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EVALUATION OF THE ANTICONVULSANT ACTIVITY OF ETHANOL EXTRACT OF *PSIDIUM GUAJAVA* (GUAVA LEAVES) IN ALBINO MICE

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ABSTRACT: Objective: To evaluate the anticonvulsant activity of ethanolic extract (200mg/kg & 400mg/kg) of *Psidium guajava* (guava leaves) in albino mice. **Methods:** Albino mice (25-30gms) of either sex were randomly selected and divided into 4 groups of 6 mice each, Group I (control) – Distilled water (vehicle) 1ml, Group II (Standard) – Valproic acid (40mg/kg), Group III - T1, ethanolic extract of *Psidium guajava* (200mg/kg), Group IV- T2, ethanolic extract of *Psidium guajava* (400mg/kg). All drugs were administered orally 1 h before the induction of seizures. The anticonvulsant activity was screened using maximal electroshock (MES) and pentylenetetrazole (PTZ) models. **Results:** The data were analyzed by one way ANOVA followed by Bonferroni's multiple comparison test. Ethanolic extract of *Psidium guajava* dose-dependently produces significant antiepileptic activity in comparison to control. In MES test, the percentage inhibition of seizure is T2 - 49% and T1 – 37% in comparison to control. In the PTZ test, the percentage of protection from seizure by T2 is 83.4%, and T1 is 50% when compared to the standard drug. **Conclusion:** Ethanolic extract of *Psidium guavajava* leaves showed significant anticonvulsant activity. A higher dose (400mg/kg) of the ethanolic extract showed better antiepileptic activity when compared to a lower dose (200mg/kg).

INTRODUCTION: Epilepsy is a group of heterogeneous neurological disorder characterized by spontaneous and recurrent seizures and is one of the most common disorders that affect 1% of the population [1]. The causes of epilepsy are many, ranging from idiopathic to infection to neoplasm and to head injury [2]. Epilepsy is a common disorder with an incidence of ~ 0.3-0.5 % throughout the world, and the prevalence has been estimated at 5-10 persons per 1000 [1].

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Despite the massive scale of the problem and much research, epilepsy remains poorly understood [3].

Presently available therapy is symptomatic, *i.e.*, the drugs inhibit seizures, but whether any of these prevent the development of epilepsy or cure are uncertain [4]. The currently available drugs fall into 3 major categories depending on their mechanism of action, *i.e.* promote the inactivated state of voltage-gated Na^+ channel, enhanced GABA mediated synaptic inhibition, and limited activation of voltage-gated Ca^{2+} channel [4]. Insights that promise to provide molecular targets for both symptomatic and preventive therapies are being researched. Indigenous medicinal plants are in use since ages for neurological disorders. The added advantage of these would include its complementary nature to conventional treatment, making them safer, well

tolerated, economical, and naturally accessible remedy. Nerve tonic herbs like Ashwagandha (*Withania somnifera*), Brahmi (*Bacopa monniera*), Jatamansi (*Nardastachys jatamansi*), Shanka pushpi (*Evolvulus alsinoides*) Tulsi (holy basil), etc. are used to pacify nervous system and to reduce instances of abnormal brain functioning including episodes of epilepsy [5].

The flower of *Abelsmoscus manihot* has been reported to have a neuroprotective effect against cerebral ischemia injury. The ethanolic extract of this plant contains isoquercitin, hyperoside, hibifolin, quercitin- 3- O glucoside, quercitin. These are found to contribute to its anticonvulsant and antidepressant activity *in-vivo*. [6]

The long history of guava use has led modern-day researchers to study guava extracts *Psidium guajava* (Linn.) popularly known as 'poor man's apple of the tropics,' has a long history of traditional use for a wide range of diseases. Guava contains a broad spectrum of phytochemicals including polysaccharides, vitamins, essential oils, minerals, enzymes, proteins, sesquiterpenoids, alcohols, triterpenoid acids, alkaloids, glycosides, steroids, flavonoids, tannins, and saponins. The leaves of guava are rich in flavonoids, in particular, quercetin. Much of guava's therapeutic activity is attributed to these flavonoids [7].

The various ethnomedical uses of guava are in the treatment of diarrhea, dysentery, skin infection, menstrual disorders, neurological disorders like epilepsy, digestive problems, cough, toothache, bacterial infections, *etc*. [8]

Quercetin is known to produce CNS depressant activity by interaction with presynaptic calcium channels in the CNS. In neurons showing intrinsic burst firing, signaling epileptic activity, there is a massive influx of Ca^{++} associated with the paroxysmal depolarizing shift and hence the influx of extracellular Ca++ into neurons is considered to be an important feature in triggering epileptic activity [9].

Another study has also shown its effect probably through NMDA receptor antagonism [1]. It was therefore considered worthwhile to evaluate the anticonvulsant activity of guava leaves in albino mice.

