Prabhakaran V and Ranganayakulu D. / *International Journal of Biological & Pharmaceutical Research.* 2014; 5(2): 125-130.

e- ISSN 0976 - 3651
Print ISSN 2229 - 7480

International Journal of Biological & Pharmaceutical Research
Journal homepage: www.ijbpr.com

IJBPR

HEPATOPROTECTIVE ACTIVITY OF *CANAVALIA GLADIATA* ROOT EXTRACT ON D-GALACTOSAMINE INDUCED HEPATIC DAMAGE

Vunta Prabhakaran and Diviti Ranganayakulu

Dept. of Pharmacology, Sri Pdmavathi School of Pharmacy, Tiruchanoor, Tirupati-517503, Andhra Pradesh, India.

ABSTRACT

Canavalia gladiata root extract was evaluated for *Invivo* hepatoprotective activity against D-galactosamine induced hepatic damage. *Canavalia gladiata* (Fabaceae) roots were extracted with hydroalcohol to prepare extract. The hepatoprotective activity of hydroalcoholic *canavalia gladiata* extract was assessed against D-galactosamine (D-GalN) at different dose levels. Then *in vivo* hepatoprotective of *canavalia gladiata* at dose 200 and 400 mg/kg body weight was carried out against D-GalN, which on dose of 400 mg/kg body weight i.p induces liver damage in rats. The phytochemical screening on *canavalia gladiata* roots revealed the presence of primary metabolites such as, carbohydrates and proteins, the secondary metabolites such as alkaloids, glycosides, saponins, flavonoids, phenolic compounds and tannins. The D-GalN intoxicated animals showed a significant increase in the levels of SGOT, SGPT, ALP, TC, TB and TGL and decrease in liver weight, TP and albumin when compared to the normal. All the biochemical parameters were attenuated to near normal level on treatment with the *canavalia gladiata* extract. These findings concluded that *canavalia gladiata* root extract protects the liver from severe damage caused by D-GalN and may serve as a useful adjuvant in several clinical conditions associated with liver damage.

Key Words: *Canavalia gladiata*, Hepatoprotective, D-galactosamine.

INTRODUCTION

Plants have been the basis of many traditional medicine systems throughout the world for thousands of years and continue to provide humankind with new remedies. India is the largest producer of medicinal plants and is rightly called the "Botanical Garden of the World" (Ward and Dally, 1999). Medicinal plants play a key role in the human health care. About 70-80% of the world's population depends upon plants for their primary health care as per WHO survey (Akerele, 1993).

The liver is a vital organ, which plays an important role in maintenance of metabolic functions and detoxification of endogenous and exogenous challenges such as drugs, chronic alcoholism, toxins, viral infections and xenobiotics. Every year 20,000 deaths occur due to Viruses, excessive drug therapy and alcohol intoxication which are main cause of liver diseases. Hepatocellular carcinoma is one of the ten most common tumors in the world with over 2,50,000 new cases every year. Drug induced liver injury is responsible for 5% of all hospital admissions and 50% of all acute liver failures (Ostapowicz *et al*., 2002). The allopathic drugs are inadequate to treat liver diseases and produces serious adverse reactions and cost is also high. Herbal drugs are in developed countries are in great demand to treat primary health care problems. Herbal drugs are preferred due to biological and medicinal values, higher safety margin and lesser cost.

Canavalia gladiata commonly called as Jack bean belonging to the family fabaceae distributed throughout India. It is occasionally cultivated in kitchen gardens in almost all parts of the India except in the hills above 1,200

Corresponding Author

Vunta Prabhakaran
Email: viniprabhakaran@gmail.com

m, for vegetable or pulse. *Canavalia gladiata* is large twiner, perinnials or biennials with glabrous stems, branches with compound leaves and leaflets. Leaflets are ovate or oblong, glabrous above, downy-hirsute below, sub-acute or apiculate. Flowers are large, white or liliac, sword shaped flattened pods, fruits are large and seeds are reddish brown. The roots of *canavalia gladiata* traditionally used in curing enlargement of liver by grounding into paste with cow's urine and rice gruel administered internally for consecutive days (Vedavathy, 1997). The fruits are used as astringent, cooling, appetizer and vitiated conditions of kapha and Pitta (Vaidyaratnam, 1993). *Canavalia gladiata* contains amino acids like cystin, tyrosin, tryptophan and alkaloids; seeds contain three crystalline globulins canavalin, concanavalin A and B etc. (Chopra RN and Nayar SL, 1992).

