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Original Research Article

Preliminary Pharmacognostical & Pharmacological Studies on The Pisonia Grandis R. Br. on Rats

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Abstract:
The plant *Pisonia Grandis* R.Br. belongs to the family Nyctaginaceae was taken up for the study by me to screening and gave a report on the possible Pharmacognostical and Pharmacological studies. The various ash values and extract values are determined. In Phytochemical screening showed the nature of the chemical constituents such as Alkaloids, Carbohydrates, Phenolic compounds, Protein and Amino acids, Saponins, Sterols, Flavonoids and Tanins present in the leaf extract of Pisonia Grandis R.Br. The LD50 of the aqueous and alcoholic extracts of Pisonia Grandis R.Br. leaves was found to be 2500 mg/kg. Therefore ED50 was calculated as 250 mg/kg. The Hepatoprotective activity was carried out by the Paracetamol induced model in rats. Administration of the aqueous and alcoholic extracts of Pisonia grandis R.Br. leaves showed significant hepatoprotective activity which was compared with the standared drug silymarin. The effect was more pronounced with the aqueous extracts Pisonia grandis R.Br., this may be probably due to the high contents of flavonoids.
Keywords: *Pisonia grandis* R.Br , Hepatoprotective, Histopathology, Paracetamol.

Introduction

India s a vault of home grown medications confirmations of spices are being utilized in the treatment of sicknesses for renewing body delivery system in practically all old progress. Plants have customarily filled in as men most significant weapons against microorganism. Home grown meds are generally utilized by all segment of the local area, whether straightforwardly as society cures or the medicaments of the different native framework as well as in current medications [1]. Therapeutic plants are our nearby legacy with worldwide significance. All around the world plants were utilized as principal wellspring of medication by predecessors. India has an old legacy of customary medication. India customary meds depend on different framework including Ayurveda, Unani, Sidha, Yoga and Naturopathy. The most established and the most broadly rehearsed is the job restorative framework in India [2]. The world wellbeing associations (WHO) gauge that around 80%of the populace living in the emerging nations depend practically restrictive on conventional meds for there essential medical care needs. In practically the customary medication, the restorative plants a significant job and constituents the foundation of the conventional medication. India may be the biggest maker of restorative spices and is called greenhouse of the world. Restorative spices have been utilized for millennia in some structure under, the native

arrangement of medication like Ayurveda, siddha and unani. In earth around 3.6 lakhs types of therapeutic plants are utilized, among these 1.4 lakhs species are in India. In the most recent study shows that 70000 plants are utilized in customary arrangement of medication. Individuals from one side of the planet to the other, in future will favor treatment in the customary arrangement of meds [3]. The justification behind this is that disregarding irrefutable realities in regards to the viability of current medication. Certain downsides have limited its future possibilities. The inconvenience of current medication have driven there searchers to search for elective framework particularly the antiquated and customary drugs [4-5]. Development of the conventional arrangement of medication in India:- The expression "Conventional medication" alludes to approach to securing and reestablishing wellbeing existed before the appearance of current medication. As term infers, these ways to deal with wellbeing have a place with the customary of every nation and have been given over from one age to another. Conventional framework overall has needed to address the issues of the neighborhood networks for some nations practically speaking [6]. The expression "Customary medication" alludes to the accompanying parts: needle therapy, conventional birth orderlies, mental healers and home grown medication. Customary medication has kept up with its prevalence in various Asian nations, like China, India, Japan and Pakistan [7-8]. Restorative plants are most seasoned realized medical care items. Their significance is as yet developing despite the fact that it different relying upon the ethnological, clinical and histological exploration and medication growing ,just when plant constituents are utilized straightforwardly remedial specialists yet additionally when they are utilized as fundamental materials for the combination of medication or as models for Pharmacologically dynamic compound [9-10].

