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Effect of Passiflora Edulis Sims on Reserpine Induced Fibromyalgia

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This study was carried out to assess the possible effect of *Passiflora edulis Sims* on reserpine-induced fibromyalgia with using different animal models *and* commonly used in the Virginia, southern Illinois, southeast Kansas and India as a folk medicine. Possible effect of extract of the plant was evaluated on reserpine-induced fibromyalgia. For evaluating the effect of this Plant leaves extract, different models were used such as tail flick, radiant heat, hot plate and inclined plane model. Evaluation of anti-depression activity, forced swim test and elevated plus maze (EPM) model were used. Investigations were shown that reserpine-treated animals responded with significantly increased sensitivity of pain in tail flick latency, decreased threshold of paw-withdrawal and immobility time and in Randall test. Whereas Plant leaves extract at different level of doses (e.g. 200 and 400 mg/kg) has shown a significant reduction in time of immobility, withdrawal latency of tail and the significant increase in mechanical and thermal hyperalgesia. The *Passiflora edulis Sims* showed inhibition of algesic condition in all the models which was dose dependent. During forced swim test extract of plant showed the significant reduce immobility time as compared with the control group, also in the plus maze method, Plant leaves extract showed increased time spend in open arm. The results were confirmed that the use of the extract of leaves of *Passiflora edulis Sims* in the traditional management of pain and enhances behavioural activity.

Keywords: Fibromyalgia, Pain, Antinociceptive, Depression, *Passiflora edulis Sims*, Reserpine.

Fibromyalgia (FM) is a chronic pain which concern with hyperalgesia and it is one of the largest group of soft tissue pain disorder. Fibromyalgia is estimated to affect 2-5% of the adult population[1]. Neurological symptoms develop if the patient persist this pain for more than 3 months such as fatigueness, nonrestorative sleep, cognitive disorder, stiffness, and mood disturbance like condotions [2].

Identification of fibromyalgia by the doctors-pharmacist and patients are looking for medical add for chronic pain symtoms and making regular visits to their registered medical practitioners[3]. Fibromyalgia involves in the neurotransmitter alteration. The production of neurotransmitters is changed then lead to a situation in which some stimuli are perceived more strongly than normal stimuli and producing the

characteristic aches and pains of fibromyalgia[4]. The pathophysiology is not completely known but a number of neuro-hormones, neurotransmitter, sensory disturbances as well as genetic tendency are responsible for its generation. Gender distribution of fibromyalgia is up to seven times more common in females than males. Fibromyalgia affects an individual's quality of life and physical functions[5]. Previous studies have reported that it occurs with depressive disorder and estimated that approximately half of the patients experience a depressive disorder during their life [6] and sleep disorders of fibromyalgia patients[7].

Herbal medicines have been used by people since ancient times and even today traditional medicines are relied upon for healing and treating of diseases in the world[8]. Secondary metabolites of the plants have shown a precious role in curing, treating and preventing various disease world widely[9]. Generally, patients need a different medicines to treat fibromyalgia due to their wide categorical symptoms and affect i.e. muscles pain, depression etc. Recent therapeutic remedies for the treatment of fibromyalgia is concern with a wide category of unwanted exhibitions with the allopathic medicines but the herbal medicines show good therapeutics without so much adverse effects that is why many of the countries have relied on traditional natural medicines[10].

Passiflora edulis Sims is a rapidly growing perennial herb from Virginia, southern Illinois and southeast Kansas. In India, it's diversified in the manly tropical region i.e. Erode (TN), a botanical garden of India (Coimbatore) and also Southern Andhra Pradesh[11]. Traditionally this plant has been reported to have possible antiseizure, antidepressant, astringent, cardiotonic, disinfectant, nervine, neurasthenic, tranquilizer, and vermifuge properties. Traditionally it is having powerful effects to treat of neurological and chronic diseases such as depression, heart disorders and also cancer. It is also used for mood disorders such as stress, anxiety, sleep disturbances, headaches, migraines, and general pain, stomach disturbances.

Native American, Indians, Aztecs, and Mayas civilizations have been using *Passiflora edulis Sims* as a pain remedy[12]. It is used for medicinal purposes as well as a food source and contains various components such as, nutrients, non-nutritive phytochemicals and acids and sugars. This research has focused on herbal cures of fibromyalgia among indigenous populations around the world.

