petroleum ether. The eluent was collected and concentrated on water bath (20 to 25°C) (Arora and Kalia, 2013).

Drugs and chemicals

All the drugs and chemicals used in the study were of analytical grade. Carbon tetrachloride was obtained from Sigma. While the standard drug Betaine Glucuronate + Diethanolamine Glucuronte + 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 to 200 g were used in this study. Animals were kept and maintained under laboratory condition of temperature (23 ± 3°C) with 12/12 h light and dark cycles and were allowed free attain of food and water and marked with their identification. This study was approved by Board of Advanced Studies and Research, University of Karachi BASR/No./0265/Pharm.

In-vitro testing of extracts and pure compound for anti-oxidant activity

Reducing power assay (RAP)

For evaluation of *in-vitro* antioxidant activity 100 mg of pure compound β-sitosterol glucoside, extracts and Ascorbic acid (standard drug) were taken in 50 ml volumetric flask and dissolved with sufficient amount of solvent (ethanol : water, 1:1). The volume was made up with as stock solution having 2000 µg/ml. Three different concentrations of pure compound, hexane, ethanol extracts and standard drug were prepared from stock solution.

1. 1000 µg/ml
2. 500 µg/ml
3. 250 µg/ml
4. 100 µg/ml

Each concentration of pure compound, extracts and standard (1 ml) was taken in test tube. To the test tube 5 ml of phosphate buffer (0.2 M, pH 6.6) and 1% potassium ferric cyanide solution was added and mixed well. This mixture was incubated at 50°C for 20 min. After incubation, 5 ml trichloro-acetic acid was added to stop the reaction and was kept for centrifugation at 3000 rpm for 10 minutes. After centrifugation 5 ml of supernatant was taken into another test tube and to this 5 ml of distilled water and 1 ml of 0.1% ferric chloride were added and left for 10 min. The absorbance was measured at 700 nm on spectrophotometer. The reducing power is linearly proportional to the concentration of the sample. Whole experiment was conducted in triplicate. The control was prepared by adding all reagents but without sample (Bukhari et al., 2008; Doss and Pugalenthi, 2012). The percentage of reduction power of test samples compared to standard (Ascorbic acid) was calculated by following formula (Rohman et al., 2010):

% of reduction power = $[1- (1-A_S/A_C)] \times 100$

A_S = absorbance of sample
A_C = absorbance of standard at maximum concentration tested

In-vivo hepatoprotective testing of extracts and pure compound

Scanning of hepatoprotective activity of pure compound, extracts and standard drug were performed in two models (Argal and Dwivedi, 2010):

i. Prophylactic study model
ii. Therapeutic study model

One hundred eighty (180) male and female rats were divided into six groups (A, B, C, D, E and F) of five animals each (n = 5) and treated orally as followed.

Group A served as tested drug group and further divided into three groups:

1. A Rats received β-sitosterol glucoside 25 mg/kg body weight for five days, on sixth day rats received CCl4 (1.5 ml/kg body weight in equal volume of olive oil (1:1))
2. A Rats received β-sitosterol glucoside 75 mg/kg body weight for five days, on sixth day rats received CCl4 (1.5 ml/kg body weight in equal volume of olive oil (1:1))
3. A Rats received β-sitosterol glucoside 100 mg/kg body weight for five days, on sixth day rats received CCl4 (1.5 ml/kg body weight in equal volume of olive oil (1:1))

Group B served as tested drug group and further divided into three groups:

1. B Rats received hexane extract 75 mg/kg body weight for five days, on sixth day rats received CCl4 (1.5 ml/kg body weight in equal volume of olive oil (1:1))
2. B Rats received hexane extract 150 mg/kg body weight for five days, on sixth day rats received CCl4 (1.5 ml/kg body weight in equal volume of olive oil (1:1))
3. B Rats received hexane extract 250 mg/kg body weight for five days, on sixth day rats received CCl4 (1.5 ml/kg body weight in equal volume of olive oil (1:1))

Group C served as tested drug group and further divided into three groups

1. C Rats received ethanol extract 75 mg/kg body weight for five days, on sixth day rats received CCl4 (1.5 ml/kg body weight in equal volume of olive oil (1:1))
2. C Rats received ethanol extract 150 mg/kg body weight for five days, on sixth day rats received CCl4 (1.5 ml/kg body weight in equal volume of olive oil (1:1))
3. C Rats received ethanol extract 250 mg/kg body weight for five days, on sixth day rats received CCl4 (1.5 ml/kg body weight in equal volume of olive oil (1:1))

Group D served as standard group and further divided into three groups:

1. D Rats received standard drug 5.37 mg/kg body weight for five days, on sixth day rats received CCl4 (1.5 ml/kg body weight in equal volume of olive oil (1:1))
2. D Rats received standard drug 10.75 mg/kg body weight for five days, on sixth day rats received CCl4 (1.5 ml/kg body weight in equal volume of olive oil (1:1))
3. D Rats received standard drug 16.13 mg/kg body weight for five days, on sixth day rats received CCl_4 (1.5 ml/kg body weight in equal volume of olive oil (1:1))

Group E served as toxicant group. Rats received distilled water for five days on sixth day rats received single dose of CCl_4 (1.5 ml/kg body weight in equal volume of olive oil (1:1))
Group F served as normal control group. Rats received only saline for five days.

