African Journal of Pharmacy and Pharmacology Vol. 7(1), pp. 8-14, 8 January, 2013
Available online at http://www.academicjournals.org/AJPP
DOI: 10.5897/AJPP12.501
ISSN 1996-0816 © 2013 Academic Journals

Full Length Research Paper

Hepatoprotective effects of *Justicia adhatoda* L. against carbon tetrachloride (CCl_4) induced liver injury in Swiss albino mice

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Accepted 5 November, 2012

Medicinal plants are believed to be a precious natural reservoir and have been continuously studied for their pharmacological activities against various ailments, liver being no exception. Leaves of *Justicia adhatoda* L. are being extensively used against many human ailments. Hepatoprotective effects of *J. adhatoda* L. leaves and flowers were investigated on carbon tetrachloride (CCl_4) induced liver damage in Swiss albino mice. Liver injury was assessed by estimation of biochemical parameters which includes liver function tests and supplemented by histopathological examination of liver. Aqueous, ethanolic and methanolic extracts of different concentrations were given orally and activities of these extracts were compared with standard drug (silymarin). The results showed that *J. adhatoda* leaves and flowers showed unambiguous hepatoprotective activity against CCl_4 induced liver toxicity. It was also concluded that different concentrations of leaves and flowers of *J. adhatoda* did not show any sign and symptoms of toxicity and mortality.

Key words: *Justicia adhatoda* L., Swiss albino mice, silymarin, hepatoprotective effects.

INTRODUCTION

Drug-induced liver injury is a major health problem that became a challenge not only for health care professionals, but also for the pharmaceutical industry and drug regulatory agencies which are in search of alternative medicines for hepatoprotection. Drug-induced liver injury accounts for more than 50% of acute and chronic liver failure, which includes about 39% hepatotoxicity caused by overdose of acetaminophen and 13% idiosyncratic liver injury triggered by other drugs (Michael and Cynthia, 2006; Kaplowitz, 2001). Isoniazid and rifampicin are considered as the first line drugs used for tuberculosis therapy and are associated with hepatotoxicity (Tasduq et al., 2005). Most of the hepatotoxic chemicals damage liver cells mainly by inducing lipid peroxidation and other oxidative damages (Shanmugasundaram and Venkataraman, 2006). Liver is a chemical factory, and i5t is accountable for the regulation, synthesization, storage and secretion of many important proteins, nutrients, chemicals and clears toxin or superfluous substances from the body. Therefore, healthy liver determines the vigor rank of an entity (Gupta et al., 2006). More than 50% of acute and chronic liver failure, which includes approximately 39% hepatotoxicity are caused by overindulgence of acetaminophen and 11% idiosyncratic liver injury triggered by other drugs (Michael and Cynthia, 2006; Kaplowitz, 2001).

Animal cells are equipped with both enzymic and non-enzymic antioxidant defense with varied efficacies that

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protect animals against oxidative stress caused by wide range of toxins including carbon tetrachloride (CCl_4) (Karbownik et al., 2001).

Organism may have an endogenous shielding antioxidant defense system against the damage of free radicals. Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) are enzymatic antioxidants that catalyze detoxification response of toxic oxygen metabolites. Bilirubin is a dominant lipophilic antioxidant that protects membranes from lipid peroxidation and protects membrane proteins from oxidation (Thomas et al., 2009). It accounts for nearly all of the antioxidant activity of human serum and predominant influence against superoxide and peroxyl radicals (Novotny and Vitek, 2003). Many folk remedies from plant origin are tested for their potential antioxidant and hepatoprotective liver damage in experimental animal model. CCl_4- induced hepatotoxicity model is widely used for the study of hepatoprotective effects of drugs and plant extracts (Qureshi et al., 2010; Yu et al., 2010). Phytochemicals from medicinal plants serve as lead compounds in drug discovery and design (Ncube et al., 2008). *Justicia adhatoda* L. syn. *Adhatoda vasica, Adhaotoda zylenica* is a very popular plant of Himalaya and throughout Northern region of Pakistan. It belongs to family Acanthaceae, subclass Asteridae, and species adhatoda generally known as bakkar and bakkas. *J. adhatoda* is an evergreen, gregarious shrub 3 to 6 m long, large leaves lanceolate 10 to 20 by 4 to 8 cm and flowers are white or purple in short, dense auxiliary pedunculates (Ahmad et al., 2009). Foremost, the uses of this plant extracts are against bronchitis. It is also used as antispasmodic, antioxidant, anti-inflammatory, anti-tussive, antipyretic and antibacterial. Diabetes is also being treated with Justicia by traditional healers in certain areas of Pakistan (Ahmad et al., 2009). The current study was undertaken to evaluate the hepato-protective effects and safety assessment studies of *J. adhatoda* L. against CCl_4-induced hepatic damage in albino mice.

