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Research Article

EVALUATION OF HEPATOPROTECTIVE EFFECT OF JASMINUM GRANDIFLORUM ETHANOLIC LEAF EXTRACT IN PARACETAMOL INDUCED HEPATOTOXICITY IN RATS

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A R T I C L E I N F O

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A B S T R A C T

Ethanolic leaf extract of Jasminum grandiflorum was evaluated for hepatoprotective activity in rats. The leaf extract (100 and 200 mg/kg, p.o.) showed a remarkable hepatoprotective activity against Paracetamol induced hepatotoxicity as judged from the serum marker enzymes and antioxidant levels. In hepatoprotective study, Paracetamol induced a significant rise in Aspartate amino transferase (AST), Alanine amino transferase (ALT), Alkaline phosphatase (ALP), total bilirubin, total proteins, Lipid peroxidation (LPO), Super oxide Dismutase (SOD (100 and 200 mg/kg) significantly ($P<0.001$) altered serum marker enzymes and antioxidant levels to near to normal against Paracetamol treated rats. The activity of the extract at dose of 200 mg/kg was comparable to the standard drug, Silymarin (100 mg/kg, i.p.) for hepatoprotective activity. Histopathological changes of liver sample were compared with that of the respective control samples. The results indicate the hepatoprotective property of Ehanolic leaf extract of Jasminum grandiflorum exhibited a significant activity against Paracetamol induced hepatotoxicity in experimental rats.

INTRODUCTION

The liver is the vital largest body**.** Hepatotoxicity is the damage of the liver cells due to some chemicals, drugs, infections etc. The agents which cause hepatotoxicity are D - Galactosamine, Carbon tetrachloride, Anti tubercular drugs etc. A major revolution in neuropharmacology and psychopharmacology was during the past years. The natural compounds have played a major role in neurological disorders. A number of herbal drugs traditionally employed in the Indian system of medicine Ayurveda. Herbal medicines are being used by about 80% of the world population primarily in the developing countries for primary health care [1].

Jasminum grandiflorum belongs to the family Oleaceae.The plant is found throughout India. It is grown in subtropical North West Himalaya, North Circars, Mahendragiri in Ganjam, Hills of Viagapatam, Western Ghats, Nilgiris, Pulneys and Hills of Tinnevelly above 5000 feet. Run wild and often cultivated [2]. Leaves are used in Ulcerative stomatitis, Otalgia, Ulcers, Wounds, Leprosy. Roots are used in Paralysis, Sterility, Mental disability, Constipation, Leprosy, Skin diseases. Flowers are used in Opthalmic Cepalopathy, Odontopathy, Pruritis, Ulcers, Dysmenorrhoea, Aphrodisiac [3]. Jasmine oil is used in Antidepressant, Aphrodisiac activity, relieves menstrual pain, for cancer, heart diseases. Jasminum Scent helps relax the mother and help to relieve the pain of childbirth and is thought to increase breast milk. Other uses are Migrane, Antibiotic, Diuretic, Emmenagogue [4]. The main aim of this study is to evaluate the hepatoprotective activity of *Jasminum grandiflorum* leaf extract.

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MATERIALS AND METHODS

Plant material

The leaves of *Jasminum grandiflorum* was collected and authentified by Prof. V. Chelladurai, Ph.D., Research officer – Botany, Tirunelveli.

Preparation of Extraction

Fresh leaves was collected, shade – dried and powdered mechanically. About 100 gm of leaf powder was extracted with 500 ml of ethanol as solvent (70 % ethanol) by maceration at room temperature for 4 h using a mechanical shaker. The extract was dried at 40oC under vacuum under reduced pressure and dried in desiccators. The plant material is defatted by using petroleum ether or Hydrochloric acid. Thus the prepared extract is used for further pharmacological evaluation.[5]

Drugs and chemicals

Paracetamol (Ranbaxy Laboratories Ltd, India), Silymarin (Sigma-Aldrich, St.Louis, MO63103, USA) were used for the study. All the solvents used for the extraction process are of Laboratory grade and they are purchased from local firms.

