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Antioxidant and Hepatoprotective Potential of Stem Methanolic Extract of *Justicia gendarussa* Burm

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Abstract: *In vitro* antioxidant activity of stem extracts of *Justicia gendarussa* Burm along with *in vivo* hepatoprotective activity of methanolic fraction was carried out to ascertain the folkloric claim of its hepatoprotective activity. The crude methanolic extract was prepared by soxhlet extraction and fractionated into pet ether, chloroform and methanol fractions. The marc remained was further extracted with doubled distilled water by refluxation in waterbath. Preliminary phytochemical tests, total phenolic and flavonoid content present in each fraction/extract was determined. All fractions and aqueous extract were evaluated for their antioxidant activity using DPPH free radical scavenging activity, hydrogen peroxide scavenging activity, reduction of ferric ion in presence and absence of EDTA. The methanolic fraction was further studied for its *in vivo* hepatoprotective activity using CCl_4 induced hepatotoxicity in albino rats. The various biochemical parameters were evaluated to asses its hepatoprotective activity. Methanolic fraction has more phenolic/flavonoid content and shows good antioxidant activity. The methanolic fraction has a good hepatoprotective activity at dose of 300 mg kg^{-1} b.wt. in albino rats. Interestingly its hepatoprotective activity decreases as the dose increases. Stem extract of JG has moderate hepatoprotective activity; it may be due to its total phenolic and flavonoid contents. These findings substantiate the ancient literature about its reports as hepatoprotective herb and inspires for further extensive study regarding its exact hepatoprotective activity.

Key words: Justicia gendarussa, hepatoprotective, CCl_4, methanolic

INTRODUCTION

Herbal medicines derived from plant extracts are being increasingly utilized to treat a wide variety of clinical diseases, through relatively little knowledge about their mode of action is available. There is a growing interest in the pharmacological evaluation of various plants used in Indian traditional system of medicine.

Justicia gendarussa Burm (JG) belonging to family Acanthaceae and it is considered as native of China. It is frequently grown in Indian gardens as hedge or border plant; it is sometimes found as an escape. It is propagated by cuttings and grows quickly. It is hardy, withstands heavy rainfall and thrives in shade. The plant is used in conditions such as inflammations, bronchitis, vaginal discharges, dyspepsia, eye diseases and fever in Ayurveda, an Indian system of medicine. A decoction of the root boiled in milk is given in rheumatism, dysentery and jaundice (Kirthikar and Basu, 2005). The stems are reported to contain a bitter and slightly toxic alkaloid. A decoction of alcoholic extract of the roots produced slight paralysis in rats in doses of 1-2 g kg^{-1} b.wt. and in doses of 10-20 g kg^{-1} it is antipyretic and depressant producing violent diarrhoea eventually death. Decoction of leaves is used to treat chronic rheumatism. It is reported that the flowering head along with the portion of stem is used as demulcent and astringent (Anonymous, 1959; Chopra *et al*., 1956; Kirtikar and Basu, 2005). It is also reported to contain β-sitosterol, friedelin, lupeol and four simple 0-substituted aromatic amines (Chakravarty *et al*., 1982). Recently, plant extracts were reported for their anti-inflammatory and antioxidant (Devprakash, 2000), reverse transcriptase inhibitory activity (Woradulayapinij *et al*., 2005), analgesic activity (Ratnasooriya *et al*., 2007), antioxidant potential of aqueous ethanolic extract of JG (Mruthunjaya and Hukkeri, 2007).

Also the plant is used in treatment of jaundice in locals in the state of Gujarat, India. The hepatoprotective activity of *Justicia gendarussa* has not been evaluated scientifically to substantiate its ancient literature regarding its usefulness as hepatoprotective herb (Pandey *et al*., 2005). So, this study was conceptualized to evaluate its hepatoprotective property. Antioxidant and free radical scavenging activities of herbs are reported to

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be responsible for the hepatoprotective activity. JG was reported to possess antioxidant activity (Mruthunjaya and Hukkeri, 2007). The hepatoprotective activity of JG may be due its free radical scavenging activity. So in the present study extracts of JG were subjected for *in vitro* antioxidant and free radical scavenging activities prior to the *in vivo* hepatoprotective activity.

MATERIALS AND METHODS

Plant material: Stem samples were collected from the Veer Narmad South Gujarat University Campus, Surat, India, in the month of September 2007 and sample was identified and authenticated by Dr. Minu Parabia, Professor and Head, Department of Bioscience. A specimen sample (KLKJG01) was deposited in the Department of Bioscience.

Preparation of the extracts: Five hundred gram of Shade dried stems powder of the plant was extracted with 5 L of methanol using Soxhlet method till the exhaustion for about 24 h (ME-13.82%). Marc obtained was further extracted with pure Doubled Distilled (DD) water using water bath by reflux (WE-1.73 %). Both the extracts were concentrated under vacuum to get the residues. The methanolic extract was further fractionated to petroleum ether (PEF-8.9%), chloroform (ChF-7.8%) and methanol (MF-83.3%).