MATERIALS AND METHODS

Plant and chemicals

J. adhatoda leaves and flowers were collected from adjoining areas of Islamabad and Rawalpindi in fine plastic bags duly labeled with numbers and date of gathering of samples. Samples were identified by taxonomist from Department of Botany, Pir Mehar Ali Shah, Arid Agriculture University, Rawalpindi, Pakistan and registered as specimen (voucher specimen numbers 215 and 216). All chemicals used in this experiment were of the analytical status and purchased from Sigma Chemicals USA.

Preparation of plant extracts

Extraction procedure of World Health Organization (WHO, 2011) was modified for this study. A total of 10 g (each) sample of leaves and flowers were weighed and dissolved in ethanol, methanol and water (1:10) and positioned in shaking incubator at 120 rpm, 25°C for 24 h. Solutions were centrifuged at 14000 rpm for 15 min and were filtered. Finally, solvents were recovered via rotary evaporator and the extracts were dried at 37°C, labeled and stored for further process.

Animals

Swiss albino mice weighing between 30 and 40 g were used in this evaluation and were obtained from National Veterinary Laboratories, Islamabad. They were housed in well ventilated stainless-steel cages at room temperature (24±2°C) in hygienic provision under natural light and dark schedule and were fed on standard laboratory diet. Food and water were given *ad libitum.*

Toxicity studies

Acute toxicity study was conducted according to the Organization for Economic Cooperation and Development (OECD) revised up and down procedure for acute toxicity testing (OECD, 2001). A dose of 400 mg/kg of the aqueous, ethanolic and methanolic extracts of the leaves and flower of *J. adhatoda* were administered to six healthy adult albino mice of either sex in each group.

Experimental induction of hepatic damage

Male Swiss albino mice were selected for experimental assays and were sorted in groups of six mice in each cage. Group I (negative control) mice were administered normal food and water for 15 days. Group II (normal saline control) received normal saline daily for 15 days p.o. Group III (induction control) received intraperitonealy, 25% CCl_4 dissolved in olive oil at dose of 1 ml/kg of body (b.w.) eight twice a week. Group IV (drug or positive control) received CCl_4 intraperitonealy twice a week and standard drug silymarin (100 mg/kg b.w.) on the remaining days of the week for 15 days. Group V to X served as herb treated groups and further divided into two subgroups like groups A and group B. Groups A and B were orally administered extracts of leaves and flower of *J. adhatoda.* Groups VA and VB received CCl_4 intraperitonealy twice a week and methanolic extracts of the leaves and flower, respectively (100 mg/kg b.w.) for the rest of 15 days. Groups VIA and VIB received CCl_4 intraperitonealy twice a week and methanolic extracts of the leaves and flower, correspondingly (200 mg/kg b.w.) up to 15 days. Groups VIIA and VIIB received CCl_4 intraperitonealy twice a week and ethanolic extracts of the leaves and flower, respectively (100 mg/kg b.w.) for 15 days. Groups VIIIA and VIIIB received CCl_4 intraperitonealy twice a week and ethanolic extracts of the leaves and flower, respectively (200 mg/kg b.w.) for 15 days. Groups IXA and IXB received CCl_4 intraperitonealy twice a week and aqueous extracts of leaves and flower, respectively (100 mg/kg b.w.) for 15 days. Groups XA and XB received CCl_4 intraperitonealy twice a week and aqueous extracts of leaves and flower, correspondingly (200 mg/kg b.w.) for 15 days.

Serum separation and liver isolations

At the end of experiment, animals were weighed and were anesthetized with 20% chloroform and blood samples were collected by carotid artery puncture allowed to coagulate at room temperature for half an hour and then centrifuged at 3000 rpm for 10 min. The serum was separated and conserved at -20°C for ensuing analysis. Animals were sacrificed under diethyl ether anesthesia at fasting state, liver tissues were promptly excised and a part of liver homogenized in normal saline (0.9%), centrifuged at 3000 rpm for 10 min and supernatants were kept at -20°C for the

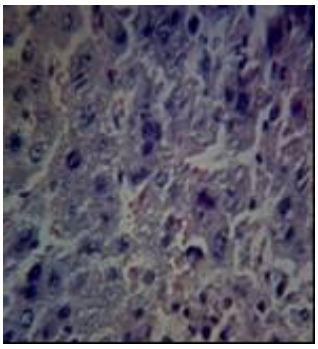

Figure 1. Microscopic view of liver tissue of normal mice.