Animals

Swiss albino rats of either sex (125 - 150 g) were maintained for 7 days in the animal house under standard conditions, temperature (24 ± 10°C), relative humidity (45-55%) and 12:12 light: dark cycle. The animals were fed with standard rat pellet and water ad libitum. The animals were allowed to acclimatize to laboratory conditions 48hr before the start of the experiment.[6]

Experimental model

The animals were divided into 4 groups (n=6), Group-I (Negative control) treated with Paracetamol (400 mg/kg), Group - II, III (test group) treated with extract (100 mg/kg and 200 mg/kg), Group - IV (Standard group) treated with Silymarin (100 mg/kg). The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC). All the experiments were conducted according to the guidelines of Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA).[7]

Biochemical parameters [8]

Estimation of Serum Aspartate Amino Transferase (AST) By Kinetic Method

Label reagent tubes as Blank, control and test. Incubate at 37oC for 5 minutes. Add 0.8 ml of AST reagent to each tube. Add 100 µl of normal, control serum to the control tube. Cap the tube and mix well by inversion. Add 100 µl of test serum sample to test tube. Cap the tube and mix well by inversion. Incubate reagent tubes at 37oC for 60 minutes. Add 0.5 ml of color developer A to each reagent tube. Cap the tubes and mix well by inversion. Let the reagent tubes stand at room temperature for 20 minutes. Add 2 ml of color developer B to each reagent tube. Cap the tubes and mix well by inversion. Let the tubes stand at room temperature for 5 minutes. Wipe the reagent tubes clean with a lint free tissue paper. Place the blank tube in the test well and adjust the photometer to zero absorbance. Place the control and test tubes in the test well and record the absorbance of the control and test samples [5].

$$\text{AST concentration} = \frac{\text{Absorbance of test sample}}{\text{Absorbance of control sample}} \times \text{mean assay value of control}$$

Estimation of Serum Alanine Transaminase (ALT)

Label reagent tubes as Blank, control and test. Incubate at 37oC for 5 minutes. Add 0.8 ml of ALT reagent to each tube. Add 100 µl of normal, control serum to the control tube. Cap the tube and mix well by inversion. Add 100 µl of test serum sample to test tube. Cap the tube and mix well by inversion. Incubate reagent tubes at 37oC for 30 minutes. Add 0.5 ml of color developer A to each reagent tube. Cap the tubes and mix well by inversion. Let the reagent tubes stand at room temperature for 20 minutes. Add 2 ml of color developer B to each reagent tube. Cap the tubes and mix well by inversion. Let the tubes stand at room temperature for 5 minutes. Wipe the reagent tubes clean with a lint free tissue paper. Place the blank tube in the test well and adjust the photometer to zero absorbance. Place the control and test tubes in the test well and record the absorbance of the control and test sample [6].

$$\text{ALT concentration} = \frac{\text{Absorbance of test sample}}{\text{Absorbance of control sample}} \times \text{mean assay value of control}$$

Estimation of Alkaline Phosphatase (ALP)

Pipette 1 ml of reagent into appropriate tubes and allow equilibrating at 37oC. Adjust spectrophotometer to zero with water at 405 nm. Transfer 0.025 ml (25 µl) of sample to reagent. Mix well. After 1 minute, measure the absorbance. Return tube to 37oC. Repeat readings every minute for next 2 minutes. Calculate the average absorbance difference per minute. The Abs/min multiplied by the factor 2187 will yield results in IU/L. Samples with values above 800 IU/L should be diluted 1:1 with saline, re assayed and the results multiplied by 2 [7].

$$\text{IU/L} = \frac{\text{Abs/min} \times 1000 \times \text{TV}}{\text{mM} \times \text{LP} \times \text{SV}} = \frac{\text{Abs/min} \times 1000 \times 1.025}{18.75 \times 1 \times 0.025}$$

Where as
Abs/min = Absorbance change per minute
1000 = Conversion of IU/ml to IU/L
TV = Total volume (1.025 ml)
mM = Mllimolar absorptivity of p – nitrophenol (18.75)
LP = light path (1 cm)
SV = Sample volume (0.01 ml)

Estimation of Total Serum Proteins

Label test tubes blank, standard, control, test. Pipette 1 ml of Biuret reagent to each tube. Add 0.02 ml (20 µl) of standard and test to appropriate tubes and mix by inversion. Let the tubes stand at room temperature at 540 nm and zero absorbance with the reagent blank. Read and record absorbance readings of each tube [8].

$$\text{Total protein (g/dl)} = \frac{\text{Absorbance of unknown}}{\text{Absorbance of standard}} \times \text{Concentration of standard}$$