The *canavalia gladiata* is traditionally used medicine for the treatment of hepatopathy. Therefore, the aim of the present study was to evaluate the protective effect of *Canavalia gladiata* which was investigated in D-galactosamine (D-GalN) induced liver damage in rats. Hence the present study was designed to investigate the hepatoprotective activity of hydroalacoholic extract of *canavalia gladiata* against D-galactosamine (D-galN) induced liver damage in experimental rats.

MATERIALS AND METHODS

Collection

Canavalia gladiata roots were collected from Surrounding areas of Tirumala-Tirupathi hills, A.P, India and was authenticated by Dr. K. Madhava Chetty, Department of Botany, S.V. University, Tirupathi, A.P, India.

Chemicals

All chemicals of analytical grade were obtained from SD Fine Chemicals, Mumbai. D-galactosamine (D-GalN) were obtained from Sigma Chemical Co, St. Louis, USA, Ecoline diagnostic kits were purchased from E-Merck, India and Silymarin a marketed formulation, (Muneesh Pharmaceuticals, Pondicherry) was used as a standard.

Animals

Healthy male Albino rats of Wister strain (150–200 g) were procured from the Raghavendra Enterprises, Bangalore, India, and were maintained under standard environment conditions (25–27 ^{o}C, 60–70% relative humidity, 12h dark: 12h light cycle) and were fed with standard rat feed (M/S Hindustan Lever Ltd., Bangalore, India) and water *ad libitum*. The experiments were conducted as per the guidelines of CPCSEA (Application approval No.1016/a/06/CPCSEA-003/2008).

Preparation of extract

The roots of *canavalia gladiata* were cleaned thoroughly with water to remove any unwanted matter, dried in shade, grinded to a coarse powder with a mechanical grinder, and passed through sieve no. 40. Further, it was extracted by cold maceration process using hydroalcohol by intermittent shaking for 7 days. The solvent was removed by distillation under reduced pressure and the resulting semisolid mass was vacuum dried using rotary flash evaporator (Rota vapor, R-210/215, Buchi, Switzerland). The semi solid material was kept in desiccators for drying.

Qualitative phytochemical screening

The hydro alcoholic root extract of *canavalia gladiata* was analyzed for the presence of major phytochemical constituents such as carbohydrates, proteins, amino acids, alkaloids, glycosides, saponins, sterols, flavonoids, phenolic compounds, fixed oil, phytosterols and tannins according to standard methods (Kokate CK, 1988).

Preparation of suspensions

The hydroalcoholic root extract of *canavalia gladiata* and the standard drug silymarin were suspended in sodium carboxy methylcellulose (CMC) (1.0%) in distilled water separately and used (Dhanabal SP *et al*., 2006)

Acute toxicity studies

Acute oral toxicity study in wistar rats was carried out as per OECD-423 guidelines. The dose level was selected from the 5 dose levels, 5, 50, 300, 2000 and 4000 mg/kg body weight. The rats were observed for mortality, morbidity and clinical signs changes daily for a period of 14 days.

Hepatotoprotective Studies

Experimental hepatotoxicity was induced by intraperitoneal (i.p.) administration of D-galactosamine (D-galN) (400 mg/kg body weight) (Ferencikova R *et al*., 2003).

Experimental protocol

The Wistar rats were divided into five groups of six animals each.

Group I: Served as the solvent control, received 1.0 % CMC (1ml/kg body weight (b.w) orally for 14 days.

Group II: Received a single dose of D-galN, 400 mg/kg on14th day intraperitoneal (i.p.).

Group III: Received Silymarin (50 mg/kg b.w.) for 14 days orally (Binduja Saraswat PKS *et al*., 1996).

Group IV and V: Received hydroalcoholic extract of *canavalia gladiata* (200 and 400 mg/kg b.w.) for 14 days orally.

