



## Evaluation of neuropharmacological profile of aqueous extract of *Pterocarpus marsupium*

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### Abstract

This study was conducted to evaluate the neuropharmacological profile of aqueous bark extract of *Pterocarpus marsupium* which is traditionally used for medicinal purpose. The general behaviour studies were assessed by the method of Irwin in wistar rats. The paralytic activity was analysed by inclined plane test in mice. Hypnotic potentiation was evaluated by thiopentone sodium induced sleeping time in albino mice. The analgesic activity was determined by using hot plate method in mice. The locomotor activity was assessed by using actophotometer in mice. The anticonvulsant activity was analysed by using maximal electroshock method by using an electroconvulsometer in rats. The statistical analysis of the data was done by anova and students 't' test. The data was expressed as Mean  $\pm$  SEM. *Pterocarpus marsupium* showed significant CNS inhibitory activity evidenced by decreased locomotor activity and hyperactivity towards external stimuli like touch and noise.

**Keywords:** pterocarpus marsupium, neuropharmacological, locomotor, Actophotometer, electroconvulsometer, CNS

### Introduction

Neuropharmacology is the study of how the drugs affect cellular function in the nervous system and the neural mechanisms through which they affect behaviour [1]. Drugs acting on the central nervous system were among the first to be discovered by the primitive humans and are still the most widely used group of pharmacological agents. The nervous system is an extraordinary complex communication system that can send and receive voluminous amounts of information simultaneously. Newer medicines are expensive and show adverse effects. Traditional Indian system of medicines have been used since olden days for the treatment of various neurological conditions like schizophrenia, alzheimers disease, Parkinsons disease and epilepsy [2]. In the nervous system the centrally acting drugs have therapeutic importance because of specific physiological and psychological effects. From the vast array of Materia Medica of the indigenous system so many plants have been recorded to have activity against CNS disorders and thus act as very useful remedies for the alleviation of human sufferings [3]. Number of investigations have been carried out on traditionally claimed medicinal plants to validate their efficacy. *Pterocarpus marsupium* is one such plant which is traditionally used for medicinal purpose. In today's life of stress and strain, there is a dire need for agents having neuroprotective and neuropharmacological activity enhancing learning and memory function of brain [4]. Stress involves complex biochemical, neural and immunological mechanisms and plays a crucial role in the progression of a variety of diseased states ranging from psychiatric disorders like depression and anxiety, immunosuppression, endocrine disorders including diabetics mellitus, male impotency and cognitive dysfunctions to cardiovascular disease, hypertension, peptic ulcers, migraine allergies, asthma, carcinoma, premature ageing, rheumatic diseases and ulcerative colitis.

Herbal drugs have been used since ancient times as Medicines for the treatment of a range of diseases. Herbal medicinal preparations are still popular in developing countries inspite of great advances observed in modern medicine in recent decade [5]. In general, plants were used in the form of concoction, infusions and decoction. With the development of scientific methods of purification and extraction, the active compounds responsible for the action can be identified [6]. *Pterocarpus marsupium* is commonly called as Bijasal or Indian Kino and almost all parts are used as medicine. Traditionally the decoction obtained from bark and resin is used as an astringent for ulcers, in diarrhoea, dysentery and as an abortifacient [7]. The heartwood is astringent, anti-inflammatory, anthelmintic, anodyne, used in elephantiasis, leukoderma, diarrhoea, rectalgia, cough, stomach ache, jaundice and in ulcers [8]. *Pterocarpus* has also been reported for pharmacological activities like CNS activity [9], antidiabetic [10], hepatoprotective [11], anti-inflammatory, antioxidant [12], anti-hyperglycemic [13], cardiotoxic [14] and antiproliferative [15]. The heartwood and root of the plant shows presence of chemical constituents [16] like carbohydrates, proteins, alkaloids, flavonoids, polyphenols, pentosans, pseudobaptogenin, pterosupin, pterostilbene, liquiritigenin, isoliquiritigenin, garbazol, 5-deoxy kaempferol, p- hydroxy benzaldehyde, b- eudesmol, erythrodiol- 3- mono acetate, marsupol, irisolodinone -7-rhamnopyranose. The present study was planned to Evaluate *Pterocarpus marsupium* for neuropharmacological actions along with other known actions like antidiabetic, anti-inflammatory etc. This will also help us to reveal any other possible side effects of the plant.

