

**IN-VITRO HEPATOPROTECTIVE ACTIVITY OF *JUSTICIA GENDARUSSA* STEM ON ISOLATED RAT HEPATOCYTES**KL Krishna<sup>1</sup>, Tejal A Mehta<sup>2</sup> and Jagruti A Patel<sup>2</sup><sup>1</sup>JSS College of Pharmacy, JSS University, Sri Shivarathreeswara Nagara, Mysore, Karnataka, India<sup>2</sup>Institute of Pharmacy, Nirma University of Science and Technology, Ahmedabad, Gujarat, India**Summary**

Isolated rat primary hepatocytes were used as *in-vitro* model to evaluate the hepatoprotective activity of methanolic extract of *Justicia gendarussa* Burm. Carbon tetrachloride was chosen as hepatotoxin and silymarin was the reference hepatoprotective agent. The isolated primary rat hepatocytes were incubated with standardized hepatotoxic dose of CCl<sub>4</sub> (10 mM), and various concentrations (10, 50 and 100µg/ml) of the extract and silymarin (100µg/ml). Trypan blue exclusion assay was used to measure the cell viability. Marker transaminase enzymes (GOT, GPT) and Total Protein (TP) in the cell culture suspension were determined. Methanolic extract has shown moderate hepatoprotective activity on isolated rat hepatocytes. The extract produces significant (P<0.05) moderate protective effect, however it is similar but less than silymarin. This result suggests that the *Justicia gendarussa* possess moderate hepatoprotective activity on carbon tetrachloride induced hepatotoxicity in isolated rat hepatocytes.

**Key words-** Justicia, hepatocytes, cell viability,**Introduction**

In our search for remedies from natural products that might be valuable in protecting the liver from toxin-induced injury, we found that, the methanolic extract of *Justicia gendarussa* Burm (JG) stem showed moderate ability to protect primary hepatocytes from the effects of CCl<sub>4</sub> exposure. JG is commonly known as Nil-niragundi in India and considered as native of China. It is commonly grown in Indian garden as hedge or border plant. The plant is being reported for its useful in bronchitis, inflammations, vaginal discharges, dyspepsia, tympanitis, eye diseases, fevers, dysentery, jaundice diaphoretic, chronic rheumatism and facial paralysis. Other ethno medicinal uses include febrifuge, emetic, stomach troubles, demulcent and astringent and antipyretic<sup>(1-3)</sup>. The plant is recently reported for its anti-inflammatory and antioxidant<sup>(4)</sup>, reverse transcriptase inhibitory activity<sup>(5)</sup>, analgesic activity<sup>(6)</sup>, antioxidant potential<sup>(7)</sup> and hepatoprotective activity<sup>(8)</sup>. Based on the recent research on this plant, the present study was designed to evaluate its hepatoprotective potential on isolated rat hepatocytes.

## Method

### *Plant materials and preparation of extract*

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. 500 g 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.

### *Isolation of hepatocytes*

Fasting albino adult rat was used for the study. Hepatocytes were isolated using modified method of Kiso *et al*<sup>(9)</sup>. The animals was disinfected with ethanol and anesthetized by Ketamine (i.p. 50mg/kg body weight). Dissection was done under aseptic condition using sterilized instruments. Midline incision was made on the abdomen of the anaesthetized animal. The portal vein was cannulated with needle no 25 connected to a 10ml disposable syringe. The needle was tied in place and the inferior vena cava was cut below the renal vein. Perfusion of the liver was started immediately with Ca<sup>2+</sup>-Mg<sup>2+</sup> free Hanks Balanced Salt Solution (HBSS). When the liver was thoroughly perfused (*i.e.* has turned white), the flow of the HBSS was stopped and the needle was removed. The liver was transferred to a sterile beaker containing Ca<sup>2+</sup>-Mg<sup>2+</sup> free HBSS and minced into small pieces, which were transferred to a sterile conical flask containing 10 ml of 0.075% Collagenase in HBSS. The cell suspension thus obtained was mixed properly and centrifuged at 2000 rpm for 10 minutes. The cells were washed with Dolbicos Modified Eagle Media (DMEM) 2-3 times and suspended in complete medium and incubated at room temperature for 1.5 hrs possibly to recover from the stress and membrane injury suffered during the isolation of cells. The isolated rat hepatocytes were cultured in DMEM at density of  $1 \times 10^6$  cells/ml<sup>(10)</sup>. Viability of the isolated cells was determined by Trypan blue exclusion assay by counting the number of stained and unstained cells. The cell viability in any case should be more than 90%.

