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Neuropharmacological screening of aerial parts of *Passiflora incarnata* Linn (Passifloraceae)

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Abstract

Methanolic extract of aerial parts of *Passiflora incarnata* Linn (Passifloraceae) was investigated for anxiolytic and antioxidant activities in animal models. Anxiolytic activity of methanol extract of aerial parts of *Passiflora incarnata* Linn was tested by Forced swimming test .The results showed a significant anxiolytic effect comparable,with diazepam in all the tested doses. *Passiflora incarnata* Linn is also showing antioxidant activity by histopathology and biochemical parameter. In Histopathology slides of Methanolic extract of aerial parts of *Passiflora incarnata* treated groups' shows prevention of degeneration of pyramidal cells, astrocytes and also shows collaterals when compared to control groups.

Keywords: *Passiflora incarnata*, Chronic Anti--anxiety, Antioxidant, Acetylcholinesterase.

Introduction

The plant *Passiflora incarnata* Linn (Family:Passifloraceae) is a fast growing perennial vine with climbing or trailing stems. It is a common wildfower in the southern United States. It is known as Passion Flower in English,Krishna kamalam in Tamil and Jhumkalata in Bengali.The fresh or dried whole plant has been used as a herbal medicine to treat nervous anxiety and insomnia[1]. Earlier reports showed that the plant possess antispasmodic, sedative[2], hypotensive, antimicrobial and antifungal activity[3].With reference to the above related activities of the plant part, the present study was carried out to investigate the chronic Anti-anxiety and antioxidant potential of methanolic extract of aerial parts of *Passiflora incarnata.*

Materials and Methods

Plant material

The aerial parts of *Passiflora incarnata* Linn was collected from universal Horticulture five fall road Kuttraliam TamilNadu in the month of December 2008 and identification of plant was done by Prof.P.Jayaraman.,PhD Plant Anatomy Research Centre, Medicinal plant Research Unit,Thambaram,Chennai-45 and it has been authentified. A Voucher specimen was deposited in the Department of Pharmacognosy (SRMCP/07/08), SRM College of Pharmacy for future reference.

Preparation of extract

The aerial parts of *Passiflora incarnata* Linn was dried in the shade and powered (≠ 60 mesh size) and 300gm of powder was defatted by using hexane for 18hr and the powder was extracted by soxhlet extraction method with methanol(70%) and water(30%)as solvent. The extract was evaporated using Rotovac apparatus the yield of powdered drug was 22gm.

Experimental Animals

Inbred adult male Wistar albino rats (150-200 g) and albino mice were obtained from the animal house of SRM College of Pharmacy. The animals were maintained in a well-ventilated room at a temperature of 25±1˚C with 12:12 hour light/dark cycle in polypropylene cages. Standard pellet feed (Hindustan lever, Bangalore) and tap water were provided *ad libitum* throughout the experimentation period. Animals were acclimatized to laboratory conditions for 10 days prior to initiation of experiments. The project proposal was approved by Institutional Animal Ethical Committee (IAEC/23/2007).

Preliminary Phytochemical analysis

The preliminary phytochemical analysis of the methanol extract of aerial parts of *Passiflora incarnata* Linn was performed by the standard method [4]. Colony inbred strains of wistar albino male rats weighing 200-250gm at the age of three months old, obtained from king institute was used for the pharmacological studies. The animals were maintained under standard condition(light, temperature, humidity and free access to water and food).the rats were then selected and randomly divided into 4groups.Group 1:control-treated with saline solution, Group II:treated with standard drug(Diazepam),Group III:treated with test drug extract 100mg/kg,group IV:treated with test drug extract 200mg/kg was administered per oral (p.o) in the dose of 100mg/kg,200mg/kg.

In-vivo Pharmacological evaluation

***Forced swimming test* (FST)**

The forced swimming test (FST) is the most widely used pharmacological model for assessing antidepressant activity. The test is also used for screening chronic anxiety model.The apparatus consisted a plastic cylindrical tub(60cm ht,40cm diameter)filled to 30cm depth with water at room temperature and forced to swim for 10 minutes.This depth of the water prevents subject from supporting themselves by touching the base of the swim tank with their hind paws.after each swim session,the tank was thoroughly rinsed in order to remove the presents of any potential alarm substances and the water changed.After testing,each animal was towel dried and returned to its homecage. Active components of forced-swim behaviour were assessed based on reported description.The behavioural

measures scored according to these criteria, where time spent for climbing,time spent for swimming, time spent immobile and latency to assume immobility.

