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Antioxidant and Hepatoprotective Activity of Leaf Extract of *Justicia gendarussa* Burm

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Abstract: The ancient literature reports *Justicia gendarussa* (JG) for its various uses in folk medicine including its hepatoprotective activity. The methanolic extract of air dried leaf was prepared by soxhlet extraction method and marc remained was further extracted with double distilled water by hot percolation. Preliminary phytochemical studies were carried out and total phenolic and flavonoid contents were determined. Both the extracts were evaluated for their antioxidant activity using DPPH free radical scavenging, hydrogen peroxide scavenging and reduction of ferric ion in presence and absence of EDTA. Methanolic extract has more phenolic and flavonoid content and shows good antioxidant activity. The methanolic extract was further studied for its *in vivo* hepatoprotective activity using CCl₄ induced hepatotoxicity in albino rats. The various biochemical parameters were evaluated to assess its hepatoprotective nature. The extract showed significant hepatoprotective activity at 300 mg kg⁻¹ body weight. Interestingly its hepatoprotective activity decreases as the dose increases. This study concludes that, leaf extract of JG has moderate hepatoprotective activity, which may be due to its antioxidant and free radical scavenging potential. High total phenolic content and flavonoid content are responsible for its antioxidant and hepatoprotective activity. Further studies are required to elucidate the exact phytochemical(s) and their mechanism responsible for hepatoprotective potential of JG.

Key words: *Justicia gendarussa*, hepatoprotective, CCl₄, DPPH

INTRODUCTION

Liver, the largest organ in vertebrate body, is the major site of intense metabolic activities. Liver injury caused by toxic chemicals and certain drugs has been recognized as a toxicological problem. Ayurvedic system of medicine assigns much importance to the pharmacological aspects of many plants (Venukumar and Latha, 2002). The hepatoprotective activity of *Justicia gendarussa* (JG) has not been evaluated scientifically to substantiate its ancient literature regarding its usefulness as hepatoprotective herb (Pandey, 2005). So, this study was conceptualized to evaluate its hepatoprotective property.

Justicia gendarussa considered as native of China and 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. It is found to be useful in bronchitis, inflammations, vaginal discharges, dyspepsia, tympanitis, eye diseases, fevers according to its ethnobotanical reports. Decoction of the root boiled in milk is given in rheumatism, dysentery and jaundice and the leaf and tender shoots are

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diaphoretic, given in the form of decoction in chronic rheumatism. Fresh leaves are used topically in edema and beriberi. It is reported that the flowering head along with the portion of leaf is used as demulcent and astringent. The leaves 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 body weight. and in doses of 10-20 g kg⁻¹ it is antipyretic and depressant producing violent diarrhea eventually death (Chopra *et al.*, 1956; Kirtikar and Basu, 2005; Publication and Information Directorate, 1959).

Chakravarty *et al.* (1982) isolated four simple o-disubstituted aromatic amines from the leaf of JG and characterized these constituents as 2-amino benzyl alcohol, 2-(2'-amino-benzylamino) benzyl alcohol and their respective O-methyl ethers 1 and 2 from ¹H NMR and mass spectral analyses of the bases and their acetates. Duke (2004) reported four compounds present in the plant namely 2-(2'-aminobenzylamino)-benzyl-alcohol, 2-(2'-aminobenzylamino)-benzyl-alcohol-o-methyl-ether, 2-amino-benzyl-alcohol, 2-amino-benzyl-alcohol-o-methyl-ether along with β-sitosterol. The various extracts of leaf were studied for its anti-inflammatory and antioxidant (Devprakash, 2000), reverse transcriptase inhibitory (Woradulayapinij *et al.*, 2005), analgesic activities (Ratnasooriya *et al.*, 2007) and antioxidant potential (Mruthunjaya and Hukkeri, 2007).

MATERIAL AND METHODS

Plant Material

Leaf 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, Bioscience Department. A specimen sample (KLKJG01) was deposited in the Department.

