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RESEARCH ARTICLE

Hepatoprotective activity of powdered leaves extract of *Adenanthera Pavinia* against Paracetamol induced liver damage in rats

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ABSTRACT

The extract of plant *Adenanthera Pavinia* was studied for hepatoprotective activity against Swiss albino rats with liver damage induced by Paracetamol. It was found that the different extract of *Adenanthera Pavinia* at a dose of 500 mg/kg body weight exhibited moderate protective effect by lowering the serum levels of alanine aminotransferase(ALT) or Serum Glutamate Pyruvate Transaminase (SGPT), aspartate aminotransferase (AST)or Serum Glutamate and Oxaloacetate Transaminase (SGOT) to a significant extent. The hepatoprotective activity was also supported by histopathological studies of liver tissue. Since results of biochemical studies of blood samples of Paracetamol treated rats showed significant increase in the levels of serum enzyme activities, reflecting the liver injury caused by Paracetamol and blood samples from the animals treated with the extracts of *Adenanthera Pavinia* showed significant decrease in the level of serum markers, indicating the protection of hepatic cells, the extract of above plant could afford significant dose-dependent protection against paracetamol induced hepatocellular injury.

INTRODUCTION

The liver has an enormous task of maintaining the body's metabolic homeostasis. This includes, the processing of dietary amino acids, carbohydrates, lipids, and vitamins; synthesis of serum proteins; and detoxification and excretion into bile of endogenous waste products and pollutant xenobiotics. Hepatic disorders have far reaching consequences, given the critical dependence of other organs on the metabolic functions of the liver. Liver injury and its manifestations tend to follow characteristic patterns. In some instances, the diseased process is primary to the liver. In others, the hepatic involvement is secondary, often to some of the most common diseases in humans, such as cardiac decompensation, alcoholism and extrahepatic infections with progression of diffused disease or strategic disruption of circulation or bile flow. Herbal drugs play a vital role in the management of various liver disorder, most of them speed up the natural healing process of liver. Numerous medicinal plants and their formulations are used in liver disorders in ethno medicinal practices as well as traditional system of medicine in India. Many unknown and lesser known plants are used are folk and tribal medical practices in India. The medicinal values of these plants are not known to the scientific world. The present work deals with hepatoprotective activity of powdered leaves of *Adenanthera pavinia* against paracetamol and ethanol induced liver damage in rats. *Adenanthera pavonina* is a species of leguminous tree, used for its timber, also known as Barbados pride**.** This tree is useful for nitrogen fixation, and it is often cultivated for forage, as an ornamental garden plant or urban tree, and as a medicinal plant.

MATERIALS AND METHODS

Collection and authentication of plant material

The leaves of selected plant were collected from in and around the local area of village Ingoriya, Ujjain, Madhya Pradesh and were identified and authenticated by safia college Bhopal.

Preparation of crude drug for extraction

The plant leaves were used for the preparation of the extract. The plants leaves were collected and dried under shade and then coarsely powdered with the help of mechanical grinder. The powder was passed through sieve No. 40 and stored in an airtight container for the extraction. (1)

Extraction of dried leaves

The collected, cleaned and powdered leaves of *Adenanthera pavinia* were used for the extraction purpose. 500 gm of powdered material were evenly packed in the soxhlet apparatus. It was then extracted with ethanol. The solvents used were purified before use. The extraction method used was continuous hot percolation and carried out with various solvents, for 72 hrs. The extracts were concentrated by vacuum distillation to reduce the volume to 1/10; the concentrated extracts were transferred to 100 ml beaker and the remaining solvent was evaporated on a water bath. Then they were cooled and placed in a desiccator to remove the excessive moisture. The dried extracts were packed in airtight containers and used for further studies. (2)

Physicochemical evaluation

The dried and stored powder of plant leaves were subjected to standard procedure for the determination of various physicochemical parameters

PHARMACOLOGICAL STUDIES

Acute toxicity studies

Acute toxicity study was carried out according to Organization for economic co-operation and development (OECD) guideline [Guideline 425, up and down method (1 animal used)] for oral acute toxicity study.

Experimental Design for Paracetamol induced hepatotoxicity

Test compounds

The Ethanol extract of leaves of *Adenanthera pavinia* and standard drug silymarin (25 mg/kg bw p.o.) were used.