***In vitro* antioxidant activities:** The extracts were subjected for the detailed preliminary phytochemical studies to know the phytochemical nature (Evans, 2005; Finar, 2006; Kokate *et al*., 1996; Onwukaeme *et al*., 2007).

Determination of total phenolics: The total phenolic content of all extracts were determined by using Folin-Ciocalteu's assay (Marinova *et al*., 2005). An aliquot (0.4 mL) of extract or standard solution of gallic acid (1, 5, 10, 15 and 20 μg mL^{-1}) was added to 10 mL volumetric flask, containing 3.6 mL of doubled distilled water. Reagent blank was prepared using doubled distilled water. Folin Ciocalteu's Phenol reagent (0.4 mL) was added to the mixture and shaken. After 5 min, 4 mL of 7% Na_2CO_3 solution was added to the mixture. The solution was diluted to volume (10 mL) with DD water and mixed. After incubation for 90 min at room temperature, the absorbance against prepared reagent blank was determined at 750 nm with an UV-Visible Spectrophotometer Shimadzu 1700. Total phenolic content of various extracts were expressed as mg Gallic Acid Equivalent (GAE)/100 g of extract. All samples were analyzed in triplicate.

Estimation of total flavonoids: Total flavonoid content present in the various extracts were determined by the aluminium chloride calorimetric assay (Marinova *et al*., 2005). An aliquot (0.5 mL) of extract or standard solution (quercetin) was added to test tube containing 2 mL of DD water and 0.15 mL of 5% Sodium nitrite. After 5 min, 0.15 mL of 10% aluminium chloride solution was added. At 6th min, 2 mL of 1 M Sodium hydroxide was added and the total volume was made to 5 mL with DD water. The solution was mixed well and the absorbance was measured against prepared reagent blank at 510 nm. Total flavonoid content of the extracts was expressed as mg of quercetin equivalent to 1 g of the extract. All experiments were done in triplicate and total flavonoid content was given in Mean±SD.

Free radical scavenging activity by DPPH method: Free radical scavenging potentials of the extracts were tested against a methanolic solution of α, α-diphenyl-β-picryl hydrazyl using the method of Choi *et al*. (2002). 2.5 mL of sample solution of different concentrations or standards were mixed with methanolic solution of DPPH (0.3 mM) and allowed to react at room temperature. Ascorbic acid, BHT and Gallic acid were used as standards. The absorbance was read after 30 min at 518 nm against methanol as blank. Percentage Radical Scavenging activity was calculated by the formula:

$$\text{Scavenging capacity (\%)} = 100 - \frac{(\text{Ab. of sample-Ab. of blank}) \times 100}{\text{Ab. of control}}$$

Reduction of ferric ions: The reaction mixture containing o-phenanthroline (0.5 mg), ferric chloride (0.2 mM) and test compounds (extracts/standard) dissolved in 0.2 mL ethanol in a final volume of 5 mL was incubated for 10 min at ambient temperature. The absorbance at 510 nm was measured. In another experiment, sodium dithionate (0.3 mM) was added instead of the test compound and the absorbance obtained was taken as equivalent to 100% reduction of all the ferric ions present (Rajakumar and Rao, 1993). All experiments were carried out in triplicate and % ferric ion reduction activities of various extracts were reported as Mean±SD.

The reduction of ferric ion in presence of EDTA also determined as the method of Mruthunjaya and Hukkeri (2007).

Hydrogen peroxide scavenging activity: Hydrogen peroxide scavenging ability of extracts was determined by simple UV spectroscopic method. All extracts were taken at the different concentration of 50 to 200 μg mL^{-1}. Volume adjusted to 3 mL with phosphate buffer and 1 mL

of 30 mM H_2O_2 was added. After 10 min, the absorbance value of the reaction mixture was recorded at 230 nm. Blank solution was containing the phosphate buffer without H_2O_2 (Kaur *et al*., 2006). The percentage of scavenging activity was calculated by using below formula:

$$\text{Percentage scavenged } [H_2O_2] = \frac{A_0 - A_1}{A_0} \times 100$$

where, A_0 is the absorbance of the control and A_1 is the absorbance of the sample.

Animal studies

Experimental animals: Forty six Wistar strain rats of either sex weighing 150-220 g were procured from the JSS Medical College, Central Animal Facility Centre, Mysore, Karnataka, India. The animals were housed in polypropylene cages and maintained in controlled temperature (27±2°C) and light cycle (12 h light and 12 h dark). They were fed with rat feed (Hindustan Animal Feeds) and water *ad libitum*. The animals were acclimatized with the laboratory conditions before the commencement of the experiment. Ethical clearance for the use of animals was obtained from the IAEC of JSS College of Pharmacy, Mysore, India. (Proposal No. 029/2008, dated 25th July 2008).

