

Hepatoprotective activity of Methanol extracts of Aerial parts of *Pergularia daemia*

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ABSTRACT

Pergularia daemia (Asclepiadaceae) is a perennial herb growing widely along the road sides of India and other tropical and subtropical regions of world. It has been used in folk medicine for the treatment of liver disorders. The present study is to evaluate the hepatoprotective effects of the methanol extracts of aerial parts of *Pergularia daemia* by carbon tetrachloride inducing in rats. The methanolic extract at an oral dose of 2ml/kg exhibited a significant ($p < 0.05$) protective effect by lowering serum transaminases (SGPT & SGOT), Alkaline phosphatase, total bilirubin and increasing levels of total protein and Albumin levels as compare to silymarin used as positive control. These biochemical observations were supplemented by histopathological examination of liver sections. The activity maybe a result of the presence of flavonoid compounds. Further more the acute toxicity of the extracts showed no signs of toxicity up to a dose level of 2000mg/kg. Thus it could be concluded that methanolic extract of *Pergularia daemia* possesses significant hepatoprotective properties.

Keywords: Hepatoprotective, *Pergularia daemia*, CCl₄.

INTRODUCTION

The world Health Organization states that approximately 85 to 90% of the world's population consumes traditional herbal medicines [1]. While the herbal drug industry has been in high growth since the late 1990s due to the growing demand in developing and developed countries [2]. The plant biodiversity in India has served as the foundation for the development of many traditional system of medicine, including Ayurveda, Unani, Siddha and Tibetan [3]. In recent years there has been a rapid increase in standardization of selected medicinal plants of potential therapeutic significance [4]. The compounds present in plants have the ability to reduce the toxicity of any chemical or a pathogen that enters the

human body [5]. Liver is the largest glandular organ in human body. It plays a major role in purifying the blood by eliminating the toxic substances. Liver is the only human organ which is capable of regenerating its lost tissue. There is no mechanism for replacing the function of liver [6]. The chemical substances that affect the function of liver are alcohol and carbon tetrachloride (CCl₄) [7-10]. *Pergularia daemia* is a perennial twining herb that belongs to milky weed family Asclepiadaceae [11]. The plant was used as laxative, anti-pyretic, expectorants and also in infantile diarrhea [12]. The latex of the leaf is used as pain killer [12]. The photochemical present in the leaves of *Pergularia daemia* are flavanoids, alkaloids, terpenoids, steroids and tannins [13]. Pharmacological studies have confirmed that *Pergularia daemia* exhibit a broad range of biological effects. The crude extract of plant has been used as a traditional medicine for the treatment of various diseases [11]. Hence, the present work is focused towards the study on the efficacy and hepatoprotective activity of crude powder of aerial parts of plant to treat the experimentally induced jaundice affected rats.

MATERIALS AND METHODS

Animals used in the present study were procured from the small animals breeding station, Vijayawada, Andhra Pradesh, India. They were housed in polypropylene cages (38 x 23 x 10cm) with six animals per cage and maintained standard environmental conditions (14h dark/10h light cycles; temp 25±2°C; 35-60% humidity, air ventilation) and were fed with standard pellet diet (M/s. Hindustan Lever Ltd., Mumbai, India) and fresh water *ad libitum*. The animals were acclimatized to the environment for two weeks prior to experiment use. Animals were fasted over night before the experimental schedule, but have free access for water *ad libitum*.

The hepatoprotective and the antioxidant activity of the methanol extract of *pergularia daemia* leaves were studied against CCl₄ induced hepatotoxicity in rat model by following method [14]. The rats were divided into 5 groups of six animals each.

Group-I was served as control and received normal water daily for 14 days.

Group-II rats were received olive oil (2 ml /kg p.o.) for 14 days. Group-III rats were received silymarin (standard hepatoprotective herbal formulation from *Silibum marianum*) every day at the rate of 50 mg/kg p.o. for 14 days. To determine the hepatoprotective dose response of methanol extract doses daily at the concentrations of 200 and 400 mg/kg p.o. were administered for 14 days to Group IV- V. On 14th day, six hours after the last dose of standard drug and test sample, CCl₄ (1:1 v/v in olive oil) was administered at the dose of 2ml/ kg bw p.o. to group II-VI. Twenty four hours after the dose of CCl₄ administration, blood samples were collected from all groups by puncturing retro-orbital plexus. The

blood samples were allowed to clot for 45 min and were centrifuged at 2500 rpm for 15 min then the serum was collected and analyzed for various biochemical estimations. After collection of blood samples, the rats were sacrificed and their liver excised, rinsed in ice cold normal saline followed by 0.15 M Tris HCl (pH 7.4), blotted dry and weighed.