On day 14th all groups were induced with hepatotoxicant D-galactosamine (D-galN) (400 mg/kg body weight by i.p. except Group I. On 15th day, Blood

sample was collected in sterile centrifuge tubes and allowed to clot. Serum was separated and used for the estimation of biochemical parameters viz., Serum glutamate pyruvate transaminase (SGPT), Serum glutamate oxalate transaminase (SGOT), Alkaline Phosphate (ALP), Triglycerides (TGL), Total Cholestrol (TC), Total Protein (TP), Albumin (ALB), and Total Bilirubin (TB) in autoanalyzer (Microlab 200, Vital Scientific, The Netherlands) using Ecoline diagnostic kits (Roy Chanchal K *et al*., 2006).

For histological investigations of the liver, slices were processed for paraffin embedding in formalin. Five-micron sections of the liver tissue stained with haematoxylin and eosin were examined for histopathological changes under a light microscope (Galigher AE *et al*., 1976).

Statistical analysis

Results of the parameters were reported as mean ± SEM for determination of significant intergroup differences. Each parameter was analyzed separately and a one way analysis of variance (ANOVA) was carried out followed by bonferroni's test using computer based fitting programme (Graph Pad Prism software). The results were judged at significant value $P < 0.001$.

RESULTS

Qualitative phytochemical screening

The phytochemical screening of hydroalcoholic root extract of *canavalia gladiata* reveals that the presence of carbohydrates, proteins, alkaloids, flavonoids, phenolic compounds and tannins. Result is recorded (Table 1).

Acute toxicity

The behavior of the treated rats appeared to be normal. No toxic effect was observed with the hydroalcoholic extract of *canavalia gladiata* upto the dose level of 4000 mg/kg b.wt therefore, the extract was found to be safe. Thus, selected effective doses are 1/10th maximum tested dose.

Hepatoprotective studies

The result reveals that intoxication with D-GalN significantly altered the biochemical parameters when compared with normal control rats ($P<0.001$).

Table 1. Phytochemical screening of hydroalcoholic extract of *Canavalia gladiate*

Test	Observation
Alkaloids	+
Flavonoids	+
Carbohydrates	+
Proteins & amino acids	+
Steroids	-
Glycosides	-
Tannins & Phenolics	+

+ indicates presence of compound
- Indicates absence of compound

Table 2. Effect of hydro alcoholic extracts of C.G on liver weight

Vehicle control	D-GalN	D-GalN + Sylimarin	D-GalN + C.G (200 mg/kg)	D-GalN + C.G (400 mg/kg)
2.41±0.34	1.95±0.06	2.36±0.05	2.0±0.21**	2.11±0.06**

** $p < 0.05$ denotes statistical significance in comparison to hepatotoxicity group

Table 3. Estimation of Biochemical Parameters of hydroalcoholic extract of *Canavalia gladiata* in D-GalN Treated Hepatotoxic Rats

S. No	Groups	SGOT (U/L)	SGPT (U/L)	ALP (U/L)	TGL (mg/dl)	Total Cholesterol (mg/dl)	Albumin (g/dl)	Total Protein (g/dL)	Total bilirubin (mg/dl)
1	Vehicle Control	28.33±1.48	18.83±0.60	197.67±6.82	79.83±0.87	48.00±1.21	5.18±0.11	6.90±0.15	0.48±0.10
2	(D-GalN 400 mg/kg b.wt)	73.33±3.40**	84.83±3.44**	373.33±17.7**	125.67±1.76**	174.0±2.96**	3.55±0.16**	4.35±0.40**	1.03±0.07**
3	Positive control Sil ymarin (50 mg/kg)	31.83±2.02[a]	23.17±0.95[b]	215.17±6.46[b]	71.17±2.27[b]	69.432±7.543**	4.42±0.14[b]	6.0±0.12[b]	0.42±0.09[b]
4	Test1 (200mg/kg b.w)	64.65±8.434**	74.543±6.432**	302.00±19.94	88.90±1.07	114.67±3.81	3.83±6.40	5.07±0.50	0.31±0.06
5	Test2 (400 mg/kg b.w)	52.42±5.76**	68.67±2.28	273.00±12.58	83.71±3.11	85.67±5.14	4.10±3.91	6.0±0.50[c]	0.36±0.08