Acute toxicity studies: Acute toxicity study of methanolic fraction of JG was done according to the acute toxic classic method (OECD guideline 425, 2001) using albino female rats. The animals were kept fasting for overnight providing only water, after which the extract was administered orally for one animal at the limit dose of 2000 mg kg^{-1} and observed for 14 days (with special attention for the first 4 h of administration followed by the next 20 h). If the animal dies, the limit test was terminated and main test was conducted. If the animal survives, four additional animals were dosed sequentially so that five animals are tested. However, if three animals died, the limit test was terminated and the main test was performed. The LD_{50} is greater than 2000 mg kg^{-1} if three or more animals survived. If an animals unexpectedly dies late in the study and there are other survivors, it is appropriate to stop dosing and observing all animals to see if other animals will also die during a similar observation period. The same method was adopted at limit dose of 5000 mg kg^{-1}.

Hepatoprotective activity: Rats were divided into 6 groups of 6 animals each as follows: Rats of group I served as control and received oral administration of 2.5% gum acacia (vehicle) at the dose of 1 ml kg^{-1} b.wt., group II received oral administration of vehicle plus CCl_4 (hepatotoxic rats), group III received standard drug silymarin at the dose of 100 mg kg^{-1} body weight plus CCl_4 as group II animals and group IV to VI received the methanolic fraction at the dose of 150, 300 and 500 mg kg^{-1} body weight plus CCl_4 as group II animals. All treatments were given once daily for seven days.

On the 7th day, all group animals except group I, were given a single dose of CCl_4 (1 mL kg^{-1} b.wt.) in 1:2 Olive oil after 6 h of last dose administration. Animals of group I were given plain doubled distilled water and olive oil in 1:2 ratio of 1 mL kg^{-1} b.wt. Animals were sacrificed 24 h after the last dose and blood was collected by carotid bleeding (Kaur *et al*., 2006).

Biochemical estimation: Blood was centrifuged; serum was separated and used for estimation of biochemical parameters. Serum Glutamic Oxaloacetic Transaminase (SGOT) and Serum Glutamic Pyruvic Transaminase (SGPT) were estimated by Thefeld, serum alkaline phosphatase (SALP) by Klin, bilirubin (total and direct) by Winsten and Cehelyk (1969) method, total protein by colour complexation with copper ion in an alkali solution (Gornall *et al*., 1949). All the determinations were carried out using standard kits (Agappe Diagnostic Pvt. Ltd, Kerala, India) by using fully automated Biochemical Analyser ChemWell, Awareness Technologies Inc, USA.

Statistical analysis: All values are expressed as Mean±SEM. Statistical analysis was performed by one-way Analysis of Variance (ANOVA) and individual comparisons of the group mean values were done using Tukey's Multiple Comparison Test, with the help of Graph Pad prism 4.0 software. The value of p lower than 0.05 were considered significant.

RESULTS

The % yield of methanolic extract and water extract were found to be 13.82 and 1.73%, respectively. Percentage yield of fractions were found to 8.9, 7.8 and 83.3%, respectively for PER, ChF and MF.

Preliminary phytochemical investigation has revealed the presence of flavonoids, phenolic compounds, Cardiac glycosides, lactones, reducing sugars and terpenoids in the methanolic fraction. PEF found to contain sterols and terpenoids, ChF found to contain sterols, terpenoids and flavonoids and WE found to contain flavonoids, saponins, carbohydrates and Cardiac glycosides.

Total phenolic content and total flavonoid content was determined to know the ratio of phenolics and flavonoids in different fractions and extracts. Phenolic content determination is based on the on the principle

that polyphenols when react with FOLIN reagent give blue colour chromogen in alkaline media, which can be measured at 760 nm. The concentration of polyphenols in extracts is calculated by using standard curve prepared with gallic acid. The total phenolic content of methanolic fraction of JG has found to be 112.66±4.04 mg equivalent to gallic acid present in 1 g of extract (sample). The Gallic acid equivalent value for PEF, ChF and WE were found to be 8.33±3.33, 9.99±1.66 and 27.77±3.46, respectively (Table 1). The principal of aluminium chloride calorimetric method is that aluminium chloride forms acid stable complexes with the C-4 keto group and either the C-3 or C-5 hydroxyl group of flavones and flavonols. In addition, aluminium chloride forms acid labile complexes with the orthodihydroxy groups in the A- or B-ring of flavonoids (Chang *et al*., 2002). The total flavonoid content of JG stem extracts were determined and reported in Table 1. The total flavonoid content of MF is more when compare to the other fractions. The value of MF was found to be 65.50+2.29 g equivalent to 1 mg of quercetin. Where as the values were found to be 17.23+0.83, 18.55+0.84 and 22.83+2.25 g equivalent to the 1 mg of quercetin respectively for PEF, ChF and WE.