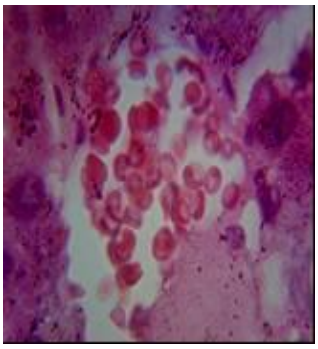

Figure 2. Microscopic view of liver obtained from CCl_4 control mice.

assay of biochemical parameters related to oxidative stress.

Determination of body weight increase and biochemical assays

Experimental animals were weighed before and after the onset of toxicity at the end of trial, change in body weight was noted down. Concentrations of aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), serum total proteins, albumin and plasma total bilirubin were estimated as described by Qureshi et al. (2010) and also using reagent kits purchased from Pioneer Diagnostics Company.

Histopathological examination of liver tissue

Small pieces of liver tissues were transferred to 10% formalin for proper fixation and were processed and embedded in paraffin wax. Sections of 5 to 6 µm in thickness were cut and stained with haematoxylin and eosin. Each liver was microscopically examined to assess the severity of lesions. The degree of liver damage was assessed and lesion grading was done as minimum, moderate and maximum.

Statistical analysis

Statistical analysis of data was performed by using the student's unpaired t-test and by analysis of variance (ANOVA) under absolutely randomized design (CRD) and the means of the treatment were compared by Duncan's Multiple Range Test (DMRT) (Anitha and Karuppasamy, 2011).

RESULTS

The effects of *J. adhatoda* leaves and flowers on serum enzymes and proteins in CCl_4 induced hepatotoxic mice are summarized in Tables 1 and 2. Administration of 25% CCl_4 (1 ml/kg) drastically reduced body weight ($P<0.05$) and the total protein and albumin concentration (2.53±0.46, 1.76±0.63). However, it increases serum enzymes, that is, ALT (264.87±5.9), AST (169.36±8.8), ALP (682.6±9.2), and total bilirubin (1.24±0.15) as compared to normal control ($P<0.001$).

The pretreatment of different extracts of *J. adhatoda* leaves and flowers (200 and 100 mg/kg) in CCl_4 administered mice significantly reduced the toxic changes. Methanolic, ethanolic and aqueous extracts of the leaves and flowers were used in this investigational study. Results in Tables 1 and 2 clearly confirmed that pretreatment of flower extracts of methanol, ethanol and water at higher dose (200 mg/kg) more considerably increased ($P<0.001$) the body weight (17.16, 12.32 and 16.64%), respectively equivalent to the effect observed with leaves extracts of *J. adhatoda* and silymarin used as standard drug. There is significant reduction ($P<0.01$) in the level of serum ALT, ALP, AST activities and total bilirubin and increase in the level of total proteins and albumin after pretreatment with *J. adhatoda* leaves and flowers as compared to toxic control. Activity of the plant extracts was similar to silymarin in which flower extracts showed more noteworthy ($P<0.05$) results as compared to silymarin.

Histopathological studies further confirmed the hepatoprotective effects of *J. adhatoda* leaves and flowers. Liver sections of normal control animals exhibited normal hepatic cells each with well defined cytoplasm, prominent nucleus and nucleolus and well brought out central vein whereas that of toxicant administered group animals showed total loss of hepatic design with centilobular hepatic necrosis, fatty changes vacuolization and blocking of sinusoids, Kepffer cell hyperplasia, crouding of central vein and apoptosis. The administration of *J. adhatoda* extracts reversed the gross disturbances observed in the liver cytoarchitecture (Figures 1 to 9).

DISCUSSION

The CCl_4 is one of the toxic chemicals used to evaluate the effects of herbal and synthetic drugs on liver injury (Yu et al., 2010). Administration of this toxicant results in the increase of liver enzymes and reduction in the activity of antioxidants in the body. When CCl_4 administered in

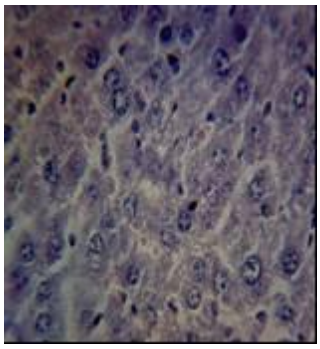

Figure 3. Liver sections from silymarin treated CCl_4 injected mice.

