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EVALUATION OF *JASMINUM GRANDIFLORUM* FOR HEPATOPROTECTIVE ACTIVITY IN ISONIAZID INDUCED LIVER DAMAGE

Netranjali Dhamal, Mihir Patel and Shrikant Pawar*

Department of Pharmacology, Sinhgad Technical Education Society's Smt. Kashibai Navale College of Pharmacy, Kondhwa, Pune-411048, Maharashtra, India

ABSTRACT

Key words:

Liver,
Jasminum grandiflorum,
Isoniazid,
Lipid profile

Correspondence to Author:

Shrikant Pawar

Assistant Professor, Department of
Pharmacology, Sinhgad Technical
Education Society's Smt. Kashibai Navale
College of Pharmacy, Kondhwa, Pune-
411048, Maharashtra, India

E-mail: shrikant_pawar12@rediffmail.com

Objectives: The study was designed to evaluate hepatoprotective effect of the ethanolic leaves extract of *Jasminum grandiflorum* (JG) in Isoniazid (INH) induced hepatotoxicity in wistar albino rats.

Materials and methods: Mature leaves of JG were collected, authenticated and subjected to successive ethanolic extraction. Hepatic damage was induced in wistar rats by administering INH (54mg/kg, p.o.) once a daily for 30 days followed by administration of JG (200mg/kg, p.o.) 1h.prior to the administration of INH (54mg/kg, p.o.) once daily. Silymarin (50mg/kg. p. o) was used as standard for 30 days.

Result: Elevated levels of Aspartate transaminase, Alanine transaminase, and Lipid profile following INH administration were significantly lowered by JG treatment. Deposition of collagen was observed in liver and found to be less in JG treated animals; Pretreatment of rats with JG significantly decreases Lipid peroxidation (LPO) and increases the antioxidant activities.

Conclusion: The study reveals the hepatoprotective activity of leaves extract of JG in isoniazid induced liver damage.

INTRODUCTION: The liver is a major detoxifying organ in vertebrate body, which involves intense metabolic activities. Certain toxic chemicals, medicines can cause liver damage, which has been recognized as a toxicological problem. However, herbal medicines are known to play an important role in the treatment of various ailments, including hepatopathy. Many traditional practitioners have claimed that numerous medicinal plants and their formulations can be effectively used for the alleviation of different types of liver diseases¹.

Liver damage is always associated with cellular necrosis, increase in lipid peroxidation and reduction in the tissue GSH levels. In addition serum levels of many biochemical markers like SGOT, SGPT, ALP and Bilirubin

are elevated. Conventional drugs used in the treatment of liver diseases are unsatisfactory and sometimes can have serious side effects².

Administration of INH produces abbreviation in liver. The INH induced hepatotoxicity is initiated by CYP-450-mediated metabolism to toxic metabolites such as acetyl hydrazine and hydrazine. Hydrazine reacts with sulfhydryl group, which result in glutathione depletion within the hepatocytes leading to cell death³.

Jasminum grandiflorum L. (Family: Oleaceae) exhibit a wide ecological range and found extensively all over India. The leaves of JG are used in the treatment of odontalgia, fixing loose teeth, ulcerative stomatitis, leprosy, skin diseases, ottorrhoea, otalgia, stangury, dysmenorrhoea, ulcers, wounds and corns.

The leaves of this species have a distinction of being used in Indian folk medicine for treating ulcers. There is however, a need to study and provide evidence for the efficacy of *Jasminum* extract in the treatment of liver disease ⁴.

MATERIALS AND METHODS:

Drugs and chemicals: INH procured from research labs and Silymarin was obtained from Microlabs. All other chemicals were of analytical grade.

Preparation of JG Alcoholic Plant Extract: The leaves of JG were collected from the fields surrounding Pune and were authenticated by Botanical Survey of India, Pune. The leaves were air dried, powdered, and then extracted with 70% ethanol by using Soxhlet method. The extract was filtered with filter paper and then the solvent was evaporated at reduced pressure by using rota-evaporator apparatus (HEIDOLPH) to get viscous mass. The yield of extract obtained was 7 %.