This experimental design was performed in duplicate, that is, prophylactic and therapeutic models keeping all the parameters same as described above.

Prophylactic study model

Acute hepatotoxicity was induced by CCl_4 in predefined experimental groups on the fifth day thirty minutes post treatment of tested drugs.

Therapeutic study model

In this investigation work, first trigger toxicity in liver by oral administration of single dose of CCl_4. After half an hour of CCl_4 administration animals were given their particular doses for 5 days regularly excluding control group.

On day six rats of both evaluation prototypes were sacrificed using anesthesia and approximately 5 ml blood sample 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 (Tasleem, 2015).

Biochemical analysis

Liver damage was assessed by the estimation of serum activities of hepatic ALP, ALT, γ-GT, DB and TB according to the reported methods by Edwards and Bouchier (1991) using commercially available kits. The enzyme activities were measured and read on a Photometric Micro lab. Liver function test was performed at 25°C, and all the reagents were ready to use (Alqasoumi et al., 2013).

Histopathological analysis of liver

Incision was made on abdomen of the animals, the liver excised, weighed and used for histological studies. Liver tissues were fixed with 10% phosphate-buffered neutral formalin, dehydrated in graded (50 to 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 (Lodhi et al., 2009).

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 determines a distinctive phase or stages necrosis or in a broader sense the damages occur in liver cells.

Criteria used to detect staging of fibrosis in portal tract are shown in Figure 1. Criteria used to detect grading of inflammation in portal tract are shown in Figure 2 (Batts and Ludwig 1995; Gelow et al., 2010).

Statistical analysis

The obtained data were statistically analyzed by one-way analysis of variance (ANOVA) followed by the Duncan multiple tests All analyses were performed using the statistical package for social science [SPSS version 20]. Values of *P < 0.001 and **P < 0.01 were considered significant (Khan and Khanum, 1994).

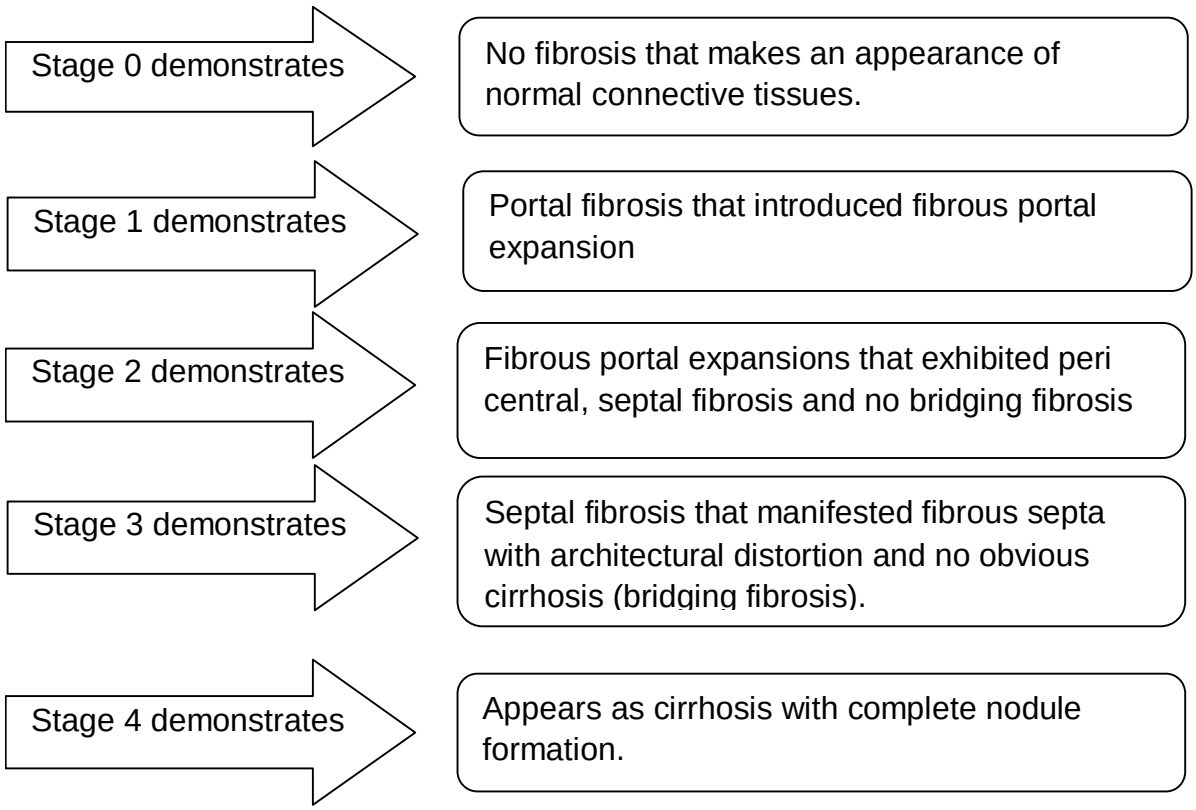

Figure 1. Criteria used to detect staging of fibrosis in portal tract.