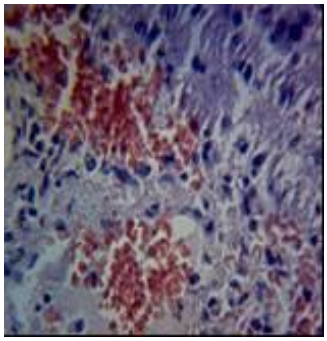

Figure 4. Liver sections from *Justicia* leaves aqueous extract treated CCl_4 injected mice.

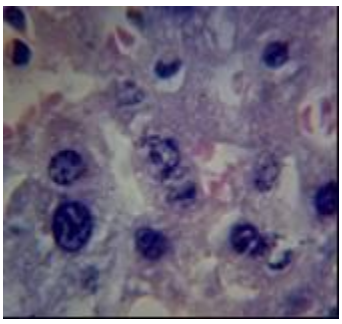

Figure 5. Micros view of liver tissue of mice treated with *Justicia* leaves ethanolic extract.

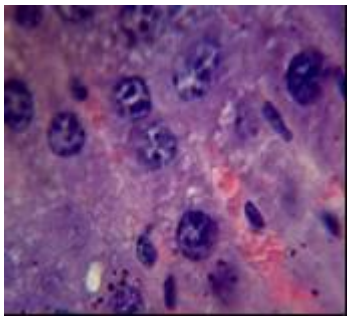

Figure 6. Micros view of liver tissue of mice treated with *Justicia* leaves methanolic extract.

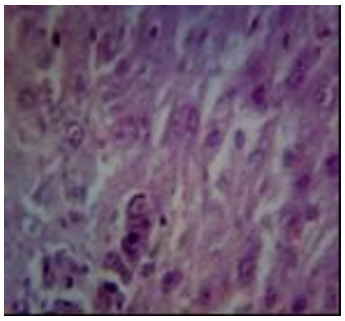

Figure 6. Micros view of liver tissue of mice treated with *Justicia* flowers aqueous extract.

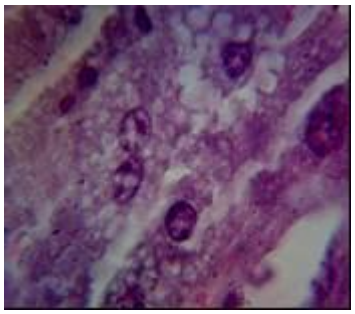

Figure 8. Micros view of liver tissue of mice treated with *Justicia* flowers ethanolic extract.

mice, it causes severe toxicity by interfering with their normal metabolic functions. Inside the cell, CCl_4 is biotransformed into trichloromethyl radical ($*CCl_3$) which join together with cellular proteins and membrane lipids and disintegrate them, there by initiating elevated level of lipid peroxidation which is the principle action of CCl_4 (Aghel et al., 2007). It is also presumed that herbal drugs inhibit the CYP2E1 enzyme activity in hepatic microsomes *in vivo* hepatocytes which are reflected as their increased levels in serum (Rajesh and Latha, 2004). Pretreatment with *J. adhatoda* leaves restored the liver enzyme parameters showing a dose dependent effect. The reduction of liver enzyme parameter (ALT) was significant and is considered as a specific marker of liver injury due to toxic drugs, alcohol and virus (Sherlock and Dooley, 2002). The protective effect may be the result of stabilization of plasma membrane, thereby preserving the

Table 1. Effects of *J. adhatoda* leaves and flowers on body weight gain in CCl_4 administered mice.