Table 1: Total phenolic and flavonoids content of the JG stem extracts (Mean±SD)

Extracts	Total phenolic content (mg GAE g^{-1} of extracts)	Total flavonoids (g mg^{-1} quercetin)
MF	112.66±4.04	65.50±2.29
ChE	9.99±1.66	17.23±0.83
PEF	8.33±3.33	18.55±0.84
WE	27.77±3.46	22.83±2.25

Free radical scavenging activity by DPPH method is shown in Table 2. MF moderately scavenged DPPH radical with the IC_{50} value of 262.91±4.38 μg mL^{-1}. The scavenging was found to be dose dependent. Where as ascorbic acid, gallic acid and butylated hydroxyl toluene (BHT) used as standards shown IC_{50} values of 4.17±0.5, 1.86±0.0 and 29.08±0.52 μg mL^{-1}, respectively. Where as the DPPH radical scavenging activity of PEF, ChF and WE were found to be very less and their IC_{50} values were found to be 481.67±5.204, 482.5±5.0 and 466.67±3.145 μg mL^{-1}, respectively.

Extracts react with Fe^{3+} to reduce and convert it to Fe^{2+}. The degree of coloration indicates the reduction potential of the extracts. The change in the absorbance produced at 510 nm has been used as a measure of reducing activity of ferric ions. Reduction of all the ferric ions is considered as 100% in presence of sodium dithionite instead of the extract. Fe^{+2} reacts rapidly with 1, 10-*O*- phenanthroline and forms red (orange) coloured complex which is exceptionally stable. This complex has a strong absorption in the visible spectrum at a wavelength of 510 nm (Rajakumar and Rao, 1993). The reduction of ferric ion in presence of EDTA also determined by the method of Mruthunjaya and Hukkeri (2007). As shown in Table 3, in absence of EDTA, MF has shown ferric ion reduction of 43.33±0.57% at 1000 μg concentration, where as PEF ChF and WE shown less % of reduction than the MF. i.e., 22.71±0.66, 20.57±0.22, 40.07±0.60%, respectively at the same concentration. IC_{50} values of all fractions were not detected, where as IC_{50} value of standards ascorbic

Table 2: Percentage of free radical scavenging activity of JG stem extracts, Ascorbic acid, Gallic acid and BHT in DPPH Method (Mean±SD)

Con (μg mL^{-1})	MF	PEF	ChF	WE	Con (μg mL^{-1})	Ascorbic acid	Gallic acid	Con (μg mL^{-1})	BHT
50	05.768±0.390	---	---	---	0.25	---	11.13±0.31	5	16.85±1.37
100	12.171±1.176	---	---	---	0.50	---	16.95±0.57	7	22.36±1.08
150	17.175±1.410	---	---	---	0.75	---	21.91±1.62	10	28.28±2.05
200	34.438±1.488	26.132±1.117	20.443±0.942	---	1.00		32.81±0.97	15	36.79±1.19
250	47.617±1.789	---	---	---	1.50		45.20±1.61	20	42.50±1.98
300	58.860±1.672	35.520±0.981	25.584±0.924	31.739±1.057	2.00	21.60±0.64	58.04±0.70	25	52.14±1.59
400	---	43.469±0.819	35.061±0.703	42.147±1.289	2.50	31.70±1.52	---	---	---
500	---	---	---	51.434±1.505	3.00	40.02±1.85	---	---	---
600	---	59.481±0.304	46.912±0.115	58.794±0.252	3.50	44.30±0.45	---	---	---
700	---	---	---	59.929±1.697	4.00	48.20±1.03	---	---	
800	---	66.907±1.635	55.779±1.465	70.939±1.004	4.50	53.99±0.37	---	---	---
1000	---	72.088±0.540	64.952±0.270	---	---	---	---	---	---
IC_{50} value	262.916±4.389	481.666±5.204	482.5±5.0	466.666±3.145		4.17±0.50	1.86±0.06		24.08±0.52

Table 3: Ferric ion reduction activity of JG stem extracts, ascorbic acid and gallic acid in percentage (Mean±SD)

Con (μg)	MF	PEF	CHF	WE	Con (μg)	Ascorbic Acid	Con (μg)	Gallic acid
200	14.18±0.52	7.11±0.22	5.72±0.15	10.89±0.52	10	22.25±0.24	2	14.66±1.51
400	20.91±0.66	10.58±0.39	9.62±0.34	17.12±0.47	20	35.15±0.75	4	32.64±0.88
600	29.00±0.77	15.06±0.55	12.55±0.49	25.74±0.86	30	53.49±1.27	6	42.88±0.51
800	36.33±0.97	18.88±0.56	15.91±0.58	32.86±1.11	40	73.64±0.41	8	60.01±1.11
1000	43.33±0.57	22.71±0.66	20.57±0.22	40.07±0.60	50	97.53±0.47	10	69.57±0.14
IC_{50} value	ND	ND	ND	ND		27.57±0.32		6.93±0.42

concentration is the total extract/standard present in the reaction mixture in μg. ND: Not detected at the tested level, No activity was detected for the extracts/standards in presence of EDTA

acid and gallic acid were found to be 27.57±0.32 and 6.93±0.42 μg, respectively.