### *Cytoprotective effect*

The primary rat hepatocytes thus obtained were used for studying the cytoprotective activity of JG extract. Fixed number of hepatocytes ( $1 \times 10^6$  cells/ml) was incubated with different concentrations (10, 50 and 100 µg/ml) of JG extracts and hepatotoxin 10 mM toxin (CCl<sub>4</sub>) in sterile test tubes. The above cell suspension was incubated for 3 hour and after 3hrs of incubation the cell viability was determined by the trypan blue exclusion assay. After wards cell suspension was centrifuged at low speed (2000 rpm) for 15 min. The supernatant solution was used for the estimation of marker enzymes like Glutamic Oxaloacetic Transaminase (GOT), Glutamic Pyruvic Transaminase (GPT) and Total Protein (TP). All determinations were done in Semi Auto analyzer (Microlab 300) using Mark diagnostic kits. In the CCl<sub>4</sub> control tubes media was added instead of extract while in normal tubes contains no CCl<sub>4</sub>. Silymarin (100µg/ml) was used as standard reference hepatoprotective.

### *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 5.0 software. The value of *P* lower than 0.05 was considered as significant.

## Results

The cytoprotective effect of JG is depicted in table 1. Isolated rat hepatocytes incubated with 10 mM CCl<sub>4</sub> resulted in induction of significant ( $P < 0.05$ ) sub maximal toxicity, which was indicated by 69.06% and 52.74 % decrease in cell viability and TP content of hepatocytes respectively. Similarly an elevation about 141.45% and 216.62% of GOT and GPT level are observed respectively upon intoxicated with CCl<sub>4</sub>. Isolated rat hepatocytes pretreated with JG in the concentration of 10-100 µg/ml showed a moderate protective effect by restoring the viability of hepatocytes (9.71- 20.82%), TP content (22.76-27.73%), GOT (12.04-31.84%) and GPT (18.75-29.52%). The maximum significant ( $P < 0.05$ ) hepatoprotective activity was observed at 100 µg/ml concentration where as no/least activity was produced at 10 µg/ml concentration. The reference standard drug silymarin showed good hepatoprotective effect by restoring viability (83.93%), GOT (91.59%), GPT (76.01%) and TP (76.48%).

**Table 1 Effect of Justicia gendarussa extract on CCl<sub>4</sub> induced hepatotoxicity in isolated rat hepatocytes**

Treatment	Viable cell (%)	GOT (IU/L)	GPT IU/L	TP g/dl
Normal	95.66±0.88	64.33±0.33	94.33±0.33	0.91±0.025
CCl <sub>4</sub> Control	29.57±0.50	155.33±0.88	298.67±1.20	0.43±0.015
Silymarin 100 µg/ml	85.05±0.58** (83.93)	72.00±1.53* (91.59)	143.33±1.45* (76.01)	0.81±0.019** (76.48)
JG 10 µg/ml	25.33±1.20 (-6.38)	144.33±1.20* (12.04)	260.33±1.33 (18.75)	0.39±0.015 (-8.27)
JG 50 µg/ml	37.33±0.67** (9.71)	134.67±1.45* (22.67)	245.66±1.45* (25.94)	0.54±0.014** (22.76)
JG 100 µg/ml	44.33±0.88** (20.82)	126.33±0.67* (31.84)	238.33±1.35* (29.52)	0.56±0.018** (27.73)