Group	Body weight (g)		
	Before experiment	After experiment	Change in weight (%)
Normal control	20.33±0.26	24.43±0.28	18.91±2.69
Solvent control Normal saline (10 ml/kg)	20.14± 0.53	22.72±0.24	12.33±1.83
Induction control (25% CCl_4, 1 ml/kg)	20.55±0.23*	18.98±0.50*	-6.61±1.52*
Drug control (Silymarin 100 mg/kg) + CCl_4	23.51±0.23$^{\lambda}$	26.07±0.16$^{\lambda}$	11.23±1.00$^{\lambda}$
*J.*l meth (200 mg/kg) + CCl_4	25.29±0.28$^{\alpha}$	28.57±0.31$^{\alpha}$	12.97±0.60$^{\alpha}$
*J.*l meth (100 mg/kg) + CCl_4	20.41±0.22	21.84±0.21	6.99±0.16
*J.*l eth (200 mg/kg) + CCl_4	25.72±0.61$^{\alpha}$	28.80±0.29$^{\alpha}$	11.98±1.59$^{\alpha}$
*J.*l eth (100 mg/kg) + CCl_4	24.99±0.29	26.66±0.41	6.29±0.71
*J.*l aq (200 mg/kg) + CCl_4	26.03±0.65	28.55±0.43	9.68±1.40
*J.*l aq (100 mg/kg) + CCl_4	28.11±0.92	29.89±0.80	6.37±1.51
*J.*f meth (200 mg/kg) + CCl_4	21.89±0.68$^{\beta\lambda}$	25.64±0.54$^{\beta\lambda}$	17.16±1.63$^{\beta\lambda}$
*J.*f meth (100 mg/kg) + CCl_4	28.82±0.86	30.94±1.14	7.35±0.90
*J.*f eth (200 mg/kg) + CCl_4	22.82±1.82$^{\alpha}$	25.74±1.59$^{\alpha}$	12.32±.16$^{\alpha}$
*J.*f eth (100 mg/kg) + CCl_4	25.66±0.56	27.52±0.39	7.26±1.71
*J.*f aq (200 mg/kg) + CCl_4	21.92±0.93$^{\beta\lambda}$	25.50±1.09$^{\beta\lambda}$	16.64±1.53$^{\beta\lambda}$
*J.*f aq (100 mg/kg) + CCl_4	27.00±0.72	28.70±0.49	6.35±2.13

Data are expressed as mean ± standard deviation (SD, n=4). Values significantly different as compared to normal control: *P<0.05. Values significantly different as compared to CCl_4-administered control: $^{\alpha}$P<0.05, $^{\lambda}$P<0.01. Values significantly different as compared to standard drug: $^{\beta}$P< 0.001. J.l meth: *Justicia adhatoda* leaves methanolic extracts, J.l eth: *Justicia adhatoda* leaves ethanolic extracts, J.l aq: *Justicia adhatoda* leaves aqueous extracts, J.f meth: *Justicia adhatoda* flowers methanolic extracts, J.f eth: *Justicia adhatoda* flowers ethanolic extracts, J.f aq: *Justicia adhatoda* flowers aquous extracts.

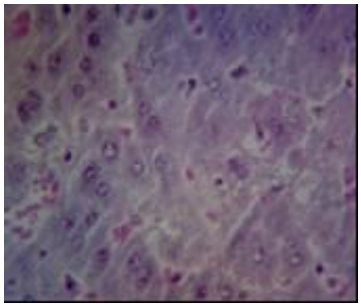

Figure 9. Micros view of liver tissue of mice treated with *Justicia* flowers methanolic extract.

structural integrity of cell as well as the repair of hepatic tissue damage caused by CCL_4 (Pari and Murugan, 2004). The lessening in cellular proteins might be due to the disruptive effect on endoplasmic reticulum, of which consequent loss of P450 enzyme will protein synthesis. Less protein merger results in the growth of triglycerides leading to fatty liver (Shenoy et al., 2001).

ALT is more selectively a liver paranchymal enzyme than AST. AST presents two isozymes, one is present in mitochondria, while the other one in cytoplasm (Sapakal et al., 2008). Another key parameter used to assess liver toxicity is bilirubin determination which is a chemical product of hemoglobin which is conjugated with glucoronic acid in hapatocytes to amplify its water solubility (Sreepriya et al., 2001; Ravi et al., 2005; Rajib et al., 2009). Rise of serum enzymes indicates the harmful effects of CCl_4 on liver of animals, because these enzymes are localized in cytoplasm and released after cellular damage (Mohan et al., 2007). The increased level of serum bilirubin might be due to inconsistent production of bilirubin due to excessive breakdown of red blood cells (RBCs) and the incapacity of animal to handle bilirubin due to liver damage which would cause either intra or extra hepatic obstruction (Ahmad et al., 2002). The increase of plasma bilirubin levels by CCl_4 further indicates that CCl_4 is a toxic agent for liver which is also in agreement with results reported by Vogel (2002), Samudram et al. (2008), Ahsan et al. (2009) and Tsala et al. (2010).

