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Phytochemical and Safety Evaluation Studies ofPithecellobium dulce (Roxb.) Benth

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Abstract

Pithecellobium dulce (Roxb.) Benth., commonly known as Manila tamarind, Madras thorn, or camachile, is a species of flowering plant in the pea family, Fabaceae, that is native to the Pacific Coast and adjacent highlands of Mexico, Central America, and northern South America. It is also sometimes known as monkeypod, but that name is also used for several other plants, including Samanea saman. It has more than a dozen vernacular names. Pithecellobium dulce is an introduced species and extensively naturalized in the Caribbean and Florida, as well as the Philippines and Guam via the Manila galleons. It has also been introduced to Cambodia, Thailand, and South Asia, It is considered an invasive species in Hawaii.

Introduction

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Pithecellobium dulce (Roxb.) Benth.

It is a spiny, nearly evergreen tree that grows up to 20 meters high, with irregular branches, grayish bark becoming rough, then furrowed. Leaves are bipinnate, each forming a single pair of leaflets, and small, sharp thorns are found in pairs at the base of each leaf.

The tree is often planted for a living fence or thorny hedge. It provides food, forage, and firewood (smoky firewood). Pods and leaves are favoured and consumed by livestock (horses, cattle, goats, and sheep). The pulp of the fruit is white or red and is very sweet and of a pleasant flavour as a human food especially the large aril. Both aril and seed are sweet with a chestnut flavour. The fruit is eaten raw or used for making sweet-sour drinks similar to lemonade. Seeds contain 20% green edible oil and a protein-rich cake (30%). The tree also produces an edible gum reminiscent of gum arabic. Flowers are attractive to bees, and they produce a good quality of honey. The bark is very high in tannins of the catechol type (up to 37%) and is used in the tanning industry in the Philippines and India. As a fodder tree, it gives crude protein content up to 29% and 17.5% of crude fiber as recorded in the foliage (leaves). The tree is often used for land rehabilitation due to its soil hardiness and drought tolerance, and as a windbreak. The flowers produce a pod, which turns pink when ripe and opens to expose the seed arils, a pink or white, edible pulp. The pulp contains black shiny seeds that are circular and flat. Pollen is a polyad of many pollen grains stitched together.

Pollen of Pithecellobium dulce:

The seed is dispersed via birds that feed on the sweet pulp. The tree is drought- resistant and can survive in dry lands from sea level to an elevation of 1,500 m (4,900 ft), making it suitable for cultivation as a street tree.

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Uses as Food and Fodder:
Names like "dulce' (sweet) and "Manila tamarind " reflect the wide use of the pods as food. Pods contain a pulp that is variously sweet and acidic. commonly white, but also red. The seed and pulp are made into a sweet drink and eaten roasted or fresh. In India. the seeds are used fresh or in curries. The pods are relished by monkeys and livestock. The flowers are attractive to bees as a source of pollen. The resulting honey is of high quality. Although the pods are attractive fodder to most animals. the leaves are browsed but not considered an important animal fodder.

Reported Traditional Uses:
In folk medicine, it is reported to be abortifacient, anodyne, astringent, larvicidal, and a remedy for convulsions, dysentery, dyspepsia, earache, leprosy, peptic ulcers, sores, toothache, and venereal diseases.
Aqueous extract of the aerial parts of Pithecellobium dulce (P. dulce) was prepared for chemical studies and Safety evaluation studies:

Chemical Compounds Identified:
Thin layer chromatographic fingerprinting with standard amino acids showed the presence of:
DL-B-phenyl alanine L-proline
DL-tryptophane DL-valine
Thin layer chromatographic fingerprinting with standard Steroids showed the presence of:
Stigma sterol B-sitosterol Lupeol
Palmitic acid is also confirmed through chromatographic methods.

Evaluation of safety studies:

Acute and toxicity studies:
Three groups of 10 mice each (a Total of 30 mice) were taken, weighed and labeled. Group I of mice was administered 10 % of the P. dulce (0.3 ml/10 g). Group II was administered 20% of the P. dulce (0.3 ml/10 g), and the III group was given distilled water, which served as a control.
The treated and control groups of animals were observed for gross behavioral signs and symptoms using a battery of tests. Body weights, before and at 24 h, and on 72 hrs after administration of green branches, were recorded. Signs and symptoms of toxicity and mortality, if any, were recorded during the seven days after the administration of the P. dulce aquous extract.
Various studies including gross behavioral activity, mortality, body weight change, locomotor activity, rectal temperature, and motor coordination.
Animals Body weight
Dose Albino mice, T/O strain 30-40 g 0.3 ml/10g. p.o.