Superscript** denotes statistical significance in comparison to solvent control group at $p < 0.01$
Superscript[b] *** denotes statistical significance in comparison to hepatotoxicant group at $p < 0.01$
Superscript [c**] denotes statistical significance in comparison to hepatotoxicant group at $p < 0.05$

Table 4. Hepatoprotective effect of hydroalcoholic root extract of *canavalia gladiata* on parameters in the tissue (Liver) of rats intoxicated with D-galactosamine

S. No	Groups	SOD (U/mg protein)	CAT (µM H_2O_2 consumed/mg protein)	Reduced GSH (µg of GSH / mg protein)	MDA (nM of MDA/mg protein)
1	Normal	5.34±0.05	7.37±0.43	8.64±0.32	0.71±0.07
2	Control (D-GalN 400 mg/kg b.wt)	1.32±0.58*	1.03±0.03*	2.34±0.31*	2.89±0.02*
3	Positive control Silymarin (50 mg/kg)	4.76±0.54**	5.53±0.53**	6.21±0.59**	0.82±0.07**
4	Test1 (200mg/kg b.w)	2.01±0.23	2.67±0.90**	2.92±0.64	2.01±0.28
5	Test2 (400mg/kg b.w)	2.99±0.49	5.42±0.60**	4.02±0.19	0.69±0.19

Superscript** denotes statistical significance in comparison to solvent control group at $p < 0.01$
Superscript[b] *** denotes statistical significance in comparison to hepatotoxicant group at $p < 0.01$
Superscript [c]** denotes statistical significance in comparison to hepatotoxicant group at $p < 0.05$

Table 5. Effect of hydroalcoholic extracts of C.G, on phenobarbotone induced sleeping time (in min)

S. No	Group	*Canavalia gladiata*
1	Vehicle	134±1.69
2	D-GalN (400 mg/kg b.wt)	203±3.91
3	Silymarin (50 mg/kg b.w)	120±2.21
4	Test-I (200 mg/kg PO)	152±1.42

Treatment with the hydroalcoholic extract of *canavalia gladiata* extract at 200 mg/kg and 400 mg/kg body weight induced significant decreases in serum levels of SGPT, SGOT, ALP, TGL, TC and TB ($P<0.001$) and a significant elevations in serum levels of the TP and albumin ($P<0.001$) when compared with D-GalN treated rats. Among the two doses 400 mg/kg dose showed better hepatoprotective activity compared with 200 mg/kg dose. Treatment with standard silymarin (50 mg/kg) exhibited similar results ($P<0.001$) as with 400 mg/kg.

DISCUSSION

D-GalN-induced liver damage is a useful model for the study on hepatic injury. It has been shown to produce liver damage resembling human viral hepatitis in morphological features (Decker K and Keppler D, 1974). D-GalN induces a rapid decrease in uracil nucleotides which ultimately causes a rapid inhibition of RNA synthesis, disturbance of biosynthesis of glycoproteins, lipoproteins and nucleic acids with alteration of cellular membrane and lekage of enzymes into serum, leads to progressive damage of cellular membranes and ultimately to spotty liver cell necrosis. This cellular damage provokes inflammatory reactions resulting in a histological picture closely resembling human viral hepatitis (Decker K *et al*., 1971). The changes in the structure of cellular membrane may stimulate lipid peroxidation due to the liberation of active free radicals.

In the present study, it was observed that D-GalN hepatotoxicity decreases the weight of liver due to the inhibition of protein synthesis, necrosis and leakage of enzymes from the liver. The extract of *Canavalia gladiata* restored the liver weight by promoting the regeneration of hepatocytes (Girish C, 2009).

Liver damage leads to high serum levels of SGOT and SGPT which are released due to the leakage of cell membrane from liver into the blood. Among the two enzymes, SGPT is a better index of liver injury, as liver SGPT activity represents 90 % of total enzyme present in the body (Achiliya GS *et al*., 2003). ALP is a membrane bound glycoprotein produced in bile duct and is another measure of liver damage and increase in its activity is due to increased synthesis in presence of increased biliary pressure (Suresh Kumar SV *et al*., 2006). Reduction in the levels of SGPT and SGOT towards their normal values is an indication of stabilization of plasma membranes as well as repair of hepatocytes damage caused by D-galN insult. This effect is in accordance with the view that serum levels of the transaminases return to normal with healing of the hepatic parenchyma and regeneration of hepatocytes and suppression of increased serum activity with concurrent reduction of abnormal bilirubin levels suggests biliary dysfunction during hepatic injury due to D-galN (Mukherjee PK, 2002).