Herbal extracts contain antioxidant phytochemicals like flavonoids and phenolic compounds and triterpenes. Due to the radical scavenging potential of these phytochemicals, toxicity level reduces and body tends back towards normalcy (Laszczyk et al., 2006). Several studies have shown the hepatoprotective effects of *Justicia* leaves (Pandit et al., 2004; Bhattacharya et al., 2005; Krishna et al., 2010), but no work has reported on flowers of *J. adhatoda*. It has been reported that due to its antioxidant potential, *J. adhatoda* ethanolic extracts pretreatment significantly prevents radiation induced chromosomal eccentricity in bone marrow cells and reduces stickiness of chromosomes (Kumar et al., 2007).

Table 2. Effects of *J. adhatoda* leaves and flower extracts on serum enzymes activities and plasma total bilirubin level.

Group	Biochemical parameters					
	ALP (U/L)	AST (U/L)	ALT (U/L)	Plasma total bilirubin (mg/dl)	Total proteins (g/dl)	Total albumin
Normal control	130.29±6.76	39.472 ± 4.24	48.367±6.74	0.146 ± 0.03	7.213±0.80	3.285±0.46
Normal saline (10 ml/kg)	248.177±2.05	41.962± 0.72	47.51±4.29	0.253± 0.01	5.018±0.76	4.010±0.35
Induction control (25% CCl_4, 1 ml/kg)	682.622±9.2*	264.87± 5.91*	169.36±8.8**	1.24± 0.15*	2.53±0.46**	1.764±0.63*
Drug control (Silymarin 100 mg/kg) + CCl_4	283.457±7.5$^{\lambda}$	78.022±1.25$^{\lambda}$	50.665±6.7$^{\alpha}$	0.24± 0.07$^{\alpha}$	5.760±0.64	4.345±0.42
*J.*l meth (200 mg/kg) + CCl_4	305.46±5.28	125.26 ± 4.58	73.522±8.11	0.372 ± 0.03	6.055±0.90	4.075±0.38
*J.*l meth (100 mg/kg) + CCl_4	394.535±9.18	179.72 ± 9.90	85.315±3.91	0.543 ± 0.05	3.784±0.35	3.405±0.31
*J.*l eth (200 mg/kg) + CCl_4	357.107±35.9	81.93 ±7.48$^{\lambda}$	55.022±6.8$^{\alpha}$	0.273 ± 0.03$^{\lambda}$	5.890±0.38	4.367±0.2$^{\alpha\beta}$
*J.*l. eth (100 mg/kg) + CCl_4	385.142±6.68	137.022±7.23	92.83±2.25	0.542 ± 0.03	4.877±0.25	2.612±0.39
*J.*l aq (200 mg/kg) + CCl_4	275.42±9.66	71.372±6.23$^{\beta\lambda}$	89.602±5.90	0.194 ± 0.01$^{\alpha}$	6.777±1.83$^{\alpha\beta}$	3.612±0.27
*J.*l aq (100 mg/kg) + CCl_4	392.567±10.1	155.022 ±9.37	113.225±2.8	0.413 ± 0.03	4.690±0.62	2.345±0.28
*J.*f meth (200 mg/kg) + CCl_4	246.275±4.2$^{\lambda}$	86.73± 4.10	57.55±6.02$^{\alpha}$	0.152 ± 0.0$^{\alpha\beta}$	6.988±1.31$^{\alpha\beta}$	4.720±0.8$^{\alpha\beta}$
*J.*f meth (100 mg/kg) + CCl_4	396.257±6.75	166.67 ± 7.4	96.55±5.51	0.676 ± 0.03	4.493±0.32	2.535±0.37
*J.*f eth (200 mg/kg) + CCl_4	209.49±10.1$^{\alpha}$	65.157±2.30$^{\alpha\beta}$	57.30±3.97$^{\alpha\beta}$	0.418 ± 0.03	6.116±0.48	3.962±0.47
*J.*f. eth (100 mg/kg) + CCl_4	308.667±8.61	137.187 ±6.92	77.297±3.00	0.696 ± 0.07	3.711±0.29	2.752±0.18
*J.*f aq (200 mg/kg) + CCl_4	221.71±3.8$^{\alpha\beta}$	90.785± 5.23$^{\lambda}$	60.223±9.0$^{\alpha\beta}$	0.523 ± 0.22	6.212±0.90	3.856±0.68
*J.*f aq (100 mg/kg) + CCl_4	383.212±9.98	166.26± 9.65	86.377±0.14	0.846 ± 0.07	4.256±0.14	2.610±0.32