RESULTS

In the present experimental study, the normal animals showed 3.20 ± 0.39 mg/dl, as the normal level of serum bilirubin. After the induction of CCl₄ (2ml/kg) in the rats, it was found to be increased to 9.40 ± 0.42 mg/dl than the normal level. After the treatment with *Pergularia daemia* crude powder at the dose of 200 mg/kg of body weight, the serum bilirubin level was decreased to 7.37 ± 0.34 mg/dl. (Table & fig) The normal level of serum alanine transaminase is 54.18 ± 0.68 U/l. After the injection of CCl₄ the serum ALT level was rapidly raised to 160.78 ± 0.82 U/l and it was reduced to the level of 120.53 ± 0.57 U/l by treating the animals with the crude extract of *Pergularia daemia*.

The level of serum aspartate transaminase is 63.59 ± 0.73 in normal animals. When the hepatotoxic CCl₄ was injected the AST level was raised to 176.40 ± 0.43 U/l in the untreated animals and the treated animals showed a significant reduction in their AST levels to about 136.14 ± 0.47 U/l by treating the animals with crude extract of *Pergularia daemia* The normal level of serum Alkaline phosphatase was found to be 61.21 ± 0.68 U/l and this level was raised to 183.53 ± 0.68 U/l when CCl₄ was injected. When the animals were treated with extract the reduction of serum ALP was to 130.07 ± 0.80 U/l.

Table: Clinical parameters of various animal groups

Groups	Total	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	Total
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	protein(g/dl)				Bilirubin (mg/dl)
Control	7.59 ± 0.21	63.59 ± 0.73	54.18 ± 0.68	61.21 ± 0.68	3.20 ± 0.39
Induced	4.69 ± 0.08	176.40 ± 0.43	160.78 ± 0.82	183.55 ± 0.68	9.40 ± 0.42
Standard drug	7.43 ± 0.06	70.47 ± 0.68	60.68 ± 0.63	68.74 ± 1.05	4.9 ± 0.39
Methanol extract 200mg	5.39 ± 0.15	136.14 ± 0.47	120.53 ± 0.57	130.07 ± 0.80	7.37 ± 0.34
Methanol extract 400mg	6.54 ± 0.08	81.05 ± 0.52	79.88 ± 0.52	83.51 ± 0.48	4.79 ± 0.31

