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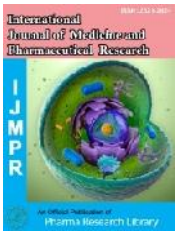

Research Article **Open Access**

Evaluation of Hepatoprotective activity of aqueous extract of *Polycarpea corymbosa* on D-Galactosamine-induced hepatotoxicity in rats

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A B S T R A C T

Hepatoprotective activity of *polycarpea corbosa* Linn. Whole plant aqueous Extract-Shade dried and coarsely powdered plant (1 kg) was extracted successively with decocted in purified boiling water in the ratio of 1:9. Polyherbal formulation (Liv-52,500mg/kg) and Silmarin (25 mg/kg), were evaluated for hepatoprotective activity using D-Galactosamine (D-GalN) induced hepatotoxicity in rats. The parameters assessed were serum levels of Serum Glutamic Oxaloacetate Trasaminase (SGOT), Serum Glutamic Pyruvate (SGPT), Trasaminase (SGOT), Alkaline Phosphatase (ALP), total protein, albumin, globulin, total cholesterol, total bilirubin and blood sugar changes in liver. Test drug also shown to suppress MDA and improved the antioxidant enzymes (superoxide dismutase, catalase, Glutathione peroxidise) and GSH levels. The treatmant with aqueous extract (500mg/kg),has shown to significantly reversed the biochemical changes induced by D-Galactosamine in rats, which is comparable to standard herbal formalution, Liv-52 and silymarin at the employed doses, which evidencing the promising potential of aquoes extract of *polycarpea corbosa* Linn for hepatoprotective activity.

Keywords: Polycarpea carbosa Linn. Polyherbal formulation Liv-52, Hepatoprotective activity, D-Galactosamine (D-GalN)-induced hepatic damage.

A R T I C L E I N F O

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1. Introduction

Liver is the largest internal organ, weighing approximately about 1.36 kg, in human body and is very much essential for survival. It is the principle organ for maintaining the body's internal environment (Sumeet Dwivedi., 2008). It is

involved with almost the biochemical pathways related to growth, fight against disease, nutrient supply, energy production and reproduction. Because of its major metabolic activity and relationship to the gastrointestinal tract, the liver is an important target organ victimed by toxic drugs, xenobiotics and oxidative stress (Harmut et al., 2002). More than 900 drugs, toxins and herbs have been reported to cause liver injury and drugs for 20% - 40% of all instance of fulminant liver failure.

The use of natural remedies for the treatment of liver diseases has a long history, starting with the Ayurvedic treatment, and extending to the Chinese, European and other system of traditional medicine. The 21st century has seen a paradigm shift towards therapeutic evaluation of herbal Products in the liver diseases by carefully synergizing the strengths of the traditional systems of medicine with that of the modern concept of evidence-based medicinal evaluation, standardization of herbal products and randomised placebo controlled clinical trials to support clinical efficacy (Thyagarajan et al., 2008). Inspite of tremendous advances in modern medicine, no effective drugs are available that stimulate liver function and offer protection to the liver from the damage or help to regenerate hepatic cells (Chattopaddhyay et al., 2003).

At present, a large number of herbal medicinal preparations are recommended for the treatment of liver disorders and quite often claimed to offer significant relief. Prevention is first care better than cure to reduce hepatotoxicity with growing interest in herbal therapy. Liv.52, a polyherbal Ayurvedic formulation available in a tablet form and shown to exhibits hepatoprotective function when tested against D-Galactosamine treated rats, through suppression of GSH and improvement of antioxidant enzymes (superoxide dismutase, catalase, Glutathione peroxidise) levels. We have to find the new herbal drug which can be used for hepatoprotective with more efficacy and least side effects**.** *Polycarpea corymbosa* belonging to Caryophyllaceaes is a small subdeciduous tree grown wild and cultivated in many parts of the India. Leaves of *Polycarpea corymbosa* has anti-inflamatory activity and used in the treatment of jaundice. The whole plant is used for the treatment of swelling, urinary calculi, srangury ulcers and hepatoprotective activity (madhava shetty et al., 2004).

2. Materials and Methods

Collection of the plant material:

It is found throughout the greater part of India, pantropical deciduous forests ascending upto 2100 m in the Himalayas and commonly distributed by the waysides and exposed slopes on the hill all over the Chittor disrict. S.V.U. Botanical garden, Tirupathi (madhavashetty et al., 2004).

Extraction: The plant material of *Polycarpea corymbosa* was obtained from the Tirumala hills. The shade dried whole plant of *Polycarpea corymbosa* was finely powdered. The fine powder was decocted in purified boiling water in the ratio of 1:9. The decoction was then filtered, weight /ml. This is considered as aqueous extract of *polycarpia corymbosa* (AEPC).