### Material and methods

The plant material (powered bark extract) was procured from Swastik formulations, Varanasi. Albino mice (Swiss)

weighing 25-35g and rats of wistar strain weighing 150-200g of either sex were used in the study. They were housed in polypropylene cages and were maintained under 12 hours dark/light cycle and fed with standard rat feed (Hindustan Lever Ltd) and water was provided ad libitum.

#### **Experimental method for evaluation of general behaviour in mice** <sup>[17]</sup>

In the experiment, albino wistar rats were randomly assigned into five groups of six Individuals each (n=6). Aqueous extract of *Pterocarpus marsupium* was administered for three groups at 100, 300, 500 mg/kg body weight respectively. The group four and five were administered Diazepam (5mg/kg) as drug control and 2% v/v tween 80 as vehicle control. The behavioural effects were assessed by the method of Irwin. After 2 hours of treatment the animals were observed for the next 4 hours for following parameters (32) Loss of righting reflex, Depression, Twitches, Lacrimation, Spontaneous Motor activity, Ataxia, Convulsions, Straubs phenomenon, Tremors, Catalepsy, Corneal reflex, Pupil diameter (constriction or dilation), Salivation, Cyanosis, Piloerection Defecation and Micturation..

#### **Experimental method for detection of paralytic activity in mice** <sup>[18]</sup>

The mice were divided into three groups of six individuals each Group-I Animals (control) were administered 1ml/kg normal saline. Group-II Animals (test) were administered aqueous solution of *Pterocarpus marsupium* extract (100 mg/kg). Group-III Animals (standard) were administered (2ml/kg) Diazepam. The activity was assessed by keeping the mice on smooth inclined screen at an angle of 30 degrees for 30 seconds on the screen. The time taken by the mice to slide from the screen was recorded for both control group and test animals. The sliding time of the test group animals was compared with the standard animals.

#### **Experimental method for determination of Hypnotic potentiation** <sup>[19]</sup>

The albino mice were divided into three groups of six individuals each. The test group received aqueous extract of *Pterocarpus marsupium* 100mg/kg body weight. Positive control was treated with diazepam (1mg/kg) and normal control treated with (1% Tween 80 in water). After thirty minutes, Thiopentone Sodium (40mg/kg) was administered to each mouse of all groups except normal control to induce sleep. The animals were placed on their backs by leaving sufficient space between two animals. Latent period (time between administration of thiopentone sodium to loss of righting reflex) and duration of sleep (time between the loss and recovery of righting reflex) were observed.

#### **Experimental method for evaluation of Analgesic activity in mice** <sup>[20]</sup>

The albino mice were divided into three groups of six individuals each. Control group were treated with 1ml/kg normal saline. Standard group were treated with 25mg/kg aqueous solution of Aspirin. Test animals were treated with 100mg/kg aqueous solution. The hotplate method described by Eddy and Leimbach was used to evaluate analgesic activity. The animals were dropped gently on a hotplate maintained at a constant temperature of 55°C. The reaction time was taken as the interval between the contact of mice

with the hotplate till the moment they lick their forepaws or jump out. A cut off time of 30 seconds is followed to avoid any thermal injury to the paws. Measurements were carried out at 30, 60 and 120 minutes. Only the mice that showed initial nociceptive response within 30 seconds were selected and used for the study.

#### **Experimental method for evaluation of Locomotor activity in mice** <sup>[21]</sup>

The locomotor activity was evaluated using actophotometer. The Swiss albino mice of either sex were divided into two groups of six individuals. Group-I Animals (control) were administered 1ml/kg normal saline. Group-II Animals (Test) were administered 100 mg/kg aqueous solution of *Pterocarpus marsupium* extract. The activity was recorded using an activity cage (Actophotometer). The actophotometer operates on photoelectric cells which were connected in circuit with a counter. When the beam of light falling on the photocell was cut off by the animal, a count was recorded. The locomotory count for each animal of both control and test groups was recorded for 10 min. The difference in the activity was recorded considering before treatment values and after treatment values.