RESULTS

A pale yellow amorphous solid of β-sitosterol glucoside was obtained from ethanol extract of *A. pavonina* leaves. Identification of isolated compound based on detection by melting point thin layer chromatography and infra red analysis. The melting point of β-sitosterol glucoside is 284°C which was confirmed by published melting point 283 to 286°C by (Arunachalam et al., 2009). Presences of β-sitosterol glucoside was detected on TLC plates as pink spot with Rf value of 0.5 which was near to the earlier reported Rf value of 0.6 by Rahmana et al. (2009). IR spectrum had absorption features at 3515 cm^{-1} this indicating that a hydroxyl group (-OH) is present. Stretching of normal aliphatic ester (>C=O) showed region of absorption at 1650 cm^{-1}. Finally, the absorption band at 815 cm^{-1} indicated the –CH stretching. This spectral data was comparable to the spectral data given by Arora and Kalia (2013).

In-vitro anti-oxidant activity

The *in-vitro* antioxidant power of test samples was analyzed by direct electron donation in reduction of Fe^{3+} to Fe^{2+} and compared with standard ascorbic acid. The product was visualized by forming the intense Prussian blue color complex. Higher absorbance value indicated a

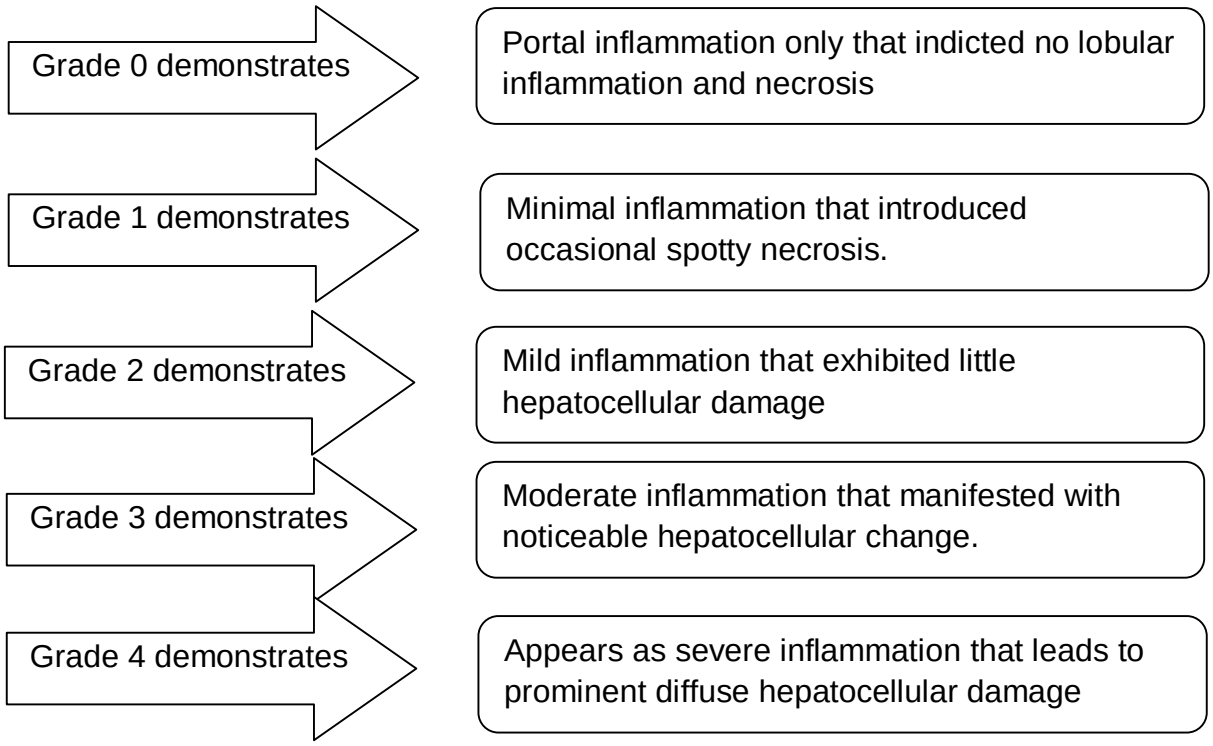

Figure 2. Criteria used to detect grading of inflammation in portal tract.