Estimation of Total Bilirubin

Label tubes as blank, standard, control, test. Each tube requires a blank tube. Dispense 1 ml of total Bilirubin reagent to all blank tubes. Prepare a working reagent (Total Bilirubin working reagent: Add 0.05 ml (50 µl) of Sodium nitrate to 1 ml of total Bilirubin reagent and mix well). Dispense 1 ml of working reagent into labeled test tubes, except blank. Add 0.1 ml (100 µl) of each standard, control and sample to its respective tubes. Mix well. Allow all tubes to stand for 5 minutes at room temperature. Set the wavelength of the instrument at 560 nm. Zero with reagent blank. Read and record absorbance of all tubes [9].

$$\text{Total Bilirubin (mg/dl)} = \frac{\text{Abs of unknown} - \text{Abs of blank}}{\text{Abs of calibrator} - \text{Abs of calibrator blank}} \times \text{Concentration}$$

Estimation of Lipid Peroxidation (LPO)

To 0.1 ml of the liver homogenate, 2.0 ml of 20% TCA was added. The contents were mixed well and centrifuged at 4000 rpm for 20 minutes. 2.0 ml of the supernatant was mixed with 2.0 ml of Thiobarbituric acid reagent. Reagent blank standards

(5-20 n moles) were also treated similarly. The contents were heated for 20 minutes in a boiling water bath. The tubes were cooled to room temperature and the absorbance was read at 532 nm. The lipid peroxide content was expressed as moles MDA per 100 mg protein [10]
Concentration = A (V/E) P

A = Absorbance, V = Volume of solution, E = Extinction coefficient (1.56 x 10-6), P = Protein content of tissue

Superoxide Dismutase (SOD) by Mark Lund Method

To 0.5 ml of tissue homogenate, 0.5 ml of distilled water was added to dilute the sample. To this 0.25 ml of ice-cold ethanol and 0.15 ml of chloroform were added. The mixture was shaken for a minute at 4^{O} C and then centrifuged. The enzyme activity in the supernatant was determined. Adreno chrome produced in the reaction mixture containing 0.2 ml of EDTA, 0.4 ml of Sodium carbonate and 0.2 ml of epinephrine in a final volume of 2.5 ml was followed at 470 nm. Transition of Epinephrine to adrenochrome was inhibited by the addition of the required quantity of enzyme. The amount of enzyme required to produce 50% inhibition of epinephrine to adrenochrome transition was taken as one enzyme unit. Activity of the enzyme was expressed as units/min/mg protein [11]

RESULTS

Table 1 Effect of JGLE on AST, ALT, ALP

Treatment	Ast (iu/l)	Alt (iu/l)	Alp (u/l)
Normal	78.34 ± 2.83	52.75± 2.56	112.58 ± 2.02
Paracetamol	178.15±2.69*	134.2±2.88*	198.52±2.82*
Paracetamol + Silymarin 100 mg/kg	82.68±2.16**	53.29±2.76**	115.8±2.93**
Paracetamol + JGLE 100 mg/kg	122.05±2.79**	89.16±2.8**	127.45±2.04**
Paracetamol + JGLE 200 mg/kg	93.26±2.31**	54.81±3.06**	114.43±2.8**

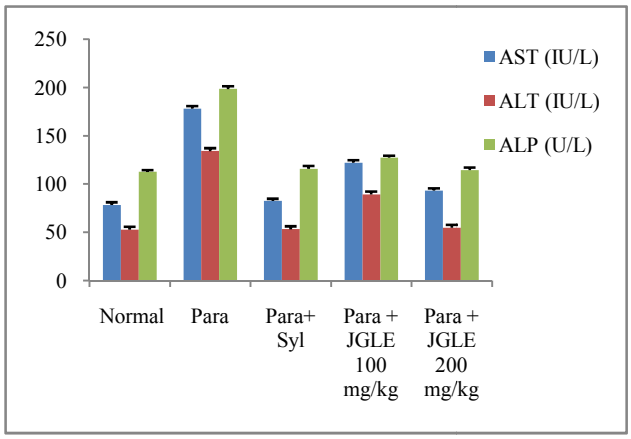

Figure No 1 Values were given as mean ± S.D. for six rats in each group. Values are statistically significant at **p < 0.001 as compared with Paracetamol group;

Table 2 Effect of JGLE on Serum proteins and Total Bilirubin levels

Treatment	Serum proteins	Total bilirubin
Normal	7.17±0.07	0.87±0.14
Paracetamol	5.65±0.02*	2.85±0.63*
Paracetamol + Silymarin 100 mg/kg	6.92±0.03**	1.04±0.14**
Paracetamol + JGLE 100 mg/kg	6.64±0.09**	1.98±0.87**
Paracetamol + JGLE 200 mg/kg	6.86±0.2**	0.96 ±0.12**