Chemicals and reagents: Paracetamol and silymarin.

Experimental design

Rats of either sex were divided into four groups of six animals in each group. (*n* = 6) (3-4)

Group I: Received water (5 ml/kg, p.o.) for 9 days once daily, and served as normal control.

Group II: Received water (5 ml/kg, p.o.) for 9 days once daily and paracetamol (1 g/kg, p.o.) on the 7th day.

Group III: Received standard drug silymarin (25 mg/kg, p.o.) for 9 days once daily and paracetamol (1 g/kg, p.o.) on the 7th day.

Groups IV: Ethanol extract (500 mg/kg) for 9 days once daily and paracetamol (1 g/kg, p.o.) on the 7th day.

On the last day, functional parameters *i.e.* onset of sleep and duration of sleep, morphological parameters *i.e.* liver weight and

liver volume, serum marker enzyme parameters *i.e.* Serum glutamic Pyruvate transaminase (SGPT), Serum Glutamic Oxaloacetic Transaminase (SGOT) and Alkaline phosphatase (ALP), (5) biochemical parameters *i.e.* Total bilirubin and Total protein (6) were analyzed according to the reported methods.

Histopathological studies (7, 8)

The animals were sacrificed and the liver of each animal was isolated and was cut into small pieces, preserved and fixed in 10% formalin for two days. Then the liver piece was washed in running water for about 12 hours to remove the formalin and was followed by dehydration with isopropyl alcohol of increasing strength (70%, 80% and 90%) for 12 hours each. Then finally dehydration is done using absolute alcohol with about three changes for 12 hours each.

Dehydration was performed to remove all traces of water. Further alcohol was removed by using chloroform and chloroform removed by paraffin infiltration. The clearing was done by using chloroform with two changes for 15 to 20 minutes each. After paraffin infiltration the liver pieces were subjected to automatic tissue processing unit. Embedding in paraffin vaccum hard paraffin was melted and the hot paraffin was poured into L shaped blocks. The liver pieces were then dropped into the molten paraffin quickly and allow cooling. Sectioning the blocks were cut using microtome to get sections of thickness of 5 microne. The sections were taken on a micro slide on which egg albumin i.e., sticking substance.

Statistical analysis

The results of the study were expressed as mean ± SEM. ANOVA was used to analyze and compare the data, followed by Dunnet's test for multiple comparisons.The value of probability less than 5 % ($P < 0.05$) was considered statistically significant. (9)

RESULT

Acute toxicity study

The results showed that there was no mortality amongst the graded dose groups of animals and they did not show any toxicity or behavioral changes at a dose level of 5000 mg/kg. This finding suggests that the extracts were safe in or non toxic to rats and belonging to category 5 (>5000).

Hepatoprotective activity of plant extract on paracetamol induced hepatotoxic rats

In paracetamol treated animals, the volume of the liver was significantly increased, but it was normalized in EthanolExtract (500 mg/kg, po)

and silymarin treated groups of animals. A significant reduction in liver volume supports hepatoprotective effects of selected extract. The results are shown in Table 1.

Effect of plant extract on physical parameters:

Liver weight

In paracetamol treated rats, enlargement of liver was observed, which was evident of increase in the liver weight. The groups treated with EthanolExtract (500 mg/kg, p.o) and silymarin showed significant restoration of liver weight nearer to normal

Liver volume

In paracetamol treated animals, the volume of the liver was significantly increased, but it was normalized in EthanolExtract (500 mg/kg, po) and silymarin treated groups of animals. A significant reduction in liver volume supports hepatoprotective effects of selected extracts. The results are shown in Table 2.

Effect of selected plant extracts on serum marker enzyme levels of paracetamol induced hepatotoxic rats

There was a significant elevation in the levels of serum marker enzymes like SGOT, SGPT and ALP in hepatotoxicated animals. Pretreatment with EthanolExtract (500 mg/kg, po) and silymarin (25 mg/kg, po) exhibited an ability to counteract the hepatotoxicity by decreasing the elevated level of serum marker enzymes. The results are shown in Table 3.

Effect of plant extract on biochemical parameter in paracetamol induced hepatotoxicity rats.

In paracetamol treated groups, there was a significant increase in total bilirubin and significant reduction in total protein. Whereas in EthanolExtract (500 mg/kg, po) and silymarin treated groups, significant reduction in total bilirubin and significant increase in total protein was observed. The results are shown in Table and Figure 4.