Biochemical assessment

Preparation of Brain homogenate

At the end of the experimental period (24 hours after the FST) after 7th and 14th day all animals were sacrificed by cervical dislocation for determination of the Bio-chemical assessment and Histopathology studies .Brain tissues were quickly removed and washed with ice-cold sterial physiological saline(0.9%).A 10% homogenate was prepared in 0.1M sodium phosphate buffer,PH 7.4,centrifuged at 10,000(4^{o}c)for 15minutes to remove cellular debris and the supernatant was used for the analysis of Acetylcholinesterase(AChE)enzyme determination[5],Mono amino oxidase enzyme determination[6] and Estimation of total protein[7]. It is also used for the estimation of antioxidants such as assay of super oxide dismutase, glutathione peroxidase [8], glutathione reductase [9] vitamin C and histopathological studies.

Statistical analysis

The statistical analysis was carried out using analysis of variance (ANOVA) followed by Dunnet's test.P value<0.05 were considered as significant and <0.01 is more significant.

Results

The preliminary phytochemical group tests were performed by the standard protocol and the results are presented in table 1. The results showed the presence of alkaloids, carbohydrates,proteins, flavonoids,glycosides,saponins and terpenes in methanol extract of aerial parts of *Passiflora incarnata* Linn.

Forced Swimming Test (FST)

The results of the anxiolytic effect of methanol extract of aerial parts of *Passiflora incarnata* Linn is presented in table 2 and 3, revealed that the immobility time increases when compared to the control group. MEPI shows an increased time latency to assume immobility. And there was reduced time for swimming and climbing of MEPI treated group when compared with control group. MEPI was effective even in learned stress period of 14 days in forced swimming test in which the swimming and climbing time was reduced well when compared to the 7 days stress and there was increased time of latency to assume inmobility.

Table No: 1 Preliminary phytochemical test for MEPI

SL.No.	Phytochemical Tests	Results
1	Test for Alkaloids	+Ve
2	Test for Carbohydrates	+Ve
3	Test for Proteins	+Ve
4	Test for Steroids	-Ve

5	Test for Sterols	-Ve
6	Test for Phenols	-Ve
7	Test for Flavonoids	+Ve
8	Test for Gums and mucilage	-Ve
9	Test for Glycosides	+Ve
10	Test for Saponins	+Ve
11	Test for Terpenes	+Ve

Key: +Ve: indicates the presence of compounds, -ve: indicates the absence of compounds

Table No: 2 Effect of MEPI on Forced swimming test for 7 days

GROUP	**I**	**II**	**III**	**IV**
SW	121.23±6.3	90.15±5.4	88.16±5.35*	70.34±4.6**
CL	115.52± 2.5	103.14±5.35	99.25±5.4*	75.56±3.5**
LAIM	108.32± 5.6	119.21±4.36	121.21±4.69*	126.65±5.3**
IM	99.45± 2.6	108.56±4.64	114.56±7.36**	119.29±5.9*

Values are expressed as mean± SEM of 6 animals.
Comparisons were made between: Group I (control), II(MEPI 100 mg/kg) and IV (MEPI 200 mg/kg). Symbol represents the statistical significance done by ANOVA, followed by Dunnet's "t" test. .* P>0.05,**P>0.01.

Table No: 3 Effect of MEPI on Forced swimming test for 14 days

GROUP	I	II	III	IV
SW	98.23±4.3	60.14±2.4	58.18±3.35*	42.54±4.9**
CL	82.52± 8.5	50.64±5.89	49.55±5.45*	33.74±1.8**
LAIM	114.32± 7.6	130.22±3.96	141.26±4.70**	136.68±7.5*
IM	120.65± 1.6	118.46±6.34*	120.54±6.98**	117.28±5.8

Values are expressed as mean± SEM of 6 animals.
Comparisons were made between: Group I (control), II(MEPI 100 mg/kg) and IV (MEPI 200 mg/kg). Symbol represents the statistical significance done by ANOVA, followed by Dunnet's "t" test. .* P>0.05,**P>0.01.