Table 1. Reducing power capacity of β-sitosterol glucoside, hexane and ethanol extracts of *Adenanthera pavonina*

Concentration (µg/ml)	Absorbance at 700 nm			
	Standard drug (ascorbic acid)	β-sitosterol glucoside	Hexane extract	Ethanol extract
100	0.106 ± 0.005	0.226 ± 0.0008	0.102 ± 0.002	0.032 ± 0.001
250	0.514 ± 0.011	0.232 ± 0.0007	0.122 ± 0.016	0.109 ± 0.127
500	1.026 ± 0.006	0.251 ± 0.008	0.125 ± 0.019	0.124 ± 0.002
1000	1.441 ± 0.094	0.279 ± 0.028	0.244 ± 0.031	0.246 ± 0.057

Key: The results are means of triplicate estimations, ± SEM.

stronger reducing power of the test samples as shown in (Tables 1 and 2).

Hepatoprotective studies

Control group

Blood serum and liver tissue sample were evaluated for biochemical and histological analysis. Serum activity of enzymes ALP, ALT, γ-GT and DB and TB were presented in Table 3 and histopathological finding of liver tissue were shown in Table 4 and Figure 3.

Toxicant group

Single dose administration of CCl_4 induced an increased hepatic enzymes activity as shown in (Table 3). Histological profile showed severally diffused portal tract, hepatocellular damage with prominent bridging fibrosis and architectural distortion, interlobular damaged and scattered fatty degeneration and highlighted in Table 4 and Figure 4.

Prophylactic model

Standard drug

Pretreatment of standard drug prevented the raised in hepatic enzymes levels and kept them near to normal when given in high concentrations such as 16.13 mg/kg body weight (Table 3). Histopathological profile of liver tissue presented no visible changes in hepatic cells.

Table 2. Percentage of reducing power capacity of β-sitosterol glucoside, hexane and ethanol extracts of *Adenanthera pavonina.*

Concentration (µg/ml)	Percentage of reducing power capacity			
	Standard drug (ascorbic acid)	β-sitosterol glucoside	Hexane extract	Ethanol extract
100	7.30	11.6	7.0	2.2
250	35.60	16.0	8.4	7.5
500	71.30	17.4	8.6	8.6
1000	-	19.3	16.9	17.0

Key: Percentage of reduction power calculated by absorbance of standard (ascorbic acid) at maximum concentration tested.

Table 3. Effect of *Adenanthera pavonina* on biochemical parameters relating to hepatoprotective properties (prophylactic model).

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)
β-sitosterol glucoside	25	5.20 ± 0.489	506.20 ± 0.004**	43.40 ± 0.001**	3.60 ± 0.066	0.20 ± 0.034	0.46 ± 0.024
	75	4.40 ± 0.400	371.60 ± 0.001**	38.60 ± 0.003**	2.60 ± 0.033	0.08 ± 0.016	0.36 ± 0.002**
	100	5.20 ± 0.480	183.40 ± 0.001**	37.40 ± 0.007**	0.60 ± 0.070	0.04 ± 0.178	0.28 ± 0.034
Adenanthera pavonina(hexane extract)	75	5.20 ± 0.800	514.00 ± 0.009**	61.80 ± 0.001**	2.40 ± 0.118	0.08 ± 0.016	0.52 ± 0.001**
	150	6.00 ± 0.001**	354.20 ± 0.008**	54.20 ± 0.005**	1.80 ± 0.105	0.08 ± 0.016	0.50 ± 0.001**
	250	4.40 ± 0.400	258.60 ± 0.001**	51.80 ± 0.010**	1.20 ± 0.070	0.08 ± 0.016	0.38 ± 0.001**
Adenanthera pavonina (ethanol extract)	75	4.40 ± 0.400	551.00 ± 0.002**	72.80 ± 0.001**	2.60 ± 0.129	0.12 ± 0.070	0.40 ± 0.002**
	150	4.80 ± 0.001**	488.14 ± 0.002**	52.00 ± 0.001**	1.40 ± 0.005**	0.12 ± 0.070	0.34 ± 0.030
	250	4.80 ± 0.001**	242.40 ± 0.005**	58.20 ± 0.002**	1.20 ± 0.109	0.12 ± 0.004**	0.32 ± 0.001**
Standard drug	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	-	.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

'Key: ALP= Alkaline phosphatase, ALT= Alanine aminotransferase, γ--GT= Gamma-glutamyl transpeptidase, DB= Direct bilirubin, TB = Total bilirubin IU/ dl= International units per liter, mg/dl = Milligram per deciliter.
All values are given in Mean ± SEM, n = 5, *P < 0.001 and **P<0.01 significant as compared to toxicant
♦Dose of toxicant in ml/kg body weight.

