Asian Journal of Pharmaceutical and Health Sciences
www.ajphs.com

Hepatoprotective Activity of *Desmodium oojeinense* (Roxb.) H. Ohashi against Paracetamol Induced Hepatotoxicity.

Jayadevaiah K.V.[a]*, Ishwar Bhat K.[b], A.B. Joshi[c], Vijaykumar M. M. J.[a], Pinkey Rawal.[a]

a Department of Pharmaceutical Chemistry, SJM College of Pharmacy,Chitradurga-577502, Karnataka, India.
b Department of Pharmaceutical Chemistry, NGSM Institute of Pharmaceutical Sciences, NITTE University, Mangalore - 575018, Karnataka, India
c Department of Phyto Chemistry, Goa College of Pharmacy, Goa, India.

ARTICLE HISTORY

Received: 23.01.2012

Accepted: 15.02.2012

Available online: 10.05.2012

Keywords:

Desmodium oojeinense(Roxb.).H.Ohashi, Paracetamol, Liver.

***Corresponding author:**

Email : jayadevkv@rediffmail.com
Tel : +919880570839

ABSTRACT

Natural remedies from medicinal plants are considered to be effective and safe alternative treatment for liver toxicity. The present study was aimed to investigate the hepatoprotective activity of ethanolic extract of *Desmodium oojeinense*(Roxb.).H.Ohashi against Paracetamol induced Hepatotoxicity in rats. Ethanolic extract showed significant ($p<0.05$) hepatoprotective effect by lowering the serum levels of various biochemical parameters such as SGOT, SGPT, ALP, total Bilirubin and by increasing the levels of total Protein, in the selected model. These biochemical observations were in turn confirmed by histopathological examinations of liver sections and are comparable with the standard hepatoprotective drug Silymarin (100mg/kg bodyweight i.p.) which served as a positive control. Our findings suggested that the ethanolic extract of *Desmodium oojeinense* (Roxb.).H.Ohashi possesses the hepatoprotective activity.

INTRODUCTION

Liver is the vital organ of metabolism and excretion[1]. It has a surprising role in maintenance, performance and regulating the homeostasis of body[2].

Liver diseases are the most serious ailment and are mainly caused by toxic chemicals (Excess consumption of alcohol, high doses of Paracetamol, Carbon-tetrachloride, chemotherapeutic agents, peroxidised oil, etc)[3]. Liver damage is always associated with cellular necrosis, increase in tissue lipid peroxidation and depletion in the tissue GSH levels. In addition serum levels of many biochemical markers like SGOT, SGPT, bilirubin, alkaline phosphatase, are elevated[4]. In spite of tremendous advances in modern medicine no effective drugs are available which stimulate liver functions and offer protection to the liver from the damage or help to regenerate hepatic cells[1]. Therefore, many folk remedies from plant origin are tested for its potential hepatoprotective liver damage in experimental animal model.

Paracetamol induced hepatoxicity model is widely used for the study of hepatoprotective effect of drugs and plants.

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[5].Herbal drugs are prescribed widely even when their biologically active components are unknown because of their effectiveness, fewer side effects and relatively low cost[6].

Desmodium Oojeinense (Roxb.) H. Ohashi, belonging to the family Fabaceae. It is found distributed in the sub and outer Himalayan valleys and slopes up to altitude of 5000 ft, from Punjab to Bhutan, Chota Nagpur, Central India, Orissa, Bombay, Marvar of Rajputan, forest of Ganjam, and Vizag.

The bark is acrid and hot, anthelmintic, astringent to the bowels, cures “kapha” and vata”,dysentery, leucoderma, ulcers, blood diseases, skin diseases, burning sensations and anaemia (Ayurveda). In the central prominence the bark is used as a febrifuge. The bark when incised furnishes a Kino-like exudation, which is used in cases of dysentery and diarrheoa[7].

Based on ancient practices and traditional uses of this plant, an effort has been made to establish the hepatoprotective activity of ethanolic extract of *D. oojeinense*.

