

Protective effect of ethanolic extract of *Psidium guajava* L. leaves in tacrine-induced orofacial dyskinesia by assessing its neurobehavioral and biochemical alterations in rats

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The present study investigated the protective effect of the ethanolic extract of *Psidium guajava* L. against tacrine-induced orofacial dyskinesia in rats. Behavioral assessments such as vacuous chewing movements, tongue protrusions, orofacial bursts, locomotion, and cognition were recorded. Forebrain of tacrine treated animals was assessed for its antioxidant levels. *P. guajava* (100 and 200 mg/kg, p.o.) significantly ($P < 0.05$) inhibited tacrine induced vacuous chewing movements, tongue protrusions, orofacial bursts and significantly ($P < 0.05$) increased locomotion and cognition. Treatment with *P. guajava* (100 and 200 mg/kg, p.o.) exhibited significant elevation in the levels of superoxide dismutase, catalase, glutathione reductase and inhibition of lipid peroxidation in the forebrain region as compared to the tacrine treated group of animals. Results suggest that *P. guajava* extract (100 and 200 mg/kg) has good potential to ameliorate tacrine induced orofacial dyskinesia.

Keywords: Orofacial dyskinesia, *Psidium guajava* L., Tacrine, Vacuous chewing movement.

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Introduction

Orofacial dyskinesia is an involuntary repetitive movement of the mouth and face. In most cases, they occur in older psychotic patients and in whom long-term treatment with antipsychotic drugs of the phenothiazine and butyrophenone groups. These dyskinesias are frequent in occurrence and characteristically are irreversible. Several biochemical mechanisms have been proposed as causes, including hypersensitivity or partially denervated brain dopamine receptors and low affinity of the offending drugs for brain muscarinic cholinergic receptors. Clinical therapy has been attempted primarily with drugs that antagonize dopamine receptors or deplete brain dopamine¹.

Tacrine is a parasymphathomimetic, anticholinesterase that is indicated for the treatment of mild to moderate dementia of the Alzheimer's disease. The drug is associated with extrapyramidal motor side effects in humans; these effects include various parkinsonian symptoms such as bradykinesia, rigidity, and tremor².

In rats, central muscarinic receptor stimulation produce a number of different orofacial movements, of which the most common is known as vacuous chewing movement (VCM) purposeless chewing. Tacrine is postulated to exert its therapeutic effect by enhancing cholinergic function. This is accomplished by increasing the concentration of acetylcholine at cholinergic synapses through reversible inhibition of its hydrolysis by acetylcholinesterase, thus it produces VCM, tongue protrusions (TPs), orofacial bursts (OBs)^{3,4}. Acute tacrine has also been reported to cause decrease in the activity of antioxidant defence enzymes, superoxide dismutase (SOD), catalase (CAT) and glutathione reductase (GSH). This supports the free radical hypothesis of orofacial dyskinesia^{5,6}.

Some of the medicinal plants used in orofacial dyskinesia are *Hibiscus rosa sinensis*⁷, roots and rhizomes of *Nardostachys jatamansi*⁸, *Evolvulus alsinoides*⁹, *Murraya koenigii*¹⁰, and *Korean ginseng*¹¹.

Psidium guajava L., belonging to the family Myrtaceae, is a fruit-bearing tree commonly known as guava. Its leaf contains copious amounts of phenolic phytochemicals which inhibit peroxidation reaction in the living body and therefore, can be

