ANTICONVULSANT AND MYORELAXATION ACTIVITY OF *PSIDIUM GUAJAVA* LINN. LEAF EXTRACT

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Summary

Objective: To study the anticonvulsant activity of ethanolic extract of *Psidium guajava* in albino mice.
Materials and methods: The anticonvulsant and myorelaxation activity of ethanolic extract of leaves of *P. guajava* (200, 400 & 600mg/kg., p.o.) was assessed using albino mice against maximum electroshock seizure (MES) test and rotarod test respectively.
Result: The ethanolic extract of *P. guajava* reduced the duration of hind limb tonic extension (HLTE) in a dose dependent manner against MES model.
Conclusion: The ethanolic extract of *P. guajava* inhibits MES-induced convulsions.

Key Words: Anticonvulsant, *Psidium guajava,* MES, Rotarod.

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Introduction

Guava (*Psidium guajava*) member of family Myrtaceae, is an important small fruit tree native to tropical America, now distributed in all tropical and subtropical areas of the world.[1] The plant is indigenously known as Amrud in Hindi, Perukah in Sanskrit, Peyara in Bengali and Perala-hunnu in Kannada.[2] Different parts of the plants are used in the traditional system of medicine for the treatment of various human ailments such as wounds, ulcers, bronchitis, eye sores, bowels, diarrhea and cholera.[3,4] Unripe fruit – used for antidiarrhoeal. Leaves – used for dysentery, diabetes, cough and cold. Flowers – anthelmintics. Guava juice may be helpful in regulating blood sugar in type 2 diabetes.[5] Infusion of the leaves are used for treating fever, for diarrhea and as a tonic of psychiatry.[6] When applied in the mouth, it was used to treat mouth sores and gum swelling, to aid wound healing and to prevent bad odour.[7] The major constituents of the leaf were identified to be tannins, β-sitosterol, maslinic acid, essential oils (mainly caryophyllene, β-bisabolene, aromadendrene, β-selinene, nerolidiol, caryophyllene oxide and sel-11-en-4-ol) and triterpenoids, including oleanolic, ursolic, crategolic and guaijavolic acids.[8]

The aim of this work is to evaluate the anticonvulsant activity of ethanolic extract of leaves of *P. guajava* in order to provide a basis for the folkloric use of the plant.

Materials And Methods

Plant material

Fresh leaves of *P. guajava* were collected from the local area of the Jhansi, India in the month of August, 2008. The plant authenticated by Dr. H. B. Singh, Scientist and Head, Raw Materials Herbarium and Museum, National Institute of Science Communication and Information Resources (NISCAIR), New Delhi and a voucher specimen (No: NISCAIR/RHM/037/68) was deposited in the herbarium.

Preparation of extract

The leaves of *P. guajava* were dried in air, crushed in coarse powder and subjected to successive extraction using ethanol in a soxhlet apparatus. The extract was concentrated under reduced pressure using rotatory evaporator at temperature not exceeding 40°C and then dried in vacuum oven. The extract was stored in desiccators at cool place and reconstituted in water for injection just before use.

Animals used

Male albino mice (20-30 g) of either sex were procured from animal house, Institute of Pharmacy, Bundelkhand University, Jhansi, India. The animals were housed in standard cages with free access of food (standard laboratory rodent's chow) and water. The animal house temperature was maintained at 23±3.0°C with a 12-h light/dark cycle. The Institutional Animal Ethics Committee approved the protocol of the study.

Drugs used

Phenytoin (Samarth Life Sciences Pvt. Ltd., Baddi, H.P., India) and Diazepam (Helios Pharmaceutical Pvt. Ltd., Baddi, H. P., India) were used in this study. The plant extract was dissolved in normal saline and subjected for anticonvulsant activity and muscle relaxant activity using MES and Rota rod models, respectively. Phenytoin and Diazepam were dissolved in normal saline (0.9% NaCl solution).

Acute toxicity study

The acute toxicity for the ethanolic extract of leaves of *P. guajava* was determined in female albino mice (20-25 g). The animals were fasted overnight prior to the experiment and fixed dose OECD guideline No.420 (Annexure 2d) method of CPCSEA was adopted for acute toxicity studies. [9] The ethanolic extract was administered in doses of 300, 2000, 5000 mg/kg. p. o. to group of mice, each containing ten animals and mortality was observed after 24 h.