Priliminary phytochemical study:

The aqueous extract was subjected to preliminary phytochemical (Harbone, 1984) analysis to assess the presence of various phytoconstituents, including flavonoids, alkaloids and glycosides.

Chemicals:

Silymarin, D-Galactosamine, Liv-52, and all other reagents used were of analytical grade. Diagnostic kits used in this study were procured from Span diagnostics Ltd, India and Excel diagnostics Ltd, India.

Experimental animals: Healthy Male albino rats of Wistar strain of about 180-200g used for the study were purchased from Raghavendra enterprises, Bangalore. The animals were caged individually and kept in air conditioned room at temperature of 22±2^{o}c with 50%±10% relative humidity with 12hrs light and dark cycle. Throughout the study animals were maintained at normal laboratory conditions. Animals were maintained at standard rat pellet diet (Pranav Agro's Ltd, India) and drinking water *ad libitum*.

Acute toxicity studies

Acute oral toxicity study was performed as per OECD-423 guidelines (acute toxic class method). Wistar albino rats of either sex were selected randomly and divided into five groups. The animals were fasted over night Aqeous extract in doses of 2000, 4000, 5000 mg/kg body weight were administered orally to II -V groups. Group I which received vehicle (water) served As normal control. The animals were observed continuously for 2 hrs, and then intermittently for 6 hr and at the end of 24 hrs, the number of deaths was noted to determine LD50 of the extract (Annie et al., 2004). Animals were also observed for behavioral, neurological and autonomic profiles simultaneously.

Experimental design:

Animals were divided in five groups of six rats each.
Group 1: Served as normal control.
Group 2: Serve as induced drug D -Galactosamine at dose of 400-mg/kg i.p. on 14th day.
Group 3: Serve as Silymarin (25 mg/kg p.o.) was used as positive standard, **Group 4:** Serve as *Polycarpea corymbosa* plant extract at a dose of 500 mg/kg, p.o as a fine suspension in water for 14 days prior to the administration of D–galactosamine, **Group 5**: Rats were pretreated with hepatoprotective herbal standard i.e Liv-52 at a dose of 500 mg/kg p.o as a fine suspension in 1% sodium carboxy methyl cellulose (CMC) for 14 days prior to the administration of D- galactosamine.

Biochemical parameters:

At the end of the treatment period all animals were fasted and the blood samples were withdrawn from the retro orbital venous plexus of rats without any coagulant for the separation of serum. After collecting the blood in the microcentrifuge tubes kept aside for 1hr at room temperature and then serum was isolated by centrifugation at 2000 rpm for 15 min and stored until analyzed for biochemical estimation of parameters these includes Serum glutamate oxaloacetate transaminase (SGOT), Serum glutamate pyruvate transaminase (SGPT), Total Bilurubin (TB), Total protein (TP) and Total cholesterol (TC) by using reagent kits obtained from Autospan diagnostic Pvt. Ltd. India. Also body weights of the animals were noted.

3. Results and Discussion

Preliminary phytochemical screening

Preliminary phytochemical screening of the plant extract revealed the presence of alkaloids, tannins carbohydrates.

Table 1: Priliminary Phytochemical Screening

Chemical test	Inference
Alkaloids	Positive
Carbohydrates	Positive
Phytosterols	Negative
Glycosides	Negative
Tannins	Positive
Resins	Negative
Terpenoides	Negative

Acute toxicity studies:

The Acute toxicity studies were performed for extracts of selected plant according to the toxic classic method as per guidelines. None of these extracts showed mortality at highest dose of 5000 mg/kg and therefore considered safe.

Effect of *P. corymbosa* on elevated serum biochemical parameters-induced by D-galactosamine in rats

There was drastic and significant increase in the SGOT, SGPT, serum total bilurubin levels in the D-Galactosamine group, when compared to the normal group. However, treatment with silymarin and Liv-52 significantly decrease the biochemical markers, as compared to D-Galactosamine group. The result was same with that of both the test and herbal standard group suggesting the healing of damaged hepatocytes and stability of bilary function, by the aqueous extract. (Table2).

