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Research Article

Phytochemical Investigation and Hepatoprotective Activity of Ripe Fruits of *Pithecellobium dulce* in *Albino Rats*

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Abstract: In the present investigation the plant *Pithecellobium dulce* Benth, (Leguminosae) commonly known as Manila Tamarind is found to exhibit a wide range of pharmacological properties. The present study was designed to evaluate the hepatoprotective activity of the ripe fruits of this herb has been demonstrated against alcohol and paracetamol in rats. Phytochemical analysis of the fruit extract revealed the presence of alkaloids, flavonoids, glycosides, saponins, phytosterols, and triterpenoids. Results of this study indicate possible protective effect of ripe fruit extract of *Pithecellobium dulce* on alcohol induced toxicity and paracetamol induced toxicity in liver.
Keywords: *Pithecellobium dulce*, AST, ALT, ALP, SGOT, SGPT, AEPD and EEPD.

INTRODUCTION

The liver is the second-largest organ of the body and the largest gland. The normal adult liver weighs about 1.5 kg; representing 2.5% of the body weight. It is involved with almost all the biochemical pathways related to growth, fight against disease, nutrient supply, energy provision and reproduction [1, 2]. The liver is expected not only to perform physiological functions but also to protect against the hazards of harmful drugs and chemicals. Inspite of tremendous scientific advancement in the field of hepatology in recent years, liver problems are on the rise. Jaundice and hepatitis are two major hepatic disorders that account for a high death rate [3]. Presently only a few hepatoprotective drugs and that too from natural sources (there is not a single effective allopathic medication), are available for the treatment of liver disorders.

Pithecellobium dulce Benth., belonging to the family of *Leguminosae* (*subfamily Mimosoideae*) locally known as Jangal Jalebe and with English name as Manila Tamarind, is a small to medium sized, evergreen, spiny woody legume tree up to 18 m height, it is native of tropical America and also found throughout India and Pakistan. The generic name refers to the curly pod that mimics an ape's earring (pithekos-ellobium) and the species name "dulce" refers to the sweet pod. *Pithecellobium dulce* is the only species among 100-200 species in the genus and has become widespread outside its origin [4, 5]. The plant is well known for its edible fruits and they have been consumed for various ailments in a traditional manner. The fruits are linear, curved legumes (Pods) that range in length from 10 to 13 cm. The pod splits along both margins. The legumes may contain 5 to 12 seeds which are reddish brown to black in colour. The fruits turn from green to reddish brown when they ripen. The pod fragments can be eaten raw or made in to a drink for its nutritive as well as therapeutic values but still most of the chemical constituents of the pods are remained unexplored and underutilized [6]. Various parts of the tree such as bark, leaves and seeds have been studied for their medicinal properties [7].

Survey of literature shows that hepatoprotective studies on *P. dulce* fruit against alcohol and paracetamol induced toxicity have not been carried out in the past. Hence, the present study was carried out to determine the hepatoprotective effect of ripe fruit extract of *P. dulce* on alcohol induced toxicity and paracetamol induced toxicity.

MATERIALS AND METHODS

Collection of plant materials

Pithecellobium dulce fresh ripe fruits were collected from Bhongir Village at Nalgonda Dist, Telangaana state, India, in the month of April 2013. The plant were identified and authentified by Botanical Survey India (BSI), Hyderabad and the voucher samples are kept in the BSI herbarium for reference (BSI/DRC/12-13/Tech. /234).

Preparation of extracts

Fresh fruits of *Pithecellobium dulce* were collected and dried under shade at room temperature for few days. The dried fruits were powdered and passed

through sieve (10/60) for coarse powder. This Shade-dried powder of ripe fruits of *P. dulce* was extracted successively with (1:6) Petroleum ether, Chloroform, Ethanol in a soxhlet apparatus for about 18 hours and aqueous extract was prepared by maceration process. After 24h the extract was filtered and the filtrate dried in a hot air oven at 45°C till solid/semisolid mass, was produced. After drying, the respective extracts were weighed and percentage yield of extracts were determined.