Evaluation of anticonvulsant activity: Maximum electroshock-induced seizures

Electro-convulsive shock, inducing Hind Limb Tonic Extension (HLTE) in 99% of the animals, was previously determined.[10] corneal electrodes were used for bilateral delivery of electrical stimulus. Electro-convulsive shock (50 mA for 0.2 Sec.) was delivered through corneal electrode to induce HLTE phase in mice. The electrical stimulus was applied using a stimulator apparatus for five groups of six each.

Group I served as control (vehicle treated, i.p.); Group II served as standard (received Phenytoin sodium 25mg/kg body weight, i.p.), Group III, Group IV and Group V were treated with ethanolic extract as 200, 400 and 600mg/kg body weight, p.o. respectively. The current was delivered after 60 min. of oral administration of ethanolic extract and after 30 min. of intraperitonial administration of control and standard. The incidence and duration of HLTE was noted.

Myorelaxation activity: Rota rod performance

The effect on motor co-ordination was assessed using Rotarod apparatus (Biocraft Scientific System Pvt. Ltd., Agra, India) Pre-selected mice (animal that stayed for at least 2 min. on the rotating bar, 24 hrs. before testing) were placed on the horizontal rotating bar (diameter 2.5 cm, 12 r.p.m.).The test was conducted on five groups of 6mice each, 60 min. after the administration of ethanolic extract (200, 400 & 600 mg/kg,p.o.) and 30 min. after the administration of diazepam(1mg/kg i.p.) and normal saline (10ml/kg, i.p..).[11]

Statistical analysis

The data was presented as mean ±SEM. The data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey multiple comparisons test.[12] A difference of $P<0.001$ was considered significant in all cases.

Results

Acute toxicity

In the acute toxicity study, the ethanolic extract of *P. guajava* was found to be safe in the doses used and there was no mortality in a dose of 2 g/kg, p.o.

Evaluation of anticonvulsant activity: Maximum electroshock-induced seizures

The ethanolic extract of *P. guajava* exhibited almost dose dependent anticonvulsant activity. The extract significantly decreased the duration of HLTE phase in MES-induced seizures. The result is shown in table 1.

Table 1. Effect of *Psidium guajava* extract on MES induced seizures in mice

S. No.	Treatment	Duration of HLTE	Mortality (%)	Recovery (%)
1.	Vehicle	15.73±0.35	80	20
2.	Phenytoin	3.73±0.08	0	100***
3.	PGE-200	14.42±0.28	0	100***
4.	PGE-400	11.05±0.32	20	80***
5.	PGE-600	7.61±0.41	0	100***

PGE-200, PGE-400 and PGE-600 - *Psidium guajava* extract dose 200, 400 and 600mg/kg body weight.
Values are mean ± SEM, n=6, ***=P<0.001 compared with control.

Myorelaxation activity: Rota rod performance

A significant dose dependent muscle relaxant effect of *P. guajava* was observed in rotarod appararus compared to that produced by diazepam. The result is shown in table 2.

Table 2. Effect of ethanolic extract of *Psidium guajava* on Rota rod test in mice.

Sr.No.	Treatment	Time of fall (Sec.)	Myorelaxation %
1.	Vehicle	290±2.303	--
2.	Diazepam	18.01±0.33***	100
3.	PGE-200	146.71±1.16***	87.67
4.	PGE-400	105.33±0.85***	82.57
5.	PGE-600	75.17±0.59***	76.0

Values are mean ±SEM mice were pretreated with Vehicle and PGE p.o. 60 min. before Rota rod model. ***=P<0.001 (n=5).

Discussion

The observation emanated in the present study indicated that the *P. guajava* was without any lethal effect in a dose upto 2 g/kg and possessed anticonvulsant activity against seizures induced by MES in a dose dependent way.

The most popular and widely used animal seizures model is the traditional MES test. The MES test is considered to be a predictor of likely therapeutic efficacy against generalized tonic-clonic seizures.[13] MES induced tonic seizures can be prevented either by drugs that inhibit voltage dependent Na^+ channels, such as phenytoin, valproate and lamotrigine.[14,15] or by drugs that block glutamatergic excitation mediated by the N-methyl-D-aspartate (NMDA) receptor such as felbamate.[16] The study showed that ethanolic extract from leaves of *P. guajava* can inhibit voltage dependent Na^+ channels as phenytoin in MES induced tonic seizures.

Thus, in conclusion, *P. guajava* possesses anticonvulsant activity against the MES induced seizures. Further research is in progress to isolate the compound responsible for the activity.

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