The present study showed that D-GalN caused significant decreased levels of total proteins and serum albumin indicating hepatic disorder (Wong, 2007). It was reported that the lowering of serum albumin levels may be attributed to reduction of albumin mRNA expression (Dabeva MD, 1993). The elevated levels of total proteins & albumin by the extract of the *canavalia gladiata* in pretreated and standard groups attenuated due to protein synthesis, (Girish C, 2009) stability of the hepatic cell membrane structure and stimulation of albumin mRNA

expression indicates hepatoprotective activity compared to control rats and restoring liver vitality.

In the present investigation D-galN induced marked liver injury as evidenced by increased serum levels of marker enzymes SGOT, SGPT and ALP indicating induction of hepatotoxicity. The damage may be caused by the direct action of toxic chemicals or may result from secondary reactions. In D-galN induced acute hepatotoxic model, pretreatment with hydroalcoholic root extract of *canavalia gladiata* offered hepatoprotection as evidenced by the mitigation of the rise in SGOT, SGPT, ALP, TGL, total bilirubin and total cholesterol levels and increase in liver weight, TP and albumin.

The activity of antioxidant enzymes SOD and CAT has significantly reduced in D-GalN insult group (Anandan R, 1999; Meena B, 2008) compared to the normal group and both enzymes are restored to near normal level in pretreated animals due to the scavenging of free radicals produced by D-GalN induced lipid peroxidation.

In this study, it was observed that GSH was depleted in D-GalN treated rats and is correlated with report (Seckin S *et al*., 1995) and is expected due to the more consumption of GSH by increased radical reactions. The depletion of GSH is inhibited in the pretreatment of rats by *Canavalia gladiata* extract and standard compared to hepatotoxicant group.

The levels of MDA in lipid peroxidation significantly increased in animals intoxicated with D-GalN (Wong, 2007). Elevation of MDA levels also shows parallel significant reduction in GSH. The decreased level of MDA was observed by the plant extract treated animals.

Barbiturates are exclusively metabolized by the hepatic microsomal enzymes. D-GalN induced hepatic injury decreased the activity of hepatic microsomal enzymes that delay barbiturate metabolism thereby prolonging the sleeping time which is restored by the plant extract.

These findings could be correlated with an earlier study where it was reported that pretreatment of rats with *Tephrosia purpurea* offered heptoprotective action against CCl_4 and D-galactosamine induced toxicity (Sree Rama murthy M *et al*., 1993) and hepatoprotective effect of *Sargassum polycystum* was evaluated in D-galactosamine induced hepatitis. It significantly reduced the diagnostic marker enzymes (SGPT, SGOT and ALP) in plasma of rats. It has also demonstrated the antioxidant activity against D-GalN induced hepatitis by inhibiting the activation of lipid peroxidation and by preserving the enzymatic and non-enzymatic antioxidant defense system at near normal. The hepatoprotectivity might be due to its antioxidant property and membrane stabilizing action (Meena *et al*., 2008).

CONCLUSION

Liver disorders remain to be one of the serious health problems. Knowledge of liver diseases has increased enormously in the past few years and has changed diagnosis as well as treatment of hepatic disorders. The reliable allopathic hepatoprotective drugs inadequate and has their own side effects. Hence, in the present study an attempt made to evaluate alternative treatment for hepatotoxicity with no/minimal adverse effects.

In the present study with D GalN induced acute hepatotoxicity model, pretreatment with hydro alcoholic extract of *Canavalia gladiata* offered hepatoprotection by potential antioxidant activity, and as evident by the inhibition of rise in AST, ALT, ALP, T.B, TGL levels and restoring the protein and Albumin synthesis and is supported by *in vivo* antioxidant studies providing hepatoprotective activity of hydro alcoholic extract of *Canavalia gladiate* mediating through scavenging of active free radicals. Therefore it can be concluded from present study that the hydro alcoholic extract of *Canavalia gladiata* have significant hepatoprotective activity.