Materials and Methods

Collection and authentication of plant material: The plants Pisonia grandis R.Br. were collected n from the Shivpuri, Madhya Pradesh, India. The plant material was taxonomically dentified from Dr. Bapi Ray Sarkar Associate Professor & HOD of Department of Pharmaceutical Technology University of North Bengal, Raja Rammohunpur, District-Darjeeling West Bengal, India Pin 734013.iAuthentication letter voucher no NBU/PHARM. TECH/002/2024

Preparation of extract: The leaves of *Pisonia grandis* R.Br. were dried under shade and then powdered with a mechanical grinder. The powder was passed through sieve no 40 and stored in an airtight container for further use [11-13].

Chemical:-

1. Petroleum ether (60-80^{0}C)
2. Ethanol (95% V/W)
3. Distilled water with chloroform(0.25%)

Extraction Procedure: The dried powdered leaves of *Pisonia grandis* R.Br. were defatted with petroleum ether (60-80^{0}C) in a Soxhlet apparatus. The defatted powder material thus obtained was further extracted with Ethanol. Aqueous extract was prepared by cold maceration process. The solvent removed by distillation under low pressure and the resulting semisolid mass was vacuum dried using rotary evaporator.

Preliminary Phytochemical Studies: The various phytochemical investigation was done, these were carbohydrates and glycosides, alkaloids, phytosterols, fixed oils, gums and mucilages, proteins and free amino acid, phenolic compounds and tannins, flavonoids etc.

Physico-Chemical Evaluation: To determine the different ashvalues and extractive values Ayurvedic pharmacopoeia procedures were used as determination of Ash Values, extractive Values etc [14-17].

Pharmacological Studies

Procurement of Animals: Wister rats (150-200gm) of either sex and of approximately the same age, used in procured from listed suppliers

of from CPCSEA listed of Shri Ramnath Singh college of Pharmacy, Gormi, Bhind, Gwalior, were used for the study. The animals are exposed to alternate cycle of 12 hrs of darkness and light 12hrs. The animals were fasted for at least 12 hrs before the onset of each activity. The animals were acclimatized to the laboratory condition for 1 week before starting the experiment. All experimental were performed during morning according to OECD guidelines for care of laboratory animals and the ethical guideline for investigation of experimental pain in conscious animals [18-21].

Acute Toxicity Studies: Organization for Economic co-operation and Development (OECD) regulates guideline for oral acute toxicity study. It is an international organization which works with the aim of reducing both the number of animals and the level of pain associated with acute toxicity testing.

Following are the main type of guideline followed by OECD

• Guideline 420, fixed dose procedure. (5 animals used)

• Guideline 423, acute toxic class. (3 animals used)

• Guideline 425, up and down method. (1 animal used)

Hepatoprotective Studies

Test compounds: The aqueous and alcoholic extracts of leaves of Pisonia grandis (250 mg/kg body weight) and standard drug silymarin (25 mg/kg body weight) were used.

Chemicals and reagents: The following chemicals were obtained from the indicated commercial Paracetamol, Silymarin (Promise labs, Sagar, M.P. India).

Experimental animal: Wister rats (100-150gm) used in the present studies were procured from CPCSEA listed of Shri Ramnath Singh college of Pharmacy, Gormi, Bhind, Gwalior, India. And Remanded by Institutional Animal Ethics Committee (IAEC) of College. The animals were fed with standard pellet diet (Animax Pharma Pvt. Ltd, Gwalior) and water ad libitum. All the animals were acclimatized for a week before use. The aqueous and alcoholic extracts of Pisonia grandis were dissolved in 10% gum acacia [22-25].

Paracetamol Induced Model: The rats were divided into 5 groups of 6 animals in each.

Group I: Received vehicle control Gum Acacia (5mg/kg.p.o)

Group II: Received Paracetamol Control (500 mg/kg p.o.) every 24h

Group III: Received Silymarin (25mg/kg) for 9 days simultaneously

Paracetamol 500mg/kg body weight every 24 h

Group IV: Received aqueous extract of leaves of Pisoniagrandis

R.Br. (250mg/kg) for 9 days simultaneously

Paracetamol (500mg/kg body weight) every 24 h

Group V: Received alcoholic extract of leaves of Pisoniagrandis

R.Br. (250 mg/kg) for 9 days simultaneously

Paracetamol (500mg/kg body weight) every 24 h

The blood samples were collected and allowed to clot and serum was separated by centrifuge at 2500rpm for 15 min and analyzed for various biochemical parameters.