Table 4. Hepatic histopathological findings of *Adenanthera pavonina* (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	
β-sitosterol glucoside	25	2	3	Intact	Intact	+ve	+ve	+ve	+ve	+ve	Intact
	75	1	1	Intact	Intact	-ve	-ve	-ve	-ve	-ve	Intact
	100	0	1	Intact	Intact	-ve	-ve	-ve	-ve	-ve	Intact
Adenanthera pavonina (Hexane Extract)	75	4	2	Damage	Lightly dilated	+ve	-ve	+ve	+ve	Mild +ve	Intact
	150	3	1	Intact	Intact	-ve	-ve	-ve	-ve	-ve	Intact
	250	1	1	Intact	Intact	-ve	-ve	-ve	-ve	-ve	Intact
Adenanthera pavonina (ethanol extract)	75	2	4	Intact	Intact	Focal	+ve	-ve	+ve	-ve	Intact
	150	2	3	Intact	Intact	-ve	-ve	-ve	+ve	-ve	Intact
	250	2	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	+ve	Intact

Key: (-) Not present, (+) present,
♦Dose of toxicant in ml/kg body weight.

Although at less concentration of 5.37 and 10.75 mg/kg body weight, the magnitude of liver enzymes in serum were noted inferior than control but less than toxicant, which was confirmed by biopsy results shown in Table 4 and Figure 5.

Adenanthera pavonina

Prophylactic effect of β-sitosterol glucoside, hexane and ethanol extracts in CCl_4 induced hepatotoxicity were noted in Table 3. The pure compound β-sitosterol glucoside at doses of 25, 75 and 100 mg/kg body weight significantly reduced ($P < 0.01$) the levels of ALP and ALT and considerable decreased in γ-GT, DB and TB, while stage 1 fibrosis of portal tract was observed in histopathological features when compared to toxicant group. However, 25 mg/kg developed stage 2 fibrosis and depicted in Figure 6 and Table 4.

Hexane extract at a dose of 250 mg/kg body weight was significantly suppressed ($P < 0.01$) the elevation of ALP, ALT and TB as compared to toxicant and control group, however γ-GT and DB were found less than toxicant but slightly extended than control group. Histopathological features demonstrated the stage 1 fibrosis and grade 1 lobular inflammation with normal endothelial cells around the central vein. Low doses 75 and 150 mg/kg body weight treatment presented the low level of safety profile against hepatotoxicity (Table 4 and Figure 7).

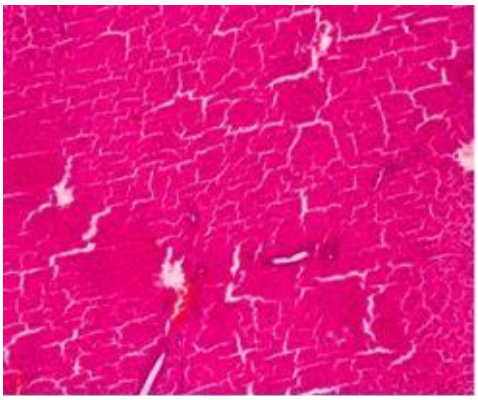

Figure 3. Histopathological micrograph of a normal control rat.

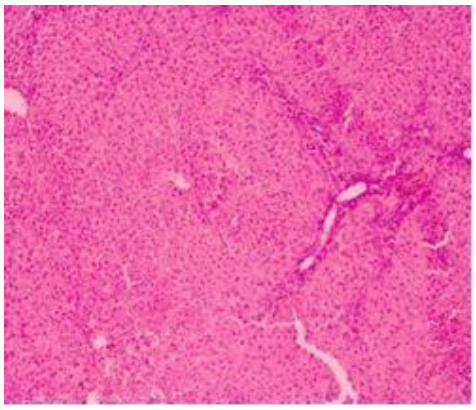

Figure 4. Histopathological micrograph of a rat treated with CCl_4.

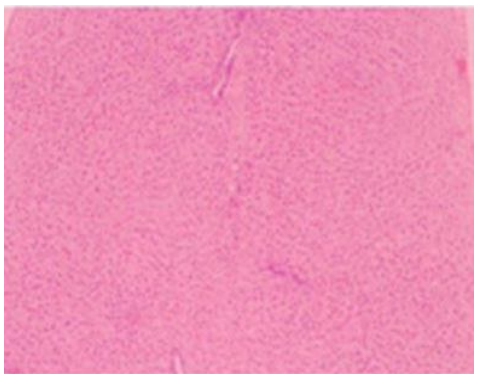

Figure 5. Histopathological micrograph of rat liver section treated with 16.13 mg/kg standard drug and CCl_4 (prophylactic model).

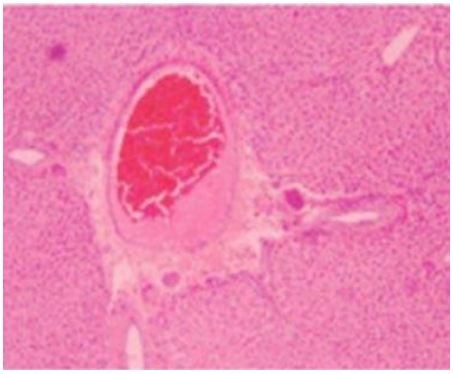

Figure 6. Histopathological micrograph of rat liver section treated with 100 mg/kg pure compound β-sitosterolglucoside and CCl_4 (prophylactic model).