The different bioactive constituents in the hydro alcoholic extract of *Canavalia gladiata* may have different mechanism of actions for curing the liver disorders. Thus there is a need to isolate and purify the active principles involved in this plant and to confirm the mechanism for providing scientific basis for its usage in the traditional system of medicine, in the management of liver disorders.

REFERENCES

Achiliya GS, Kotgale SG, Wadodkar AK, Dorle AK. Hepatoprotective activity of Panchagavya gritha in carbontetrachloride-induced hepatotoxicity in rats. *Ind J Pharmacol*. 2003; 35: 311.

Akerele O. Summary of WHO guidelines for the assessment of herbal medicine. *Herbalgram*. 1993; 28: 13-19.

Anandan R, Deavaki T. Hepatoprotective effect of *Picrorrhiza kurroa* on tissue defense system in galactosamine induced hepatitis in rats. *Fitoterapia*. 1999; 70(1): 54-7.

Binduja Saraswat PKS, Dayal Visen R, Agarwal DP, Patnaik GK. Protective action of Ursolic acid against chemical induced hepatotoxicity in rats. *Ind J Pharmacol*. 1996; 28: 232-239.

Dabeva MD, Shafritz DA. Activation, proliferation and differentiation of progenitor cells into hepatocytes in the D-GalN model of liver regeneration. *Am J Pathol*. 1993; 143(6): 1606-20.

Decker K, Keppler D, Rudigier J, Domschke W. Cell damage by trapping of biosynthetic intermediates, the role of uracil nucleotides in experimental hepatitis. *Hoppe Seylers Z Physiol Chem*. 1971; 352: 412-418.

Decker K, Keppler D. Galactosamine hepatitis, Key role of the nucleotide deficiency period in the pathogenesis of cell injury and cell death. *Rev Physiol Biochem Pharmacol*. 1974; 71: 78-105.

Dhanabal SP, Syamala G, Satish Kumar MN, Suresh B. Hepatoprotective activity of the Indian medicinal plant Polygala arvensis on D-galactosamine-induced hepatic injury in rats. *Fitoter*. 2006; 77: 472–474.

Ferencikova R, Cervinkova Z, Drahota Z. Hepatotoxic effect of D-Galactosamine and protective role of lipid emulsion. *Physiol Res*. 2003; 52: 73-78.

Galigher AE, Koyloff EN. Essentials of Practical Microtechnique. 2nd ed. New York: Lea and Febiger, 1976: 210.

Girish C, Koner BC, Jayanthi S, Rao KR, Rajesh B, Pardhan SC. Hepatoprotective activity of six polyherbal formulations in carbon tetra chloride induced kiver toxicity in mice. *Indian J Experimental Biol*. 2009; 47: 257-63.

Kokate CK. Practical pharmacognosy, 2nd ed. New Delhi: Vallabh Prakasham, 1988: 111-113.

Meena B, Ezhilan ER, Rajesh R, sheik HA, Ganeshan B, Anandan R. Antihepatotoxic potential of *Sargassum polycystum* (Phaeophyceae) on antioxidant defense status in D-galactosamine induced hepatitis in rats. *Afr J Biochem res*. 2008; 2(2): 51-5.

Mukherjee PK. Quality Control of Herbal Drugs. 1st ed. New Delhi. Business Horizons Pharmaceutical Publications, 2002: 531.

Sree Ramamurthy M, Srinivasan M. Hepatoprotective effect of *Tephrosia purpurea* in experimental animals. *Ind J Pharm.* 1993; 25: 34-36.

Suresh Kumar SV, Sugantha C, Syamala J, Nagasudha B, Mishra SH. Protective effect of root extract of Operculina turpethum Linn. against paracetamol induced hepatotoxicity in rats. *Ind J Pharm Sci*. 2006; 68:1: 32-35.

Wong MC, Portmann B, Sherwood R, Niemela O, Koivisto H. The cytoprotective effect of alpha-tocopherol and diadzien against D-GalN induced oxidative damage in the rat liver. *Metabolism*. 2007; 56(7): 865-75.