The purpose of the research, investigating traditionally claimed pharmacological activity of analgesic and antidepressant. The title plant has been used as a medicinal plant for the ancient time in India to treat many diseases because this plant possesses medicinal properties to improve ill condition. India has ethnic and biological diversity since ancient times and widely used vast variety of plants resources for medicine. Selection of this plant was based on ethnomedicinal uses and followed up of existing literature on the use of this species.

MATERIALS AND METHODS

Collection and authentication

The *Passiflora edulis Sims* was collected from area of Erode in India, authenticated by the Dr. G.V.S. Murthy, Agricultural University, Coimbatore, India. The identification number is BSI/SRC/5/23/2010-11/162.

Extraction of plant

Dried leaves of plant properly crushed and taken approximately 400gm in the form of coarse powder, and then taken in to soxhlet apparatus and extracted with pet- ether till the solvent became colourless. Marc was taken out and dried. After drying, the powdered marc was weighed and then again packed. Then marc was extracted with ethanol solvent (70%) till it became colorless. The leaves extract was concentrated by the distillation process and evaporated, to obtain a greenish mass[13].

Preparation of reserpine solution

Glacial acetic acid was taken, reserpine mixed properly in it and diluted upto a fix concentration of 0.5 % with distilled water and administered to experimental animals with the dose of 1 ml /kg subcutaneously.

Experimental animals

Healthy wistar rats (wt. 200-250 g) were used in this study. Animals of either sex were divided into different group. The animals were kept in standard experimental conditions such as room temperature maintained (25±1°C), relative humidity (50±5%) with 12 h light:dark cycle. Experimental animals were fed with standard rodent feed (Modern Scientific, Gwalior, India)

with water ad libitum. Experiments were performed on experimental animal (wistar rats) in according to the guidelines of CPCSEA, 891/PO/RE/S/OS/ CPCSEA is the registration number, approval of the experimental regime by the institutional animals ethical committee. Proposal number of experiment was SRCP/IAEC/07/12-03-2018.

Selection of dose

Plant leaves extract dose was selected on the basis of previous research a reference which was calculated on the basis of OECD-425 guidelines[14]. The calculated therapeutic doses were 200 mg/kg & 400 mg/kg per body weight for rats. Thus ethanolic leaves extract doses 200 and 400 mg/kg (Passiflora Ethanolic leaves extract - PEE-1 & PEE-2) were selected for the study. Gum acacia (2%) was used as a vehicle for administration of Plant leaves extract to animals. Diclofenac, Ibuprofen, Diazepam and Fluoxetine used as standard drugs at the different respective doses according to earlier literature.

Evaluation of analgesic property

Thermal stimuli

Tail flick model using radiant heat[15]

This process is based on that observation in which drug is able to prolong the reaction time of tail-flick withdrawal while dipping of the tail in hot water at temperature of 55 degree Celsius. The lower part of the tail has been marked at 5 cm; this part of the tail is actually immersed in a water bath. The rat shows withdraw the tail. Response time is recorded in the minimum units with the help of stopwatch after every measurements and tail was dried with proper caring. Response time was calculated before and after the time of the oral administration of standard & extract drug. Percentage increases in reaction point in time of pain responses were calculated. Percentage increase in reaction time = [Rt-Rb]÷Rb ×100 Where, Rt = Reaction time of treated drug; Rb = Basal reaction time.

Grouping of animals-

Group 1: Normal control: normal saline received (10 ml/kg).

Group 2: Fibromyalgia control: received 10 ml/ kg normal saline.

Group 3: Standard drug: received Diclofenac sodium (20 mg/kg, i.p.)

Group 4: Plant leaves extract: (PEE-1) received with dose- 200 mg/kg p.o.

Group 5: Plant leaves extract: (PEE-2) received with dose- 400mg/kg p.o.