MATERIALS AND METHODS:

Collection of plant material

Bark was collected from the medicinal garden of Sri.Ragavendra Ayurvedic Medical College Malladihalli, Karnataka, India. The plant was authenticated by Prof.Gopal Krishna Bhatt, Department of Botony,Poornaprajana college, Udupi, Karnataka,India. A voucher specimen(no.106a) was

Fig.1 : Effect of ethanolic extract of plant *D.oojeinense* on Paracetamol induced histopathological changes in rat liver

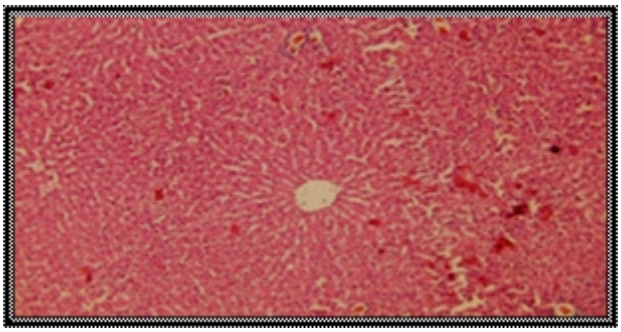

Fig 1a. Section of liver showing normal hepatic cells.

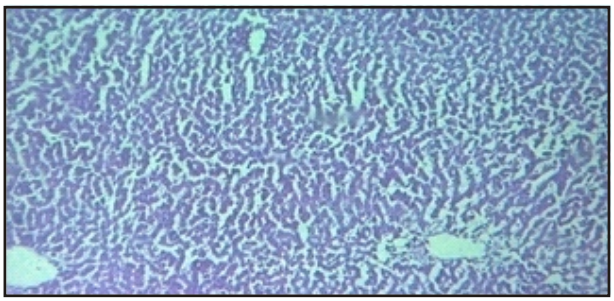

Fig 1c. Silymarin treated rat: Section of liver showing normalcy of hepatic cells, central vein and portal triad nuclei.

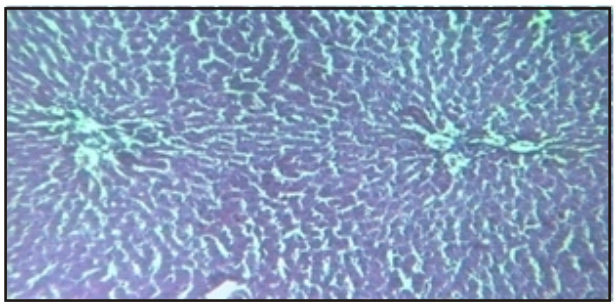

Fig 1b. Paracetamol treated liver showing marked fatty changes around portal tract as well as around central vein. Hepatocytes are with fat vacuoles and showing peripherally pushed.

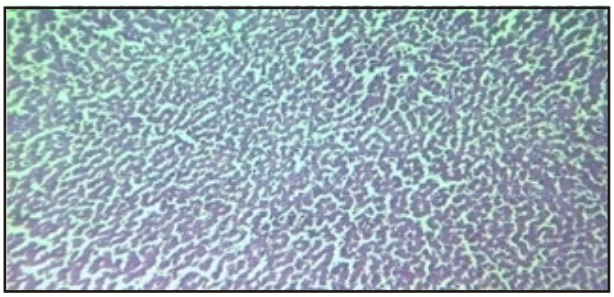

Fig 1d. *D.oojeinense* treated rats: Section of liver showing normalcy hepatic cells

deposited in NGSM Institute of Pharmaceutical Sciences, Paneer, Mangalore-5, Karnataka.

Preparation of the ethanolic extract

The dried powder material of stem bark of *D.oojeinense* was extracted with ethanol(95%) in a soxhlet extractor .The process was repeated for six times. The solvent from the total extract was distilled off and the concentrate was evaporated on a water bath to a syrupy consistency and then evaporated to dryness**.**

Preliminary Phytochemical Screening

The ethanolic extract of stem bark of *D. oojeinense* was subjected to systematic qualitative analysis for the identification of various plant phytoconstituents.

Experimental animals

Albino Wistar rats of either sex (180-260g) were obtained time to time from the laboratory of K.S Hegde Medical Academy (KSHEMA), Deralakatte, Mangalore. Four animals were housed in a cage in a climate-controlled room under standard conditions with 12:12 h light/dark cycles and free access to water and food. All the experiments were performed within the guidelines of the institutional ethical committee of KSHEMA, Deralakatte, Mangalore KSHEMA/AEC/NO.051/2007

Acute toxicity studies

The acute toxicity study was carried out in adult female albino rats weighing about 150-200 g by up and down method as per OECD 425 guidelines [8]**.** Overnight fasted animals received test drug at a dose of 2000 mg/kg body weight orally. Then the animals were observed continuously once in half an hour for next 4 h and then after 24h for general behavioural, neurological, autonomic profiles and to find out mortality.