H_2O_2 in the phosphate buffer has λ_{max} of 230 nm. In control tubes the absorbance will be only due H_2O_2. In presence of extracts the reduction of absorbance at 230 nm indicates scavenging or breakdown of H_2O_2. When H_2O_2 is scavenged or breakdown occurs the absorbance λ_{max} will be changed from 230 nm. As shown in the Table 4, MF has scavenged the hydrogen peroxide to the extent of 30.71±4.14% at 200 μg mL^{-1} concentration, where as PEF, ChF and WE have shown 10.64±1.59, 8.12±0.43 and 23.43±0.61% at the same concentration. The PEF and ChF were not shown any activity up to 100 μg mL^{-1} concentration.

In acute toxicity studies the methanolic fraction of JG extract has not shown any mortality at the limit dose of 2000 and 5000 mg kg^{-1} b.wt. MF was found to be safe even at the higher concentration, based on this, the dose for the hepatoprotective activity were chosen.

The results obtained in the *in vivo* hepatoprotective activity are presented in Table 5. The hepatoprotective effect of methanolic fraction of JG was assessed using *in vivo* model of CCl_4 induced hepatotoxicity by the acute oxidative stress. CCl_4 afflicts acute oxidative injury to the liver causing oxidative damage and other changes around the central vein in the liver that leads to the leakage of the marker enzymes such as AST and ALT in the serum (Recknagel, 1967).

The activities of SGOT and SGPT after 24 h of oral administration of CCl_4 at the dose of 1 mL kg^{-1} b.wt. are depicted in Table 5. It is observed that, CCl_4 administration resulted in a significant elevation in the level of both SGOT (427.76% of the vehicle treated control) and SGPT (676.09% of the vehicle treated control). Administration of JG methanolic fraction at the 150 and 300 mg kg^{-1} b.wt. for 7 days prior to CCl_4 administration dose dependently restored the activities of AST and ALT (Fig. 1, 2). The maximum reduction in the elevated biomarker enzymes (AST/ALT) were found to be at dose level II (300 mg kg^{-1}) and percentage of reduction was 53.29 and 25.11%, respectively. The methanolic fraction at dose level of 500 mg kg^{-1} b.wt. has shown less hepatoprotective activity than the lower doses. These results indicate JG stem possesses moderate (maximum) antihepatotoxic activities at dose level II.

The total and direct bilirubin content present in the serum after 24 h of CCl_4 exposure is given in the Table 5. As shown in the Fig. 3 and 4 the MF at the dose of 300 mg kg^{-1} has shown maximum activity in reducing the amount of bilirubin in the serum. The percentage of reduction was found to be 70.10 and 19.51%, respectively for total and direct bilirubin, where as the value for the silymarin were found to be 71.64 and 56.09%, respectively.

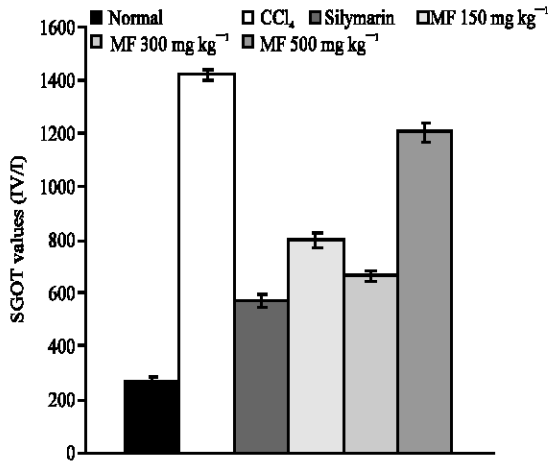

Fig. 1: Shows the effect of JG stem extract on the Serum Glutamic Oxaloacetic Transferase on CCl_4 induced hepatotoxicity. Values are Mean±SEM n = 6 animals in each group, a: Highly significant, reduction compared to hepatotoxin (CCl4) ($p<0.001$), b: Significant reduction compared to hepatotoxin (CCl_4) ($p<0.05$)

Table 4: Percentage of hydrogen peroxide scavenging activity of JG stem extracts (Mean±SD)