MATERIALS AND METHODS: Albino mice of either sex of average weight 25-30 gm aged 3-4 months were bred in central animal house. They were housed in groups of three in clean polypropylene cages with 12 h light/dark cycle at 25 ± 2 ºC and 65 ± 5% humidity. They had access to food (standard pellet diet, Hindustan Lever Ltd) and water. All experiments were carried out between 11 AM and 3 PM. Ethical clearance was obtained from the Institutional Animals Ethical Committee for the experimentation.

Distilled water (Control group), Standard drug valproic acid (40 mg/kg) were administered orally. Test drug, ethanolic extract of *Psidium guajava* – 200 mg/kg and 400 mg/kg were administered orally 1 hour before MES and PTZ tests.

Mice were randomly divided into six groups, with six animals in each group. These six groups were used for MES test. After a wash period of 2 days, the same six groups were used for PTZ test. Test drugs, vehicle, and standard drug were administered orally 1 hour before conducting MES and PTZ tests.

Evaluation of Antiepileptic Activity:

Maximal Electroshock-induced Seizures: [10]Electrical stimulation is applied via ear electrodes with a current strength of 50 mA for 0.2 sec. The resultant seizure passes through various phases; phase of tonic limb flexion (1.5-sec duration), phase of tonic limb extension (10-sec duration), finally followed by a variable short clonic interval which may lead to asphyxial death in some animals. Twenty-four hours before testing of anticonvulsants (to avoid any possible kindling effect), the animals are pre-screened for their ability to develop full tonic extension in the maximal electroshock test.

Suppression of tonic hind limb extension is taken as a measure of efficacy in this test.

Pentylenetetrazole (PTZ) Induced Convulsion [11]: 1 hour after test drugs and standard drug administration, 70 mg/kg pentylenetetrazole is injected intraperitoneally. Each animal is placed into an individual plastic cage for observation lasting 30 min. Within 30 min they develop a sequence of excitement, myoclonic jerks, clonic seizures, one or more maximal tonic seizures

followed by recovery or some animals may succumb to death. Seizure latency and the abolition of clonic seizures with loss of righting reflex will be taken as an index of protection.

Statistical Analysis: The effect of *Psidium guajava* extract in two different doses on MES, and PTZ models of seizure induction were expressed as mean ± SD. Percentage inhibition of seizure was calculated, respectively. Data were analyzed using one-way ANOVA followed by Bonferroni's multiple comparison tests. P values <0.05 were considered significant.

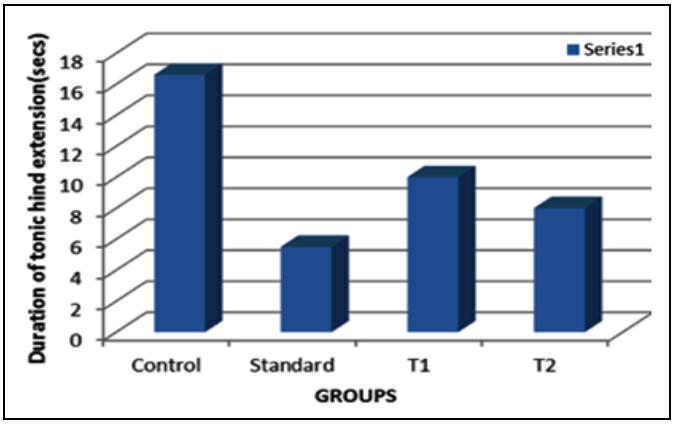
RESULTS:

Maximum Electroshock-Induced Seizures: The ethanolic extract of *Psidium guajava* produced significant dose-dependent antiepileptic activity in comparison to control group. The extract at a dose of 400 mg/kg produces marked reduction in duration of tonic hind limb extension – 8 ± 1.09 sec in comparison to the dose of 200 mg/kg – 10 ± 1.89 sec. The duration of tonic hind limb extension and the percentage inhibition of seizure by the respective groups are depicted in **Table 1** and **Graph 1**.

TABLE 1: EFFECT OF ETHANOLIC EXTRACT OF *PSIDIUM GUAJAVA* ON DURATION OF TONIC HIND LIMB EXTENSION ON MES MODEL

Groups	Duration of tonic hind limb extension (secs)	% inhibition of seizure
Control	16.6 ± 0.51	0
Standard	5.5 ± 0.54	66.8
T1	10 ± 1.89	37
T2	8 ± 1.09	49

Values are expressed as mean ± SD. Statistical analysis of data was carried out by one way ANOVA followed by post hoc Bonferroni' s multiple comparison tests. *p-value < 0.05 - significant.