The effective dose of 50% ethanolic extract of *D.oojeinense* was decided as 1/10 of the maximum dose (2000mg/kg). So we used the 50% ethanolic extract as such 100, 200 and 400mg/kg body weight p.o. for hepatoprotective activity**.**

Assessment of hepatoprotective activity

Paracetamol induced Hepatotoxicity model [9]

Wistar rats (200-250gm) of either sex were used, the rats were maintained under standard environmental conditions with free access to feed and water. Rats were divided into six groups of six animals each in a group.

Group I: received 0.6% CMC (10ml/kg, p.o.) as normal control for 9 days.

Group II: received a single dose of Paracetamol (2g/kg, p.o) suspended in 0.6%w/v CMC, as treated control group on the 9th day.

Group III: received silymarin(100mg/kg)suspended in 0.6% CmC. Orally once daily as standard reference for 9 days followed by a single dose of Paracetamol (2g/kg, p.o) suspended in 0.6%w/v CMC on 9th day.

Group IV, V, VI received the test drug at three dose level orally once daily for 9 consecutive days followed by a single dose of Paracetamol (2g/kg, p.o) suspended in 0.6%w/v CMC on the 9th day. Food was withdrawn 12 hour before Paracetamol administration to enhance the liver damage in animals of groups (I, II, III, IV, V & VI).

After 24 h**,** the animals were sacrificed by chloroform

anesthesia and the blood was collected by heart puncture. The blood sample of each animal were taken and allowed to clot for 45 minutes at room temperature. Serum was separated by centrifugation and subjected for assessment of various biochemical parameters like total protein, total bilirubin, SGOT, SGPT and ALP. The liver was quickly dissected out, washed with saline and preserved in 10% formalin solution for histopathological investigation.

STATISTICAL ANALYSIS

The data were expressed as Mean ± SEM and analyzed by using one way analysis of variance (ANOVA), followed by Tukey's fair test. P<0.05 was considered as statistically significant.

RESULTS AND DISCUSSION

Preliminary Phytochemical investigation

Qualitative tests for identification of Phytoconstituents revealed the presence of Alkaloids, Carbohydrates, Steroids, Triterpenoids and Flavonoids.

Paracetamol induced hepatotoxicity model

The results of hepatoprotective effects of ethanolic extract of plant *D. oojeinense.*on paracetamol intoxicated rats are shown in Table 1. Intoxication of rats with Paracetamol significantly ($p<0.05$) altered the biochemical parameters when compared with normal control rats. In the Paracetamol treated group serum SGOT, SGPT, ALP, and total bilirubin levels were significantly elevated. Groups treated with alcoholic extract of plant *D. oojeinense* showed significant dose dependent decrease in serum SGOT,SGPT,ALP, and total bilirubin levels ($p<0.05$) when compared with Paracetamol treated rats. Standard drug silymarin also exhibited similar results significantly ($p<0.05$).

Histopathological Observations

The histopathological observations basically support the results obtained from serum enzyme assays. Histology of the liver section of normal control animals showed normal hepatic cells with well preserved cytoplasm, prominent nucleus and nucleolus and well brought out central vein. The liver sections of Paracetamol intoxicated rats showed massive fatty changes, necrosis,ballooning degeneration and broad infiltration of the lymphocytes and kupffer cells around the central vein and the loss of cellular boundaries .

The histological architecture of liver sections of rats treated with ethanolic extract of plant *D.oojeinense* showed more or less normal lobular pattern with a mild degree of fatty changes, necrosis and lymphocyte infiltration almost comparable to the normal control and the silymarine treated group.

Hepatic cells appear to participate in a variety of enzymatic metabolic activities. Paracetamol produced marked liver damage at the given doses as expected. Paracetamol in larger doses produces liver necrosis after undergoing bioactivation to a toxic electrophile, N-acetyl-p-benzoquinone-imine (NAPQI) by cytochrome P-450 monooxygenase. NAPQI binds to macromolecules and cellular proteins, and also oxidizes lipids and alters homeostasis of calcium after depletion of glutathione. Pretreatment with ethanolic extract of plant *D.oojeinense brought* down the elevated levels of SGOT, SGPT, ALP, Total bilirubin. These biochemical restorations may be due to the inhibitory effects on cytochrome P-450 or promotion of its glucorination[10].

CONCLUSION

On the basis of results obtained above, it can be concluded that alcoholic extract of plant *D. oojeinense* seems to possess a significant hepatoprotective activity. The Phytoconstituents like steroids, triterpenoids, flavonoids which are present in this plant may be responsible for the hepatoprotection.