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expected to prevent various chronic diseases such as diabetes, cancer, and heart-disease¹². Furthermore, decrease in free-radicals has antioxidantizing effect in the body, meaning these guava leaf polyphenols can prevent arterial sclerosis, thrombosis, cataract and inhibit senescence of the body and skin¹³. Many people habitually take medicinal decoction of guava leaf for long treatment of diarrhoea¹⁴ and the safety of guava leaves has empirically been confirmed¹⁵. People in China use guava leaf as anti-inflammatory and hemostatic agent¹⁶. Guava is rich in tannins, phenols, triterpenes, flavanoids, essential oils, saponins, carotenoids, lectins, vitamins, fibre, and fatty acids. It has been reported that the leaves of *P. guajava* L. contain an essential oil rich in cineol, tannins, and triterpenes. In addition, three flavanoids (quercetin, avicularin, and guaijaverin) have been isolated from the leaves¹⁷. In view of these, the present study investigated the protective effect of ethanolic extract of *Psidium guajava* leaf in tacrine induced orofacial dyskinesia by assessing its neurobehavioral and biochemical alterations in rats.

Materials and Methods

Drugs and chemicals

Tacrine was purchased from Sigma- Aldrich, Mumbai and all other chemicals used for the study were purchased locally.

Experimental animals

Wistar albino male rats weighing (150-200 g) were procured from Mahaveera Enterprises, Hyderabad, India. They were housed in colony cages and maintained at a temperature of 24 ± 2 °C with a 12 h light/dark cycle and 55 ± 1 % RH, maintained on standard pellet and water *ad libitum* throughout the experimental period. The animals were acclimatized for a period of one week. The experiments conducted were according to the guidelines of the committee for the purpose of control and supervision of experiments on animals (CPCSEA), New Delhi, India and approved by the Institutional Animal Ethical Committee (PDCPS/CPCSEA/001/2013-14). The rats were randomly divided into four groups of five in each group¹⁸: i) Vehicle treated group (10 mL/kg), ii) Tacrine (2.5 mg/kg, i.p.) treated group, iii) Ethanolic extract of *P. guajava* (100 mg/kg, p.o.) daily for 15 days and Tacrine (2.5 mg/kg, i.p.) on day-15 after one hour of administration of the extract, and iv)

Ethanolic extract of *P. guajava* (200 mg/kg, p.o.) daily for 15 days and Tacrine (2.5 mg/kg, i.p.) on day-15 after 1 h of administration of the extract.

Plant material

The fresh leaves of *P. guajava* were collected during September 2013 from Sainikpuri, Hyderabad and authenticated by the Department of Pharmacognosy, Priyadarshini College of Pharmaceutical Sciences, Hyderabad (Voucher No. PDCPS/081/2013-14).

Plant material extraction

The leaves were cleaned, shade dried, and grounded to coarse powder. The dried powder (100 g) obtained was defatted by maceration with petroleum ether (60-80 °C). The marc was dried and extracted with ethanol. Finally, ethanolic extracts were air dried at room temperature and the yield obtained was 5.6 % w/v. The extract obtained was used for phytochemical analysis. The test samples of ethanolic extract were made in appropriate concentrations using distilled water prior to its use for animal studies.

Phytochemical screening¹⁹

The preliminary phytochemical screening of ethanolic extract was carried out as per methods of Kokate *et al.*

Neurobehavioral parameters

After tacrine administration, rats were placed in a Plexiglass observation box (22 cm × 22 cm × 22 cm) for a 10 min habituation period. All rats were observed for 1 h and the number of VCM, TP, and OB were carefully recorded¹⁸. VCM was defined as a single mouth opening in the vertical direction not directed towards any physical material. TP was noted as visible extension of tongue outside of mouth. The observations during grooming period were not taken into account. The effect of *P. guajava* on locomotion was evaluated using actophotometer and open-field apparatus. The total counts were recorded in actophotometer. The number of squares traversed and number of rearings was recorded in open field apparatus for 5 min. Cognitive behavior was assessed using elevated plus maze learning task. Transfer latency, the time in which the animal moved from the distal end of the open arm to the centre was utilized as an index of learning process. Animals were placed individually at open arm and the transfer latency was recorded.