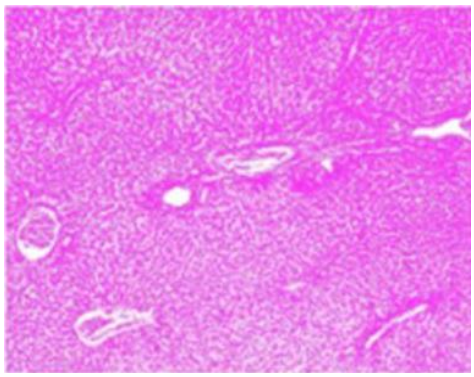

Figure 7. Histopathological micrograph of rat liver section treated with 250 mg/kg hexane extract of *Adenanthera pavonina* leaves and CCl_4 (prophylactic model).

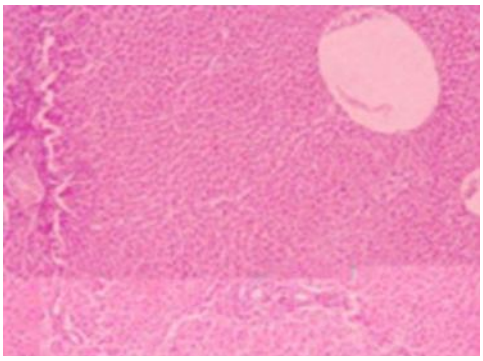

Figure 8. Histopathological micrograph of rat liver section treated with 250 mg/kg ethanol extract of *Adenanthera pavonina* leaves and CCl_4 (prophylactic model).

Pretreatment of ethanol extract at a dose of 250 mg/kg body weight showed the significant reduction ($P < 0.01$) of all tested enzymes when compared to toxicant group but slightly less than the control group. Histopathological examination of liver section presented stage 2 fibrosis and grade 1 inflammation of portal tract. Hepatoprotective effect of ethanol extract tested at doses of 75 and 150 mg/kg body weight was found similar to hexane extract with mild stage of fibrosis but severe degree of inflammation was noted (Figure 8 and Table 4).

Table 5. Effect of *Adenanthera pavonina* on biochemical parameters relating to hepatoprotective properties (therapeutic model).

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)
β-sitosterol glucoside	25	5.20 ± 0.489	501.60 ± 0.015	39.80 ± 0.005**	3.00 ± 0.023	0.14 ± 0.052	0.40 ± 0.003**
	75	4.40 ± 0.400	215.40 ± 0.004**	33.40 ± 0.014	1.80 ± 0.053	0.14 ± 0.025	0.44 ± 0.001**
	100	4.40 ± 0.400	191.00 ± 0.001**	30.20 ± 0.001**	1.20 ± 0.109	0.04 ± 0.178	0.14 ± 0.052
Adenanthera pavonina (Hexane Extract)	75	5.330 ± 0.015	324.50 ± 0.001**	97.33 ± 0.003**	2.60 ± 0.098	0.166 ± 0.067	0.68 ± 0.004**
	150	4.80 ± 0.001**	452.4 ± 0.016	54.20 ± 0.024	2.00 ± 0.066	0.08 ± 0.099	0.46 ± 0.008**
	250	5.20 ± 0.003**	246.20 ± 0.002**	52.80 ± 0.001**	2.00 ± 0.047	0.08 ± 0.016	0.33 ± 0.010
Adenanthera pavonina (ethanol extract)	75	4.40 ± 0.440	474.60 ± 0.004**	61.60 ± 0.008**	2.80 ± 0.066	0.12 ± 0.033	0.38 ± 0.003**
	150	4.80 ± 0.001**	426.60 ± 0.010	61.80 ± 0.002**	2.00 ± 0.061	0.10 ± 0.034	0.280 ± 0.002**
	250	6.80 ± 0.489	205.60 ± 0.011**	58.60 ± 0.001**	1.80 ± 0.037	0.04 ± 0.178	0.12 ± 0.070
Standard drug	5.37	6.000 ± 0.005**	275.200 ± 0.003**	77.000 ± 0.007**	4.000 ± 0.041	0.400 ± 0.041	0.520 ± 0.012
	10.75	5.500 ± 0.002**	263.000 ± 0.008**	63.600 ± 0.001**	3.600 ± 0.029	0.200 ± 0.116	0.360 ± 0.001
	16.13	5.500 ± 0.002**	228.000 ± 0.012	59.400 ± 0.002**	0.600 ± 0.070	0.120 ± 0.004**	0.160 ± 0.056
Control	-	5.00 ± 0.003	258..2 ± 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

'Key: ALP= Alkaline phosphatase, ALT= Alanine aminotransferase, γ--GT = Gamma-glutamyl transpeptidase, DB = Direct bilirubin, TB= Total bilirubin IU/ dl= International units per liter, mg/dl = Milligram per deciliter.
All values are given in Mean ± SEM, n=5, *P<0.001 and **P<0.01 significant as compared to toxicant.
♦Dose of toxicant in ml/kg body weight.