Phytochemical Study: Preliminary phytochemical screening of the powdered leaves was performed for the presence of alkaloids, phenolics, flavonoids, saponins, carotenoids, carbohydrates and glycoside.⁵

Test	Result
Flavonoids	+ve
Phenolics	+ve
Carotenoids	+ve
Steroids	+ve
Saponin	- ve
Vit. C	+ve

- ve = absent, +ve = present

Acute Toxicity Studies: In LD50 studies, it was found that the animals were safe upto a maximum dose of 2000 mg/kg body weight. There were no changes in normal behavior pattern and no signs and symptoms of toxicity and mortality were observed. The biological evaluation was carried out at doses of 200 mg/kg body weight ⁶.

Determination of Total Phenolic Content: Total phenolic content of JG extract was measured by Folin-Ciocalteu reagent as an oxidizing agent and Gallic acid as standard. To 0.5ml of extract solution (2mg/ml) in water, 2.5ml of Folin-Ciocalteu reagent and 2ml of sodium carbonate solution were added. After 20 min, incubate at room temperature. The absorbance was measured at 760nm using UV Visible spectrophotometer. Total phenolic were quantified by calibration curve obtained from measuring known concentration of Gallic acid ⁷.

$$\text{Absorbance} = 0.0015x - 0.0117$$

Experimental Animals: Albino Wistar rats (150-200g) were obtained from animal house facility of Smt. Kashibai Navale College of Pharmacy, Pune and were housed in clean cages and in a well ventilated facility with free access to standard feed and drinking tap water. The animals were kept at room temperature (28±2 °C) with a 12 h daylight/ dark cycle under humid tropical conditions. All procedures involving the use of laboratory animals were in accordance to the CPCSEA and were approved by the institutional animal ethical committee.

Treatment Protocol:

INH induced Hepatotoxicity: Animals were divided into four groups containing six animals in each. Group I (control) rats were fed with standard diet and administered with saline solution of once daily for period of 30 days. In group II (INH control), rats were treated with INH (54mg/kg p. o.) once daily for period of 30 days. Group III was treated with ethanolic extract of JG at (200mg/kg p. o.) and simultaneously received INH (54mg/kg p. o.) 1h. after administration of JG extract once daily for a period of 30 days. Group IV rats were treated with standard drug Silymarin (50mg/kg p. o.) and received INH (54mg/kg p. o.) 1h. after administration of standard drug once daily for a period of 30 days. At the end of the experiment, rats were sacrificed and blood was collected for the biochemical estimations. ⁸.

Assessment of Lipid Peroxidation (LPO): Lipid peroxidation by TBRS method carried out by using MDA equivalents derived from tetraethoxypropane. MDA and other aldehyde have been identified as products of LPO that reacts with Thiobarbituric acid to

give pink colored species that absorbs at 532nm. The method involved heating of biological sample with TBA reagent for 20 min in a boiling water bath. TBA reagent contains 20% Trichloroacetic acid, 0.5% Thiobarbituric acid and 2.5% N HCl. After cooling, the solution was centrifuged at 2000rpm for 10 min and precipitate obtained was removed. The absorbance of the supernatant was determined at 532nm against the blank. 9.

Assessment of liver damage: Blood was collected under ether anesthesia 24 h after last dose and analyzed for serum AST, ALT and Lipid profile. The liver tissue was dissected out immediately washed with ice

cold saline and used for determination of liver weight, liver index and collagen content.

Histopathological investigations: Liver tissue were dissected out and fixed in 10% formalin. The paraffin sections were prepared and stained with hematoxylin, eosin and examined using light microscopy.