ACKNOWLEDGEMENT

The authors are thankful to Dr.Shivamurthy Murugha Sharanaru, President, SJM Vidya Peetha for providing all necessary facilities through the Principal, SJM college of

Table-1: Effect of ethanolic extract of plant *D.oojeinense* on serum biochemical parameters of Paracetamol intoxicated rats

Groups	Treatment	Total Bilirubin (mg/dl)	Total protein (gm%)	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)
Groups-I	Control	1.18 ± 0.07	7.03 ± 0.08	138.28 ± 5.09	76.49 ± 6.17	133.61 ± 5.14
Groups-II	Paracetamol	3.22 ± 0.11	5.38 ± 0.11	410.72 ± 6.56	401 ± 3.64	277.4 ± 2.52
Groups-III	Paracetamol+Silymarin	1.34 ±0.03**	5.79 ± 1.02**	141.16±1.54**	113.32 ±2.3**	157.5 ± 3.46**
Groups-IV	Paracetamol+Ethanolic extract(100mg)	2.85 ± 0.08	5.45 ± 0.08*	429.23 ± 69.2	473.49 ±48.98	296.28 ± 5.58
Groups-V	Paracetamol+Ethanolic extract(200mg)	1.83 ± 0.13	5.52 ± 0.07*	335.41±15.81*	188.7 ±26.67*	258.16 ± 8.16*
Groups-VI	Paracetamol+Ethanolic extract(400mg)	0.97±0.05**	5.97 ± 0.26**	149.19 ± 5.12*	101.20 ±3.84*	145.19 ± 4.76*

All the values are expressed as (mean ± S.E.M) (n=6)
** p <0.001, * p <0.05, when compared to toxic control.

Pharmacy ,Chitradurga, Karnataka. The authors are also thankful to the Principal, NGSMIPS, Mangalore and NITTE educational trust.

REFERENCES

1. Suman Patanayak,Siva Sankar Nayak,Durga Prasad Panda,Subas Chandra Dinda,Vikas Shende, Amol Jadav, Hepatoprotective Activity of Crude Flavonoids Extract of Cajanus scarabaeoides (L) in Paracetamol Intoxicated Albino Rats. Asian J Pharm Biol Res.2011: 22-27.

2. Mahendra Singh Yadav, Anurag Kumar, Ajeet Singh, Uma Shankar Sharma, Niranjan Sutar. Phytochemical investigation and Hepatoprotctive activity of Cissampelos pareira against carbon tetrachloride induced hepatotoxicity. Asian J Pharm & health sci. 2011; 1(3): 106-110.

3. C.Maheswari, R.Maryammal and R. Venkatanarayanan. Hepatoprotective Activity of Orthosiphon stamineus on Liver Damage Caused by Paracetamol in Rats. Jordan J Bio Sci. 2008; 1(3) : 105-108.

4. Manoj Soni, P. K. Mohanty, and Y.A. Jaliwala, Hepatoprotective Activity of Fruits Of Prunus domestica. Int J pharm and bio.sci. 2011; 2(2): 439-453.

5. Malaya Gupta, Upal Kanti Mazumder, Thangavel Siva Kumar, Periyasamy Gomathi, Ramanathan Sambath Kumar.Antioxidant and Hepatoprotective Effects of Bauhinia racemosa against Paracetamol and Carbon Tetra-chloride Induced Liver Damage in Rats. Iranian J pharmacol & therapeutics. 2004; 3(1): 12-20.

6. Rajesh Kumar, Sushil Kumar, Arjun Patra, S.Jayalakshmi. Hepatoprotective activity of aerial parts Plumbago zeylanica Linn against carbon tetrachloride induced hepatotoxicity in rats, Inter.J. of pharm and Ph.ceutical.Sci. 2009; 1(1): 171-175.

7. Kirthikar KR, Basu BD. Indian Medicianl Plants. Vol-I. Allahabad: 1935, 755-756.

8. OECD 425 guidelines. OECD guidelines for testing of chemicals. 2001 Dec; 1/26:1-26.

9. Chanchal K Roy, Jagaelish Kamath, Mohammed Asad. Hepatoprotective activity of Psidium guajava Linn leaf extract. Ind J Expt Biol. 2006; 44: 305-311.

10. Baheti JR, Goyal RK, Shah GB. Hepatoprotective activity of Hemidesmus indicus in rats. Ind. J Expt. Biol. 2006; 44: 399-402.