#### **Experimental method for Evaluation of anticonvulsant activity in rats** <sup>[22]</sup>

The rats were divided into three groups of 6 each individual. Group-I Animals (control) were administered with 1ml/kg normal saline. Group-II: Animals (Test) were administered with 100 mg/kg aqueous solution of *Pterocarpus marsupium*. Group III: Animals (standard) were administered 25mg/kg Phenytoin.

Maximal electroshock method was used to induce convulsions in rats. A Convulsometer was used for the purpose. The animals were held properly and electroshock of 150mA for 0.2 sec was applied with the help of ear electrodes. The different stages of convulsions (i.e) tonic flexion, tonic extensor phase, clonic convulsions, stupor and recovery or death were noted. The time spent by the animal in each phase was also recorded. The standard group which was treated with phenytoin were subjected to electroshock to induce convulsions. The reduction in time or abolition of tonic extensor phase of the maximal electroshock convulsions was recorded. The test group animals which were administered *Pterocarpus* extract were subjected to electroshock to induce convulsions and the different stages of convulsions are monitored. The time spent by the animal in each phase was noted which were compared with the standard animals.

#### **Statistical analysis**

The data obtained from the above experiments was subjected to statistical analysis and analysed by ANOVA and student's t test. The data were expressed as mean  $\pm$  SEM.

#### **Results**

##### **Results of *Pterocarpus marsupium* on general behaviour in mice**

The mice did not exhibit loss of righting reflex, depression and tremors after administration of *Pterocarpus marsupium* extract in the dose up to 500 mg/kg. The pupil diameter was normal and animals showed corneal reflex. There was no salivation, cyanosis and piloerection. There was a decrease in the spontaneous motor activity. Also lacrimation, urination and defecation were not seen. (Table: 1)

**Table 1:** Effect of *Pterocarpus marsupium* on General behaviour in mice

GrossActivity	Pterocarpus marsupium		
	100 mg / kg	300 mg / kg	500 mg / kg
Loss of righting reflex	-	-	-
Depression	-	-	-
Tremors	-	-	-
Corneal reflex	+	+	+
Pupil diameter	-	-	-
Salivation	-	-	-
Cyanosis	-	-	-
Piloerection	-	-	-
Staubs Phenomenon	-	-	-
SMA	+	+	+
Lacrimation	-	-	-
Defecation and Urination	-	-	-

Effect Present: (+)

Effect Absent:(-)

**Results of *Pterocarpus marsupium* on paralytic activity, hypnotic activity, analgesic activity, locomotor activity, anticonvulsant activity in mice**

There was paralytic activity shown. The test group animals stayed on the inclined screen for a longer time period ( $2.83 \pm 0.31$ ), while the standard group showed a lower value of ( $2.16 \pm 0.17$ ). The results are summarized and graphically depicted. *Pterocarpus marsupium* considerably potentiated the sleeping time induced by thiopentone sodium. *Pterocarpus marsupium* exhibited analgesic activity ( $5.50 \pm 0.42$ ) compared to control ( $4.00 \pm 0.25$ ) group. The results are summarized and depicted.

*Pterocarpus marsupium* did not show reduction in extensor phase of the convulsions, indicated that it did not possess anticonvulsant activity. In the standard group there was absence of the extensor phase of convulsions. The results are tabulated in Table 2