Preparation of the Extracts

Shade dried leaves of about 500 g was subjected for size reduction to coarse powder. The powder was extracted with 5 L methanol (analytical grade) using soxhlet apparatus till the exhaustion for about 24 h (MLE). Marc obtained after methanolic extraction was extracted with pure distilled water by reflex (WE). Both the extracts were concentrated under vacuum to get the residues. The percentage yield of methanolic leaf extract and water extract were found to be 12.72 and 1.83%.

In vitro Antioxidant Activities

The extracts were subjected for the preliminary phytochemical studies to know the phytochemical nature of the extracts (Finar, 2006; Khandelwal, 2006; Kokate *et al.*, 1996; Onwukaeme *et al.*, 2007; Evans, 2005).

Determination of Total Phenolics

The total phenolic content of both the 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 double distilled water. Reagent blank using double distilled water was prepared. Folin Ciocalteu's phenol reagent (0.4 mL) was added to the mixture and shaken. After 5 min, 4 mL of 7% Na₂CO₃ solution was added to the mixture. The solution was diluted to volume (10 mL) with double distilled water and mixed. After incubation for 90 min at room temperature, the absorbance against 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.

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). Stock solutions (1 mg mL^{-1}) of both the extracts were diluted to final concentrations of 50 to $300 \mu\text{g mL}^{-1}$ in case of MLE and 300 to $800 \mu\text{g mL}^{-1}$ in case of WE in methanol. A total of 0.3 mM methanolic solution of DPPH was added to 2.5 mL of sample solution of different concentration and allowed to react at room temperature. After 30 min, the absorbance values were recorded at 518 nm and converted into the percentage of scavenging capacity using the following equation:

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

Methanol (1 mL) and extract solution (2.5 mL) was used as a blank, while DPPH solution plus methanol was used as a negative control. The positive controls were DPPH solution plus 1 mL of standard flavonoids (Ascorbic acid, Butylated Hydroxy Toluene and Gallic acid).

The EC_{50} values were calculated by linear regression of plot, where the abscissa represents the concentration of tested plant extract or flavonoids and the ordinate represents the average percentage scavenging capacity from three replicates.

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 percentage ferric ion reduction activities of various extracts were reported as Mean \pm 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. Different concentrations of extracts in the range of 50 - $200 \mu\text{g mL}^{-1}$ were taken. 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:

$$\text{Scavenged (\%)} [\text{H}_2\text{O}_2] = [(A_0 - A_1) / A_0] \times 100$$

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

Estimation of Total Flavonoids

Total flavonoid content present in both the extract was determined by the aluminium chloride colorimetric assay. An aliquot (0.5 mL) of extract or standard solution of quercetin was added to test tube containing 2 mL of double distilled water. To the test tube was added 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 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 reported as Mean \pm SD (Marinova *et al.*, 2005).

Animal Studies

Experimental Animals

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

Acute Toxicity Studies

Acute toxicity study of MLE of JG was done as per OECD guideline 425 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 LD₅₀ 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 six groups of six animals each as follows: Group 1 served as control and received oral administration of 2.5% gum acacia (vehicle) at the dose of 1 mL kg⁻¹ body weight. Group 2 is hepatotoxic rats and received oral administration of vehicle plus CCl₄ (1 mL/kg b.wt. in 1:2 Olive oil). Group 3 received silymarin at the dose of 100 mg kg⁻¹ body weight plus CCl₄ as group 2 animals and considered as standard drug treated animals. Group 4, 5 and 6 received the MLE at the three (I, II, III) dose level of 150, 300 and 500 mg kg⁻¹ body weight plus CCl₄ as group 2 animals. All treatments were given once daily for seven days. Samples were prepared as suspensions using 2.5% gum acacia.