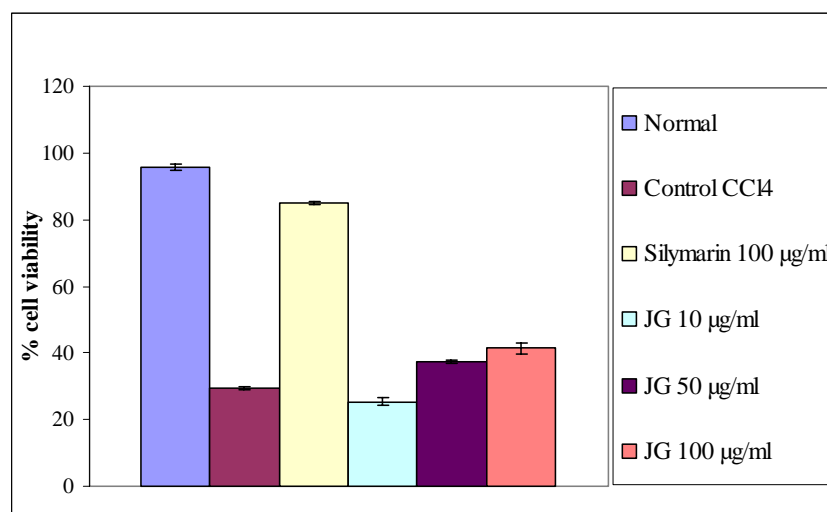
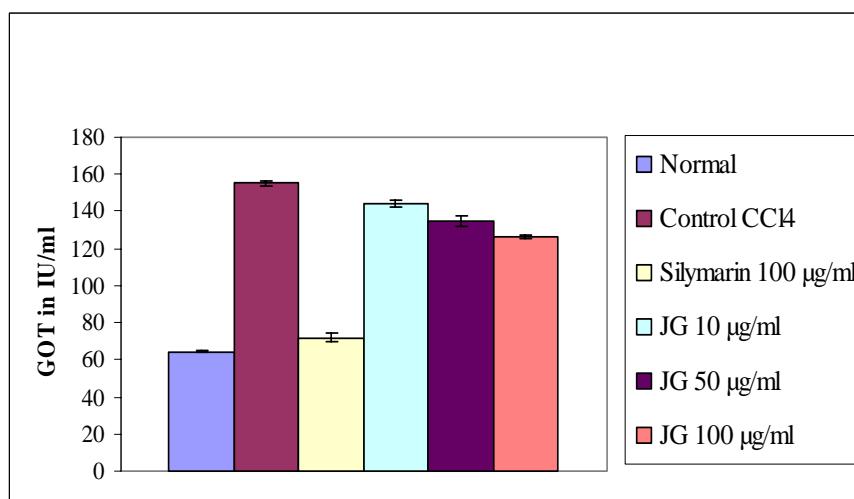
Data represents the Mean±SEM of three values, JG: Justicia gendarussa extract.

\*significant reduction compared to hepatotoxin (CCl<sub>4</sub>) ( $P < 0.05$ )

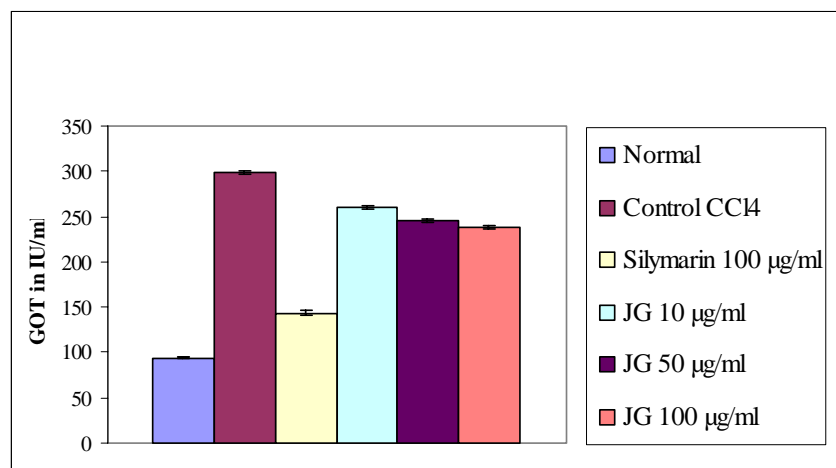
\*\* significant increase compared to hepatotoxin (CCl<sub>4</sub>) ( $P < 0.05$ )

## Discussion

The present study was performed to assess the hepatoprotective activity in isolated rat hepatocytes against carbon tetrachloride induced acute hepatic injury. CCl<sub>4</sub> induced hepatic injury is commonly used as an experimental method for the study of hepatoprotective effect of medicinal plants. The hepatotoxicity induced by CCl<sub>4</sub> is due to its metabolite CCl<sub>3</sub><sup>\*</sup>, a free radical that binds to lipoprotein and leads to peroxidation of lipids of the endoplasmic reticulum<sup>(11)</sup>. 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 an index of its protective effects. Although transaminase enzymes levels are not a direct measure of hepatic injury, they show the status of the liver. The lowering of marker liver enzymes is a definite indication of hepatoprotective action of the drug. The transaminase enzymes GOT and GPT levels are reliable markers of liver function<sup>(12)</sup>.