	Scavenging activity (%)			
Conc. (μg mL^{-1})	MF	PEF	ChF	WE
50	6.04±0.61	ND	ND	3.12±0.53
100	13.47±3.20	ND	ND	8.74±0.97
150	21.40±1.45	3.43±0.49	2.18±0.51	15.30±1.32
200	30.71±4.14	10.64±1.59	8.12±0.43	23.43±0.61

Table 5: Show the hepatoprotective activity of methanolic fraction of JG stem on CCl_4 induced hepatotoxicity

Group	SGOT (IU L^{-1})	SGPT (IU L^{-1})	Total bilirubin (mg dL^{-1})	Direct bilirubin (mg dL^{-1})	ALP (IU L^{-1})	Total protein (gm dL^{-1})
Normal (2.5% gum acacia)	269.50±17.36	119.83±4.43	0.41±0.06	0.22±0.02	463.83±21.36	6.65±0.31
CCl_4 (1 mL kg^{-1})	1422.33±19.9	930.33±35.70	1.94±0.08	0.41±0.30	858.16±21.75	5.00±0.21
Silymarin (100 mg kg^{-1})	574.33±26.50*	440.66±26.36*	0.55±0.04*	0.18±0.03*	546.83±22.81*	6.08±0.38**
MF (150 mg kg^{-1})	802.66±28.68*	834.66±29.53**	0.733±0.025	0.41±0.03**	853±38.78**	5.76±0.04
MF (300 mg kg^{-1})	664.33±18.92*	696.66±15.57*	0.58±.03*	0.33±0.02**	589±24.25*	6.03±0.02**
MF (500 mg kg^{-1})	1202.33±37.68	856.33±19.22	0.75±0.05	0.41±0.03	797.16±48.96	5.53±0.16

Values are Mean±SEM n = 6 animals in each group. *Highly significant reduction compared to hepatotoxin (CCl_4) ($p<0.001$), **Significant reduction compared to hepatotoxin (CCl_4) ($p<0.05$)

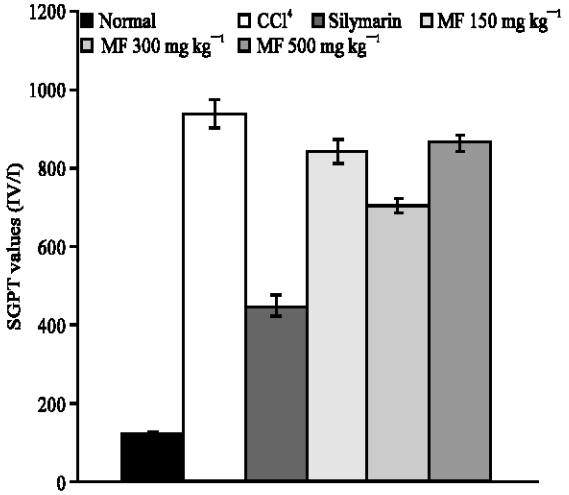

Fig. 2: Shows the effect of JG stem extract on the Serum Glutamic Pyruvic Transferase on CCl_4 induced hepatotoxicity. Values are Mean±SEM n = 6 animals in each group, *Highly significant reduction compared to hepatotoxin (CCl_4) ($p<0.001$), **Significant reduction compared to hepatotoxin (CCl_4) ($p<0.05$)

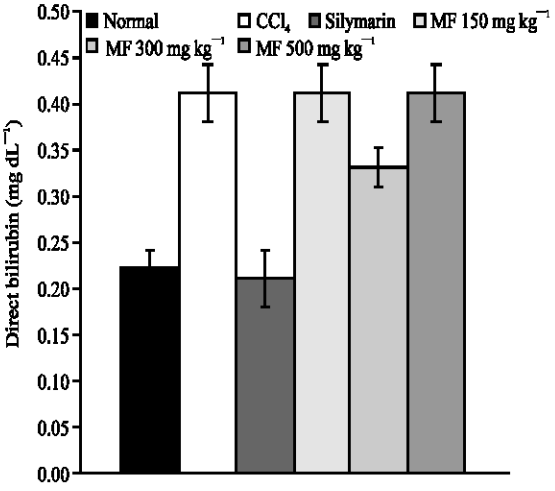

Fig. 4: Shows the effect of JG stem extract on the direct bilirubin on CCl_4 induced hepatotoxicity. Values are Mean±SEM n = 6 animals in each group, *Highly significant reduction compared to hepatotoxin (CCl_4) ($p<0.001$), **Significant reduction compared to hepatotoxin (CCl_4) ($p<0.05$)