On the 7th day, all group of animals except group 1 were given a single dose of CCl₄ (1 mL kg⁻¹ b.wt.) in 1:2 Olive oil after 6 h of last dose administration. The group 1 animals were given 2.5% gum acacia and olive oil in 1:2 ratio of 1 mL kg⁻¹. Animals were sacrificed 24 h after the last dose and blood was collected by carotid bleeding (Kaur *et al.*, 2006). The liver was isolated and weighed for the weight variation studies among different groups.

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 (1974), method serum alkaline phosphatase (SALP), bilirubin by Winsten and Chelyk (1969) method and total protein by colour complexation with copper ion in an alkali solution (Gornall *et al.*, 1949). All the determinations were carried out using standard diagnostic kits (Agappe Diagnostic Pvt. Ltd, Kerala, India) by using fully automated Biochemical Analyzer ChemWell, manufactured by Awareness Technologies Inc, USA.

The wet weight of liver of all animal were calculated and compared with that of vehicle and CCl₄ treated animal.

Statistical Analysis

All values are expressed as Mean±SEM. Statistical analysis were 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 as significant (P is probability).

RESULTS AND DISCUSSION

Preliminary phytochemical investigation has revealed the presence of flavonoids, phenolic compounds, cardiac glycosides, reducing sugars and terpenoids in MLE.

Total Phenolic Content

This is based 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 MLE of JG was found to be 164.16±3.00 mg gallic acid equivalent per g of extras. The value for WE was found to be 29.44±3.46 mg gallic acid equivalent per g of extract (Table 1), the amount of polyphenols present in the WE is very less when compared to the MLE.

Total Flavonoid Content

The total flavonoid content of MLE is more when compare to WE. The value of MLE was found to be 54.33±4.50 mg equivalent of quercetin per g of extract. Where as the values was found to be 26.17±0.94 mg equivalent of quercetin per g of extract for WE. The potent antioxidant activity of MLE may be due to the presence of more amount of flavonoid content (Table 1).

Free Radical Scavenging Activity by DPPH Method

As shown in the Table 2, MLE strongly scavenged DPPH radical with the IC₅₀ value of 222.68±1.26 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 were shown IC₅₀ value of 4.17±0.5, 1.86±0.0 and 29.08±0.52 µg mL⁻¹, respectively. DDPH radical react with suitable reducing agents then losing colour stiochiometrically with the number of electrons consumed which is measured spectrophotometrically at 517 nm. 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 compared to the pure compounds, IC₅₀ value of 222.68±1.26 of MLE of JG is moderately high and shows that JG is moderately potent DPPH free radical scavenger. Where as the DPPH free radical scavenging activity of WE was found to be very less (455.0±5.0 µg mL⁻¹), this may be due the presence of more phenolic/flavonoid content of the MLE than in the WE.

Table 1: Total phenolic and flavonoid content of JG leaf extracts

Extracts	Total phenolic content (mg GAE/g of extracts)	Total flavonoids mg/g quercetin
MLE	164.16±3.00	54.33±4.50
WE	29.44±3.46	26.17±0.94

Values are Mean±SD

Table 2: Percentage free radical scavenging activity of JG leaf extracts, Ascorbic acid, Gallic acid and BHT in DPPH Method

Conc. ($\mu\text{g mL}^{-1}$)	MLE	WE	Conc. ($\mu\text{g mL}^{-1}$)	Ascorbic acid	Gallic acid	Conc. ($\mu\text{g mL}^{-1}$)	BHT
50	12.81±2.53	---	0.25	---	11.13±0.31	5	16.85±1.37
100	17.03±2.74	---	0.50	---	16.95±0.57	7	22.36±1.08
150	21.92±1.28	---	0.75	---	21.91±1.62	10	28.28±2.05
200	41.32±1.27	---	1.00	---	32.81±0.97	15	36.79±1.19
250	57.31±0.89	---	1.50	---	45.20±1.61	20	42.50±1.98
300	69.30±1.23	38.31±2.35	2.00	21.60±0.64	58.04±0.70	25	52.14±1.59
400	---	40.09±1.68	2.50	31.70±1.52	---	---	---
500	---	56.04±2.38	3.00	40.02±1.85	---	---	---
600	---	60.53±0.73	3.50	44.30±0.45	---	---	---
700	---	68.14±0.09	4.00	48.20±1.03	---	---	---
800	---	71.44±1.35	4.50	53.99±0.37	---	---	---
1000	---	---	---	---	---	---	---
IC ₅₀ value	222.68±1.26	455.0±5.0	466.66±3.14	4.17±0.50	1.86±0.06	---	29.08±0.52