Animals

Albino Wistar strain rats weighing between 150-200gm and adult Swiss albino mice (20-25g) of either sex, obtained from Srivenkateshwara enterprises, Bangalore were used for the hepatoprotective study. Throughout the study animals were maintained at normal laboratory conditions and were given standard animal feed. Before performing the experiment the ethical clearance was obtained from institutional animal ethics committee (IEAC). IAEC No.-769/2010/CPCSEA.

Chemicals and Other Reagents

Bradford reagent, protein estimation kit, disodium hydrogen phosphate buffer (Na2HPO4) or Tris buffer solution, sodium nitrate(NaNo3), ethylene diamine tetra acetic acid (EDTA), glacial acetic acid, hydrogen peroxide (H2O2), nicotinamide adenine dinucleotide reduced (NADH), sodium dihydrogen phosphate (NaH2PO4), Bromocresol green, ethanol, methanol and other reagents, Drugs(Silymarin and paracetamol).

Phytochemical investigations

The dried powder of fruits of *Pithecellobium dulce* was subjected to systematic phytochemical screening. The extracts were subjected for phytochemical investigation by Qualitative chemical identification tests [8-12].

Determination of acute toxicity

The acute toxicity of *Pithecellobium dulce* extract was determined by using albino mice (20-25g) those maintained under standard husbandry conditions. The animals were fasted 12h prior to the experiment and acute oral toxicity as per OECD guideline no. 420 was determined.

Animals were administered with single dose of 2000 mg/kg extract and observed individually with special attention given during the first 2 hours and for a total of 8 days. During this period the mortality and/or the moribund status of the animals were noted [13].

Experimental Design for Hepatoprotective Activity

Albino rats (Wistar Strain) of either sex weighing 150-200g were selected and divided into seven groups of six animals each. This experimental design for both cases like alcohol and paracetamol induced heptotoxicity.

Group I: Vehicle treated rats were kept on normal diet and served as control for 15 days.

Group II: Rats orally received 30% alcohol (1.5 ml/rat / twice a day) for 15 days.

Group III: Rats orally received Silymarin (25 mg/kg b.w/day) and alcohol as group II, for 15 days.

Group IV: Rats orally received *AEPD* (200 mg/kg b.w/day) and alcohol as group II, for 15 days.

Group V: Rats orally received *AEPD* (400 mg/kg b.w/day) and alcohol as group II, for 15 days.

Group VI: Rats orally received *EEPD* (200 mg/kg b.w/day) and alcohol as group II, for 15 days.

Group VII: Rats orally received *EEPD* (400 mg/kg b.w/day) and alcohol as group II, for 15 days.

The blood was collected from the retro orbital plexus of the rats of all groups 24 h after the last dose administration, under light anesthetic ether. The blood samples are centrifuged at 3000 rpm/30 min to separate the serum. The serum was analyzed for various biochemical parameters such as AST, ALT, ALP, ALB, BIT and BID. Their percentage protection was calculated, using Autoanalyser. Liver was dissected out and subjected for morphological study such as liver weight and liver volume of each animal. Further the liver was placed in 10% formalin solution for histopathological study.

Note: Follow the same experimental design for paracetamol induced toxicity in place of alcohol [14].

Estimation of serum bio-chemical parameters

The principle, details of the kits and methodology used in the estimation of the various bio-chemical parameters by Autoanalyser in the present investigation are as follows [15-18]

- Estimation of AST
- Estimation of ALT
- Estimation of ALP
- Estimation of bilirubin
- Estimation of albumin (Bromocresol green, end point assay)

Histopathological studies

On the 16th Day, after sacrification of rats by cervical dislocation, liver samples were dissected out and washed immediately with ice-cold saline to remove as much blood as possible. A portion of liver tissue in each group was preserved in 10% formaldehyde solution for histopathological studies. Haematoxylin and eosin were used for staining and later the microscopic slides of the liver tissue were photographed at magnification 40X.

