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Original article

Evaluation of antipyretic, muscle relaxant and neurobehavioural activities of various leaf extracts of *Pongamia pinnata* L.

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Abstract

Different leaf extracts of *Pongamia pinnata* L., *i.e.*, aqueous, alcoholic, acetone and chloroform were investigated for antipyretic activity, muscle relaxant activity and neurobehavioural activity at the dose rate of 50 mg/kg and 100 mg/kg in wistar rats. Antipyretic activity was evaluated using *E. coli* LPS (2 µg/kg). For muscle relaxant activity, rota-rod apparatus was used and fall-off time was noted. For the assessment of neurobehavioural profile, elevated plus-maze was used and anxiolytic responses produced by the extracts were noted. Rodents (rats and mice) have an aversion for open spaces and prefer enclosed ones, therefore, spend more time in enclosed spaces (thigmotaxis). Time spent in open and closed arms was automatically recorded by the camera attached to the computer through software. In evaluation of antipyretic activity, aqueous extract @ 100 mg/kg showed maximum response when compared to control. However, present study revealed no muscle relaxant and neurobehavioural activity of different leaf extracts of *P. pinnata*.

Key words: *Pongamia pinnata* L., antipyretic, muscle relaxant, neurobehavioural

1. Introduction

There are hundreds of medicinal plants that have a long history of curative properties against various diseases and ailments (Subramoniam, 2014; Mohammad Yunus, 2015; Rais-ur-Rahman, 2017; Zulfa Nooreen *et al*., 2018). Alternative system of medicine is getting more popular for treatment of various diseases. Many medicinal plants provide relief comparable to that obtained from allopathic medicines. Traditional medicine is an important source of potentially useful new compounds for the development of chemotherapeutic agents. The first step towards this goal is the screening of plants used in popular medicine. Plant drugs are frequently considered to be less toxic and free from side effects than the synthetic ones (Momin, 1987).

India is one of the 12 mega biodiversity centers, having 45,000plant species; its diversity is unmatched due to the 15 different agroclimatic zones, 10 vegetative zones, and 15 biotic provinces (Nayanabhirama, 2016). More than 80% population in the rural areas of the world is dependent on plant based traditional medicines including traditional systems of medicine such as Ayurveda, Unani and traditional Chinese medicine (Majeed, 2017).These concepts forced the scientists to discover modern medicines where plants are used as the source due to presence of active chemical constituents (Dang, 2018).

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Pongamia is a genus having one species only, *Pongamia pinnata* (L.) [Syn. *Pongamia glabra* (Vent); *Derris indica* (Lamk.)] (also known as Indian Beech) which belongs to the family Leguminosae and sub-family Papillionaceae (Meera *et al.*, 2003). It is a medium sized glabrous, perennial tree which grows in the littoral regions of South Eastern Asia and Australia (Satyavati *et al.*, 1987; Allen and Allen, 1981). The tree is planted for shade and is grown as ornamental tree and is one of the few nitrogen fixing trees producing seeds containing 30-40% oil. It is commonly known as Karanj in Hindi (Rangari 2002). Other common names include Honge/ Karajata in Kannada, Pungai in Tamil, Kanuja in Telugu, Karach in Bengali, Naktamala in Sanskrit and Sukhchain in Urdu. *P. pinnata* is a preferred species for controlling soil erosion and binding sand dunes because of its dense network of lateral roots. Root, bark, leaves, flower and seeds of this plant have medicinal properties and so; it is traditionally used as medicinal plant. All parts of the plant have been used as crude drug for the treatment of tumors, piles, skin diseases, wounds and ulcers (Tanaka *et al*., 1992). In the traditional system of medicines such as Ayurveda and Unani, the *P. pinnata* plant is used for anti-inflammatory, antiplasmodial, anti-nonciceptive, antihypergly-camic, antilipid peroxidative, antidiarrhoeal, antiulcer, anti-hyperammonic and antioxidant activity (Chopade *et al*., 2008). In the light of above, the present study investigated the potential of different leaf extracts of *P. pinnata* as an antipyretic, muscle relaxant and neurobehavioural agent in rodents.

2. Material and Methods

2.1 Plant material

Based on ethno-pharmacological information, leaves were collected from campus of Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, Punjab, India and were authenticated by the

botanist of Collaborative Ayurveda Research Centre, GADVASU, Ludhiana. Immediately after collection, leaves were washed and dried under sunlight. The dried leaves were finely grounded into powder, weighed and kept for further analysis.