Hot plate model

Animals were placed on a hot plate and temperature maintained with 55°C ±1. Placement time of the animals on the hot plate and various events like licking of the hind paw, shaking or jumping off from the surface were recorded as the response latency. The Wistar rats weighed between 200-250 grams were used to assess the analgesic activity and divided into five groups. The animals were fasted before starting of the experiment for 12 hr, given only water. Standard drug was given orally after 60 minutes, animals were kept on hot plates and comments were recorded and at intervals of 1,2,4,6 and 8 hours.

Group 1: Normal control: dose-10 ml/kg normal saline.

Group 2: Fibromyalgia control: received-10 ml/ kg normal saline.

Group 3: Standard drug: received ibuprofen with 100 mg/kg i.p. dose.

Group 4: Plant leaves extract: (PEE-1) received with dose- 200 mg/kg p.o.

Group 5: Plant leaves extract: (PEE-2) received with dose- 400mg/kg p.o.

The animals gently dropped on the hot plate. The time of latency of pain responses in term of paw jumping or paw licking was observed. Cut off reaction time of 10 sec was chosen in order to avoid physical injury. The basal reaction time and reaction time were recorded. The percentage increase in reaction time of pain responses were calculated by the following formula. The percentage increase of reaction time = [Rt-Rb]÷Rb ×100 Where, Rt = Reaction time of treated drug Rb = basal reaction time

Pain state model using mechanical stimuli

Inclined plane model

Pain state model was using mechanical stimuli inclined plane / Randall Sellito apparatus. The plane has two rectangular wooden board which is connected to one end and another is freely movable to various degrees. The second running plane is fixed on two wooden side panels with a marked degree on their surface. The height of 0.2 cm rubber mat, the inclined plane is set at 65 degrees. The test and standard groups of 4 rats of each were administered orally after, 60 and 90

minutes, the rats were placed in the upper part of the tilted plane and it has been given 30 seconds or hanging or falling. After 4,11,19,26 min., the experimental animals were placed on upper part of the inclined plane for thirty sec to hang on or to fall off.

Group 1: Normal control group: received-10 ml/kg normal saline.

Group 2: Fibromyalgia control: received-10 ml/kg normal saline.

Group 3: Standard drug: received Diazepam (20 mg/kg i.p.).

Group 4: Plant leaves extract: (PEE-1) received with dose- 200 mg/kg p.o.

Group 5: Plant leaves extract: (PEE-2) received with dose- 400mg/kg p.o.

After thirty minutes, animals were placed on the inclined plane at 45° angle for two min, maintain the ability to remain at such inclination was noted.

Evaluation of antidepressant activity

Porsolt forced swim test model[16].

Animals were placed in a plastic tank size measured 45 × cm × 35 cm × 60 cm (l+w+h) for 30 minutes (pretest session) a day before the test. After 24 hr (the test session), five-minute session was recorded as the total duration of non-continuity in the score (in seconds). Rats were decided to be stable when the legs of the feet were not moving forward and the rat went ahead (a temporary condition). The change in the immobility period was calculated after administering drugs to the groups as mentioned in the table.

Group 1: Normal control: received 2% gum acacia.

Group 2: Fibromyalgia control: received 2% gum acacia.

Group 3: Standard drug: received fluoxetine with 20mg/kg, i.p dose.

Group 4: Plant leaves extract: (PEE-1) received with dose- 200 mg/kg p.o dose.

Group 5: Plant leaves extract: (PEE-2) received with dose-400mg/kg p.o dose.

Elevated plus maze model (EPM)[17]

Plus maze model was used to assess neurological like anxiety and motor behaviour of the animals. The EPM made up of four arms, 40 cm in length and 20 cm in width, arranged in such a way that the two arms are opposite to each other. The floor of the maze elevated at 50 cm. Two arms were enclosed by walls with 30 cm high and the other two arms were exposed. Groups of rats for force swimming test were:-

Group 1: Normal control group: received 2% gum acacia.

Group 2 Fibromyalgia control group: received 2% gum acacia.

Group 3: Standard drug group: received diazepam with the dose of 20 mg/kg i.p.

Group 4: Plant leaves extract group: (PEE-1) received with dose-200 mg/kg p.o.

Group 5: Plant leaves extract group: (PEE-2) received with dose- 400 mg/kg p.o.