Gross behavioral studies:
Both treated and control groups of animals were observed for gross behavioral signs and symptoms using a battery of tests.

Autonomic:	Salivation, nasal discharge, diarrhea, urination, and piloerection.
Behavioral:	Sedation, drooping head, sitting position with head up, depression, Restlessness, excessive preening, irritability, aggressive behavior. Righting reflex, sensitivity to pain, hind limb reflex, sensitivity to Sound and touch.
Neuromuscular:	Decreased and increased activity, tremors, weakness, muscle tone, ataxia, convulsions, prostration, and hind limb weakness
Cardiovascular:	Increase or decrease heart rate
Ocular:	Lacrimation, ptosis
Gastrointestinal:	Salivation, diarrhea, bloody stool, constipation, defecation.
Cutaneous:	Piloerection, alopecia, edema, swelling, necrosis.

Mortality:
The group of the mice treated with 10% and 20% of the aqueous extract of green branches was observed for mortality of the animals.

Body weight change:
Bodies' weights, initially, at 24 and 72 hrs after administration of test sample, were recorded.
Locomotor activity:
Locomotor activity was monitored in treated and control animals using the Columbus Activity Meter. The same animals administered for the toxicity test were tested for locomotor activity. Four animals each group was introduced into the compartment of the equipment and was monitored for 60 minutes. The 'total', 'Ambulatory' and 'Vertical' activities were recorded by the in-built system on the compartments. Distance traveled, resting time, ambulatory time and stereotypic time were recorded by the PC connected to the equipment. The locomotor activity data obtained in the treated groups of the animals were compared with that of control group.
Motor coordination:
The test to evaluate the motor impairment, ataxia, or sedation, the motor coordination was assessed using a treadmill (Rota-rod) apparatus. Mice were placed on a rotating rod (3 cm diameter, 25 rpm and were observed for 1 minute and the number of mice falling off the rota rod was recorded. The treated and control mice were tested for their ability to remain on the rotating rod (or number of animals that fell down) in the treated and control groups.
Rectal temperature measurement:
The rectal temperature was measured using a rectal probe and compared with that of the control group.
Blood pressure and heart rate:
Rats were anaesthetized with urethane at a dose of (1 g/kg i.p.) and the carotid artery was cannulated. The blood pressure was measured directly from the cannula using a transducer-amplifier-recorder assembly. Blood pressures were recorded at different time intervals after the administration of the aqueous extract of green branches (10% 1ml/100g body weight, intragastric). The blood pressure and heart rate were also measured in another rat using 20% aqueous extract of green branches (0.2 .03 & 0.5 ml administered i.v.).
Results:

- The results of the acute toxicity study showed no overt signs and symptoms of toxicity.
- The treated animals did not show salivation, nasal discharge, diarrhea, lacrimation, or tremors.
- No death was recorded at 24 hrs and 72 hrs of the observation period following the treatment. No tremors, loss of righting reflex observed.
- The aqueous extract of green branches (10%) showed reduced locomotor activity as compared to the control values. Reduction in motor coordination was also recorded. No change in rectal temperature was noticed.
- No change in body weights after 24 h 72 hrs of the treatment period were recorded.
- The animals showed no changes in blood pressure and heart rate on intragastric administration. However, the intravenous administration of the aqueous extract of the green branches showed a transient reduction in blood pressure. The survival of the animal following the intragastric as well as intravenous administration showed that the test substance is devoid of toxicity in the present experiment.

Conclusion:
The result of acute toxicity indicated that the test sample (Aqueous extract of Pithecellobium dulce) showed no overt signs and symptoms of toxicity and caused no mortality at the doses tested. However, the reductions in motor coordination and locomotory activity were observed in the treated animals.
The treated animals showed no significant change in the mean body weights of treated animals recorded at 24 h, 72h of treatment, as compared to the control animals.
To further confirm the toxicity, the animals administered the aqueous extract of the P. dulce (through the intragastric and intravenous route) did not cause mortality in the treated animals.
The present data of the study revealed that the aqueous extract of the aqueous extract fresh Pithecellobium dulce tested at two dose levels (10% and 20%) is safe on acute administration in albino mice.
To ensure further safe evaluation, daily prolonged treatment (90 days) treatment of the aqueous extract of the plant is recommended.
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