Statistical Analysis: Data were computed for statistical analysis using graph pad prism software. The results were expressed as the mean \pm SEM. The results were analyzed by one-way ANOVA followed by Dunnett multiple comparison test

RESULTS:

TABLE 1: EFFECT OF ETHANOLIC LEAVES EXTRACT OF JG ON LIVER ENZYMES IN INH INDUCED HEPATOTOXICITY (n=6)

Parameter (I.U./L)	Control	INH Control (54mg/kg)	JG treated (200mg/kg)	Silymarin std. (50mg/kg)
AST	17.67 \pm 10.70	323.5 \pm 7.719	271.2 \pm 11.24**	244.2 \pm 11.6***
ALT	31.17 \pm 14.46	79.17 \pm 4.658	64.5 \pm 5.353*	52.33 \pm 1.687**

Values expressed as mean \pm S.E.M, n=6. *P< 0.05, **P< 0.01, ***P< 0.001 compared to INH control group,

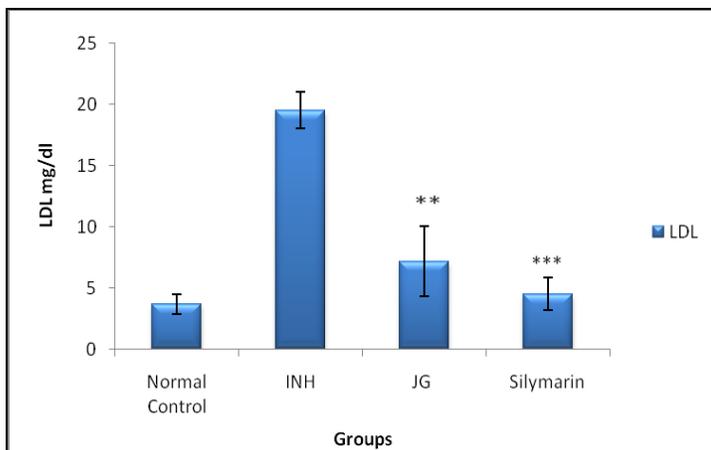


FIGURE 1: EFFECT OF JG AT THE DOSE OF 200mg/kg ON LDL LEVEL. Values expressed as mean \pm S.E.M, n=6. ***P< 0.001 compared to INH control group.

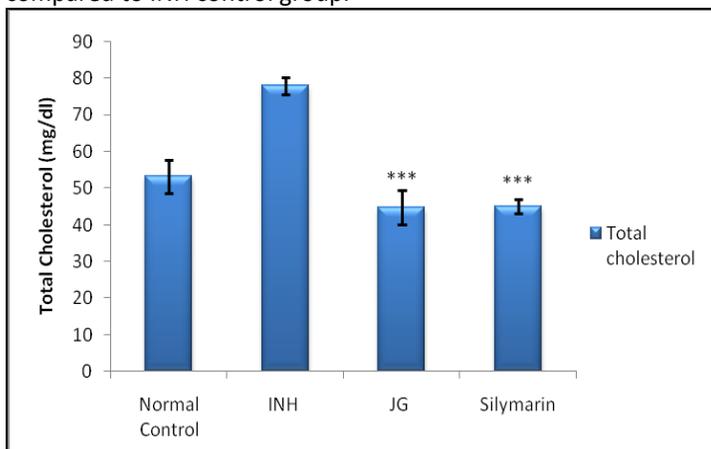


FIGURE 2: EFFECT OF JG AT THE DOSE OF 200mg/kg ON TOTAL CHOLESTEROL LEVEL. Values expressed as mean \pm S.E.M, n=6. **P< 0.01 compared to INH control group.

Effect on liver weight, liver index, Collagen estimation, Lipid peroxidation: There was significant decrease in liver weight and liver index, collagen estimation in treated groups.

