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ASSESSMENT OF HEPATOPROTECTIVE ACTIVITY OF *SYMPLOCOS RACEMOSA* BARK AGAINST PARACETAMOL INDUCED LIVER DAMAGE IN RATS AND ITS POSSIBLE MECHANISM OF ACTION

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ABSTRACT

Symplocos is a genus of flowering plants in the order Ericales, containing about 250 species native to Asia, Australia and the Americas. Current study is conducted to evaluate the hepatoprotective activity and its possible mechanism of action of *Symplocos racemosa* bark extract. Single dose of paracetamol (750mg/kg) was used to induce the hepatic damage in rats. Methanolic bark extract of *Symplocos racemosa* (200mg/kg) and silymarin (25mg/kg) were administered orally once daily for 7 days. Serum hepatic markers (SGOT, SGPT, SALP, serum bilurubin and total protein) and antioxidant parameters (GSH, SOD, CAT and LPO) were estimated. Silymarin and methanolic bark extract of *Symplocos racemosa* with paracetamol reversed the elevated levels of SGOT, SGPT, SALP, Serum bilurubin, LPO and restored the decreased levels of total protein, SOD, CAT and GSH. The effect produced by the bark extract of *Symplocos racemosa* was comparable with that of silymarin. From the result it was concluded that, The methanolic bark extract of *Symplocos racemosa* exhibited hepatoprotective and the probable mechanism of action may be due to its antioxidant property.

Key words: *Symplocos racemosa*, Hepatoprotective, Paracetamol and Antioxidant.

INTRODUCTION

Liver is a vital organ which plays major role in metabolism and excretion of xenobiotics from the body. Liver injury or its dysfunction is a major health problem that challenges not only health care professionals but also the pharmaceutical industry and drug regulatory agencies. Chronic liver disease and cirrhosis is the 12th leading cause of death. Liver cell injury caused by various toxic chemicals, certain chemotherapeutic agents, carbon tetrachloride, excessive alcohol, overloaded iron, NSAIDs is well-studied.

Oxidative stress, which results due to imbalance between the antioxidant defense system and the formation of reactive oxygen species (ROS), may induce damage to hepatocellular biomolecules such as proteins, carbohydrates, lipids, RNA and DNA through oxidative modification and contributing to the pathogenesis of human diseases (Singh *et al*., 2009). In view insight of synthetic drugs, causes severe adverse side effects. There is growing hub to develop more safer drugs which may raise the therapeutic benefits for patients. A large number of medicinal plants have been tested and found to contain active principles with therapeutic properties against hepatotoxicity. Plants contain a variety of chemical constituents like phenols, carotenoids, glycosides, flavonoids. organic acids, lipids and alkaloids which showed hepatoprotective activity. Medicinal plants containing phytochemicals with antioxidant potential have strong protective effect against hepatotoxicity (Singh *et al.*, 2008). Herbal remedies are very promising and

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valuable alternative options for treatment of liver complaints.

Symplocos racemosa Roxb. is an evergreen Ayurvedic plant widely distributed in the tropics and subtropics of Asia, Australia and America. *Symplocos racemosa* is a small, evergreen tree, upto 6-8.5 m tall found in the plains and lower hills throughout North and East India, ascending in the Himalayas up to an elevation of 1400 m, Bengal, Assam and Chota Nagpur. Traditionally the bark of *Symplocos racemosa* was used in various ailments like inflammation, constipation, eye diseases, bleeding gums, asthma, arthritis, ulcer, tumours, leprosy, filariasis, gonorrhea, hepatic damage, haemorroids and skin diseases (Kirthikar and Basu, 1999, Anonymous, 2006).

Symplocos racemosa bark reported to have analgesic and anti-inflammatory activity (Sharma *et al*. 2013), Antioxidant activity, Antibacterial (Devmurari 2010), Anthelmintic activity (Rao *et al*., 2011), Anti-angiogenic activity (Hussain *et al*., 2009), Anticancer activity (Raval *et al*., 2009) and Hepatoprotective activity (Wakchaure *et al*., 2010). It also used in the treatment of Alzheimer's disease (Rashid *et al*., 2008) and Peptic ulcer disease (Krishna *et al*., 2013). Current study is conducted to evaluate the mechanism of *Symplocos racemosa* bark as a potent hepatoprotective against paracetamol induced hepatic damage in rats by its free radical scavenging property.

Plant Material

The barks of *Sympolcos racemosa* Sathuragiri hills and it was identified and authenticated as *Sympolcos racemosa's* bark by Scientist 'F' Botanical survey of India, Southern Regional Centre, Tamilnadu Agriculture University, Coimbatore. The Voucher specimen (BSI/SRC/5/49/14-15/Tech - 656) has been deposited in department for further references.

Preparation of Extract

The collected barks were, shade dried and then ground into coarse powder. The powder was then subjected to exhaustive extraction by a maceration process using 90% methanol as a solvent at room temperature for 7 days. The methanolic extract was concentrated by vacuum distillation to dry. The collected extract was stored in a desiccators and used for further pharmacological study.

Animals

Male Wistar albino rats weighing between 150 – 220 gm were used for this study. The animals were obtained from animal house, Karpaga Vinayaga Institute of Medical Sciences, Kancheepuram, Tamilnadu, India. The animals were placed at random and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24±2^{o}C and relative humidity of 30 – 70 %. A 12:12 light: day cycle was followed. All animals were allowed to free access to water and fed with standard commercial pelleted rat chaw (M/s. Hindustan Lever Ltd, Mumbai). All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics Committee.