Assessment of liver function: The liver was removed and weighed. Biochemical parameters i.e., Serum Glutamic Pyruvate Transaminase (SGPT), Serum Glutamic Oxaloacetic Transaminase (SGOT), Alkaline Phosphatase (ALP), Gamma-Glutamyl Transpeptidase (GGTP) were analyzed according to the reported methods [26].

Histopathological Studies: Liver slices fixed for 12 h in Bouin's solution were processed for paraffin embedding following standard micro technique.885µ section of the livers stained with alum haematoxyl in and eosin, were observed microscopically for histopathological changes i.e. normal liver, damaged and recovered liver were studied and compared.

Histopathological Report of Rat Liver Sample (Paracetamol Induced)

Histopathological studies: Rats were sacrificed, livers excised, cleaned with saline and they were transferred into 10% neutral

formalin solution, after one week liver tissues were dehydrated with a sequence of ethanol solutions, embedded in paraffin, cut n to 5µm section, stained with haematoxylin-eosin dye and then observed under photomicroscope [27-28].

Results and Discussion

The plant Pisonia grandis R.Br. is an ndigenous tree which was chosen for this study. The plant belongs to the family Nyctaginaceae. The scanty availability of nformation on this plant facilitates the study on it.

General characters of family Nyctaginaceae: Nyctaginaceae is a family of around 33 genera and 290 species of flowering plants, widely distributed in tropical and subtropical regions, with a few representatives in temperate regions. The family has a unique fruit type, called an "anthocarp", and many genera have extremely large (>100 µm) pollen grains.

The family has been almost universally recognized by plant taxonomists. The APG II system assigns it to the order Caryophyllales in the clade core eudicots. A more recent study by Douglas and Manos clarified the relationships among almost all of the genera in the family and demonstrated that a substantial diversification of herbaceous genera has occurred in arid North America. Many genera of Nyctaginaceae possess unusual characters.

Habitat: India, pacific oceans

Parts Used: Leaves, Bark

Medicinal uses: Inflammation, wound healing, algesia, ulcer

An evergreen tree, all parts glabrous or the young shoots minutely puberulous. Leaves ovate-oblong to oblong, 15-25cm. Long, usually unequal and obtuse at the base, on a 1.3-3.3cm. Long petiole, shortly acuminate to acute and blunt thick-membranous, glabrous or minutely puberulous in the axils of the nerves. Flowers dioeciously, in peduncled rather large puberulous cymes: perianth about 3mm. long funnel shaped, grayish puberulous. Fruits in squarrose stiff puberulous of glabrous panicles, similar to those of the former linear-club-shaped, about 1.3cm long, truncate, 5-cornered, the corners with a single row of sharp and little recurved acute prickles [29-31].

Figure 1: *Pisonia Grandis* R. Br. (whole plants, leaves)

The attempt is made to study the Pharmacognostical, phytochemical and pharmacological activities of leaves of Pisonia grandis. The study was divided nto three major parts i.e.

- Pharmacognostical studies
- Phytochemical screening
- Pharmacological studies.

Physicochemical analysis of crude drug: The physicochemical analysis of the leaves powder was carried out as total ash value, acid insoluble ash value, water soluble ash value and Sulphated ash value was found to be 3.0232% w/w, 2.6932% w/w, 1.3992 % w/w and 3.4236 % w/w respectively. The extractive values (water soluble and alcohol soluble extractive values)

were determined. The alcohol soluble extractive value (16.72% w/w) is more then that of water soluble extractive value (12.92% w/w). The water soluble and alcohol soluble values indicate the presence of amount of constituents which are water and alcohol soluble.

Table 1: Ash values of *Pisonia grandis* R.Br.

Name of the plant	Total ash (%w/w)	Acid insoluble ash (%w/w)	Water soluble ash (%w/w)	Sulphated ash (%w/w)
Pisonia grandis R.Br.	3.0232	2.6932	1.3992	3.4236

Table 2: Table Extractive values of *Pisonia grandis* R.Br.