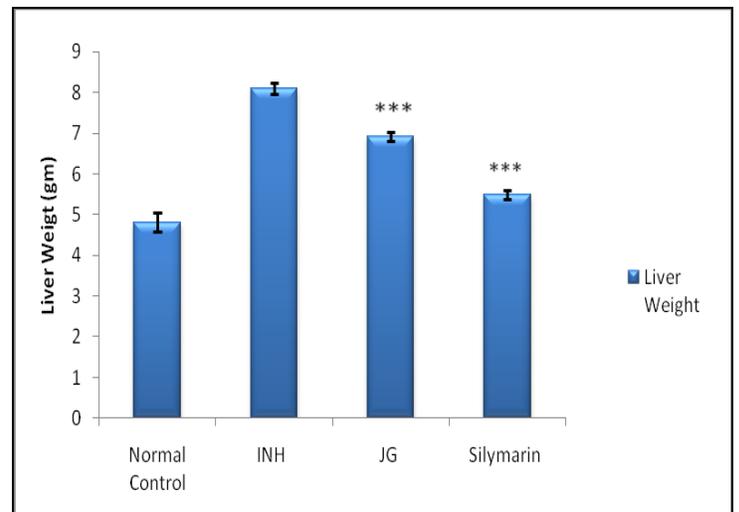


FIGURE 3: EFFECT OF JG AT THE DOSE OF 200mg/kg ON LIVER WEIGHT. Values expressed as mean \pm S.E.M, n=6. ***P< 0.001 compared to INH control group.

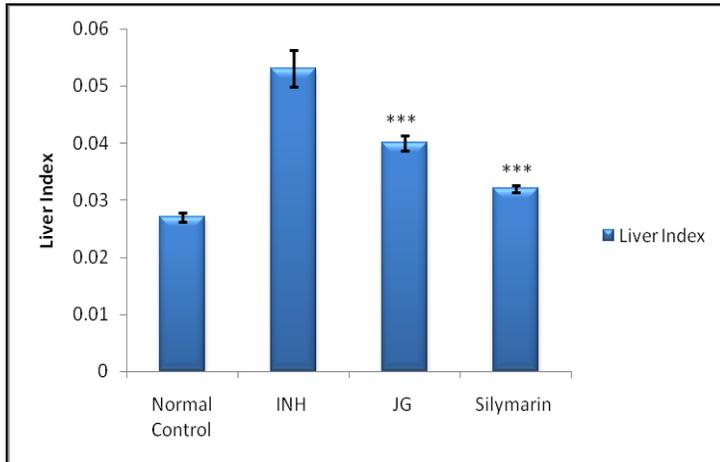


FIGURE 4: EFFECT OF JG AT THE DOSE OF 200mg/kg SIGNIFICANTLY AFFECT ON LIVER INDEX. Values expressed as mean \pm S.E.M, n=6. ***P< 0.001 compared to INH control group.

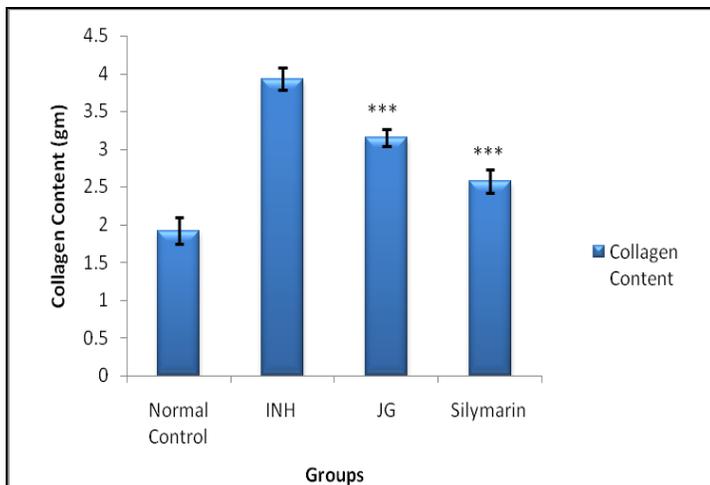


FIGURE 5: EFFECT OF JG AT THE DOSE OF 200mg/kg ON COLLAGEN CONTENT ESTIMATION. Values expressed as Mean \pm S.E.M, n=6. ***P< 0.001 compared to INH control group.