CONCLUSION

In present study, coarsely powdered shade dried plant material selected for the hepatoprotective activity was subjected for extraction. The extract after concentration is first subjected for preliminary physical and phytochemical investigation to assess the quality of plant material and understand the nature of active constituents present. Pretreatment with ethanol extract (500 mg/kg, p.o.) and silymarin (25 mg/kg) significantly increases the onset of sleep in the experimental animals while decreases the duration of sleeping time, which is an indirect

evidence of their hepatoprotective effect. The groups treated with ethanolextract (500 mg/kg, p.o) and silymarin (25 mg/kg, p.o) showed significant restoration of liver weight and liver volume nearer to normal. The isolated livers from the hepatotoxicant treated animals exhibited increase in liver weight and liver volume. Indeed, extracts treated animals exhibited decrease in the values of above physical parameters as an indication of hepatoprotection. (11). Finally based on improvement in serum marker enzyme levels, physical parameters, functional parameters and histopathological studies, it is concluded that EthanolExtract possesses hepatoprotective activity and thus supports the traditional application of the same under the light of modern science.

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TABLES AND FIGURE

Table1: Effect of plant extract on functional parameters in paracetamol induced hepatotoxic rats.

S.No.	Treatment/ Dose	Onset of sleep (Sec.)	Duration of sleep (Min.)
1	Normal	170.0 ± 2.06	110.2 ± 2.80
2	Induced (Paracetamol)	98.4 ± 6.28*	255.8 ± 5.90*
3	Standard (Silymarin)	176.6 ± 4.48***	140.2 ± 4.49***
4	EthanolExtract (500mg/kg)	121.5 ± 4.80**	228.2 ± 5.02**

Values are mean ± SEM, n = 6. (One way ANOVA Followed by Dunnette multiple comparisons test). Statistically significance of ** P<0.01, *** P<0.001, when compared with Paracetamol induced group and * P<0.05, when compared with normal group.

Table: 2 Effect of plant extract on physical parameters in paracetamol induced hepatotoxic rats.

S.No.	Treatment/ Dose	Liver weight (wt./100gm b.w.)	Liver Volume
1	Normal	6.84 ±0.06	6.97 ± 0.05
2	Induced (Paracetamol)	8.84 ± 0.48*	9.02 ± 0.49*
3	Standard (Silymarin)	7.02 ± 0.46***	7.36 ± 0.49***
4	EthanolExtract (500mg/kg)	8.68 ± 1.26**	8.89 ± 1.28**

Values are mean ± SEM, n = 6. (One way ANOVA Followed by Dunnette multiple comparisons test). Statistically significance of ** P<0.01, *** P<0.001, when compared with paracetamol induced group and * P<0.05, when compared with normal group.

Table 3: Effect of plant extract on serum enzyme parameter in paracetamol induced hepatotoxicity rats.

S.No.	Treatment/ Dose	SGPT U/L	SGOT U/L	ALP U/L
1	Normal	62.0 ± 3.71	168.04 ± 2.80	190.0 ± 8.01
2	Induced (Paracetamol)	154.8 ± 8.64*	248.4 ± 9.24*	360.20 ± 8.82*
3	Standard (Silymarin)	86.86 ± 8.63***	176.16 ± 8.17***	166.35 ± 4.27***
4	EthanolExtract (500mg/kg)	131.28 ± 8.84**	245.28 ± 8.44**	260.0 ± 7.89**

Table: 4 Effect of plant extract on biochemical parameter in paracetamol induced hepatotoxic rats.

S.No.	Treatment/ Dose	Total Bilirubin mg/dl	Total Protein gm/dl
1	Normal	0.38 ± 0.06	9.57 ± 0.24
2	Induced(Paracetamol)	5.42 ± 0.11*	5.42 ± 1.46*
3	Standard (Silymarin)	0.45 ± 0.82***	9.21 ± 1.26***
4	EthanolExtract (500mg/kg)	0.68 ± 0.62**	6.28 ± 0.12**

Values are mean ± SEM, n = 6. (One way ANOVA Followed by Dunnette multiple comparisons test). Statistically significance of ** P<0.01, *** P<0.001, when compared with paracetamol induced group and * P<0.05, when compared with normal group.