Biochemical parameters

After observing behavioral parameters, the animals were sacrificed and the brain was isolated. The forebrain was dissected out and rinsed with isotonic saline and weighed. A 10 % (w/v) tissue homogenate was prepared in phosphate buffer (pH 7.4). The homogenate was centrifuged at 10 000 rpm for 20 min and the supernatant was used for estimation of anti-oxidant levels- SOD²⁰, CAT²¹, GSH²², and the extent of lipid peroxidation (LPO)²³. UV spectrophotometer (ELICO, SL-159) was used for the assessment of biochemical parameters.

Statistical analysis

Results were expressed as the mean±SEM. For statistical analysis of the data, group means were compared by one-way analysis of variance (ANOVA) followed by Dunnett's posthoc test, $P < 0.05$ was considered significant.

Results

Phytochemical Analysis

The Phytochemical analysis of ethanolic extract of *P. guajava* revealed the presence of flavonoids, alkaloids, tannins, and saponins.

Neurobehavioral assessments

Administration of tacrine (2.5 mg/kg, i.p.) significantly ($P < 0.05$) induced VCM, TP, and OB in rats. Prophylactic treatment with ethanolic extract of *P. guajava* (100 and 200 mg/kg) for a period of 15 days significantly ($P < 0.05$) and dose dependently decreased tacrine induce VCMs, TPs and OBs (Table 1). Tacrine administration significantly decreased the locomotor activity, *P. guajava* (100 and 200 mg/kg) attenuated tacrine induced hypolocomotion and restored

Table 1—Effect of ethanolic extract of *Psidium guajava* (100 and 200 mg/kg) on tacrine induced orofacial movements in rats

Treatment (mg/kg)	VCM	TP	OB
Vehicle (1)	96.2±7.04	12.22±2.78	2.2±1.2
Tacrine (2.5)	2329±176.8*	149.2±10.68*	102.6±11.16*
Ethanol extract (100)	1313±14.6**	82.2±7.172**	55.2±7.78**
Ethanol extract (200)	727.2±51.01**	38.2±3.74**	19.6±2.619**
F value	65.15	75.06	35.56

n=5, values are Mean±SEM, * $P < 0.05$ compared to control, ** $P < 0.05$ compared to tacrine treated group, VCM-vacuuous chewing movement, TP- tongue protrusion, OB- orofacial burst.

locomotion significantly ($P < 0.05$) (Table 2, 3). *P. guajava* (100 and 200 mg/kg) also decreased the transfer latency (Table 4).

Biochemical assessments

Tacrine treated animals showed a significant ($P < 0.05$) increase in the levels of LPO and a significant ($P < 0.05$) decrease in the levels of antioxidant enzymes like SOD, CAT, GSH suggesting exaggerated free radical generation. Treatment with *P. guajava* (100 and 200 mg/kg) significantly ($P < 0.05$) raised the levels of antioxidant enzymes like SOD, CAT, GSH, and decreased the levels of lipid peroxidation suggesting its antioxidant potential (Table 5).

Table 2—Effect of ethanolic extract of *Psidium guajava* (100 and 200 mg/kg) on tacrine induced locomotor dysfunction using actophotometer.

Treatment (mg/kg)	Counts
Vehicle (1)	255.8±23.29
Tacrine (2.5)	69.6±17.08*
Ethanol extract (100)	139.2±12.33**
Ethanol extract (200)	222.8±13.99**
F value	23.92

n=5, values are Mean±SEM, * $P < 0.05$ compared to control, ** $P < 0.05$ compared tacrine treated group.

Table 3—Effect of ethanolic extract of *Psidium guajava* (100 and 200 mg/kg) on Tacrine induced locomotor dysfunction using open field apparatus.

Treatment (mg/kg)	No. of squares traversed	Rearings (self+assisted)
Vehicle (1)	12.2±3.023	6.4±1.5
Tacrine (2.5)	1.6±0.92*	1.6±0.81*
Ethanol extract (100)	13.42±3.234**	3.8±1.46
Ethanol extract (200)	22.4±2.396**	3.8±0.802
F value	9.81	1.58

n=5, values are Mean±SEM, * $P < 0.05$ compared to control, ** $P < 0.05$ compared to tacrine treated group.