Name of the plant	Alcohol soluble extractive values (%w/w)	Water soluble extractive values (%w/w)
Pisonia grandis R.Br.	16.72	12.92

Extraction of plant material: The dried and coarse powdered leaves of Pisonia grandis was extracted with solvents of increasing polarity successively by soxhlet apparatus whereas aqueous extract was obtained by cold maceration. The percentage yield of dried and coarse powdered leaves of Pisonia grandis was found to be 9.4%, 7.9%. 1.72% and 2.64% respectively with alcohol, aqueous, chloroform and petroleum ether. The percentage yield of the aqueous extract of leaves of Pisonia grandis was found to be lower than alcoholic extract.

Table 3: Data showing the percentage yield value of various extractions of *Pisonia grandis* R.Br. leaves

Plant name	Part used	Method of Extraction	Yield in Percentage w/w			
			Petroleum ether	Chloroform	Alcohol	Water
Pisonia Grandis R.Br.	Leaves	Continuous hot Percolation	2.64	1.72	9.4	7.9

Phytochemical screening: The various extract of the plant of Pisonia grandis were subjected to phytochemical screening which reveals the presence of various pharmacological active compounds showing in table below. Petroleum ether extract- Carbohydrates, fixed oils, Flavonoids Chloroform extract - Alkaloids and Flavonoids Alcoholic extract- Alkaloids, Phenolic compounds, Tanins, Protein and Amino acids, Saponins, Sterols and Flavonoids Aqueous extract–Carbohydrates and Tanins.

Table 4: Phytochemical studies of various extracts of Pisonia grandis R.Br. leaves

S.No	Plant Constituents	Identification Test	Pet. ether extract	Alcoholic extract	Aqueous extract
1	Alkaloids	Mayer's Test	-	+	-
		Hager's Test	-	+	-
		Dragendroff's Test	-	+	-
		Wagner's Test	-	+	-
2	Carbohydrate	Molisch Test	+	-	+
		Fehling Test	+	-	+
3	Glycosides	Borntrager's Test	-	-	-
		Legal's Test	-	-	-

4	Phenolic compounds	Fecl3 Test	-	+	-
		Lead acetate Test	-	+	-
5	Tanins	Fecl3 Test	-	+	+
		Alkaline reagent Test	-	+	+
		Lead acetate Test	-	+	+
6	Protein and aminoacids	Million's Test	-	+	-
		Ninhydrin Test	-	+	-
		Biuret Test	-	+	-
7	Saponins	Foam Test	-	+	-
8	Gums and Mucilage	Ppt. with 90% alcohol	-	-	-
9	Sterols	LibermannburchardTest	-	+	-
10	Fixed oils and fats	Spot Test	+	-	-
11	Flavonoids	Shinoda's Test	+	+	+
		Alkaline reagent Test	+	+	+

Pharmacological studies

Determination of LD50: The alcoholic and aqueous extracts of plant of *Pisonia grandis* R.Br. Were screened for acute toxicity study by OECD guideline for getting LD50. The results showed that the LD50 was found to be 2500 mg/kg. Therefore its ED50 is 250 mg/kg.

Table 5: Acute Toxicity study

Groups	No. of animals	Dose (mg/kg)	Result
1	3	5	No death
2	3	50	No death
3	3	300	No death
4	3	2000	1 death

LD50- 2500mg/kg ED50- 250mg/kg

Hepatoprotective studies (Paracetamol Induced Model): The result of biochemical parameters revealed that the elevation of enzyme level in Paracetamol treated group, are almost restored to the normal in level in the extract treated group.

Effect on SGPT: Aqueous and alcoholic extract of leaves of *Pisonia grandis* R.Br. showed significant Hepatoprotective activity as they reduced SGPT to 31.600±0.678 and 41.80±0.800 as compared to the hepatotoxic control 65.40±0.748. The results of treated with extract of Pisoniagrandis R.Br. are comparable with hepatotoxic control 65.40±0.748 which are tabulated in Table No. 7 and hence the extract of *Pisonia grandis* R.Br. showed significant Hepatoprotective activity.