MLE: Methanolic leaf extract, WE: Aqueous extract, As: Acid-ascorbic acid, BHT: Butylated hydroxy toluene

Table 3: Percentage Ferric ion reduction activity of JG leaf extracts, ascorbic acid and gallic acid

Conc. (μg)	MLE	WE	Conc. (μg)	As acid	Conc. (μg)	Gallic acid
200	15.01±0.58	12.39±0.57	10	22.25±0.24	2	14.66±1.51
400	23.71±0.28	20.88±0.51	20	35.15±0.75	4	32.64±0.88
600	31.40±1.27	28.41±1.24	30	53.49±1.27	6	42.88±0.51
800	39.24±1.88	36.64±1.89	40	73.64±0.41	8	60.01±1.11
1000	50.92±0.23	48.00±0.15	50	97.53±0.47	10	69.57±0.14
IC ₅₀ value	983.86±1.27	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

Ferric Ion Reduction Potential

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 Ferric ions reducing activity. The reduction is measured taking Sodium dithionite instead of the extract and considered as equivalent to 100% reduction of all the ferric ions present. Fe^{2+} reacts rapidly with 1, 10- O-phenanthroline and forms 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, MLE has shown 50.92±0.23% activity at 1000 μg concentration with IC₅₀ value of 983.86±1.27, where as WE has less % of reduction, 48.00±0.15% at the same concentration. IC₅₀ value of WE was not detected, where as IC₅₀ value of standards ascorbic acid and gallic acid were found to be 27.57±0.32 and 6.93±0.42 μg , respectively. But in the presence of EDTA neither the extract nor the standards were shown ferric ion reduction activity. These result indicates that the JG extracts were reduced the ferric ions moderately, when compared to the standard ascorbic acid and gallic acid.

Hydrogen Peroxide Scavenging Activity

H_2O_2 in phosphate buffer has the λ_{max} of 230 nm. In control tubes the absorbance will be only due to 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. MLE has scavenged the hydrogen peroxide to the extent of 35.92±1.55% at 200 $\mu\text{g mL}^{-1}$ concentration, where as WE has shown 27.18±0.88% at the same concentration. These results show that, JG extracts are mild hydrogen peroxide scavenger.

Table 4: Percentage of hydrogen peroxide scavenging activity of JG leaf extracts

Conc. ($\mu\text{g mL}^{-1}$)	MLE	WE
50	8.75 \pm 1.11	4.99 \pm 1.40
100	20.63 \pm 1.23	11.55 \pm 1.36
150	30.30 \pm 1.27	19.39 \pm 2.54
200	35.92 \pm 1.55	27.18 \pm 0.88

Table 5: Show the hepatoprotective activity of MLE of JG leaf on CCl_4 induced hepatotoxicity