Statistical Analysis

Results were expressed as mean ± SEM. Statistical significance was determined by one way ANOVA (Analysis of Variance) with dunnet's test.[18].

RESULTS

Tail flick model using radiant heat

The tail flick latency of fibromyalgia of control group, significantly decreased ($p< 0.001$) from 15 to 90 min onwards, as compared with normal control groups. Oral intaking of Diclofenac sodium significantly increased ($p<0.001$) tail withdrawal latency on 15, 30, 60 and 90 min,

Table 1. Evaluation of Analgesic property of Passiflora Ethanolic leaves extract using Tail flick model

Groups	Dose mg /kg/p.o	Reaction Time (Min.) 15	30	60	90
Fibromyalgia Control Group	2 ml	13.05±0.51***	12.25±0.29***	12.25±0.33***	13.36±0.27***
StandardDiclofenac Sodium	20	9.41±0.14	6.05±0.23*	4.36±0.16**	3.13±0.21***
PEE-1	200	11.38±0.14	10.98±0.28	8.83±0.21*	7.43±0.12*
PEE-2	400	10.11±0.25	8.41±0.26*	6.11±0.27**	5.6±0.28**

Expressing of values have done as mean ± SEM, Data was analysed by one- way ANOVA followed by Dunnetts't' tests *$p< 0.05$, ** $p < 0.01$ and ***$p< 0.001$ as compared with fibromyalgia control group, ***$p < 0.001$ as compared with normal control group.

respectively, compared with a fibromyalgia control group (Table-1). PEE 1 and PEE 2 significantly increased in tail withdrawal latency ($p< 0.05$ and $p< 0.01$) respectively increases time .when compared with a fibromyalgia control group (Table-1).

Hot Plate model

The paw withdrawal latency of fibromyalgia control group was significantly ($p< 0.001$) decreased on 1,4,6 and 8 hr. as compared with the normal control groups. Ibuprofen treated rats showed significantly increased ($p< 0.001$) in paw withdrawal latency on 8 hr as compared with fibromyalgia control group (Table-2). Whereas treatment with extract 200 mg/kg and 400 mg/kg showed significant increase ($p< 0.05$ and $p< 0.01$) respectively in paw withdrawal latency respectively, as compared with fibromyalgia control group (Table-2).

Inclined plane / Randall apparatus

Paw withdrawal threshold of all groups of animal on day 0 was found to be in range 240-264. No significant difference was found in paw withdrawal threshold among groups on 0 days. The paw withdrawal threshold reduced after reserpine administration induced fibromyalgia. The fibromyalgia control group showed significantly ($p<0.001$) reduction in paw withdrawal threshold from the day 11th onwards when compared with normal control group. Diazepam treated group was found to significantly ($p< 0.001$) increased the paw threshold in the post-treatment period on day 26th when compared to the fibromyalgia control group (Table-3). Plant leaves extract with the dose of 200 mg/kg has shown significant ($p<0.05$) elevation of paw withdrawal threshold on day 19th and 26th respectively, compared with the fibromyalgia control group. Oral administration of the extract (400 mg/kg) was shown significantly ($p<0.01$) decreased paw withdrawal latency on 11th, 19th, and 26thdays, compared with the fibromyalgia control group (Table-3).

Table 2. Evaluation of Analgesic property of Passiflora Ethanolic leaves extract using Hot Plate model

Groups	Dose mg/ml/kg p.o	1sthr	2nd hr	4thhr	6thhr	8thhr
Fibromyalgia Control Group	2 ml	13.14±0.40	13.23± 0.51	13.31± 0.25	13.60± 0.44	13.73± 0.43
Standard Ibuprofen	100	12.60±0.44***	9.41±0.14***	8.05± 0.23 ***	6.01±0.17***	4.25±0.23***
PEE-1	200	13.21±0.27	10.66±0.30	9.05±0.37	7.33±0.11*	7.0±0.23*
PEE-2	400	13.06±0.35	9.51±0.25*	8.41±0.26**	7.0±0.27**	6.2±0.30**

Expressing of values have done as mean ± SEM, Data was analysed by one- way ANOVA followed by Dunnetts't' tests *$p< 0.05$, ** $p < 0.01$ and ***$p< 0.001$ as compared with the fibromyalgia control group, $p < 0.001$was found to be significant as compared with a normal control group.