Biochemical assessment

Table No: 4 Effect of MEPI on Brain enzyme levels

Treatment	Group I	Group II	Group III	Group IV
Ach Micro mole/min/mg Protein(seconds)	15.55±1.89	15.95±0.99	16.29±0.93 *	16.33±0.72 **
MAO	23.57±1.23	24.16±1.03	27.56±1.47 *	28.29±1.05 **
Protein Mg/dl Tissue	5.45±2.5	5.98±2.40	6.59±1.73 **	6.15±2.34 *
SOD Units/min/mg Protein	18.18±2.40	21.59±1.73 *	22.45±2.34 **	18.45±2.5
GPx Units/min/mg Protein	35.15±1.34	28.46±0.65 *	24.26±1.20 **	33.28±1.36
GR Units/min/mg Protein	22.52±0.25	28.59±0.53 *	31.51±0.85 **	24.53±0.49
Vitamin-c Micro gm/mg Protein	0.321±0.045	0.441±0.019	0.789±.023 **	0.432±0.069 *

Histopathological Studies

In histopathological studies the effect of MEPI treated group shows that the cells are prevented from degeneration and necrosis when compared to the control group due to its antioxidant property. MEPI (200mg/kg) it shows very good prevention of degeneration of cell treated for 7 and 14 days treated group.

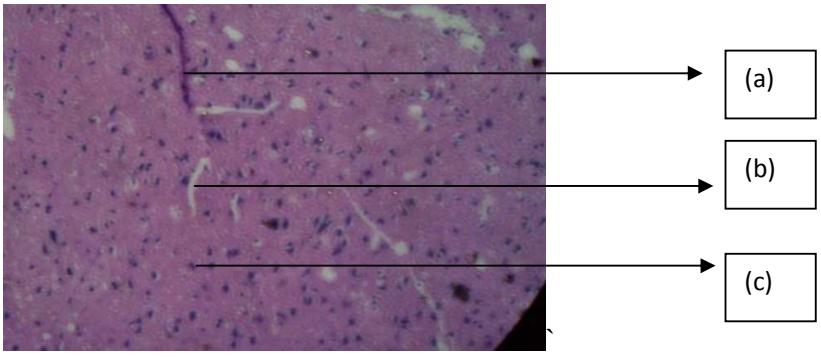

Fig :1 Normal fore brain of rat brain (a)Collateral (b) Pyramidal cell (c) Astrocytes

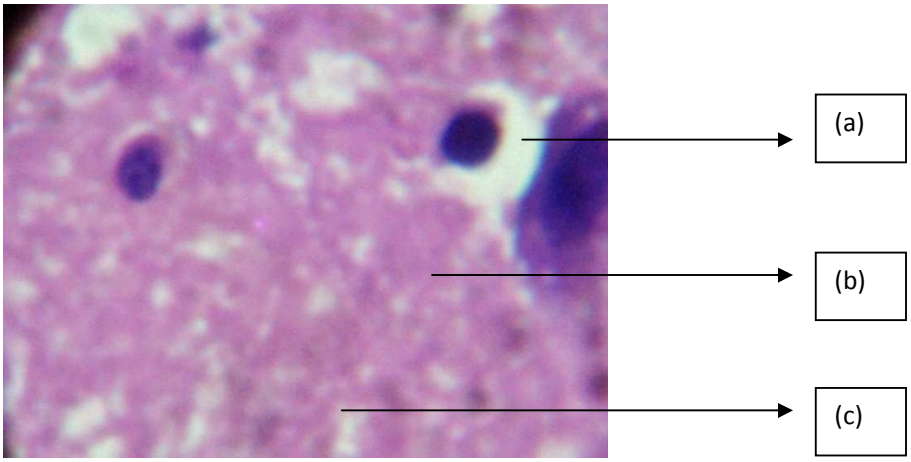

Fig : 2 Stress induced control rat brain after 7 days (a) Necrosis (b) Degeneration of Pyramidal cell and (c) Loss of astrocytes

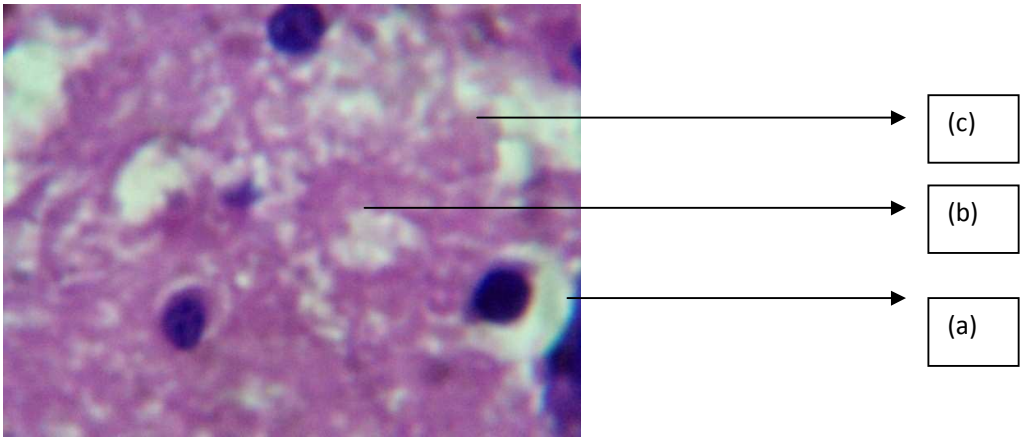

Fig : 3 Stress induced control rat brain after 14 days More (a) Necrosis (b) Degeneration of Pyramidal cell and (c) Loss of astrocytes