Therapeutic model

Standard drug

Treatment with standard drug after liver intoxication by CCl_4 caused significant reduction in the levels of hepatic enzymes in dose dependent manner which were compared to toxicant group (Table 5). Histopathological examination results presented in Table 6 and Figure 9 also indorsed therapeutic profile of standard drug.

Adenanthera pavonina

Hepatoprotective potential of β-sitosterol glucoside, hexane and ethanol extracts were evaluated against intoxication of CCl_4 and the results were presented in (Table 5). Treatment of β-sitosterol glucoside with 100 mg/kg body weight resulted significant reduction, dose of 75 mg/kg body weight was effectively minimized the extended levels of hepatic enzymes, however low dose of 25 mg/kg body weight possessed mild hepatoprotection when compared to toxicant group, which was confirmed by microscopic description of pathology report presented in Table 6 and Figure 10.

Hexane extract at doses of 75, 150 and 250 mg/kg body weight exhibited poor hepatoprotective activity against CCl_4 induced liver impairment compared with control group. But significant reduction ($P < 0.01$) of ALP and ALT levels at a dose of 250 mg/kg body weight were found when compared to toxicant group. Liver

Table 6. Hepatic histopathological findings of *Adenanthera pavonina* (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	
β-sitosterol glucoside	25	2	3	Intact	Intact	Mild	-ve	-ve	+ve	+ve	Intact
	75	2	2	Intact	Intact	-ve	-ve	-ve	-ve	-ve	Intact
	100	1	2	Intact	Intact	-ve	-ve	-ve	-ve	-ve	Intact
Adenanthera pavonina (hexane extract)	75	2	3	Intact	Intact	-ve	-ve	-ve	+ve	+ve	Intact
	150	1	3	Intact	Intact	-ve	-ve	-ve	Minimal +ve	Minimal +ve	Intact
	250	1	3	Intact	Intact	+ve	+ve	-ve	+ve	+ve	Intact
Adenanthera pavonina (ethanol extract)	75	1	2	Intact	Intact	-ve	+ve	-ve	+ve	-ve	Intact
	150	1	1	Intact	Intact	-ve	-ve	-ve	-ve	-ve	Intact
	250	0	1	Intact	Intact	-ve	+ve	-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

Key: (-) Not present, (+) present,
♦Dose of toxicant in ml/kg body weight

tissues exhibited the grade 3 hepatic inflammation and stage 3 fibrosis (Figure 11 and Table 6).

Similarly, at doses of 75, 150 and 250 mg/kg body weight, the ethanol extract when administered at a dose of 250 mg/kg body weight decreased the elevated activities of ALP, γ-GT and bilirubin when compared to toxicant group. Although it was slightly comparable with control group and histopathological examination indicated the grade 1 inflammation. At doses of 75 and 150 mg/kg body weight ethanol extract inhibited the activity of serum liver enzymes when compared to toxicant group and liver tissue analysis showed stage 1 hepatic fibrosis (Figure 12 and Table 6).

DISCUSSION

The present work has been an attempt to demonstrate therapeutic and prophylactic action of *Adenanthera pavonina* leaves hexane and ethanol extracts along with a pure compound β-sitosterol glucoside against CCl_4 induced liver injury in rats by recording morphological parameters (liver weight), determination of serum activities of enzymes and histological changes. Choice of the plant has been based on the traditional usage to treat inflammation and rheumatism.

The preliminary phytochemical screening of

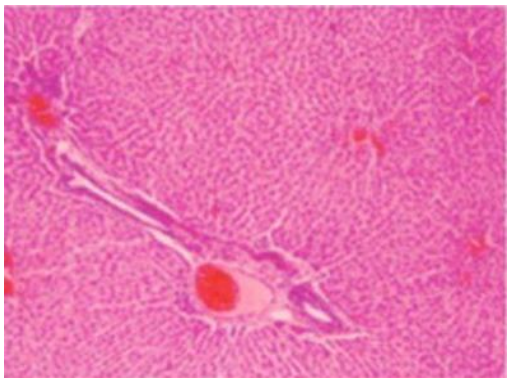

Figure 9. Histopathological micrograph of rat liver section treated with 16.13 mg/kg standard drug and CCl_4 (therapeutic model).

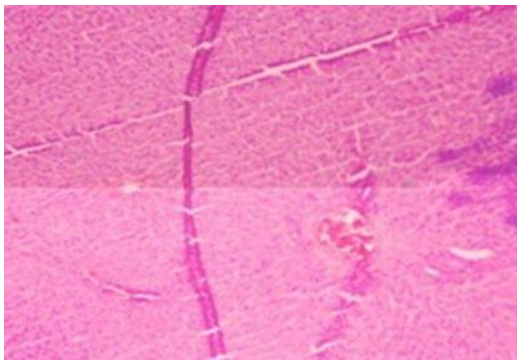

Figure 11. Histopathological micrograph of rat liver section treated with 250 mg/kg hexane extract of *Adenanthera pavonina* leaves and CCl4 (therapeutic model).