Table 4—Effect of ethanolic extract of *Psidium guajava* (100 and 200 mg/kg) on Transfer Latency using elevated Plus Maze apparatus.

Treatment (mg/kg)	Transfer latency (S)
Vehicle(1)	7.85±0.23
Tacrine(2.5)	16.23±1.56*
Ethanol extract (100)	10.39±1.22**
Ethanol extract (200)	8.22±0.95**
F value	13.86

n=5, values are Mean±SEM, * $P < 0.05$ compared to control, ** $P < 0.05$ compared tacrine treated group.

Table 5—Effect of ethanolic extract of *Psidium guajava* (100 and 200 mg/kg) on tacrine induced changes in antioxidant levels in rat forebrain.

Treatment (mg/kg)	SOD (U/mg protein)	CAT (U/mg protein)	GSH (nmols/mg protein)	LPO (μM/mg protein)
Vehicle (1)	2.073±0.09	18.49±4.69	34.58±8.62	1.66±0.254
Tacrine (2.5)	1.128±0.255*	3.63±2.86*	6.07±2.30*	8.45±2.7*
Ethanol extract (100)	2.014±0.107**	10.365±0.93**	23.95±3.72**	2.64±0.253**
Ethanol extract (200)	2.078±0.098**	17.04±4.75**	33.09±4.51**	1.89±0.51**
F value	8.91	3.77	7.76	5.44

n=5, values are mean±SEM, *P <0.05 compared to control, **P <0.05 compared to negative control (tacrine treated group).

Discussion

The present study showed that tacrine significantly induces orofacial dyskinesia, locomotor, and cognitive dysfunction in rats. Treatment with *P. guajava* (100 and 200 mg/kg) significantly inhibited these changes and also reversed the antioxidant status induced by tacrine.

It has been suggested that vacuous jaw movements could represent a rat model of parkinsonian tremor²⁴. Cholinomimetics (Tacrine) induces orofacial movements in rats. The predicted mechanisms involved are excitotoxicity and oxidative stress. Tacrine results in increased levels of Acetylcholine in the ventrolateral striatum leading to increased muscarinic receptor stimulation. This results in manipulation of Gamma amino butyric acid and glutamine transmission which may lead to excitotoxicity^{25,26}. It has also been reported that increased turnover of monoamines results in its oxidative metabolism during this hyper activation implying excessive free radical generation and thus, oxidative stress leading to neurotoxic dopamine depletion manifested as orofacial dyskinesia and other parkinsonian symptoms.

In the present study, Tacrine treated animals showed increase in the levels of lipid peroxidation and also exhibited low levels of detoxifying enzymes such as SOD, CAT, and GSH supporting the free radical hypothesis. *P. guajava* (100 and 200 mg/kg) dose dependently protected tacrine treated rats against the increase in LPO, decrease in GSH, CAT, and SOD levels. *P. guajava* (100 and 200 mg/kg) significantly attenuated Tacrine induced orofacial dyskinesia, locomotor, and cognitive dysfunctions. These findings support the earlier reports of antioxidant property of *P. guajava* leaves^{27,28}. This antioxidant effect of *P. guajava* leaf extract may be explained on the basis of antioxidant action of the phenolic and flavonoid constituents. The observed beneficial effects of *P. guajava* (100

and 200 mg/kg) in tacrine induced behavioural and biochemical changes may be attributed to its diversified chemical constituents like quercetin, avicularin, and guaijaverin¹⁷. Further investigations of the mechanism(s) of action of the plant extract and the active substance(s) responsible for its biological actions are necessary.

Conclusion

The leaves of *P. guajava* (100 and 200 mg/kg) has proved to have a beneficial role in the treatment of tacrine induced orofacial dyskinesia in wistar rats.

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