Pongamia pinnata L. fruit *Pongamia pinnata* L. leaves

2.2 Extraction

Different types of extracts of the plant were prepared using various menstruum, *viz.*, water, ethanol, acetone and chloroform by maceration technique. 100 gram of powdered leaves was soaked in 1 litre menstruum at room temperature for 48-72 h and stirred at frequent time intervals. After maceration, the extract was initially filtered using muslin cloth and then re-filtered again using Whatman filter paper No 1. The filtrate obtained was evaporated in oven at a temperature of 40°C. The residue obtained was lyophilized and kept at 4°C in air tight bottles until used. Percent yield for different solvents, *viz.*, water, ethanol, acetone and chloroform was found to be 8%, 3.8%, 3% and 2.8%, respectively.

2.3 Drugs and chemicals

Paracetamol, *E.coli* endotoxin, carboxymethyl cellulose sodium and diazepam were used in experiments.

2.4 Animals

The present investigation was conducted on 50 rats aged 3-4 months, weighing 180-200 gram, at Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana. The animals were purchased from small animal colony, GADVASU. The animals were acclimatized to the environment for ten days before starting experiment and kept in cages under standard laboratory conditions of temperature (27 - 30ºC), with a 12 h light cycle. All animals were fed commercial rat pellets, procured from Ashirwad industries, Mohali (Punjab). Feed and water were provided *ad libitum* to the animals. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) vide reference No. VMC/13/1786-1806 dated 4/4/13 and was conducted in accordance with ethical committee guidelines. Animals were divided into ten groups of five animals each. Group I served as control, group II as positive control and remaining groups served as test groups**.** Groups III and IV were administrated with aqueous extract of concentration 50 mg/kg and 100 mg/kg orally, respectively. Groups V and VI were administered with alcoholic extract at concentration of 50 mg/kg and 100 mg/kg, respectively. Similarly, groups VII and VIII with acetone extract and groups IX and X with chloroform extract having concentrations of 50 mg/kg and 100 mg/kg, respectively.

2.5 Antipyretic activity

In this experiment, *E.coli* endotoxin @ 2 µg/kg was administrated orally to all groups. Group II was given paracetamol @50 mg/kg orally. Rest of groups were treated as described above. Fever was measured at duration of 1 h, 2 h, 3 h, 4 h, 5 h and 6 h, respectively.

2.6 Muscle relaxant activity

In the study, the animals were trained to maintain balance for 40-60 sec on the rota rod rotating at a speed of 25 rpm. Only those rats which could balance themselves for 45-60 sec were selected for study. Each rat was placed individually on the rota rod, and time spent on rota rod was noted. The control group was treated with distilled water. In the positive control group, diazepam @ 10 mg/kg was administered orally as a standard drug. In group III, group V, group VII and group IX, the aqueous, alcoholic, acetone and chloroform extracts were given, respectively, @ 50 mg/kg orally whereas in group IV, group VI, group VIII and group X, respectively; these extracts were given @ 100 mg/kg orally. Fall off time was noted at 0 h, 1 h and 2 h, respectively.

2.7 Neurobehavioural activity

For the study, animals were divided into five groups of five animals each. Control group was given DW. Group II was given aqueous extract @ 100 mg/kg orally. Group III was given alcoholic extract @ 100 mg/kg orally. Group IV was given acetone extract @ 100 mg/kg orally. Similarly, Group V was given chloroform extract @ 100 mg/kg orally. Time spent in open and closed arms was automatically recorded by the camera attached to the computer through software.

3. Results and Discussion

3.1 Antipyretic activity

The results of the antipyretic activity of various extracts of *P. pinnata* in wistar rats are shown in Table 1 and Figure 1. *E. coli* endotoxin was administered orally @ 2 µg/kg to induce fever in all the groups. Group I served as control and in group II, paracetamol (standard drug) was injected @ 50 mg/kg orally. In groups III, V, VII and IX, aqueous, alcoholic, acetone and chloroform extracts were given, respectively @ 50 mg/kg orally whereas in groups IV, VI, VIII and X, these extracts were given @ 100 mg/kg orally. In group I, temperature started to decrease after 4 h and did not achieve normalcy even after 6 h, whereas in the standard group, the temperature started to decrease after 1 h and came to normal value after 4 h of *E. coli* endotoxin administration. Almost all the extract groups demonstrated the dose dependent antipyretic activity as compared to the control group with maximum activity shown by group IV in which temperature started to decrease after 2 h and became normal at 6 h. Also in group IV, the increase in body temperature after administration of the endotoxin was lowest when compared to all other groups which can be attributed to the maximum antipyretic effect of the extract. In group VI also, significant antipyretic activity was observed as the temperature started to decrease after 1 h and returned to its normal value at around 5 h but the initial rise in temperature was higher than the group IV. Antipyretic action of paracetamol is due to the inhibition of the enzyme prostaglandin synthetase, thereby impeding the biosynthesis of prostaglandins (PG) in the hypothalamus (Clark and Cumby, 1975). The antipyretic action of the extracts can be attributed to their interference with PG synthesis in the brain hypothalamus. Also, in the study conducted by Srinivasan *et al.* (2003), maximum antipyretic activity was shown by 70 % ethanolic extract of *P. pinnata* leaf extract against yeast induced pyrexia model in wistar rats.