Statistical Analysis

The statistical analysis was carried by one way ANOVA followed by Dunnet's multiple "t" test. p values < 0.05 (95% confidence limit) was considered statistically significant, using software Graph Pad Prism5.

RESULTS AND DISCUSSION

The % yields of Ethanolic and Aqueous extracts are 24.35 and 15.42 respectively. The preliminary phytochemical analysis of petroleum ether, chloroform, ethanolic, and aqueous extracts of fruits of *Pithecellobium dulce* revealed the presence of various phytoconstituents which are presented in Table No.1

Table1: Phytochemical evaluation of different extracts of *Pithecellobium dulce*

Sl. No.	Tests	Petroleum ether	Chloroform	Ethanol	Water
1	Alkaloids	-	-	+	+
2	Carbohydrates	-	-	+	+
3	Flavonoids	-	-	+	+
4	Fied oils	+	+	-	-
5	Saponins	-	-	+	+
6	Sterols	+	+	+	+
7	Tannins	-	-	+	+
8	Glycosides	-	-	+	+

Acute toxicity test

Acute toxicity study was conducted for Aqueous and Ethanolic extracts fruits of *Pithecellobium dulce* as per OECD guidelines 420 using albino mice. Each animal was administered aqueous and ethanolic extracts by oral route. 5 mice were orally administered with 2000mg/kg of extract Observed for 8 days, no mortality was observed with 2000mg/kg. Based on the above study $1/10^{th}$ (200mg/kg) and $1/5^{th}$ (400mg/kg) doses were selected.

Hepatoprotective activity

Alcohol and paracetamol treatments in rats resulted in enlargement of liver which was evident by increase in the liver weight and volume. The groups treated with Silymarin showed good restoration of liver weight and liver volume where as test groups treated with EEPD and AEPD showed significant effect on liver weight and liver volume compared to toxic control group.

Table 2: Liver weight and liver volume in alcohol and paracetamol induced hepatotoxic rats

Group	Alcohol		Paracetamol	
	Liver weight gm/100gm	Liver volume ml/100gm	Liver weight gm/100gm	Liver volume ml/100gm
Control	3.68±0.08	6.9±0.15	3.11±0.11	5.6±0.15
Toxic control	4.8±0.10	8.9±0.05	4.55±0.18	9.65±0.18
Silymarin	3.81±0.08**	7.2±0.12**	3.23±0.12**	6.06±0.10**
EEPD(200mg)	4.21±0.13*	7.58±0.11**	3.7±0.10**	7.05±0.08**
EEPD(400mg)	4.11±0.13**	7.48±0.10**	3.5±0.16**	6.57±0.08**
AEPD(200mg)	4.23±0.20*	7.63±0.14**	3.85±0.10**	7.25±0.06**
AEPD(400mg)	4.13±0.09**	7.51±0.04**	3.58±0.11**	6.76±0.08**

Values are expressed as mean ± SEM; n=6

* p≤0.05, **p≤0.01 and ***p<0.001, Comparison with toxic control

Bio chemical parameters

Effect of AEPD and EEPD fruits on AST, ALT & ALP levels in alcohol and paracetamol induced hepatotoxic rats

Rats treated with alcohol and paracetamol developed a significant hepatic damage observed as elevated serum levels of hepatospecific enzymes like AST, ALT and ALP when compared to normal control.

Treatment with Silymarin had showed good protection against alcohol and paracetamol induced toxicity to liver. Groups treated with ethanolic and aqueous extracts of fruits of *Pithecellobium dulce* showed significant effect which can be comparable with toxic control.

Dunnet's test indicates a significant reduction in elevated serum enzyme levels with extract treated animals compared to toxic control animals.

