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Original Research Article

Hepatoprotective Activity of Ethanolic Seed Extract of *Lawsonia inermis* Against Paracetamol Incudced Liver Damage in Rats

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Abstract: Aim of the study was to evaluate the hepatoprotective activity of ethanolic seed extract of *Lawsonia inermis* against paracetamol induced liver damage in rats. Wistar albino rats of either sex were divided in to 5 groups of 6 each. The rats were intoxicated with paracetamol (750mg/kg) at every 72 hrs for 21 days. Silymarin (50mg/kg) and *Lawsonia inermis* (200 & 400 mg/kg) were orally administered once daily for 21 day by suspending in 0.1% Carboxy Methyl Cellulose solution. On 22nd day blood was collected through retro orbital puncture and the serum was subjected to liver function tests like, SGOT, SGPT, SALP, biliubin and total protein. *Lawsonia inermis* (400mg/kg) showed significant significant increase in SGPT ($P<0.05$), SGOT, SALP, total protein ($P<0.05$) and significantly decrease the total bilirubin ($P<0.01$) level. From the above result it was concluded, that *Lawsonia inermis* seed extract exhibited hepatoprotective activity against paracetamol induced hepatic damage in rats.
Keywords: *Lawsonia inermis*, Paracetamol, Silymarin, Hepatoprotective activity

INTRODUCTION

Chronic liver disease occurs throughout the world irrespective of age, sex, region or race. Cirrhosis is an end result of a variety of liver diseases characterized by fibrosis and architectural distortion of the liver with the formation of regenerative nodules and can have varied clinical manifestations and complications. Chronic liver disease involves a wide range of liver pathologies that include fatty liver, hepatitis, fibrosis, cirrhosis, and hepatocellular carcinoma. Although there have been remarkable progress in discovering treatment of chronic liver diseases over the last several decades, most of the therapies still do not yield satisfactory outcomes in patients [1].

In view of the scarce treatment options and significant adverse effects incurred by conventional chemical agents, novel prophylactic and therapeutic agents against chronic liver disease are urgently needed. Over recent decades, an increasing number of herbal products, including medicinal herbs and phytochemicals, have been used for treating chronic liver diseases worldwide due to the high abundance, long-lasting curative effects and few adverse effects. According to the previous studies, medicinal herbs and phytochemicals could protect the liver by several mechanisms such as eliminating virus, blocking fibrogenesis, inhibiting oxidative injury and suppressing tumorigenesis [2]. As a chronic disease, most liver injuries need long-term treatment, thus, reducing side-effects of the therapy is critical when developing novel hepatoprotective agents.

Lawsonia inermis Linn. (syn. Lawsonia alba, Lythraceae) known as henna is a glabrous, much branched shrub or small tree with greyish-brown bark. Leaves are opposite, sub-sessile, elliptic or broadly lanceolate, entire, acute or obtuse, 2-3 cm long and 1-2 cm wide. Flowers are small, about 1 cm across, numerous, white or rose colored with four crumbled petals, and fragrant. Fruit is a small brown coloured round capsule opening irregularly and is many seeded. Seeds are about 3 mm across, smooth, pyramidal, hard and thick seed coat with brownish colour [3]. This plant is native of North Africa and South-West Asia and nowadays is widely cultivated in India, Middle East and along the African coasts of the Mediterranean Sea [4].

Traditionally in India, *Lawsonia inermis* leaves paste is applied to hands and feet, which symbolizes fertility and produced cooling effect. *Lawsonia inermis* leaves, flowers, seeds, stem bark and roots are used in traditional medicine to treat a variety of ailments as rheumatoid arthritis, headache, ulcers, diarrheoa, leprosy, fever, leucorrhoea, diabetes, cardiac disease, and hepatoprotective [5].

The leaves of *Lawsonia inermis* is used for alleviating jaundice, skin diseases, venereal diseases, smallpox and spermatorrhoea. An infusion of the flowers is a valuable application to bruises and used as an emmenagogue. The seeds were effective in dysentery and liver disorders. The bark is applied in the form of a decoction to burns and scalds. It is given internally in a variety of affections, such as jaundice, enlargement of the spleen, calculus, as an alternative in leprosy and obstinate skin affections. Root is considered as a potent medicine for gonorrhea, herpes infection, hysteria and nervous disorders.

Although this plant has been widely used traditionally in various symptoms and diseases, however few pharmacological studies such as, Immunomodulatory [6], antidiabetic [7], antioxidant [8], analgesic [9], anti-inflammatory [10], wound healing [11] have been reported. So for no literature supports the hepatoprotective effect of *Lawsonia inermis* seeds. Substantiate Based on the above, effort has been taken to evaluate the hepatoprotective activity of *Lawsonia inermis* seeds against paracetamol induced liver damage in rats.