**Table 2:** Effect, Activity and Treatment of *Pterocarpus marsupium* in mice

Group	Treatment(mg/kg)	Time in sec Mean±SEM	
Paralytic activity			
Control	Normal saline 1ml/kg, i.p	3.66±0.49	
Test	<i>Pterocarpus marsupium</i> extract 100mg/kg, i.p	2.83±0.31	
Standard	Diazepam(2mg/kg); i.p.	2.16±0.17	
Hypnotic activity			
Standard	Thiopentone sodium 40 mg/kg	25.5±4.06	
Test	<i>Pterocarpus marsupium</i> extract 100mg/kg 30 min before thiopentone sodium 40mg/kg.	61.6±9.58***	
analgesic activity			
Control	Normal saline 1ml/kg, i.p	4±0.25	
Test	<i>Pterocarpus marsupium</i> extract 100mg/kg	5.5±0.42	
Standard	25mg/kg aqueous solution of aspirin, i.p	8±0.57	
locomotor activity			
Control	Normal saline 1ml/kg i.p	186.5±16.492	
Test	<i>Pterocarpus marsupium</i> extract 100mg/kg	67±5.273	
anticonvulsant activity			
Phase:			
Control	Normal saline 1ml/kg i.p	Flexon	19±3.005
		Extensor	18±2.108
		Clonus	25.5±5.165
		Stupor	12±1.202
Test	<i>Pterocarpus marsupium</i> extract 100mg/kg	Flexon	6±0.4014
		Extensor	14±2.386
		Clonus	1.5±0.2236
		Stupor	5±0.3651
Standard	Phenytoin 25 mg/kg, i.p	Flexon	15±1.826
		Extensor	0±0.000
		Clonus	17±1.922
		Stupor	6±1.054

Values are the mean ± SEM of six mice analysed by ANOVA.  $p > 0.01$ .\*\*\* Significance  $p < 0.001$ , compared to Thiopentone sodium (standard) group.Student's 't' test values are the mean ± SEM of six mice/treatment  $p > 0.001$ , compared to control group ANOVA.  $P < 0.001$ ; significant, Students 't' test**Discussion**

In the present study the neuropharmacological activity of *Pterocarpus marsupium* was assessed. The activity was CNS inhibitory in nature as evident by the behavioural assessment<sup>23</sup>. The aqueous extract does not show any signs of neurotoxicity. The locomotor activity is the most important action on CNS in mice. *Pterocarpus marsupium* showed decreased locomotor activity and hyper activity of animals towards external stimuli like touch and noise. The decrease in locomotion indicated depressive effect on CNS. The flavonoids present in the extract may be responsible for CNS depressant activity. This can also predict anxiolytic activity of the extract. In hotplate test *Pterocarpus*

*marsupium* exhibited mild analgesic activity. From the results it can be concluded that the extract exhibits analgesic activity by central mechanism. In the results of *Pterocarpus marsupium* treatment on thiopentone sodium induced sleep and spontaneous locomotor activity were related with behavioural assessment. Thiopentone Sodium, a barbiturate produces sedative hypnotic effect at a particular dose because of its interaction with gamma amino butyric acid receptors which enhances GABAergic transmission. It potentiates the GABA activity thereby allowing chloride to enter the neuron by prolonging duration of chloride channel opening. Thiopental may block the excitatory glutamate receptors. These molecular activities may lead to decreased

neuronal activity which supports the findings obtained for the reference drug diazepam which is a CNS depressant drug that decreases the time of onset of sleep and or prolongs the length of sleep or both [24]. *Pterocarpus marsupium* significantly prolonged the thiopentone sodium induced sleeping time. The prolongation of thiopentone sodium induced sleeping time may be attributed to inhibition of thiopentone sodium metabolism or central mechanisms involved in the regulation of sleep. Diazepam which was used to induce sleep in this study, believed to act at specific binding sites that are closely linked to GABA receptors, binding of benzodiazepines enhancing GABA – ergic transmission. Many flavonoids and steroids were found to be ligands for the GABA receptors in the Central Nervous System, that can act as benzodiazepine like molecules [25]. The potentiation of thiopentone sodium induced sleep and decrease in spontaneous locomotor activity suggest central depressant effect. *Pterocarpus marsupium* showed no effect on the extensor phase of maximal electroshock-induced seizures. Probably there can be involvement of neurotransmitters which are able to augment or decrease neuropharmacological effects on repeated administration of the extracts.

### Conclusion

From the results it can be concluded that the extract exhibited mild analgesic activity. From the results of *Pterocarpus marsupium* treatment on thiopentone sodium induced sleep and spontaneous locomotor activity which were related with behavioural assessment it can be concluded that *Pterocarpus marsupium* extract has CNS inhibitory effect in mice which can be explored in future research.

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