SGPT is a cytosolic enzyme primarily present in the liver. The level of SGPT in serum increase due to leakage of this cellular enzyme into plasma by paracetamol induced hepatic injury. Serum level of SGPT can increase due to damage of the tissue producing acute hepatic necrosis. Since the extract of Pisoniagrandis R.Br. significantly reduced the level of SGPT, this suggests that the extract possesses significant Hepatoprotective activity.

Effect on SGOT: Aqueous and alcoholic extract of leaves of *Pisonia grandis* R.Br. showed significant Hepatoprotective activity as they reduced SGOT to 39.00±0.157 and 43.00±0.230 as compare to the hepatotoxic control 61.74±0.229. The results of treated with extract of Pisoniagrandis R.Br. are comparable with hepatotoxic control 61.74±0.229 which are tabulated in Table No. 7 and hence the extract of

Pisoniagrandis R.Br. showed significant Hepatoprotective activity.

SGOT is a mitochemical enzyme released from heart, liver, skeletal muscle and kidney. Liver toxicity elevated the SGOT level in serum due to the damage to the tissue producing acute necrosis such as viral hepatitis and acute cholestasis. Since the extract of *Pisonia grandis* R.Br. showed significantly reduced the level of SGOT. This suggests that the extract possesses significant hepatoprotective activity.

Effect on ALP: Aqueous and alcoholic extract of leaves of *Pisonia grandis* R.Br. showed significant Hepatoprotective activity as they reduced ALP to 65.00±0.707 and 97.80±0.583 as compared to the hepatotoxic control 125.6±0.878. The results of treated with extract of *Pisonia grandis* R.Br. are comparable with hepatotoxic control 125.6±0.878 which are tabulated in Table No. 7 and hence the extract of *Pisonia grandis* R.Br. showed significant Hepatoprotective activity.

In case of toxic liver, alkaline phosphatese levels are very high, which may be due to defective hepatic excretion or by increased production of ALP by hepatic parenchyma or duct cells. Since the extract of Pisoniagrandis R.Br Significantly reduced the level of ALP, this suggests that the extract possesses significant Hepatoprotective activity.

Effect on total bilirubin: Aqueous and alcoholic extract of leaves of *Pisonia grandis* R.Br. showed significant Hepatoprotective activity as they reduced total bilirubin to 0.616±0.018 and 0.620±0.017 as compared to the hepatotoxic control 0.656±0.200. The results of treated with extract of Pisonia Grandis R.Br. are comparable with hepatotoxic control 0.656±0.200which are tabulated in Table No. 7 and hence the extract of Pisonia Grandis R.Br. showed significant Hepatoprotective activity.

E. Effect on direct bilirubin

Aqueous and alcoholic extract of leaves of *Pisonia Grandis* R.Br. showed significant Hepatoprotective activity as they reduced direct bilirub into 0.300±0.005 and 0.366±0.008 as compared to the hepatotoxic control 0.408±0.006. The results of treated with extract of *Pisonia Grandis* R.Br. are comparable with hepatotoxic control 0.408±0.006 hence the extract of Pisonia Grandis R.Br. showed significant Hepatoprotective activity.

Table 6: Effect of aqueous and alcoholic extracts of leaves of *Pisonia grandis* on Paracetamol induced hepatotoxicity in rats

Design of treatment	Dose (mg/kg)	Total Bilirubin (%mg)	Direct Bilirubin (%mg)	SGOT U/l	SGPT U/l	Alkaline Phosphate U/l
Control	-	0.500±0.011	0.200±0.007	25.88±0.208	32.60±1.077	52.80±0.589
Hepatotoxic (Paracetamol)	500	0.656±0.200	0.408±0.006	61.74±0.229	65.40±0.748	125.6±0.878
Silymarin (Standard)	25	0.600±0.007	0.208±0.008**	27.00±0.22**	33.60±0.92**	55.20±0.860**
Alcoholic Extract	250	0.620±0.017	0.366±0.008***	43.00±0.230***	41.80±0.860***	97.80±0.583***
Aqueous extract	250	0.616±0.018	0.300±0.005***	39.00±0.157***	31.60±0.678***	65.00±0.707***