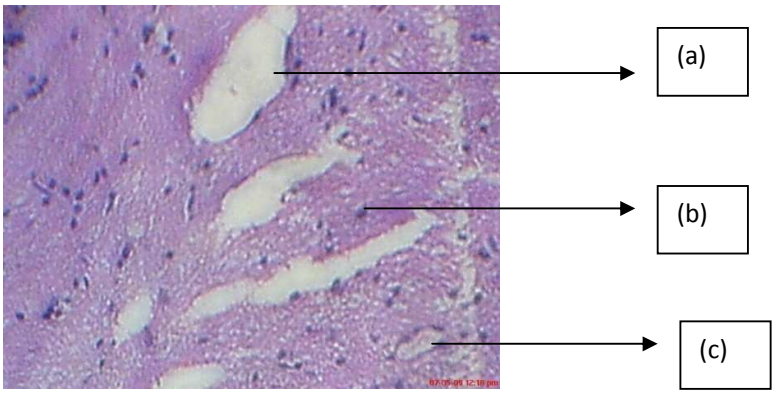

Fig : 4 MEPI(100mg/kg) treated group after 7days mild degeneration of (a) Pyramidal cell, (b) Astrocytes (c) Necrosis

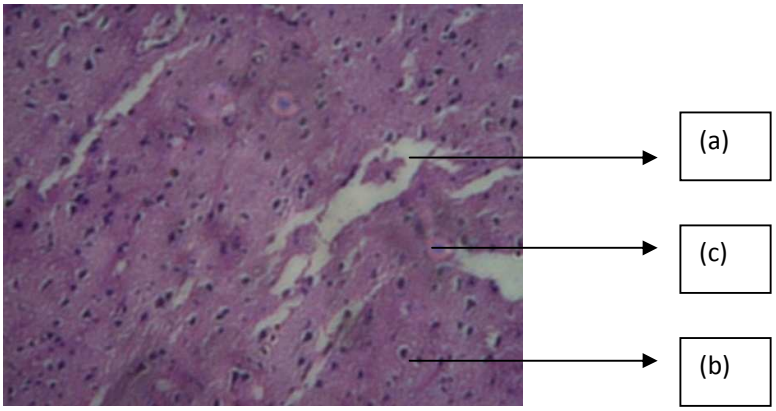

Fig : 5 MEPI(100mg/kg) treated group after 14days mild degeneration of (a) Pyramidal cell, (b) Astrocytes (c) Less Necrosis

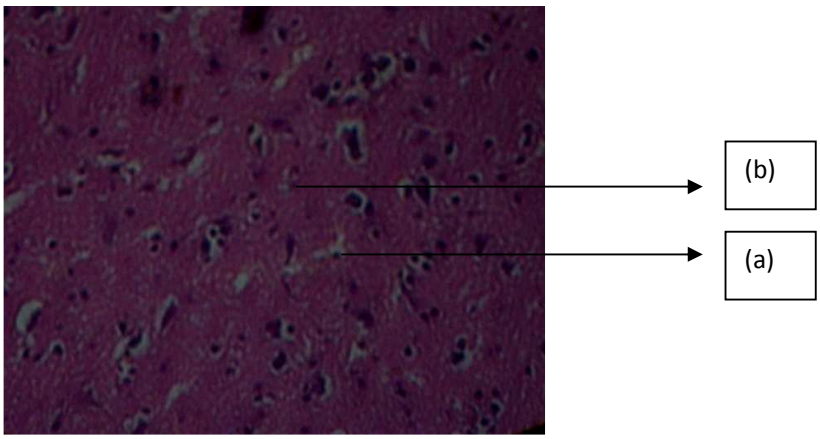

Fig : 6 MEPI(200mg/kg) treated group after 7days prevention of degeneration of (a) Pyramidal cell, (b) Astrocytes

The present study has revealed that MEPI was anxiolytic by stress induced by Forced Swimming Test in male rats. *Passiflora incarnata* is a medicinal plant with antioxidant properties. When stress is induced by FST there was a decrease in Ache and MAO level .MAO and AChE was increased in MEPI treated animals. The MEPI shows very good antioxidant activity due the presence of the iso flavonoids. Presently our study with regards of non enzymatic antioxidant estimated the amount of vitamin C present in the whole brain homogenate. We found increase activity of vitamin C in the MEPI treated group. It was observed that treatment with MEPI increases the time interval of Latency to assume immobility when rats are forced to swim in FST. Standard drug diazepam treated animals were floating and immobile by stretching their body after few seconds only the animals are started to swim because diazepam is also a centrally acting skeletal muscle relaxant hence their swimming time is less when compared with the MEPI treated animals.