Table 3: AST, ALT & ALP levels in alcohol and paracetamol induced hepatotoxic rats

Group	Alcohol			Paracetamol		
	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
Control	113.15±5.05	106.11±4.13	130.75±3.97	109.20±2.66	96.49±5.35	96.68±5.21
Toxic control	396.93±0.93	391.77±7.87	378.62±3.59	388.98±2.49	302.46±7.49	379.94±4.79
Silymarin	148.22±1.79**	135.39±6.88**	181.04±11.49**	123.86±4.67**	106.83±2.21**	128.64±1.87**
EEPD(200mg)	205.24±3.56**	190.47±8.11**	220.35±10.05**	178.46±3.72**	171.92±1.83**	191.19±11.51**
EEPD(400mg)	177.30±9.56**	162.25±3.67**	206.69±2.18**	152.71±2.23**	141.81±6.41**	156.16±6.20**
AEPD(20mg)	220.08±15.80**	200.78±5.67**	242.27±6.05**	194.62±2.19**	193.6±1.6**	201.75±8.67**
AEPD(40mg)	185.24±6.06**	178.91±9.05**	224.52±11.91**	161.93±2.30**	150.93±1.83**	165.38±5.66**

Values are expressed as mean ± SEM; n=6

* p≤0.05, **p≤0.01 and ***p<0.001, Comparison with toxic control

Effect on total bilirubin

The total bilirubin concentration was found to increase in animals with liver damage by alcohol and paracetamol. In standard group, Silymarin administration reduced total bilirubin and animals treated with EEPD and AEPD have exhibited dose dependent significant reduction in total bilirubin compared to toxic control group.

Effect on direct bilirubin

Alcohol and paracetamol treated groups significantly elevated direct bilirubin concentration in animals by inducing hepatic damage compared to normal animals. But treatment with standard drug Silymarin showed good reduction in direct bilirubin concentration. Groups treated with EEPD and AEPD significantly reduced direct bilirubin level in respective groups.

Effect on albumin

Induction of liver damage by administration of alcohol and paracetamol significantly reduced serum albumin level in positive control group animals when compared to normal animals. But the treatment with Silymarin has shown significant increase while EEPD and AEPD have shown dose dependent increase in serum albumin level compared to toxic control group.

Table 4: BIT, BID & ALB levels in alcohol and paracetamol induced hepatotoxic rats

Group	Alcohol			Paracetamol		
	BIT (mg/dl)	BID (mg/dl)	ALB(g/dl)	BIT (mg/dl)	BID (mg/dl)	ALB(g/dl)
Control	0.63±0.09	0.30±0.06	4.64±0.22	0.66±0.03	0.29±0.01	4.67±0.25
Toxic control	2.04±0.15	1.98±0.17	2.18±0.11	2.95±0.48	2.08±0.32	2.38±0.07
Silymarin	0.75±0.07**	0.41±0.02**	4.38±0.47**	0.7±0.01**	0.39±0.02**	4.41±0.43**
EEPD(200mg)	1.03±0.05**	0.82±0.05**	3.81±0.29**	1.16±0.03**	0.96±0.01**	3.75±0.04**
EEPD(400mg)	0.92±0.05**	0.68±0.05**	4.13±0.14**	0.99±0.05**	0.67±0.01**	4.10±0.16**
AEPD(200mg)	1.13±0.03**	1.08±0.11**	3.405±0.30**	1.24±0.02**	1.07±0.01**	3.62±0.29**
AEPD(400mg)	0.99±0.04**	0.75±0.04**	4.03±0.02**	1.08±0.01**	0.79±0.01**	3.95±0.45**

Values are expressed as mean ± SEM; n=6

* p≤0.05, **p≤0.01 and ***p<0.001, Comparison with toxic control.