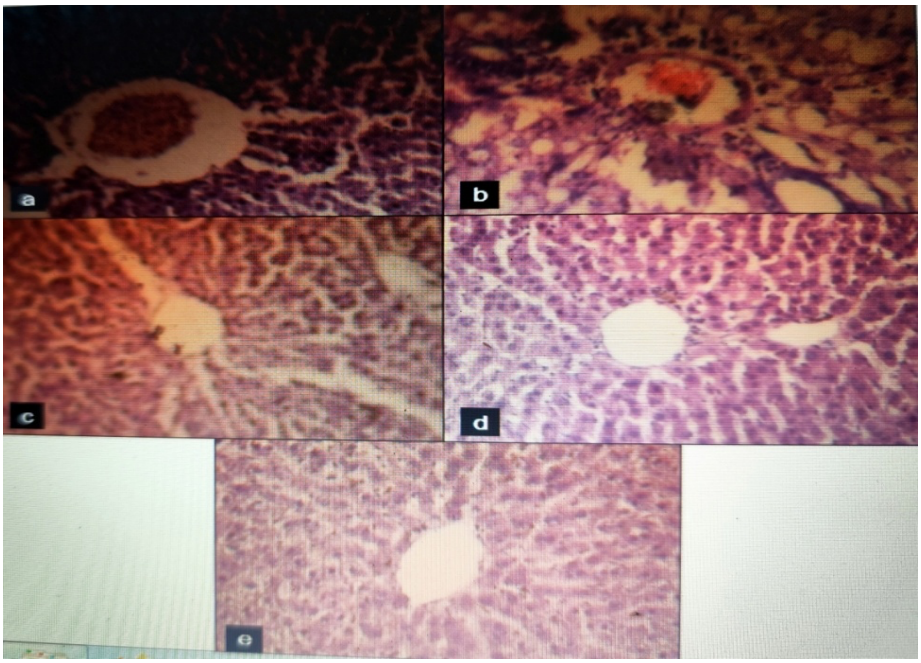

Figure 3: Histopathological studies

Histopathological studies: Photomicrographs of rat liver (hematoxylin and eosin) under low power (×100), (A) shows normal hepatic architecture; (B) shows hepatic necrosis; (C, D and E) show varying degrees of hepatic regeneration

a) Section shows liver: The architecture is maintained. The central veins, sinusoids and portal triads appear normal. The hepatocytes show moderate cytoplasm and round to oval nuclei.

b) Section shows liver: The architecture is normal. The central veins show mild congestion. The are normal. The portal triads show mild peri-portal inflammation composed of lymphocytes.

c) Section shows liver with dilated and congested central veins. The hepatocytes show feathery degeneration. The portal triads show peri-portal inflammation composed of lymphocytes.

d) Section shows liver: There is chronic venous congestion of the central veins. The hepatocytes show patchy necrosis. The portal triads show peri-portal inflammation composed of lymphocytes.

e) Section shows liver: The architecture is normal. There is mild congestion of the central veins. The hepatocytes are normal with moderate eosinophilic cytoplasm and round to oval nuclei. The portal triads are normal. There is no evidence of peri-portal inflammation.

Summary & Conclusion: The plant Pisonia Grandis R.Br. belongs to the family Nyctaginaceae was taken up for the study by me to screening and gave a report on the possible Pharmacognostical and Pharmacological studies. The various ash values and extract values are determined. In Phytochemical screening showed the nature of the chemical constituents such as Alkaloids, Carbohydrates, Phenolic compounds, Protein and Amino acids, Saponins, Sterols, Flavonoids and Tanins present in the leaf extract of Pisonia Grandis R.Br. The LD50 of the aqueous and alcoholic extracts of Pisonia Grandis R.Br. leaves was found to be 2500 mg/kg. Therefore ED50 was calculated as 250 mg/kg. The hepatoprotective activity was carried out by the Paracetamol induced model in rats. Administration of the aqueous and alcoholic extracts of Pisonia grandis R.Br. leaves showed significant hepatoprotective activity which was compared with the standared drug silymarin. The effect was more pronounced with the aqueous extracts

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