3.2 Muscle relaxant activity

The results depicting the muscle relaxant activity of various leaf extracts of *P. pinnata* on wistar rats are given in Table 2 and Figure 2. Group I served as control and group II served as standard. In the standard, diazepam @ 4 mg/kg was administered orally. In .group III, group V, groups VII and IX, the aqueous, alcoholic, acetone and chloroform extracts were given, respectively @ 50 mg/kg, whereas in group IV, group VI groups VIII and X, respectively; these extracts were given @ 100 mg/kg orally. Rats were placed on rota rod and fall off time was noted. In standard group, there was significant decrease in fall off time after 1 h and remained upto 2 h after drug administration. In the control group, there was no significant decrease in fall off time. All other groups showed non-significant decrease in fall off time. Hence, present study showed no muscle relaxant activity of *P. pinnata* leaf extracts.

In contrast to the present study, Shetty (2017) reported significant muscle relaxant activity of leaf extract of *Chromoleana odorata*. Also, Prathib (2015) reported that the ethanolic extract of *Ichnocarpus frutescens* leaves possesses significant skeletal muscle relaxant activity.

3.3 Neurobehavioural activity

The effect of various extracts of *P. pinnata* on neurobehavioural activity of wistar rats are given in Table 3. Group I served as control. Group II was given aqueous extract @ 100 mg/kg orally, Group III was given alcoholic extract @ 100 mg/kg orally and Group IV was given acetone extract @ 100 mg/kg orally. Similarly, group V was given chloroform extract @ 100 mg/kg orally. Time spent in open and closed arms was recorded automatically. It was observed that in all the extract groups, there was no significant change in the time spent in open or closed arms as compared to control group which revealed that none of the extracts modify the normal thigmotaxic behaviour of rodents.

In contrast to the present study, Shajib *et al*. (2015) reported significant anxiolytic activity of pulp extract of *Phoenix sylvestris*.

4. Conclusion

In this study, all extracts showed dose dependent antipyretic activity but maximum activity was seen in the aqueous extract of *P. pinnata* @ 100 mg/kg. However, the study revealed no muscle relaxant activity of different leaf extracts of *P. pinnata*. Also, no significant change was observed in the neurobehavioural profile of the animals when compared to the control group revealing no anxiolytic effect of the plant extracts. Further studies may be conducted in near future for separating the bioactive compounds responsible for antipyretic activity of the plant extract, thereby helping in development of plant derived drugs, thus curbing the adverse effects caused due to the use of NSAIDS and other synthetic antipyretic drugs.

Table 1: Effect of different leaf extracts of *P. pinnata* on *E. coli* endotoxin (2 µg/kg) induced fever in rats

		Increase in temperature (°C) in comparison to normal (0 hour) value					
Group	**Dose (mg/kg)**	**1 h**	**2 h**	**3 h**	**4 h**	**5 h**	**6 h**
Aqueous	50	0.80 ± 0.11^{cd}	0.96 ± 0.10^{b}	1.02 ± 0.11^{c}	0.48 ± 0.10^{dep}	0.16 ± 0.047^{de}	0.04 ± 0.03^{c}
Aqueous	100	0.76 ± 0.13^{d}	0.84 ± 0.12^{bc}	0.72 ± 0.05^{d}	0.36 ± 0.07^{efg}	0.24 ± 0.09^{de}	0.08 ± 0.04^{c}
Alcoholic	50	$1.04 \pm 0.15b^{cd}$	1.00 ± 0.17^{c}	0.88 ± 0.15^{cd}	0.68 ± 0.16^{cd}	0.28 ± 0.05^{de}	0.12 ± 0.04^{bc}
Alcoholic	100	1.20 ± 0.18^{ab}	0.92 ± 0.05^{c}	0.64 ± 0.04^{d}	0.24 ± 0.07^{fg}	0.16 ± 0.07^{de}	0.12 ± 0.04^{bc}
Acetone	50	0.88 ± 0.08^{bcd}	1.08 ± 0.07^{c}	1.08 ± 0.07^{c}	0.87 ± 0.08^{c}	0.56 ± 0.09^{c}	0.28 ± 0.05^{c}
Acetone	100	1.12 ± 0.05^{abcd}	0.92 ± 0.04^{c}	0.88 ± 0.04^{cd}	0.56 ± 0.03^{de}	0.36 ± 0.03^{cd}	0.16 ± 0.03^{bc}
Chloroform	50	1.44 ± 0.07^{a}	1.72 ± 0.10^{a}	1.64 ± 0.09^{b}	1.60 ± 0.06^{b}	0.92 ± 0.05^{b}	0.28 ± 0.04^{b}
Chloroform	100	0.88 ± 0.04^{bcd}	1.08 ± 0.05^{a}	1.08 ± 0.05^{c}	0.64 ± 0.03^{cd}	0.24 ± 0.07^{de}	0.08 ± 0.04^{c}
Standard	50	1.16 ± 0.18^{abc}	0.56 ± 0.15^{c}	0.12 ± 0.05^{e}	0.12 ± 0.05^{g}	0.08 ± 0.04^{e}	0.08 ± 0.04^{c}