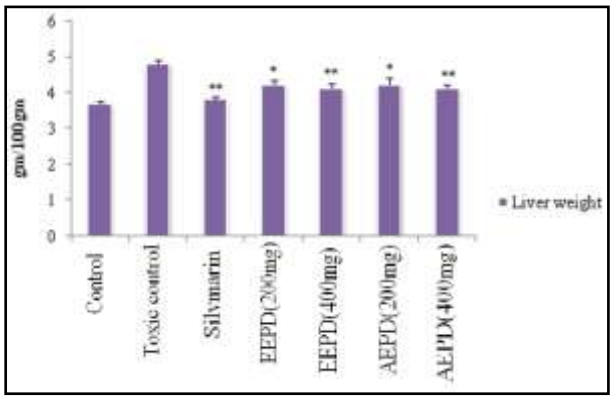


Fig. 1: Liver weight in alcohol and paracetamol induced hepatotoxic rats

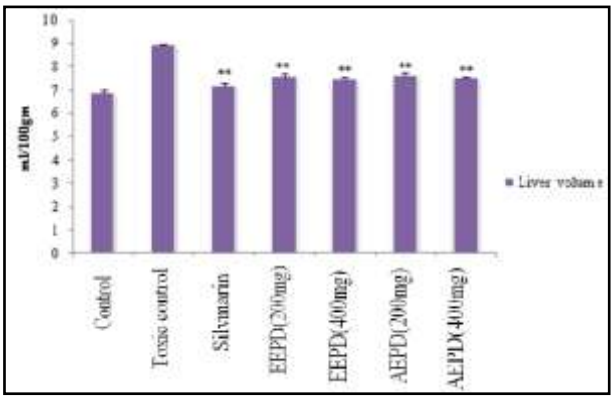


Fig. 2: Liver volume in alcohol and paracetamol induced hepatotoxic rats

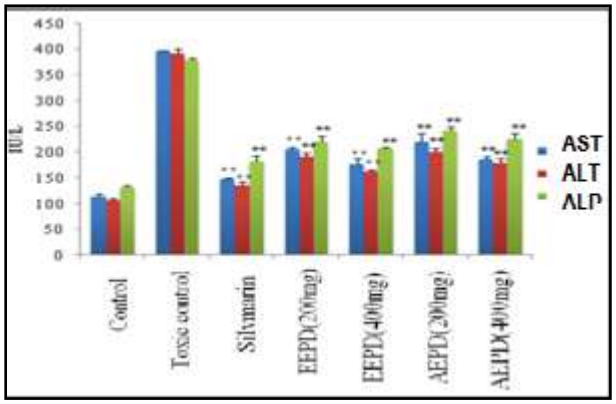


Fig. 3: AST, ALT & ALP levels in alcohol and paracetamol induced hepatotoxic rats