Values expressed as means ± standard deviation (SD, n=4). Values significantly varied comparable to normal control: *P<0.001, **P<0.01. Values significantly varied comparable to CCl_4-administered control: $^{\alpha}$P<0.01, $^{\lambda}$P<0.05. Values significantly varied comparable to drug control: $^{\beta}$P<0.05. J.l meth: *Justicia adhatoda* leaves methanolic extracts, J.l eth: *Justicia adhatoda* leaves ethanolic extracts, J.l aq: *Justicia adhatoda* leaves aqueous extracts, J.f meth: *Justicia adhatoda* flowers methanolic extracts, J.f eth: *Justicia adhatoda* flowers ethanolic extracts, J.f aq: *Justicia adhatoda* flowers aquous extracts.

The results indicated that *J. adhatoda* leaves and flowers show explicit hepatoprotective activity against CCl_4 induced liver toxicity. Among these flower extracts of *J. adhatoda* showed more prospective activity at dose of 200 mg/kg b.w.

ACKNOWLEDGEMENTS

The authors are thankful to the staff of Arid University, Rawalpindi, Pakistan for their sincere hard work and cooperation to materialize this study.

REFERENCES

Aghel N, Rashidi I, Mombeini A (2007). Hepatoprotective Activity of *Capparis spinosa* Root Bark against CCl4 Induced Hepatic Damage in Mice. Iran. J. Pharm. Res. 6(4):285-290.

Ahmad S, Garg M, Ali M, Singh M (2009). A Phyto-pharmacological overview on *Adhatoda vasica* medic. Nat. Prod. Radiansce 8(5):549-554.

Ahmad A, Pillai KK, Najmi AK, Ahmad SJ, Pal SN, Balani DK (2002). Evaluation of hepatoprotective potential of jigrine post-treatment against thioacetamide induced hepatic damage. J. Ethnopharmacol. 79(1):35-41.

Ahsan R, Monir ul Islam KM, Musaddik A, Haq E (2009). Hepatoprotective activity of methanol extract of some plants against CCL_4 induced hepatotoxicity in albino rats. Global J. Pharmacol. 3:116-122.

Anitha U, Karuppasamy R (2011). Hepatoprotective activity of sesame meal on high fat fed wistar rats. Int. J. Pharm. Sci. Res. 2(12):205-211.

Bhattacharya D, Pandit SU, Jana S, Sen S, Sur TK (2005). Hepatoprotective activity of *Adhatoda vasica* aqueous leaf extract on d-galactosamine-induced liver damage in rats. Fitoterapia 76:223-225.

Gupta AK, Chitme H, Dass SK, Misra N (2006). Hepatoprotective activity of *Rauwolfia serpentina* rhizome in paracetamol intoxicated rats. J. Pharmacol. Toxicol. 1:82-88.

Kaplowitz N (2001). Drug-induced liver disorders: Implications for drug development and regulation. Drug Saf. 24:483-490.

Karbownik M, Lewinski A, Reiter RJ (2001). Anticarcinogenic action of melatonin which involves antioxidatcive processes:

Comparison with other antioxidants. Int. J. Biochem. Cell Biol. 33:735-743.
Krishna KI, Mruthunjaya K, Patel JA (2010). Antioxidant and Hepatoprotective Potential of stem Methanolic Extracts of *Juticia gendarussa* Burn. Int. J. Pharm. 6(2):72-80.
Kumar M, Samarth R, Kumar M, Selvan SR, Saharan B, Kumar A (2007). Protective Effect of *Adhatoda vascia* Nees Against Radiation-Induced Damage at Cellular, Biochemical and Chromosomal Levels in Swiss Albino Mice eCAM 4(3):343-350.
Laszczyk M, Jager S, Simon-Haarhaus S, Scheffler A, Schempp CM (2006). Physical, chemical and pharmacological characterization of a new oleogel-forming triterpene extract from the outer bark of birch (betulae cortex). Planta Med. 72:1389-1395.
Michael PH, Cynthia J (2006). Mechanisms of Drug-Induced Liver Injury. The AAPS J. 8(1):48-54.
Mohan KG, Pallavi E, Kumar ER, Ramesh M, Venkatesh S (2007). Hepatoprotective activity of *Ficus carica* Linn. leaf extract against carbon tetrachloride-induced hepatotoxicity in rats. DARU 15(3):162-166.
Novotny L, Vitek L (2003). Inverse relationship between serum bilirubin and atherosclerosis in man: A metaanalysis of published studies. Exp. Boil. Med. 228(5):568-571.
Ncube NS, Afolayan AJ, Okoh A (2008). Assessment techniques of antimicrobial properties of natural compounds of plant origin: Current methods and future trends. Afr. J. Biotechnol. 7(12):1797-1806.
OECD (2001). OECD Guideline 425: Acute Oral Toxicity—Up-and-Down Procedure. In: OECD Guidelines for the Testing of Chemicals Vol.2 Organization for Economic Cooperation and Development. Paris, France.
Pandit S, Sur TK, Jana U, Debnath PK, Sen S, Bhattacharyya D (2004). Prevention of Carbon Tetrachloride Hepatotoxicity in rats by *Adhatoda vasica* leaves. Ind. J. Pharmacol. 36:312-320.
Pari L, Murugan P (2004). Protective role of tetrahydrocurcumin against erythromycin estolate-induced hepatotoxicity. Pharmacol. Res. 49:481-486.
Rajesh MG, Latha MS (2004). Preliminary evaluation of the antihepatotoxic activity of Kamilari, a polyherbal formulation. J. Ethnopharmacol. 91:99-104.
Rajib AM, Monirul IK, Jahan BI, Ashik MM, Ekramul H (2009). Hepatoprotective activity of methanol extract of some medicinal plants against carbon tetrachloride-induced hepatotoxicity in rats. Eur. J. Sci. Res. 7:302-310.
Qureshi MN, Bhanudansh SK, Nadeem AL, Majid AH (2010). *In-vitro* Antioxidant and *In-vivo* Hepatoprotective activity of *Leucas ciliata* leaves. Rec. Nat. Prod. 4(2):124-130.
Ravi KV, Shivashangari KS, Devaki T (2005). Hepatoprotective activity of *Tridax procumbens* against D-galactosamine/lipopolysaccharide-induced hepatitis in rats. J. Ethnopharmacol. 101:55-60.
Samudram P, Rajeshwari H, Vasuki R, Geetha A, Sathiya P (2008). Toprotective activity of Bi - herbal ethanolic extract on CCl_4 induced hepatic damage in rats. J. Afr. Biochem. Res. 2(2):61-65.
Sapakal VD, Ghadge RV, Adnaik RS, Naikwade AS, Magdum CS. (2008). Comparative hepatoprotective activity of Liv-52 and livomyn against carbon tetrachloride induced hepatic injury in rats. Int. J. Green. Pharm. 2:79-82.
Shenoy KA, Somayaji SM, Bairy KL (2001). Hepatoprotective effects of Ginkgo biloba in carbon tetrachloride induce hepatic injury in rats. Ind. J. Pharmacol. 33:260-266.
Sherlock S, Dooley J (2002). Drugs and the liver. In: Diseases of liver and Biliary system. 11 ed. Oxford: Blackwell Sci. Pub. pp.322-356.
Sreepriya M, Devaki T, Balakrishna K, Apparanantham T (2001). Effect of Indigofera tinctoria Linn on liver antioxidant defense system during D-galactosamine/endotoxin-induced acute hepatitis in rodents. Indian. J. Exp. Biol. 39(2):181-184.
Tasduq SA, Peerzada K, Koul S, Bhat R, Johri RK (2005). Biochemical manifestation of anti-tuberculosis drugs induced hepatotoxicity and the effect of Silymarin. Hepatol. Res. 31:132-135.
Tsala DE, Penlab BVNN, Mendimi NJ, Kouamouo J, Dimo T (2010). Protective activity of the stem bark methanol extract of *Alafia multiflora* against carbon tetrachloride-induced hepatotoxicity in rats. Int. J. Pharm. Sci. Rev. Res. 3:157-163.
Thomas W, Sedlaka MS, Daniel S, Higginsonb BD. Paulb KR. Julurib SHS (2009). Bilirubin and glutathione have complementary antioxidant and cytoprotective roles. PNAS 106(13):5171–5176
Yu C, Wang CL, Jin X, Wu WK, Chan, Mckeehan ML (2010). Increased CCl_4 induced liver injury and fibrosis in FGFR4-deficient mice. Am. J. Pathol. 161:2003-2010.
WHO (2011). Quality Control methods for herbal materials. Geneva, Switzerland.