Table 2: Effect of different leaf extracts of *P. pinnata* on muscle relaxant activity in rats

		Fall off time (sec)		
Group	**Dose (mg/kg)**	**0 h**	**1 h**	**2 h**
Aqueous	50	48.2 ± 0.80^{a}	47.8 ± 0.40^{a}	48.2 ± 0.73^{a}
Aqueous	100	48.4 ± 0.81^{a}	47.4 ± 1.63^{a}	47.6 ± 1.03^{a}
Alcoholic	50	47.6 ± 1.21^{a}	47.2 ± 0.86^{a}	48.2 ± 0.86^{a}
Alcoholic	100	47.8 ± 0.86^{a}	47.0 ± 1.05^{a}	48.0 ± 0.70^{a}
Acetone	50	46.8 ± 0.97^{a}	47.2 ± 1.20^{a}	47.4 ± 0.51^{a}
Acetone	100	46.6 ± 1.44^{a}	46.8 ± 0.80^{a}	48.0 ± 1.10^{a}
Chloroform	50	46.8 ± 1.16^{a}	47.2 ± 0.86^{a}	47.6 ± 0.81^{a}
Chloroform	100	46.4 ± 1.16^{a}	46.6 ± 1.81^{a}	47.2 ± 1.46^{a}
Standard (Diazepam)	2	47.4 ± 0.51^{a}	6.2 ± 0.58^{b}	5.8 ± 0.40^{b}
Control		47.8 ± 0.86^{a}	46.4 ± 1.03^{a}	46.8 ± 1.24^{a}

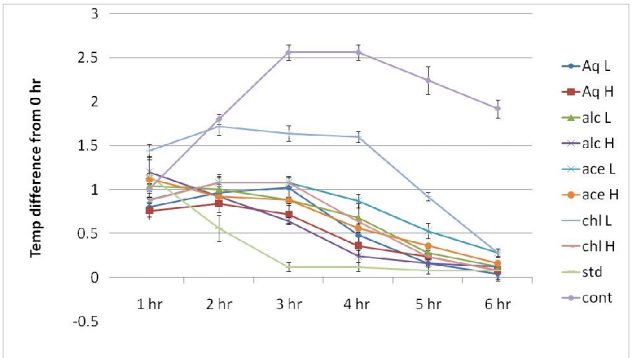

Figure 1: Effect of different leaf extracts of *P. pinnata* on *E. coli endotoxin* (2 µg/kg) induced fever in rats.

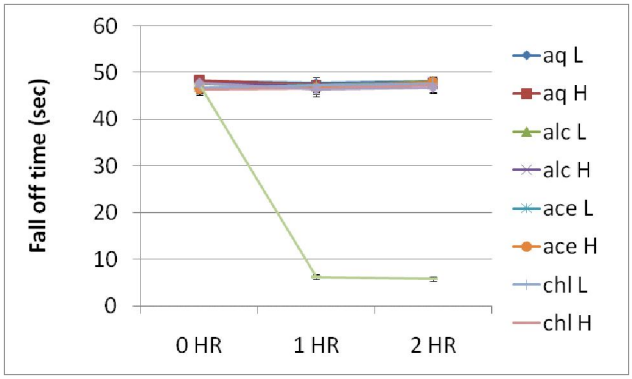

Figure 2: Effect of different leaf extracts of *P. pinnata* on muscle relaxant activity in rats.

Table 3: Effect of different leaf extracts of *P. pinnata* on neurobehavioural activity of rats

	Time spent in open arms (s)	Time spent in closed arms (s)
Control	6.28 ± 0.3[a]	883.8 ± 2.1[a]
Aqueous	6.36 ± 0.3	886.9 ± 2.6
Alcoholic	7.02 ± 0.5[a]	884.8 ± 3.1[a]
Aceetone	6.98 ± 0.5[a]	886 ± 3.5[a]
Chloroform	6.94 ± 0.4[a]	887.4 ± 2.3[a]

Conflict of interest

We declare that we have no conflict of interest.

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