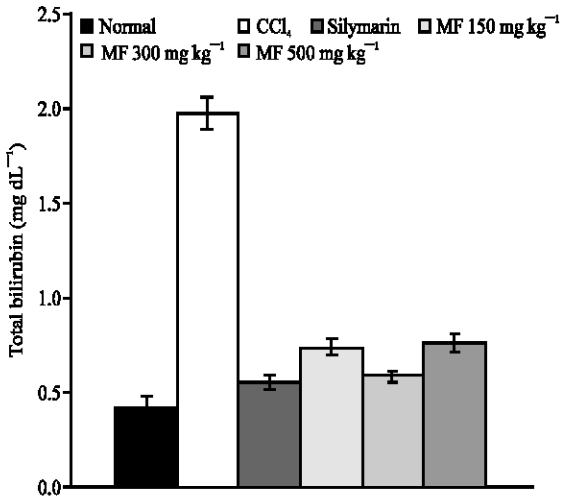

Fig. 3: Shows the effect of JG stem extract on the total bilirubin on CCl_4 induced hepatotoxicity. Values are Mean±SEM n = 6 animals in each group, *Highly significant reduction compared to hepatotoxin (CCl_4) ($p<0.001$), **Significant reduction compared to hepatotoxin (CCl_4) ($p<0.05$)

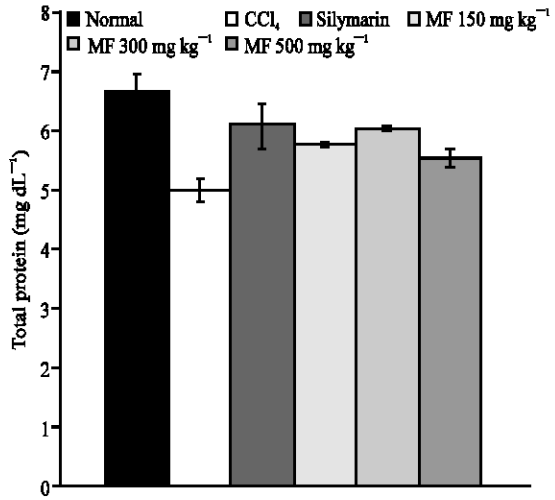

Fig. 5: Shows the effect of JG stem extract on the total protein on CCl_4 induced hepatotoxicity. Values are Mean±SEM n = 6 animals in each group, *Highly significant reduction compared to hepatotoxin (CCl_4) ($p<0.001$), **Significant reduction compared to hepatotoxin (CCl_4) ($p<0.05$)

The Table 5 shows that decrease in the total protein content after CCl_4 administration to about 24.81 % as compared to the vehicle treated animal group in the hepatotoxic animals. The MF has restored the protein content by maximum of 20.6% at dose level II (Fig. 5).

The Serum Alkaline Phosphatase (SALP) is the one more parameter used to assess the hepatoprotective activity of MF. As given in the Table 5 the SALP has increased significantly in the CCl_4 treated group when compare to the vehicle treated group. The MF has

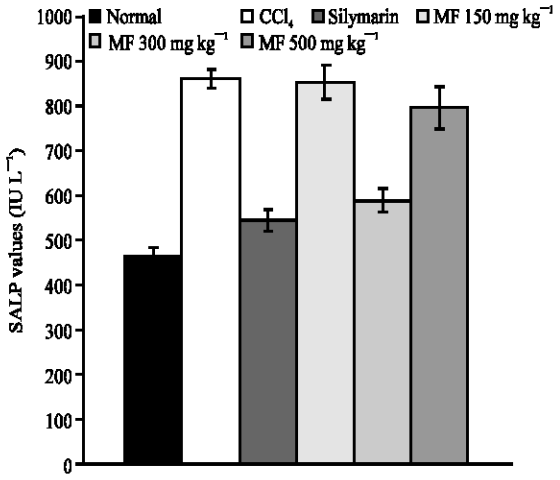

Fig. 6: Shows the effect of JG stem extract on the serum alkaline phosphatase on CCl_4 induced hepatotoxicity. Values are Mean±SEM n = 6 animals in each group, *Highly significant reduction compared to hepatotoxin (CCl_4) ($p<0.001$), **Significant reduction compared to hepatotoxin (CCl_4) ($p<0.05$)

decreased the SALP level in the dose dependent manner and maximum was found to at 300 mg kg^{-1} b.wt. (Fig. 6).

DISCUSSION

Preliminary phytochemical investigation was carried out to know the presence of various phytochemicals and their distribution in different fractions and extracts. Since, it is found that phenolics and flavonoids are the major constituents of the plant total phenolic and flavonoid contents of different fractions and extracts were determined. Determination of phenolic content shows that, very less phenolics are present in PEF, slightly higher in ChF. MF and WE found contains high phenolic content. It is evident that phenolics being polar in nature very less amount of phenolics were extracted in PEF followed by ChF. Methanol being highly polar most of phenolics have come into the MF. But WE which is prepared by extraction of marc left after extraction with methanol also shows good phenolic content, which may be due to the presence of water soluble flavonoid glycosides (Amic *et al*., 2003; Harbone, 1984). Even the flavonoid content of different fractions is also in the same pattern. i.e., flavonoid content from highest to lowest in different fractions was found to be in the order MF, WE, ChF and PEF (Table 1).