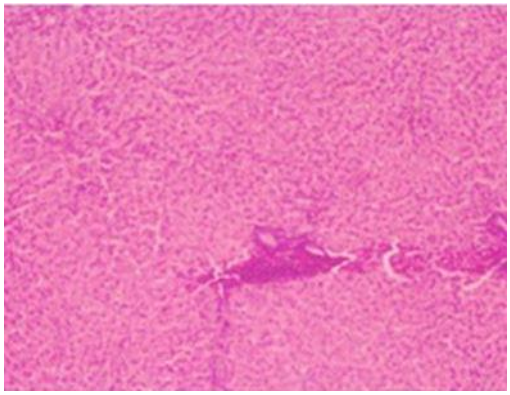

Figure 10. Histopathological micrograph of rat liver section treated with 100 mg/kg pure compound β-sito sterolglucoside and CCl_4 (therapeutic model).

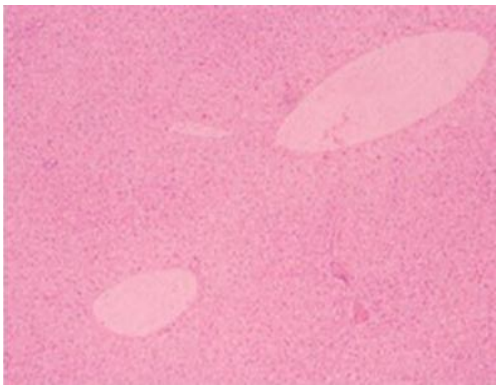

Figure 12. Histopathological micrograph of rat liver section treated with 250 mg/kg ethanol extract of *Adenanthera pavonina* leaves and CCl_4 (therapeutic model).

ethanol extract of *A. pavonina* leaves, revealed the presences of ten important phytoconstituents, alkaloids, carbohydrates, glucosides of β-sitosterol, glycosides, flavonoids, saponins, steroids, stigma sterol, tannins and terpenoids (Tasleem, 2015).

In-vitro anti-oxidant analysis usually provided a reliable method to evaluate phytopharmaceuticals to validate their usage and application to cure inflammatory conditions. In the present study, the tested plant was found to be able to donate electrons to reactive radicals, reducing them into more stable and un reactive species because of the hydroxyl group present in major phytochemicals (Walia et al., 2011). It was observed that percentage of reduction power increased with increase in concentration but hexane and ethanol extracts exhibited less degree of reduction of ferric cyanide complex to ferrous cyanide complex Fe^{2+} reduction than the β-sitosterolglucoside. However standard drug was found to be higher than all test samples. This reducing power may be due to biologically active compounds which can donate an electron to free radicals, which leads to neutralization of the radical.

Various studies noted that intoxication of CCl_4 causes dissolution of fat destroying the selective permeability of tissue membranes (Clarke et al., 1981). Mc Sween and Whaley (1992) reported the toxic effect of CCl_4 on liver cells is attributed to its metabolism in the smooth endoplasmic reticulum by the cytochrome P450 system with the production of highly reactive tri-chloromethyl radical CCl_3. This moiety reacts with the double bonds of unsaturated fatty acids in the membrane of the smooth endoplasmic reticulum producing chloroform and a lipid radical. This reacts with oxygen to initiate the autocatalytic reaction which results in the destruction of the endoplasmic membrane system. During hepatic damage, serum hepatic enzymes ALP, ALT and bilirubin present in liver cells leak into the serum (Mukherjee et al., 1997). Hepatocellular necrosis, massive fatty changes broad infiltration of the lymphocytes and kupffer cells around the central vein and loss of cellular boundaries

were accompanied (Lim et al., 2000).

Biochemical and histopathological analysis of tested samples demonstrated significant ($P < 0.01$) recovery in dose dependent manner after five days treatment in CCl_4 intoxicated models when compared to the control group but less than standard drug. In comparison β-sito sterolglucoside at a dose of 100 mg/kg body weight significantly reduced serum activity of ALP and ɣ-GT in prophylactic model that suggested the stabilization of the biliary function and endoplasmic reticulum, However serum activity of ALT and TB significantly decreased in therapeutic model of treatment indicated the stabilization of the plasma membrane as well as the repair of hepatic tissue damage caused by CCl_4. indicated highest level of hepatoprotective activity than hexane and ethanol extract. The results of histopathological studies also provided supportive evidence for biochemical analysis.

Hepatoprotective potential of tested samples mainly results from the chain of phytoconstituents present in plants. These phytoconstituents are well documented for their pharmacological activities and may counter act with free radicals to avoid their destructive effect by increasing capability of anti-oxidant enzymes.

Conclusion

In conclusion, pure compound β-sito sterolglucoside, hexane and ethanol extracts of *A. pavonina* leaves had hepatoprotection ability against CCl_4 induced liver injury mainly due to presences of phytochemicals that are well known for their potent anti-oxidant defense system.

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