Group	SGOT (IU L^{-1})	SGPT (IU L^{-1})	Total bilirubin (mg dL^{-1})	Direct bilirubin (mg dL^{-1})	SALP (IU L^{-1})	Total protein (g dL^{-1})
Normal 2.5% gum acacia	269.50 \pm 17.36	119.83 \pm 4.43	0.41 \pm 0.06	0.22 \pm 0.02	463.83 \pm 21.36	6.65 \pm 0.31
CCl_4 (1 mL kg^{-1})	1422.33 \pm 19.90	930.33 \pm 35.70	1.94 \pm 0.08	0.41 \pm 0.30	858.16 \pm 21.75	5.00 \pm 0.12
Silymarin 100 mg kg^{-1}	574.33 \pm 26.50*	440.66 \pm 26.36	0.55 \pm 0.04*	0.21 \pm 0.03*	546.83 \pm 22.81*	6.08 \pm 0.38*
MLE 150 mg kg^{-1}	683.50 \pm 31.77*	799.33 \pm 39.45	0.63 \pm 0.03	0.35 \pm 0.04**	774.33 \pm 48.79**	5.85 \pm 0.06**
MLE 300 mg kg^{-1}	591.00 \pm 19.99*	644.66 \pm 18.69	0.51 \pm 0.03*	0.28 \pm 0.03**	551.16 \pm 24.18*	6.06 \pm 0.23*
MLE 500 mg kg^{-1}	1095.50 \pm 41.84	814.00 \pm 17.15	0.78 \pm 0.05**	0.38 \pm 0.04	773.83 \pm 45.21	5.86 \pm 0.10

Values are Mean \pm 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) ($p < 0.05$)

Acute Toxicity Test

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

CCl_4 Induced Hepatotoxicity

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 indicators 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 SGOT, SGPT, bilirubin and alkaline phosphatase in the hepatic cells and a raised level in serum. The present study revealed a significant increase in the activities of SGOT, SGPT, SALP and serum bilirubin after exposure to the CCl_4 , indicating considerable hepatocellular injury.

The MLE has not shown any mortality at maximum acute dose of 5000 mg kg^{-1} in the acute toxicity studies. The maximum dose selected for the *in vivo* hepatoprotective activity was one tenth (500 mg kg^{-1}) of the maximum acute dose tested.

The results obtained in the biological experiment are presented in Table 5 and 6. The effect of MLE 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

Table 6: Shows the effect of JG leaf extract on wet liver weight on CCl₄ induced hepatotoxicity

Group	Weight of liver per 100 g body weight
Normal	4.58±0.15
CCl ₄	6.14±0.12
Silymarin	4.84±0.12*
MLE 150	5.10±0.17*
MLE 300	4.92±0.14*
MLE 500	5.44±0.09**

Values are Mean±SEM. n = 6 animals in each group, *Highly significant reduction compared to hepatotoxin (CCl₄) (p<0.001), **Significant reduction compared to hepatotoxin (CCl₄) (p<0.05)

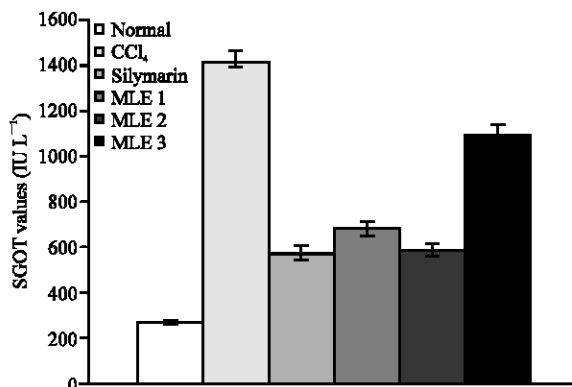


Fig. 1: The effect of JG leaf extract on the serum glutamic oxaloacetic transferase on CCl₄ induced hepatotoxicity

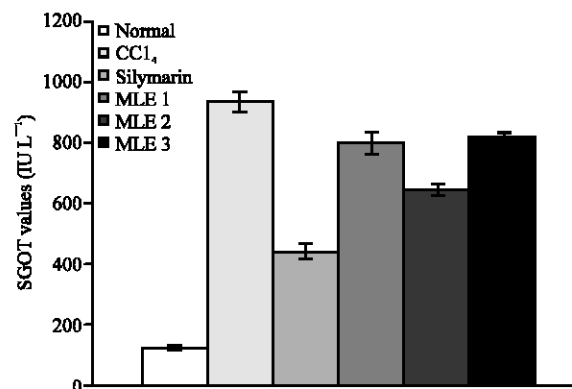


Fig. 2: The effect of JG leaf extract on the serum glutamic pyruvic transferase on CCl₄ induced hepatotoxicity