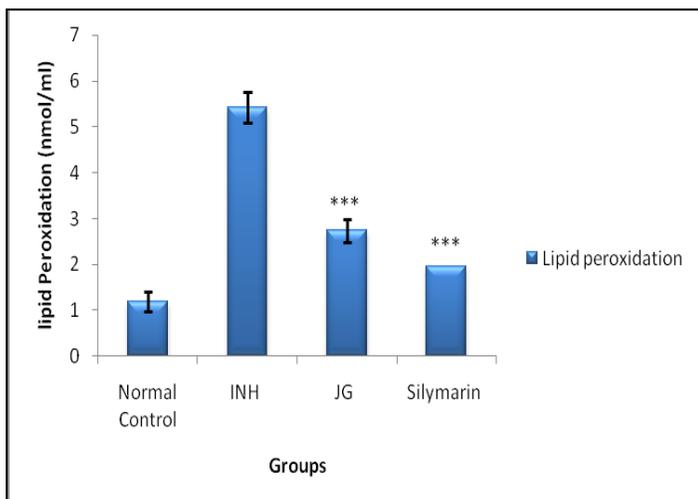


FIGURE 6: EFFECT OF JG AT THE DOSE OF 200mg/kg ON LIPID PEROXIDATION. Values expressed as mean \pm S.E.M, n=6. ***P< 0.001 compared to INH control group.

Phytochemical Screening: Our preliminary phytochemical study confirmed the presence of carbohydrates, triterpenoids, flavonoids, Phenolics, and carotenoids

Estimation of Total Phenolic Content: Phenolics are very important plant constituents because of their scavenging capability due to their hydroxyl groups. It has been revealed that various phenolic antioxidants such as flavonoids, tannins, coumarins, xanthenes and more recently procyanidins scavenge radicals dose dependently, thus they are viewed as promising therapeutic drugs for free radical pathologies. In the present study total phenolic compound of ethanolic extract was found to 1220 mg/gm gallic acid equivalent.

Histopathology of Liver: Histopathological results of control group showed normal lobular architecture, normal histology, whereas in INH control group mild periportal fibrosis was observed. Normal lobular architecture was observed in JG treated rats.