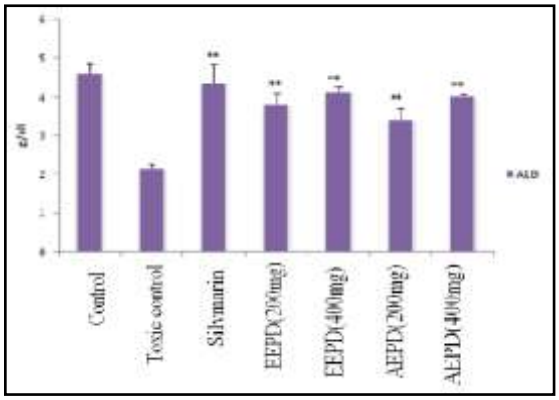


Fig. 5: ALB levels in alcohol and paracetamol induced hepatotoxic rats

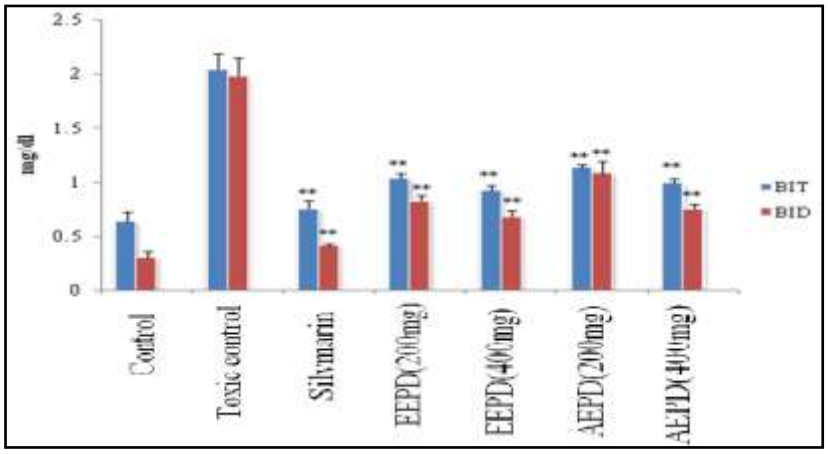
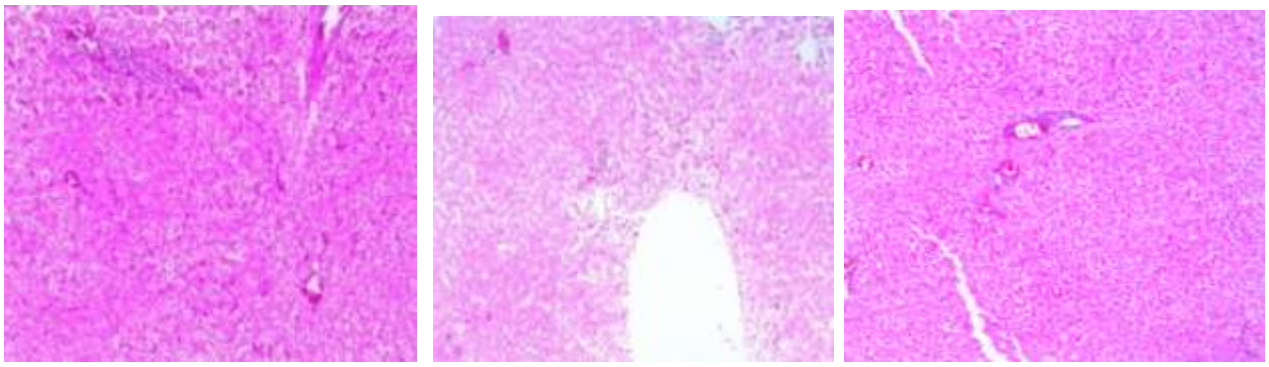
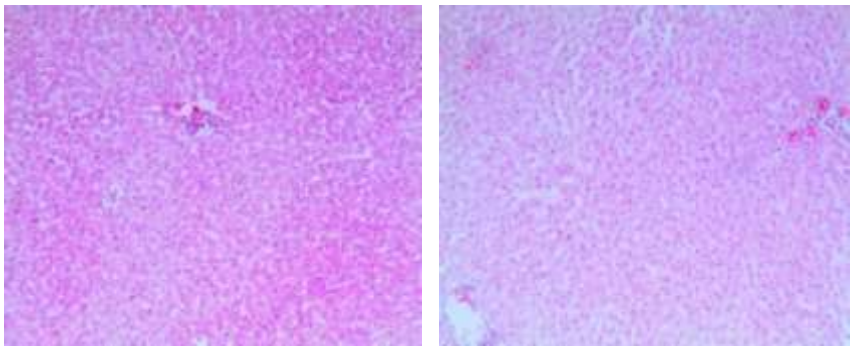


Fig 4: BIT & BID levels in alcohol and paracetamol induced hepatotoxic rats



Normal control **Alcohol/Paracetamol treated** **Silymarin + Alcohol/Paracetamol treated**



EEPD fruit (400mg) + Alcohol/Paracetamol **AEPD fruit (400mg)+Alcohol/Paracetamol**

Fig. 6: Histopathological studies of the liver in alcohol and paracetamol induced hepatotoxic rats

CONCLUSION

In present study, it is concluded that both the ethanolic and aqueous extracts of fruits of *Pithecellobium dulce* exhibits significant hepato-protective activity against alcohol and paracetamol induced hepatotoxicity in rats. And phytochemical constituents like tannins, flavonoids saponins and alkaloidal compounds are already reported for their hepato-protective activity and both the extracts contained the above mentioned constituents. However, the exact mechanism responsible for activities is currently unclear. Therefore, further investigations need to be carried out to isolate and identify specific compounds present in the plant extract responsible for these activities and exact mechanism.

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