marker enzymes such as SGOT and SGPT in the serum (Recknagel, 1967). The activities of SGOT and SGPT after 24 h of oral administration of CCl₄ at the dose of 1 mL kg⁻¹ body weight are depicted in Table 5 and 6. As can be seen, CCl₄ administration resulted in a significant elevation in the level of both SGOT (527.76% of the vehicle treated control) and SGPT (776.09% of the vehicle treated control). Administration of MLE at the 150 and 300 mg kg⁻¹ body weight for seven days prior to CCl₄ administration dose dependently restored the activities of SGOT and SGPT (Fig. 1, 2). The maximum reduction in the elevated biomarker enzymes (SGOT/SGPT) were found to be at dose level II (300 mg kg⁻¹) and percentage of reduction was 58.45 and 30.71%, respectively. The extract at dose level of

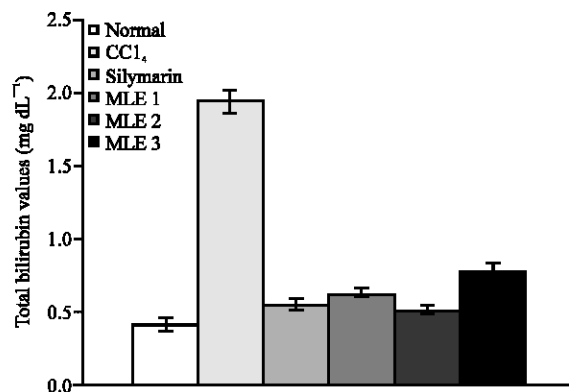


Fig. 3: The effect of JG leaf extract on the total bilirubin on CCl₄ induced hepatotoxicity

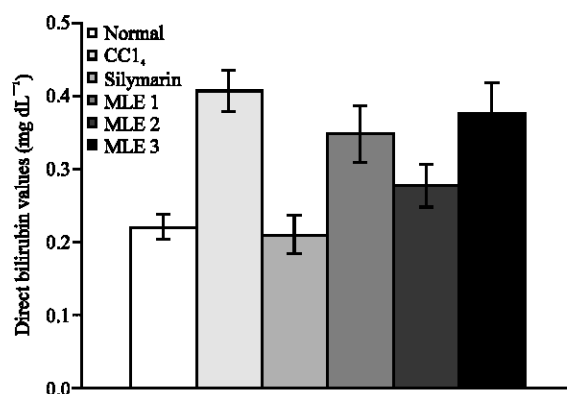


Fig. 4: The effect of JG leaf extract on the direct bilirubin on CCl₄ induced hepatotoxicity

500 mg kg⁻¹ body weight, shown the reduction in the hepatoprotective activity and further studies are required to elucidate the exact mechanism. These results indicate JG leaf possesses moderate antihepatotoxic activities at dose of 300 mg kg⁻¹. The total and direct bilirubin content present in the serum after 24 h of CCl₄ exposure is given in the Table 5. As shown in the Fig. 3 and 4 the MLE 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 73.71 and 31.70%, respectively for total and direct bilirubin, where as the value for the Silymarin were found to be 71.64 and 48.78%, respectively. The liver is the site for the production of various physiological proteins. On exposure to the hepatotoxin like CCl₄ the amount of total protein present in the serum decreases due to less activity of the hepatocytes. This can be seen from the Table 5, the total protein was decreased after CCl₄ administration to about 24.81% as compared to the vehicle treated animal group. The MLE has restored the protein content by maximum of 21.2% at dose level II (Fig. 5). The serum alkaline phosphatase (SALP) is the one more parameter used to assess the hepatoprotective activity of MLE. As given in the Table 5 the SALP has increased significantly in the CCl₄ treated group when compare to the vehicle treated group. The MLE has decreased the SALP level in the dose dependent manner at 150 and 300 mg kg⁻¹ dose level and maximum protective effect was found at 300 mg kg⁻¹ body weight and the effect was declined as the