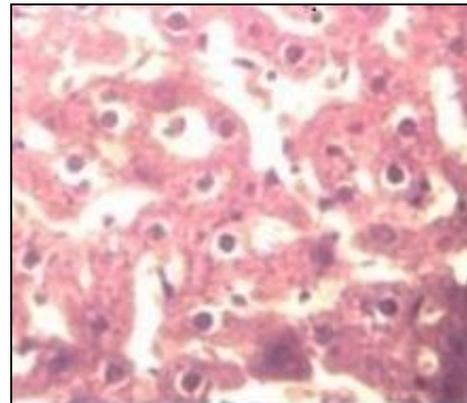


FIG. 1: SECTION OF NORMAL LIVER OF RAT

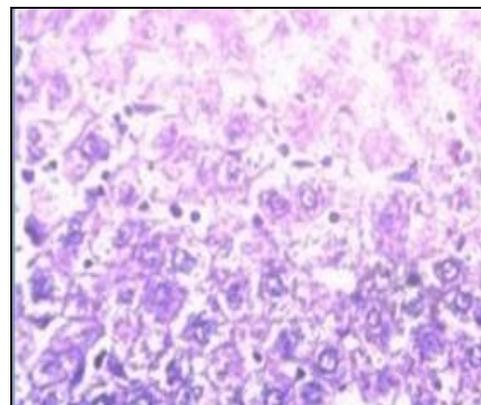


FIG. 2 SECTION OF JG TREATED LIVER OF RAT

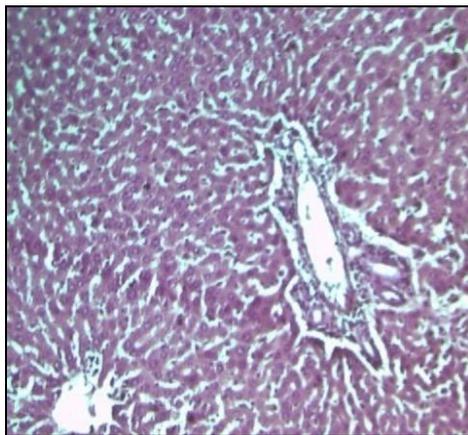


FIG. 3: SECTION OF INH TREATED LIVER OF RAT

RESULT: The effects of phytoconstituents on INH induced rats showed a significant ($p < 0.001$) decrease in serum AST and ALT levels when compared with JG control. Isoniazid treated animals showed a marked increase in the level of total cholesterol whereas, JG treated animals showed a significant ($p < 0.001$) decrease in total cholesterol level. Level of serum triglyceride increased in INH induced rats was reversed in JG treated animals significantly ($p < 0.005$). Isoniazid treated animals showed increased in the level of HDL were in JG treated animals showed significant ($p < 0.001$) decreased in the level.

In JG treated animals, no significant decrease in LDL level was observed. In case of VLDL level, JG induced rats show significant ($p < 0.005$) decrease when compared with INH treated rats. In INH treated animals, there was significant ($p < 0.001$) increase in liver weight, liver index and collagen content. The JG treatment succeeded in decreasing significantly the liver weight and collagen content deposition in liver.

DISCUSSION: Liver injuries induced by INH are the best characterized system of drugs induced hepatotoxicity and commonly used models for the screening of anti-TB and hepatoprotective activities of drugs. One of the major functions of the liver is detoxification of xenobiotics and toxins.

Among various cytochrome diverse families, CYP 450 is an important enzyme involved in metabolism of many drugs and it is abundantly found in liver with majority in numbers. INH is metabolized into bioactive metabolites. AST and ALT are enzymes originally present in high concentration in the cytoplasm.

When liver cells are in injury, these enzymes leak into the blood stream and manifest significantly elevated serum levels. Pretreatment with ethanolic extract of JG, the level of these marker enzymes in serum were near normal, this may be consequence of the stabilization of plasma membrane as well as repair of hepatic tissue damage cause by isoniazid¹⁰.

In the present study, the level of total cholesterol was higher in INH administered rats, indicating that the antitubercular drugs having capacity to induce hypercholesterolemic condition. The increase in cholesterol levels in the liver might be due to increased uptake of LDL from the blood by the tissues. The levels of protective HDL-cholesterol were also slightly increased when animals treated with JG, the probable mechanism responsible may be due to the decrease in the biosynthesis of cholesterol in the liver or by inhibiting enzymes responsible for the synthesis of cholesterol¹¹.

In case of INH treated rats there is increase in total liver weight. Because of enhanced hyaluronan deposition and its associated water of hydration appears to be associated with this increase in liver size¹². Accumulation of connective tissue proteins, especially collagen, has been reported in INH-induced liver injury, because of this there may be any obstruction in blood flow due to accumulation of collagen in liver. This accumulation of collagen was decrease by treatment with JG¹³. MDA level in liver tissue was used to determine the extent of lipid peroxidation in hepatocytes.

In the present study, elevation of lipid peroxidation in the liver of rats treated with INH was observed. The increases in MDA level in liver suggest enhanced lipid peroxidation leading to tissue damage. Treatment with JG significantly reversed these changes. Hence it is possible that the mechanism of hepatoprotection of whole plant of JG may be due to its antioxidant activity¹⁴.

CONCLUSION: The herbal drug JG at a dose (200 mg/kg) may found to act via CYP 450 metabolic pathway, as it lead to protection to the liver and significant decrease in the level of liver marker enzymes, lipid peroxidation, liver weight, liver index, and collagen content.

The dose (200 mg/kg) can be the optimal dose showing hepatoprotective activity. In conclusion, the results of this study showed that JG has therapeutic potential in the management of drug induced liver toxicity.

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