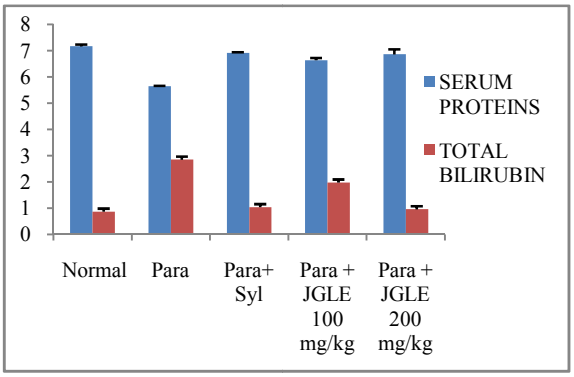

Figure No 2 Values were given as mean ± S.D. for six rats in each group. Values are statistically significant at **p < 0.001 as compared with Paracetamol group

Table 3 Effect of JGLE on antioxidant parameters

Treatment	LPO	SOD
Normal	12.46±1.75	30.45±1.09
Paracetamol	24.65±2.02*	21.05±1.63*
Paracetamol + Silymarin 100 mg/kg	13.54±1.13**	29.36±1.75**
Paracetamol + JGLE 100 mg/kg	16.92±1.09**	26.18±2.07**
Paracetamol + JGLE 200 mg/kg	14.01±1.02**	28.30±2.17**

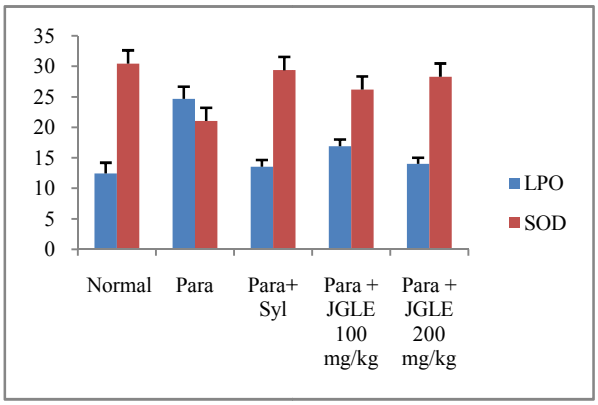

Figure No 3 Values were given as mean ± S.D. for six rats in each group. Values are statistically significant at **p < 0.001 as compared with Paracetamol group

Histopathology

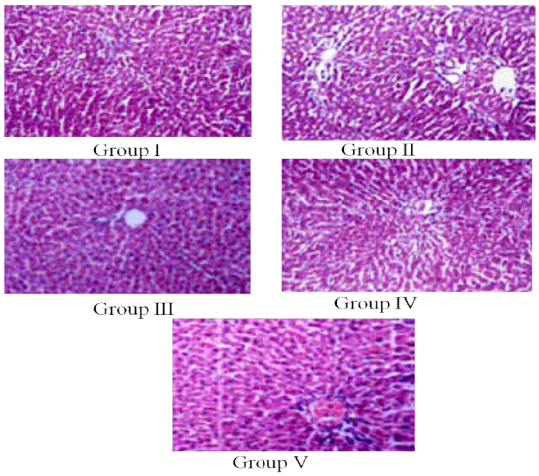

Figure No 4 Histopathology of liver sections of animals (x100),
Group II: Paracetamol treated group showing centrilobular degeneration, necrosis of hepatic cells,
Group III: Paracetamol + JGLE 100 mg/kg had shown mild degeneration and reverting to regeneration,
Group IV: Paracetamol + JGLE 200 mg/kg had shown maximum regeneration and almost near to normal architecture of hepatocytes,
Group V: Paracetamol + Silymarin 100 mg/kg showing complete regeneration and normal architecture of hepatocytes.

DISCUSSION AND CONCLUSION

The present study reveals that leaf extract of *Jasminum grandiflorum* exhibit a significant Hepatoprotective activity by reducing the biochemical parameters like AST, ALT and ALP. The plant extracts of 100 and 200 mg/kg has decreased the serum protein and total bilirubin levels. They have decreased the Lipid peroxidation levels and increased the protective Superoxide Dismutase levels in order to prevent further injury. Both the concentrations of the extract showed a significant activity when compared to that of standard drug Sylimarin. Hence *Jasminum grandiflorum* can be used as Hepatoprotective agent. Further studies are required to find out the active ingredient which is responsible for the Hepato protection and usage of this less side effct drug for treatment of liver disorders.

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