**Fig 1 Effect of JG on CCl<sub>4</sub> induced hepatotoxicity on isolated rat hepatocytes (% hepatocytes viability)****Fig 2 Effect of JG on CCl<sub>4</sub> induced hepatotoxicity on isolated rat hepatocytes (GOT level)**

Isolated hepatocytes have become valuable tools to evaluate the possible protective effect of drugs in the recent past. The techniques for high yield isolation of rat hepatocytes are made it as useful model<sup>(13)</sup>. Hepatotoxin like CCl<sub>4</sub>, paracetamol, thioacetamide etc have been shown to result in the reduction of cell viability as well as elevation in marker enzymes, similar changes in the present study confirms these changes and indicates satisfactory standardization of our isolation and culture procedure. In the present study, the hepatotoxin employed reduces cell viability possibly due to injury to plasma membranes of hepatocytes resulting in the leakage of cellular enzymes. Incubation of isolated hepatocytes with extract of JG moderately restored their viability as well as altered biochemical parameters induced by hepatotoxin (Table 1). In this study we have observed significant toxicity after 3hr incubation with hepatotoxin. The JG at the dose of 100 µg/ml has produced significant ( $P < 0.05$ ) protection against CCl<sub>4</sub> induced hepatotoxicity as shown in Fig 1-3, however the extract has shown no protective effect at 10 µg/ml concentration. The result obtained from this study supports our previous hepatoprotective activity on this plant extract<sup>(8)</sup>. It may be hypothesized that, the flavonoids, which are present in JG extract, could be considered responsible for the hepatoprotective activity.

Fig 3 Effect of JG on CCl<sub>4</sub> induced hepatotoxicity on isolated rat hepatocytes (GPT level)

### Acknowledgments

One of the authors Mr. KL Krishna is highly thankful to Dr. H.G. Shivakumar, Principal, JSS College of Pharmacy, JSS University, Mysore, for his constant encouragement & support and JSS University, Mysore, for providing the required infrastructure to carryout the research activities.

### References

1. Kirtikar KR. Basu BD. Indian medicinal plants. Dehra Dun, International book distributors. India.
2. Chopra RN. Nayar SL. Chopra IC. Glossary of Indian medicinal plants. CSIR. New Delhi.
3. Anonymous. The Wealth of India, Raw materials. Publication & information Directorate, CSIR, New Delhi.
4. Devprakash. 2000. Lignans and other chemical components of *Justicia gendarussa* and *Justicia simplex* and their bioactivity studies [Dissertation]. Manipal: MAHE deemed university.
5. Woradulayapinij W. Soonthornchareonnon N. Wiwat, C. In vitro HIV type 1 reverse transcriptase inhibitory activities of Thai medicinal plants and *Canna indica* L. rhizomes. J Ethnopharmacol 2005; 101: 84–89.
6. Ratnasooriya WD. Deraniyala SA. Dehigaspiya DC. Antinociceptive activity and toxicological study of aqueous extract of *Justicia gendarussa* Burm. in rats. Phcog Mag 2007; 3(11):145-155.
7. Mruthunjaya K. Hukkeri VI. Antioxidant and free radical scavenging potential of *Justicia gendarussa* Burm. Leaves *in vitro*. Natural Product Sciences 2007; 13 (3): 199-206.
8. Krishna KL. Mruthunjaya K. Jagruti A Patel. Antioxidant and Hepatoprotective Potential of Stem Methanolic Extract of *Justicia gendarussa* Burm. International Journal of Pharmacology 2010; 6 (2): 72-80.
9. Kiso Y. Tohkin M. Hikino H. Assay method for antihepatotoxic activity using carbon tetrachloride induced cytotoxicity in primary cultured hepatocytes. Planta Med 1983; 49: 222-225.
10. Mohammed Ibrahim. Mohammed Nane Khaja. Anjum Aara, et al. Hepatoprotective activity of *Sapindus mukorossi* and *Rheum emodi* extracts: *In vitro* and *in vivo* studies. World J Gastroenterol 2008; 14 (16): 2566-2571.
11. Recknagel R. Carbontetrachloride hepatotoxicity. Pharmacologica Reviews 1969; 19: 145-196.
12. Mulander DW, Wrublewski F, La Due JS. Transaminase compared with cholinesterase and alkaline phosphatase an index of hepatocellular integrity. Clinica Research Proceedings 1955; 3: 20-24.
13. Skett P. Problems in using isolated and cultured hepatocytes for xenobiotics metabolism based toxicity testing solution. Toxicol in Vitro 1994; 3: 491-504