The effect on serum total protein (TP) and **serum total cholestero (TC)**: There was significant reduction in the serum TP Level, while, serum TC levels were significantly rised in D-Galactosamine group, compared to normal group. However, treatment with silymarin, Test and herbal standard have significant reversed the pathological changes observed in D-Galactosamine control group, which probably suggesting that an improvement of regeneration process of the Liver. (Table 2)

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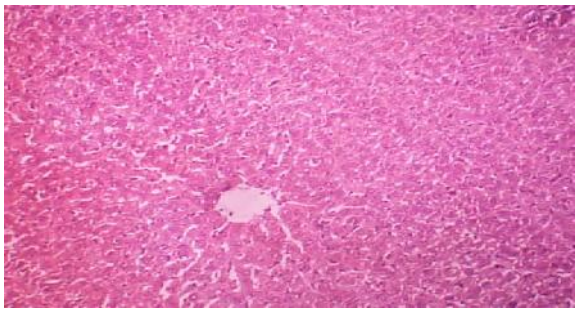

Figure 1: Liver tissue of normal group

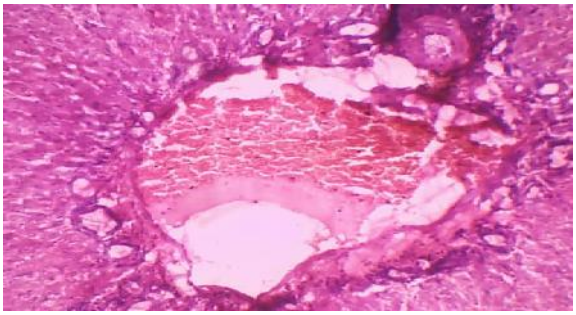

Figure 2: Liver tissue of diseased control group.

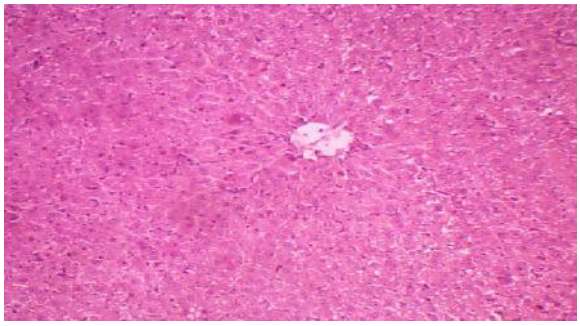

Figure 3: Liver of Standard group

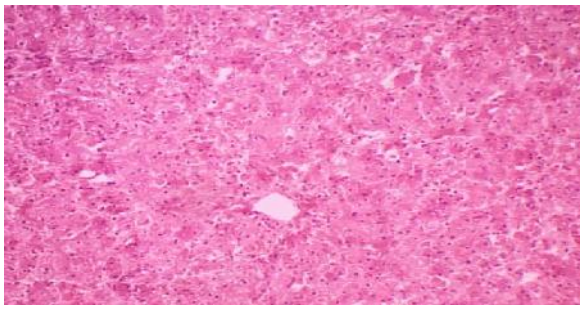

Figure 4: Liver tissue of treated group

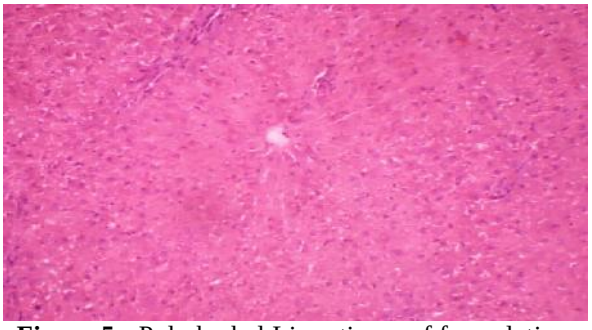

Figure 5: Poly herbal Liver tissue of formulation (Liv-52) treated group

Table 2: Effect of aqueous extracts of *Polycarpea corymbosa* (p.o) on serum biochemical parameters

S.No	Group	SGOT (IU/L)	SGPT (IU/L)	TB (mg/dl)	TP (gm/%)	TC mg/dl
1	Group-1	98.65±4.642	87.73±4.93	0.042±0.004*	0.69±0.0532	29±6.590
2	Group-2	472.98±5.657	320.67±6.32*	0.094±0.004*	0.45±0.0322*	184.34±9.451*
3	Group-3	142.87±9.694**	91.529±9.420**	0.053±0.005**	0.976±0.0132**	58.76±6.482**
4	Group-4	323.65±8.434**	174.54±6.43**	0.082±0.003**	0.590±0.025**	86.67±9.870**
5	Group-5	195.42±5.76**	114.543±6.432	0.062±0.003**	0.791±0.0043**	69.43±7.543**

Values are mean ± SEM for six observations, *p<0.05, when compared to the normal group**, p<0.05, when compared to the control group, **SGOT**-serum glutamate oxaloacetate transaminase, SGPT-serum glutamate pyruvate trans aminase, **TB**-total bilirubin, **TP-**total protein, **TC**- total cholesterol