Table 3. Evaluation of Analgesic property of Passiflora Ethanolic leaves extract using a mechanical stimuli-Inclined Plane model

Groups	Dose mg/ml/kg p.o	0 day	4th day	11th day	19th day	26th day
Fibromyalgia Control	2ml	256.16±2.48	258.66±3.98	261.5±3.48	264.21±2.11	266.83±3.57
Standard Diazepam	30	230.5±2.48	198.83±3.21	166.33±4.12***	107.00±3.95***	63.50±3.35***
PEE-1	200	252.33±0.27	230.50±3.34	181.16±5.43	117.83±7.18*	79.13±3.96*
PEE-2	400	248.83±3.31	205.83±3.13	170.50±4.45**	112.4.48**	70.00±4.51**

Expressing of values have done as mean ± SEM, Data was analysed by one- way ANOVA followed by Dunnetts't' tests *$p< 0.05$, ** $p < 0.01$ and ***$p< 0.001$ as compared with fibromyalgia control group, $p < 0.001$was found significant value as compared with normal control group.

Forced swim test

Standard drug fluoxetine (20 mg/kg i.p) showed significantly *(*p< 0.01 and p< 0.001) decreased respectively in immobility time on 19th and 26th day respectively as compared with the fibromyalgia control group (Table-4). Treatment with the PEE-1 (200 mg/kg), PEE-2 (400mg/kg) showed the significant (p<0.05 and p<0.01) decrease respectively immobility time on the 26th day as compared with the fibromyalgia control group (Table-4).

Elevated plus maze Test

Treatment with diazepam showed significantly (p< 0.01, p< 0.001) increased in mobility time and also time spent in open arm respectively, as compared with the fibromyalgia control group. Treatment with the ethanolic leaves extract at the dose of 200 mg/kg and 400 mg/kg significantly (p< 0.05 and p < 0.01) increased time spend in open arm (Table-5).

DISCUSSION

Passiflora edulis Sims has many of the secondary metabolites which is responsible for healing of various discomforts. In this study the ethanolic leaves extract of *Passiflora edulis Sims* exhibited dose-dependent inhibition of algesic & depressive condition in all the models. These models assessed neuroprotective, nociceptive effect in experimental animals induced by reserpine of *Passiflora edulis Sims*. In present study, reserpine-treated rats performed increase sensation of pain, decreased paw-withdrawal threshold and immobility time by randall test. Rats treated with the ethanolic leaves extract (200 and 400 mg/kg) has shown a significantly reduced duration of immobility and increase withdrawal latency of tail and a significant increase in mechanical hyperalgesia and thermal hyperalgesia.

The paw of rat is very sensitive to heat thus taken proper care when hot plate model was

Table 4. Evaluation of anti-depression property of Passiflora Ethanolic leaves extract using Forced swim test

Groups	Dosemg/kg/po	0 day	4th day	11thday	19th day	26th day
Fibromyalgia Control	2ml	24±1.23	61.16±1.24	64.33±1.42	66.16±1.07	72.66±1.66
StandardFluoxetine	20	24.5±1.17	60±1.12	55.16±0.74	49±1.54**	45.16±0.90***
PEE-1	200	21.16±1.60	61.16±1.55	65.16±1.60	63.83±0.83*	62.33±0.33*
PEE-2	400	22.83±0.98	62.33±1.14	60.50±1.05	55.83±0.70**	52.33±0.84**

Expressing of values have done as mean ± SEM, Data was analysed by one- way ANOVA followed by Dunnetts't' tests *$p< 0.05$, ** $p < 0.01$and ***$p< 0.001$ as compared with the fibromyalgia control group, $p < 0.001$ was found significant value as compared with normal control group.