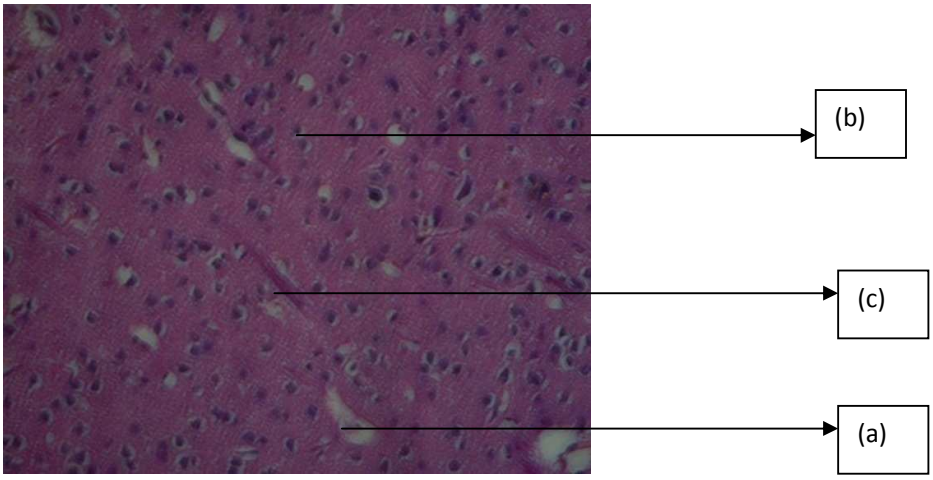

Fig : 7 MEPI(200mg/kg) treated group after 14days prevention of degeneration of (a) Pyramidal cell (b) Astrocytes (c) Collateral cells.

Diazepam treated animals are sinking due to its CNS depressant activity. Due to the CNS depressant activity the animals are shaking their heads to avoid sinking inside the water. The AChE activity has been shown to be decreased in anxiety. The calcium reflux followed by oxidative stress is involved in the decrease in activity of AChE induced by anxiety, increasing cell membrane order and ultimately leading to the exposure of less active enzyme. The observation that anxiety decreases AChE activity. The AChE activity in the brain was increased in rats treated with MEPI when compared with the control.

Histopathology studies also results that the MEPI treated animal showing antioxidant activity when compared with the control animals. Fig : 1 Normal fore brain of rat showing the pyramidal cell, astrocytes, and collateral. Fi*g*:2,3 Stress induced control animal showing the necrosis and cellular degeneration of Pyramidal cells and loss of astrocytes. Fig : 4,5 MEPI(100mg/kg) 7^{th} and 14^{th} day treated group showing the prevention of degeneration of pyramidal cells and astrocytes. Fig : 6,7 MEPI(200mg/kg) 7^{th} and 14^{th} day treated group showing the more prevention of necrosis and degeneration of pyramidal cell and astrocytes and it also showing collaterals like normal rat brain.

Conclusion

Passiflora incarnata is traditionally used for sedative, nervine, antispasmodic and analgesic. It is rarely found in India. The leaves of *Passiflora incarnata* is used as tea in Spain, Italy, US. It is also having anxiolytic activity. The work was about to treat the chronic anxiety that is learned stress. The animals were forced to swim. The first day the stress was induced by FST and later 7^{th} and 14^{th} day of FST the animals are showing more time period for the LAIM because the animals have learned the stress and the IM time period is less due to CNS depressant activity of MEPI. After the FST, the animals were very calm and sleeping when compared to the control animals. MEPI treated animals showing antioxidant activity by the biochemical parameters like AchE, MAO, protein, SOD, GPX, GR and vitamin-c. Histopathological study also shows antioxidant activity when compared with control animals.

MEPI treated animal showing prevention of degeneration of cells when compared with the control animals.Thus the study can be concluded that the Methanolic extract of *Passiflora incarnata* possess significant dose dependant anti- anxiety and anti-oxidant property as confirmed by the parameters.

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