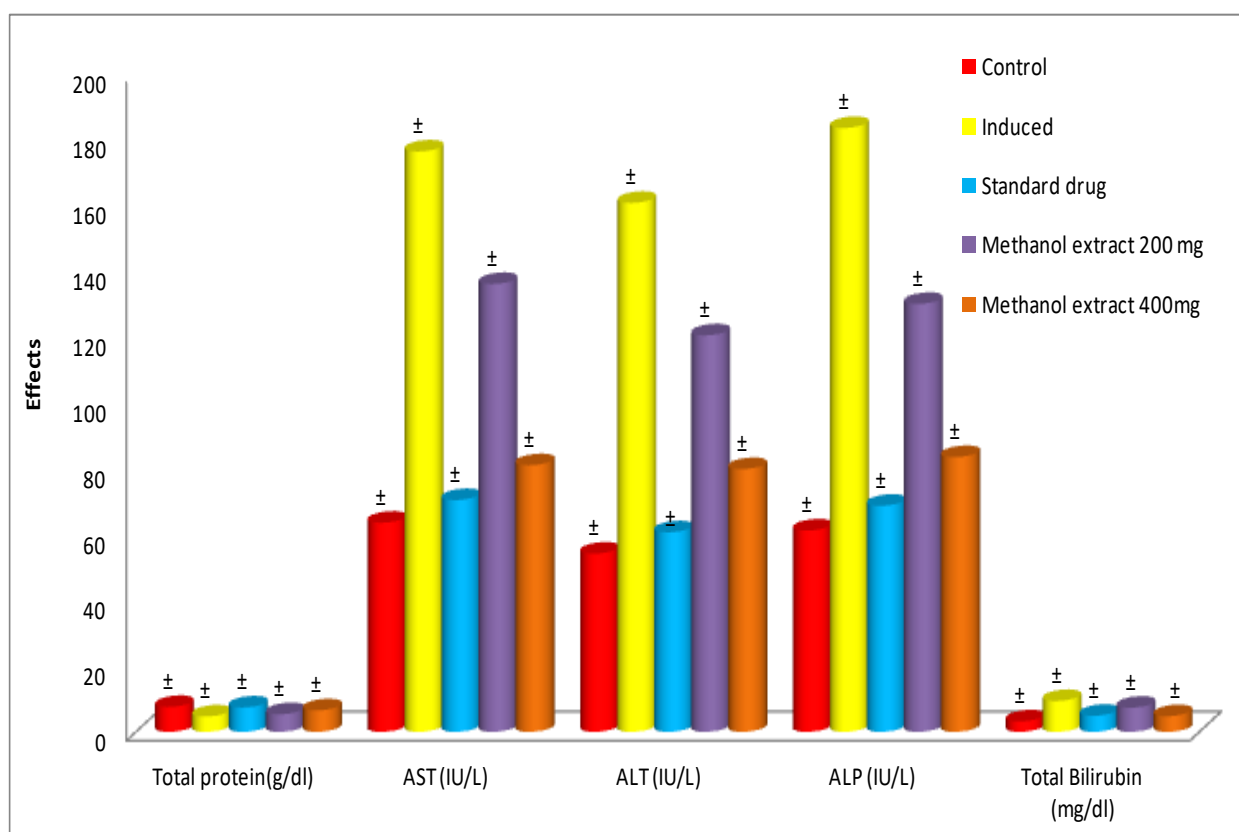


Fig: Effect of methanolic extracts of *Pergularia daemia* leaves on serum biochemical markers.

DISCUSSION

Liver disease is the devastating disease throughout the world especially in India. The injection of CCl₄ to the healthy rats caused some effects like damage to the liver membrane or liver tissues, loss of structural integrity in the liver cells and dysfunction of hepatic cells [15]. Liver diseases arise due to various other reasons as well. There are plenty of synthetic molecules available to treat liver diseases. However, these synthetic molecules cause adverse events. Moreover, many alcoholic compounds like carbon tetrachloride (CCl₄) have the ability to cause liver diseases. In CCl₄, the active metabolite namely trichloromethyl radical induces the lipid per oxidative degradation of the membranes of liver [16]. This influences the increase of serum markers like total bilirubin, ALT, AST and ALP. Among these serum markers, ALT, AST are widely used in the detection of damage to the liver. The AST and ALT diffuses out of the liver [17]. Thus, these serum markers get distributed throughout the body and are found in increased levels in the serum. Hence these are better markers to detect the liver injury. The ALP serum marker is associated with the hepatic cell function. If there is an increase in biliary pressure, then there will be an increase in the ALP level [18]. The intra hepatic biliary obstruction leads to the increase in γ -GT levels in serum. The increase in LPO is due to the loss of structural integrity in hepatocytes [19]. The present study showed that the oral administration of the crude extract of *Pergularia daemia* helps to treat and protect the hepatic cells and resulted in the decreased levels of these serum markers which were statistically significant. The similar results were reported by Jain.S.C et.al. [20]. In the present study, the obtained results are assumed to be due to the presence of flavanoids in the crude extract of the plant and it was reported in the earlier study [21]. We have studied the effect of CCl₄ on the liver of rats and the treatment of crude extract of *Pergularia daemia* on the affected rats. This study can be done on other organisms and the effect of the crude extract on those organisms can also be studied.

CONCLUSION

In the present study, methanol leaf extract of *Pergularia daemia* was analyzed for its hepatoprotective activity against model hepatotoxicant CCl₄ in rats by studying the activity of AST, ALT, ALP, and total bilirubin. The extract showed hepatoprotective activity which was dose-dependent and maximum beneficial effect was observed at a dose of 400 mg/kg. The results were comparable with that of standard drug, silymarin used in the study. The hepatoprotective action is perhaps related to its potent antioxidant activity. Further investigations are required to characterize the active hepatoprotective agent and mechanism of action.

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