GRAPH 1: EFFECT OF ETHANOLIC EXTRACT OF PSIDIUM GUAJAVA ON DURATION OF TONIC HIND LIMB EXTENSION ON MES MODEL

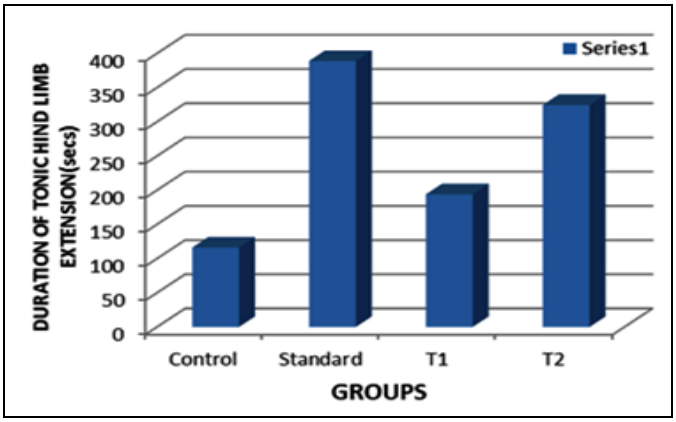
PTZ Induced Seizures: The ethanolic extract of *Psidium guajava* produced significant dose-dependent antiepileptic activity in comparison to control group. The seizure latency produced by the extract at a dose of 400 mg/kg (T2) is 324.3 ± 4.13 in comparison to the dose of 200 mg/kg(T1) – 193.6 ± 3.44.

The duration of seizure latency and the percentage of protection from seizure by the respective groups is depicted in **Table 2** and **Graph 2**.

TABLE 2: EFFECT OF ETHANOLIC EXTRACT OF *PSIDIUM GUAJAVA* ON DURATION OF SEIZURE LATENCY IN PTZ MODEL

Groups	Seizure latency (sec)	% protection from seizures
Control	115.8 ± 2.63	30
Standard	388.6 ± 18.18	100
T1	193.6 ± 3.44	50
T2	324.3 ± 4.13	83.4

Values are expressed as mean ± SD. Statistical analysis of data was carried out by one way ANOVA followed by post hoc Bonferroni' s multiple comparison tests. *p-value < 0.05 - significant.



GRAPH 2: EFFECT OF ETHANOLIC EXTRACT OF *PSIDIUM GUAJAVA* ON DURATION OF SEIZURE LATENCY IN PTZ MODEL

DISCUSSION: Epilepsy is a major public health issue in many nations, and the frequency of epilepsy and seizures increases in later life. Due to the heterogenicity of the disease and our limited understanding of it, discovery and development of anti-epileptic drugs have been especially difficult. In the above study, the ethanolic extract of *Psidium guajava* produced a significant antiepileptic effect in a dose-dependent manner. The percentage inhibition of seizure produced by T2 is 49%, and T1 is 37% in MES test. And the percentage of protection from seizure by T2 is 83.4% and T1 is 50% in PTZ test. Hence, the higher dose

(400mg/kg) has better antiepileptic activity in comparison to the lower dose (200mg/kg).

A thorough survey of the literature reveals that several plants such as *Acorus calamus, Crocus sativus, Emblica officinalis, Ginkgo biloba, Hypericum perforatum, Matricaria recutita, Panax ginseng, Passiflora incarnata, etc*. have been reported to exhibit anticonvulsant activity. Various classes of phytoconstituents such as alkaloids, lipids, terpenes, triterpenoids, flavonoids, and coumarins have been reported to possess anticonvulsant activity. The hexane extract of leaves and its active fractions produced antiepileptic activity by blockade of extracellular Ca^{2+} [11]. Quercetin exhibited mild anticonvulsant activity in MES-induced convulsion test at 50 mg/kg, p.o. [12]. Another study showed that quercetin administration (40 mg/kg/day; i.p.) inhibited amygdala electrical kindling, including its seizure severity and duration.

Neuroprotective effect of quercetin against neurotoxin-induced damage has already been reported in the central nervous system. Quercetin, the flavonoid commonly present in guava leaves could reduce neuroplastic changes in neural circuits and also augmented excitability in certain sited involved in epilepsy. On the other hand, quercetin and its derivatives can selectively inhibit NMDA receptor functionality.

The neuroprotective effect of quercetin in addition to its intrinsic antioxidant effect could be least partly due to modulation of XIAP expression, hints to a possible target for antiepileptic interventional measures [13]. Pretreatment with 50mg/kg of quercetin could attenuate seizure severity from the beginning of the kindling experiment by lowering the mean seizure stages in PTZ model [14].

Quercetin was found to have sedative effects and while traditionally being used as a nerve-calming agent was found to have MAO-A inhibitory activity. Quercetin also was found to affect neuroinflammation [15].

Quercitin (100mg/kg) significantly increased generalized tonic-clonic seizure onset and decreased generalized tonic-clonic seizure duration compared with control. Hence found to have effective anticonvulsant activity [16].

CONCLUSION: The ethanolic extract of *Psidium guajava* leaves produced significant antiepileptic activity. The flavonoid quercetin present in the extract is the main phytoconstituent contributing to its antiepileptic activity. However, further studies are required to determine the exact mechanism of the antiepileptic action of *Psidium guajava*.

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CONFLICT OF INTEREST: Nil

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