Hepatoprotective Activity (Araya *et al.*, 1987)

Animals were randomized and divided into four groups (I-IV) of six animals in each group. Group I served as untreated control and fed orally with 0.1% CMC 1ml/kg body weight daily for seven days. Group II rats were similarly treated as group I. Group III was treated as reference control which received the standard drug Silymarin (25mg/kg). Group IV was treated with 200mg/kg body weight of the *Sympolcos racemosa* extract. The extract was administered once daily for seven days through oral route. All the test drugs were administered orally once daily for 7 days. On the seventh day, paracetamol (750mg/kg, body weight) suspension was given by oral route, to all rats except the rats in group I. The biochemical parameters were estimated after an 18h past following the last test dose.

The blood was collected from all animals by puncturing retro-orbital plexus under anaesthesia using thiopentone sodium. The blood samples were allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 2500rpm at 30 ^{O} C for 15 min and utilized for the estimation of various liver function tests (SGOT, SGPT, SALP, serum bilirubin and total protein)

Serum Hepatospecific Markers

Activities of serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) were estimated by the method of (Reitman and Frankel, 1957). Based on the method of (King and Armstrong, 1934) alkaline phosphatase (SALP) activity was assayed using disodium phenyl phosphate as substrate.

Serum total bilirubin level was estimated based on the method of Malloy and Evelyn, 1937 and serum total protein level was estimated based on the method of Gornall *et al*. (1949).

Antioxidant Activity

After 24 hrs all the animals were sacrificed and the liver was rapidly excised, rinsed in ice-cold saline, and a 10% w/v homogenate was prepared using 0.15M KCI, centrifuged at 800 g for 10 min at 4°C. The supernatant obtained was used for the estimation of antioxidants like Glutathione (Ellman *et al.,* 1959), Superoxide dismutase (Kakkar *et al.,* 1984), Catalase (Sinha., 1972) and Lipid peroxidase (Romero *et al.,* 1998).

Statistical Analysis

The values were expressed as mean ± SEM. The statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Dunnet's'*t*' – test using graphpad version I. *P* values <0.05 were considered significant.

Table 1. The table shows the effect of *Sympolcos racemosa* bark extract on paracetamol induced liver damage in rats

Drug Treatment	Liver Function Test				
	SGOT (IU/L)	SGPT(IU/L)	SALP(IU/L)	Serum Bilirubin (mg/dl)	Total Protein (mg/dl)
Group I Vehicle Control 0.1% CMC	41.450 ±0.667***	57.238 ±1.888***	39.620± 1.241***	1.570 ±0.023***	7.916 ±0.144***
Group II Paracetmol (750 mg /kg)	183.48 ±3.555	110.07 ±1.931	197.66 ±2.890	4.832 ±0.1099	4.072 ±0.238
Group III Silymarin (25mg/kg)	46.468 ±1.864***	62.414 ±1.424***	46.886 ±1.331***	2.024 ±0.085***	6.678 ±0.294**
Group V *Sympolcos racemosa* Extract 200	58.260 ±1.962***	79.274 ±1.125***	65.018 ±0.969***	3.258 ±0.138***	5.926 ±0.369*

Values are in mean ± SEM (n=6), *P<0.05 , **P<0.01, ***P<0.001 Vs Paracetamol Control.

Table 2. The table shows the antioxidant effect of *Sympolcos racemosa* bark extract on paracetamol induced liver damage in rats

Drug Treatment	GSH µg of GSH consumed/min/mg protein	SOD U/mg of Protein	CAT µM of H_2O_2 consumed/min/mg protein	LPO Mm/100 g of Tissue
Group I Vehicle Control 0.1% CMC	0.74±0.013	1.65±0.021	0.89±0.002	0.28± 0.003
Group II Paracetmol (750 mg /kg)	0.34±0.015	0.90±0.010	0.54±0.018	0.59± 0.003
Group III Silymarin (25mg/kg)	0.69±0.022 **	1.41±0.004***	0.81± 0.016***	0.24± 0.012***
Group IV *Sympolcos racemosa* Extract 200mg/kg	0.56±0.003**	1.29±0.005***	0.75± 0.007***	0.31± 0.013***

Values are in mean ± SEM (n=6), *P<0.05 , **P<0.01, ***P<0.001 Vs Paracetamol Control

RESULT AND CONCLUSION

The results of hepatoprotective activity of Plant Extract (200 mg/kg) on Paracetamol treated rats are shown in Table 1. The hepatic enzymes ALT, AST, ALP in serum and total bilirubin were significantly (P <0.001) increased in paracetamol treated animals when compared to vehicle control. The silymarin significantly (P <0.001) reduced the hepatic enzymes and total bilirubin & total protein in paracetamol induced hepatic injured groups. The plant extract treated groups, 200mg/kg significantly (P <0.001) reversed the levels of SGOT, SGPT, SALT and Serum bilurubin as compared to paracetamol treated groups. In the biochemical parameters, total protein was significantly enhanced by silymarin (P<0.01) and plant extract (P<0.05) as compared to paracetamol control.

Table 2, represents the antioxidant activity of methanolic bark extract of *Sympolcos racemosa* in liver homogenate of paracetamol induced liver damage in rats. The administration of paracetamol results in liver damage which was evidenced by decrease in the activities of SOD, catalase, glutathione and enhanced the activity of lipid peroxidase in the liver homogenate. Combined administration of *Sympolcos racemosa* bark extract of *Sympolcos racemosa* markedly reversed the change in antioxidant enzymes brought by paracetamol in rats. Reference control silymarin treated group also significantly increased the level of glutathione, SOD, catalase and decreased the lipid peroxidase level in the paracetamol challenged animals. From the above it was concluded that, *Sympolcos racemosa* bark extract exhibited hepatoprotective activity by scavenging the generation of free radicals.

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