MATERIALS & METHODS

Plant Collection

The dry seeds of *Lawsonia inermis* along with plant was collected from the out skirts of Pondicherry. The plant was identified as *Lawsonia inermis* and authenticated by Scientist 'F' Botanical survey of India, Southern Regional Centre, Tamilnadu Agriculture University, Coimbatore. The Voucher specimen (BSI/SRC/14/46/15-16/Tech - 55) has been deposited in department for further references.

Preparation of Extract

The collected seeds of *Lawsonia inermis* was shade dried and pulverized to get coarse powder using mechanical blender. The coarsely powdered seeds were defatted with petroleum ether and then subjected to extraction by a maceration process using 90% ethanol as a solvent at room temperature for 7 days with occasional shaking. The ethanolic extract was concentrated to dry. The collected extract was stored in desiccators and used for further pharmacological study.

Animals

Wistar rats weighing between 150 – 220 gm (both sex) were used for this study. The animals were obtained from animal house, Chengalpattu Medical College, Chengalpattu. On arrival, 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 and were in accordance with the Institutional ethical guidelines.

Paracetamol-induced hepatotoxicity in rats

Hepatoprotective Activity [12]

The animals were divided into five groups of six animals in each group. Group I served as control received vehicle 0.1% Carboxy Methyl Cellulose (CMC) solution (1ml/kg). Group II received paracetamol (750 mg/kg) at every 72 hrs for 21 days through oral route. Group III served as reference control, received silymarin 50 mg/kg for 21 days through oral route and simultaneously administered paracetamol 750 mg/kg every 72 hrs. Group IV and V received 200 and 400 mg/kg of ethanolic seed extract of *Lawsonia inermis* respectively, for 21 days through oral route and simultaneously administered paracetamol 750 mg/kg every 72 h. All the test drugs were administered orally using gastric gavage by suspending in 0.1% CMC. On 22nd day, blood was collected through retro orbital sinus puncture under anaesthesia using thiopentone sodium.

The collected blood samples were centrifuged for 10 minutes at 2000 rpm and serum was separated. The separated serum was subjected to various biochemical tests like Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT) [13], Serum Alkaline Phosphate (SALP) [14], serum bilurubin [15] and total protein [16].

Statistical Analysis

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

RESULTS

The results of hepatoprotective activity of *Lawsonia inermis* seed extract on paracetamol treated rats was shown in Table 1. The hepatic enzymes SGOT, SGPT, SALP in serum and total bilirubin were increased and total protein was decreased in paracetamol treated animals when compared to vehicle control. The reference control silymarin reversed the levels of serum enzymes, total bilurubin and total

protein on the paracetamol induced hepatic injury by significantly (P <0.001) reduced the serum hepatic enzymes, total bilirubin and decreasing the total protein.

Lawsonia inermis 200 mg/kg significantly increase SGOT, SALP (P<0.05) and total protein (P<0.01). It does not produce any significant change in SGPT and total bilirubin. 400mg/kg of *Lawsonia inermis* showed significant increase in SGPT (P<0.05), SGOT, SALP, total protein (P<0.05) and significantly decrease the total bilirubin (P<0.01) level.

Table: 1. Hepatoprotective activity of ethanolic seed extract of *Lawsenia inermis* paracetamol induced liver damage in rats.

Drug Treatment	Liver Function Test				
	SGOT (IU/L)	**SGPT(IU/L)**	**SALP(IU/L)**	**Total Bilirubin (mg/dl)**	**Total Protein (mg/dl)**
Group I (Vehicle Control) 0.1% CMC	48.51±3.62	65.61±5.94	46.87±2.22	2.45±0.14	9.98 ±0.67
Group II(Paracetmol) (750 mg /kg)	198.33 ±5.54	118.63±8.47	202.54±5.43	6.43 ±0.53	3.62 ±0.22
Group III (Silymarin) (50mg/kg)	52.38±3.18***	67.69±4.99***	53.64±4.32***	4.22±0.05***	8.45±0.32***
Group IV(*Lawsonia inermis)* Extract (200mg/kg)	132.5±7.84*	90.24±6.92	123.51±7.82*	2.89±0.18	6.73±0.38**
Group V(*Lawsonia inermis)* Extract (400mg/kg)	94.29±4.33**	72.87±5.22*	97.67±6.31**	3.89±0.24**	5.40±0.22**

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

CONCLUSION

From the above result it was concluded that, the ethanolic seed extract of *Lawsonia inermis* exhibits hepatoprotective activity. Further study may require to elucidate its possible mechanism of action and the active principal responsible for its hepatoprotective activity.

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