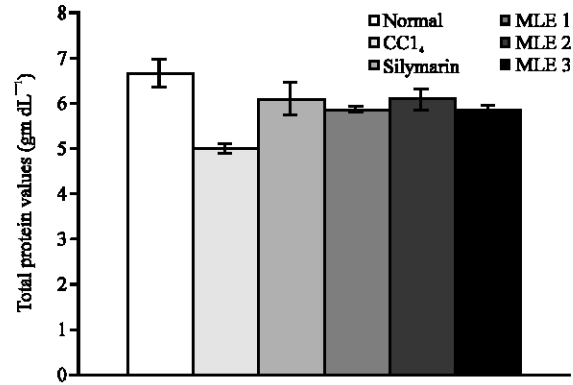


Fig. 5: The effect of JG leaf extract on the total protein on CCl₄ induced hepatotoxicity

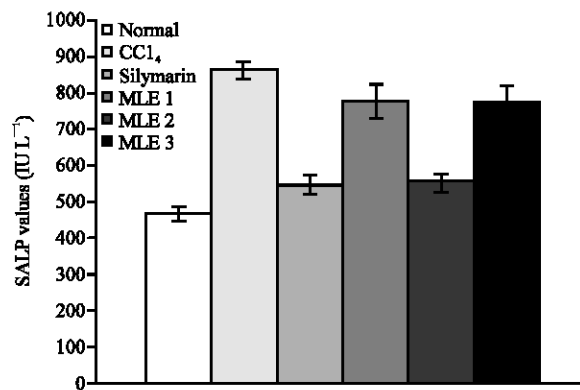


Fig. 6: Shows the effect of JG leaf extract on the serum alkaline phosphatase on CCl₄ induced hepatotoxicity

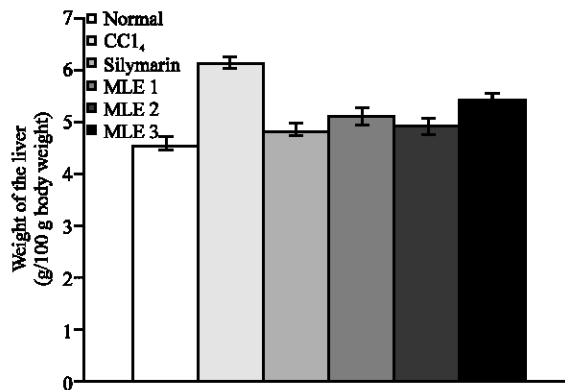


Fig. 7: Shows the effect of JG leaf extract on the wet liver weight on CCl₄ induced hepatotoxicity

dose increased to 500 mg kg⁻¹ b.wt. (Fig. 6). All these studies show that, the leaf extract of JG has a moderate hepatoprotective activity, which can be correlated to its antioxidant and free radical scavenging activity.

To access the effect of MLE on the liver weight variation after CCl_4 intoxication, the wet liver weight was calculated for each group of animals soon after the sacrifice. The results are given in Table 6. The effect of JG extract on the wet liver weight is shown in the Fig. 7.

CONCLUSION

By these studies it could be concluded that, JG leaf extract bear a moderate antioxidant activity. Their constituents scavenge free radical, reduce metal ions and exert protective effect against oxidative stress. JG extract also protects against oxidative injury induced by CCl_4 *in vivo*, the extract is capable of preventing the damage caused by the CCl_4 intoxication. The preliminary phytochemical studies of the extracts, has shown the presence of a number of polyphenols, which may be responsible for the antioxidant and hepatoprotective activities. Isolation of these compounds is in progress.

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