Table 5. Evaluation of anti-depression property of Passiflora Ethanolic leaves extract using Elevated plus maze model

Groups	Dose Mg/kg. po	Number of Entries in Open Arm	Time Taken in Open Arm
Fibromyalgia Control	2 ml	4.35± 0.35	3.58± 0.35
StandardDiazepam	20	8.27± 0.57**	7.49± 0.45***
PEE-1	200	4.95±0.41	5.49±0.84*
PEE-2	400	5.57±0.41*	7.29±0.33**

Expressing of values have done as mean ± SEM, Data was analysed by one- way ANOVA followed by Dunnetts't' tests *$p< 0.05$, ** $p < 0.01$and ***$p< 0.001$ as compared with the fibromyalgia control group, $p < 0.001$ was found significant as compared with normal control group

used. The responses of animal like jumping, paw withdrawal and licking of the paws were observed. Withdrawal latency of the responses significantly increased at two different doses of ethanolic leaves extract of plant leaves. The abdominal spasm responses were induced by Reserpine which is sensitive procedure to examine peripherally acting analgesics. When various endogenous substances like serotonin histamine, prostaglandins (PGs), bradykinins, are liberated then they causes pain, but resepine also causes pain it administered in to body. It postulated that the local receptors are to be taken part in the abdominal constrictions response.

What is pain?...Pain is always defined as an unpleasant, unbearable, uncontrolled sensory experience associated with the damaging of particular part of body. It gives warning signal to us that primarily protection should be taken. Harmful stimuli activate nociceptors through the release of chemical mediators[19]. The analgesic effect of the ethanolic leaves extract of plant may be showed via central mechanisms/receptor systems or peripheral mechanisms in which cell mediators are involved as a key player in pain. The unpleasant feelings are to be associated with acute tissue damage which causes pain. Analgesic effect against thermal stimuli may be through opioid receptors or modulation of neurotransmitters. From the above results, it can be concluded that the plant *Passiflora edulis Sims* possesses analgesic activity

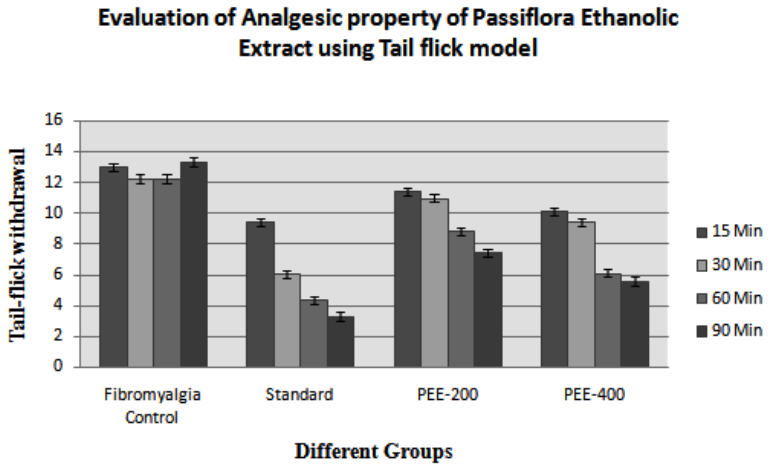

Fig. 1. Dose-response comparison of different dose with different groups of Passiflora edulis extract at different time intervals by the radiant heat tail-flick method

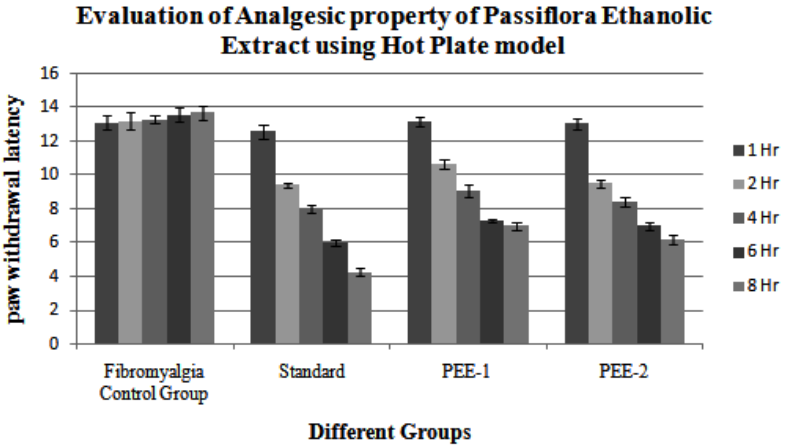

Fig. 2. Dose-response comparison of different dose with different groups of Passiflora edulis extract at different time intervals by the hot plate method