As shown in the Table 2, MF moderately scavenged DPPH radical with the IC_{50} value of 262.91± 4.38 μg mL^{-1}. Where as ascorbic acid, gallic acid and Butylated Hydroxyl Toluene (BHT) used as standards shown IC_{50} values of 4.17±0.5, 1.86±0.0 and 29.08±0.52 μg mL^{-1}, respectively. Ascorbic acid is a potent free radical scavenger and BHT is known antioxidant and is used as preservative (Singh *et al*., 2002; Mathew and Abraham, 2006). So, when compare to such potent pure compounds, IC_{50} value of 262.91+4.38 of MF of JG is moderately high and shows that JG is a potent DPPH free radical scavenger. The results obtained are found in accordance with total phenolic content and total flavonoid content. i.e., fraction (MF) showing highest phenolic/flavonoid content showed highest free radical scavenging activity and fraction (PEF) showing lowest phenolic content showed lowest free radical scavenging activity. But though the TPC (total phenolic content) of WE was found to be more than ChF and PEF, its free radical scavenging activity found to be less than them. This is may be due the presence of phenolic or flavonoid glycosides which may not be potent radical scavengers (Amic *et al*., 2003; Harbone, 1984). Similarly, in ferric ion reduction capacity of different fractions the results obtained are found in accordance with total phenolic content. i.e., fraction (MF) showing highest phenolic/flavonoid content showed highest ferric ion reduction and fraction (PEF) showing lowest phenolic content showed lowest ferric ion reduction activity as in case of free radical scavenging activity. This again proves that ferric ion reduction activity of different fractions is due to the presence of phenolics and flavonoids. But in the presence of EDTA neither the extract nor the standards were shown ferric ion reduction activity. This result indicates the JG fractions and extracts reduce of ferric ions moderately, when compared to the standard ascorbic acid and gallic acid. Even in hydrogen peroxide scavenging activity, though the hydrogen peroxide scavenging activity of different fractions are less, the activity is similar to Ferric ion reduction and DPPH radical scavenging activity. i.e. as total phenolic content/flavonoid was increased hydrogen peroxide activity was found increased. These *in vitro* results showed that MF is a potent free radical scavenger and antioxidant among the other fractions and this activity is due to flavonoids and phenolics. Since, MF was found to be potent antioxidant it was selected for *in vivo* hepatoprotective activity.

The results obtained from the present study indicate that the MF exhibited significant ($p<0.001$) hepatoprotective effect against CCl_4 induced liver damage especially at 300 mg kg^{-1} b.wt. by normalizing the elevated levels of the hepatic enzymes.

The ability of a hepatoprotective drug to reduce the injurious effects or to preserve the normal hepatic physiological mechanisms, which have been disturbed by a hepatotoxin, is the index of its protective effect. Protection of hepatic damage caused by carbontetrachloride administration has been widely used as an indicator of liver protective activity of drugs in general (Clauson, 1989). CCl_4 mediated hepatotoxicity was chosen as the experimental model. It has been established that, CCl_4 is accumulated in hepatic parenchyma cells and metabolically activated by cytochrome P_{450} dependent monooxygenases to form a trichloromethyl radical (CCl_3). The CCl_3 radical alkylates cellular proteins and other macromolecules with a simultaneous attack on polyunsaturated fatty acids, in the presence of oxygen, to produce lipid peroxides, leading to liver damage (Bishayee *et al*., 1995). Thus antioxidant or free radical generation inhibition is important in protection against CCl_4 induced liver lesion (Castro *et al*., 1974). Serum Glutamic Oxaloacetic Transferase, Serum Glutamic Pyruvic Transaminase, alkaline phosphatase, total and direct Bilirubin in plasma have been reported to be sensitive indicator of liver injury (Molander *et al*., 1955). The disturbance in the transport function of the hepatocytes as a result of hepatic injury causes the leakage of enzymes from cells due to altered permeability of membrane. This results in decreased levels of AST, ALT, Bilirubin and alkaline phosphatase in the hepatic cells and a raised level in serum. The present study revealed a significant increase in the level of SGOT, SGPT, SALP and serum bilirubin after exposure to the CCl_4, indicating considerable hepatocellular injury.

It has been hypothesized that one of the principal causes of CCl_4 induced liver injury is lipid peroxidation induced by free radical derivatives of CCl_4. Thus, antioxidant activity or the inhibition of the generation of free radicals is important in the protection against CCl_4 induced liver injury (Castro *et al*., 1974). As MF was found to be potent antioxidant its hepatoprotective activity was found to be due its antioxidant and free radical scavenging activity. All these studies show that, the MF of *Justicia gendarussa* has a significant hepatoprotective activity, which can be correlated to its antioxidant and free radical scavenging activity.

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