Table 3: Effect of aqueous extracts of *Polycarpea corymbosa* (p.o) on tissue biochemical parameters

S.No	Group	SOD (U/mg protein	CAT (uM H_2O consumed/mg protein	Reduced GSH (ug of GSH / mg protein)	MDA (nM of MDA/mg protein)
1	Normal	4±0.50	5.53±0.60	8.64±0.32	0.71±0.07
2	D-Galactosamine (400 mg/kg.b.w,i.p)	1.32±0.58*	1.03±0.03*	2.34±0.31*	1.89±0.02*
3	Silymarin (25 mg/kg.b.wt), D-Galactosamine (400 mg/kg.b.wt,i.p)	7.34±0.54**	7.37±0.53**	6.21±0.59**	0.82±0.07**
4	Test (500 mg/kg.b.wt) + Galactosamine (400 mg/kg.b.wt,i.p)	2.45±0.07**	1.67±0.90**	3.85±0.01**	1.20±0.8**
5	Liv-52 (500 mg/kg .b.wt+) Galactosamine (400 mg/kg.b.wt,i.p)	4.761±0.05**	5.67±0.43**	4.63±0.54**	0.01±0.09**

Values are mean ± SEM for six observations.*, p<0.05, when compared to the normal group. **, p<0.05, when compared to the control group, **SOD:** superoxide dismutase, CAT**:** Catalase, **GSH:** Gltathione, **MDA:** Malondialdehyde.

Discussion

Liver plays a major role in detoxification and excretion of many endogenous and exogenous compounds, any injury or impairment of its function may lead to several Implications on one's health. Management of liver diseases is still a challenge to modern medicine (Handa, 1991; Kirtikar et al., 1995). Convential drugs used in the treatment of liver diseases are often inadequate. It is therefore necessary to search alternative drugs of doubtful efficacy and safety. Morbidity and mortality resulting from chronic liver diseases such as cirrhosis is a one of the major health problem (Meena et al., 2008). The use of rats as experimental animals for hepatoprotective activity is mainly because of the structural homology of rat TNF (Burke et al., 1994). Exogenous administration of D-Galactosamine has been found to induce liver damage, which closely resembles human viral hepatitis (Taniguchi H et al., 2004 and Decker K and Keppler D, 1972). The toxicity of D-Galactosamine results from inhibition of RNA and protein synthesis in the liver (Endo Y et al., 1992 and Manabe A et al., 1996). The metabolism of D -Galactosamine may deplete several uracil nu-cleotides including UDP-glucose, UDP Galactose and UTP (Tsai CC et al., 1997) which are trapped in the formation of uridine-diphospho-galactosamine. Accumulation of UDP-sugar nucleotides (Mitra SK et al., 1998) may contribute to the changes in the rough Galactosamination of membrane structures is thought to be responsible for loss in the activity of ionic pumps. The impairment in the calcium pump, with consequent increase in the intracellular calcium is considered to be responsible for cell death.

An evidence of hepatic injury is leakage of cellular enzymes into the plasma. When liver cell plasma membrane is damaged, a variety of enzymes normally located in the cytosol are released into the blood stream. Their estimation in the serum is a useful quantitative marker of the extent and type of hepatocellular damage. The acute toxicity study revealed the absence of lethality among the tested animals when the extract P.O was asingle dose (5,50,300 and 5000mg/kg). There were no signs of any gross behavioural chanes extract at a dose of 500 mg/kg.

Administration of hepatotoxins D-Galactosamine elevated the serum levels of SGOT, SGPT, ALP, TB and TC. As well as decreases total serum proteins (TP) significantly. The rise in serum enzymes level and bilurubin has been attributed to the damaged structural integrity of the liver, because they are cytoplasmic in location and released into

circulation after cellular damages. Test drug also shown to suppress MDA and improved the antioxidant enzymes (superoxide dismutase, catalase, Glutathione peroxidise) and GSH levels. The treatmant with aqueous extract (500mg/kg),has shown to significantly reversed the biochemical changes induced by D-Galactosamine in rats, which is comparable to standard herbal formalution, Liv-52 and silymarin at the employed doses, which evidencing the promising potential of aquoes extract of *polycarpea corbosa* Linn for hepatoprotective activity.

4. Conclusion

It clearly indicates that the plant *Polycarpia corymbosa* has the moderate hepatoprotective activity. The activity of the extract is due to the chemical constituents present in it. Phytoconstituents like alkaloids (Vijayan et al., 2003), tannins are Possess hepatoprotective activity. Along with these, the oxidants and prooxidants in our plant might be responsible for its hepatoprotective activity.

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