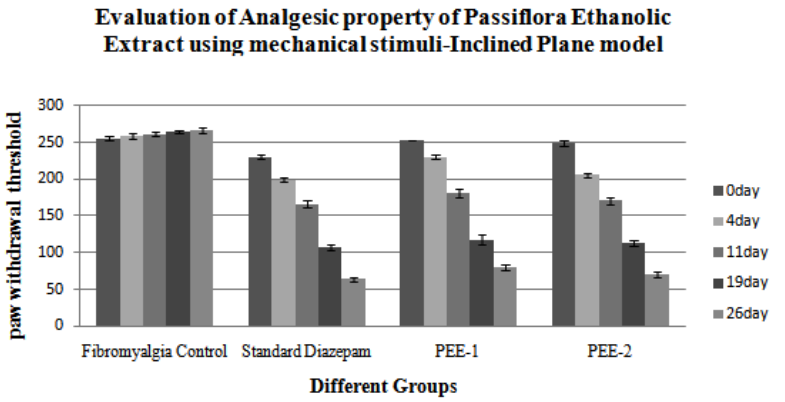

Fig. 3. Dose-response comparison of different dose with different groups of Passiflora edulis extract at different time intervals by mechanical stimuli inclined plane method

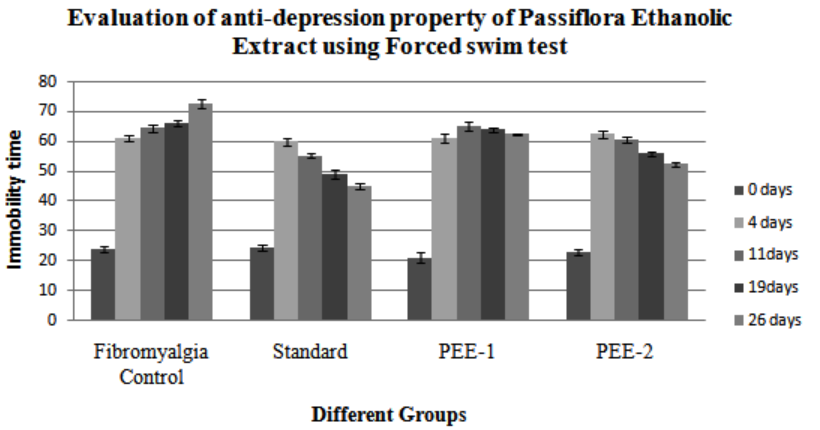

Fig. 4. Dose-response comparison of different dose with different groups of Passiflora edulis extract at different time intervals by the forced swim test method

and better results which were obtained from ethanol extractof leaves. The analgesic property may be interfering of active principles of the leaf extract of *Passiflora edulis sims* with the release of pain mediators. In an Inclined Plane method, the study showed significant decreases in falling from an inclined plane when it compared with the control group, the effects of the extract at the dose of 400 mg/kg is significantly decreased paw withdrawal latency on 11th, 19th, and 26thdays compared with the fibromyalgia control group.

Force swimming and elevated plus maze are most commonly used model for antidepressant screening.

In the forced swimming test, immobility period observed after administration of extract which was significantly changed which indicate that this plant has the property of antidepression. In this test animals were placed into an inescapable cylinder of water then animal reflects the cessation of persistent escape-directed behaviour[20]. The results of the present study indicate that administration of ethanolic leaves extract of *Passiflora edulis Sims* at the two different doses (200 mg/kg and 400 mg/kg) has significant antidepressant activity compared to control and standard drug Fluoxetine.

CONCLUSION

This study confirms the uses of the *Passiflora edulis Sims* in the traditional management of pain and enhances behaviour able functions. It may be concluded that the plant possessed various chemical constituents that revealed above

pharmacological properties. We can do more detail work and explore the lead molecules for the development of new drugs for the treatment of fibromyalgia without any adverse effect. Isolation of the chemical constituents is required for further research concern about pharmacological studies on the healing action of the drug on muscular pain. The investigation on the mode of action may pave the way for the establishment of a newregimen for the treatment of fibromyalgia.

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