TO STUDY THE EFFECT OF ETHANOLIC EXTRACT OF *PASSIFLORA EDULIS* SIMS ON RESERPINE INDUCED FIBROMYALGIA

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Abstract

This study was carried out to assess the possible effect of *Passiflora edulis Sims* on reserpine-induced fibromyalgia with using different animal models. 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. 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 behavioral activity.

KEYWORDS: - *Passiflora edulis Sims*, animal study

1. INTRODUCTION

Fibromyalgia is defined as chronic widespread pain with allodynia or hyperalgesia to pressure pain, and is classified as one of the largest group of soft tissue pain syndromes.[1,2] Fibromyalgia is a chronic widespread pain disorder estimated to affect 2% to 5% of the US adult population.[3,4]

Fibromyalgia (FM) disease is described as a common, bright and pleasant symptom with a variety of symptoms, including sleep, fatigue, and pain.[5,6]

Fibromyalgia is chronic, many symptoms and no effective treatment. It affects 2% of the US population and affects healthcare and healthcare and economic use.[7,8] Besides sleeping sleep, mental and emotional problems, symptoms of complete illness and fatigue For fibromyalgia and therapist, fatigue is severe, multifactylic and complex (76%) complicated and stagnant, for as long as it has been for more than five years, despite severe depression, fatigue is not as severe as it is for many reasons. First, there is no established nomenclature with which to describe the multiple types and manifestations of fatigue.[9,10,11]

Herbal medicine is oldest form of health care known to mankind. Herbs are used in all cultures throughout the ages. A value for first person and great variety of plants is observed. Plants food, clothing, shelter and supply of medicine.[12,13] The use of medicinal plants has been developed by observation of wildlife, as well as trial and error. Over time, the surface of each of their knowledge, their medicinal healing powers are added. They collect information about herbs methodically designed, well defined herbal pharmacopeia. In fact, as well as the medical and medical pharmacology of the 20th century, many local people with herbal lore.[14,15,16]

Herbal medicine is very effective in comparison to the conventional & allopathic form of medication. It's affordable way and easy preparation unlike other forms of medication which can create a big hole in your wallet.[17,18,19]

2. MATERIALS AND METHODS

2.1 Collection of plant

Passiflora edulis sims plants were collected in and around Erode.

2.2 Plant extraction

About 400 gm of air-dried powdered plant material was taken in 1000 ml Soxhlet apparatus and extracted with petroleum ether till the solvent became colorless. At the end of extraction process marc was taken out and it was dried. After drying, the powdered marc was weighed & again packed in soxhlet apparatus. Then the marc was extracted with ethanol till it became colorless. After that extract was concentrated with the process of distillation. The final solution was evaporated, to obtain a syrupy greenish mass.

2.3 Preparation of reserpine solution

Reserpine was dissolved in glacial acetic acid and was diluted to final concentration of 0.5 % with distilled water.

2.4 Experimental animals

Healthy adult male Wistar rats (250-300 g) were used for the study. The animals were housed at standard experimental conditions of temperature (25±1°C) with relative humidity 50±55% under 12 h light: dark cycle. They were fed standard rodent chow (Ashirwad brand, Chandigarh, India) and water *ad libitum*. Experiments were performed in accordance with the guidelines of Committee for the Purpose of Control and Supervision of Experimental Animals (CPCSEA) after the approval of the experimental protocol by the Institutional Animals Ethical Committee (IAEC).

2.5 Justification for Dose Selection

Dose of ethanolic extract of Passiflora edulis sims was selected for rats in the present study is therapeutic dose calculated on the basis of available therapeutic human dose. The calculated therapeutic dose is 200 mg/kg & 400 mg/kg body weight for rats. Hence the dose of 200 and 400 mg/kg were selected for the study.

A. Evaluation of Analgesic property

I. Pain state model using thermal stimuli

- **Tail flick model using radiant heat**
  - **Group 1:** Normal control: normal saline received (100 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**
  - **Group I**: Vehicle treated rats; received 2% gum acacia.
  - **Group II**: (Reference standard) received Ibuprofen (100 mg/kg, i.p.).
  - **Group III:** Plant leaves extract: (PEE-1) received with dose- 200 mg/kg p.o.
  - **Group IV:** Plant leaves extract: (PEE-2) received with dose- 400mg/kg p.o.
- **Pain state model using mechanical stimuli**
  - **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 (30mg/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
- **Forced swim test**
  - **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: (PEE1) received with dose- 200 mg/kg p.o dose.
  - **Group 5:** Plant leaves extract: (PEE2) received with dose-400mg/kg p.o dose.
- **Elevated plus-maze**
  - **Group 1:** Normal control group: received 2% gumacacia.
  - **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.

2.6 STATISTICAL ANALYSIS:

Results were expressed as mean ± SEM. Statistical significance were determined by one way Analysis of Variance (one way ANOVA) followed by Dunnett's 't' test with level of significance set at $P < 0.05$.

3. RESULT AND DISCUSSIONS

3.1 Tail flick model using radiant heat

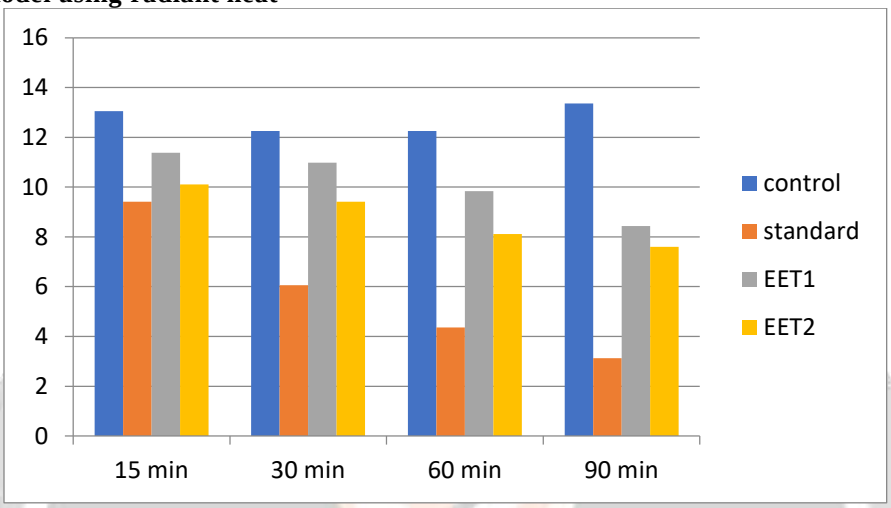

Figure 1. Tail flick model

The tail flick latency of fibromyalgia control group was significantly decreased *(p<0.001)* from the 15 to 90 min onwards, as compared to normal control groups. Oral administration of diclofenac significantly increased *(p<0.05 p<0.001, p<0.001)* tail withdrawal latency on 15,30,60,90 min respectively when compared with fibromyalgia control group. Extract 1 & Extract 2 significantly decreased tail withdrawal latency *(p< 0.01, p< 0.001* respectively) respectively increases time . when compared to fibromyalgia control group.

3.2 Hot Plate model

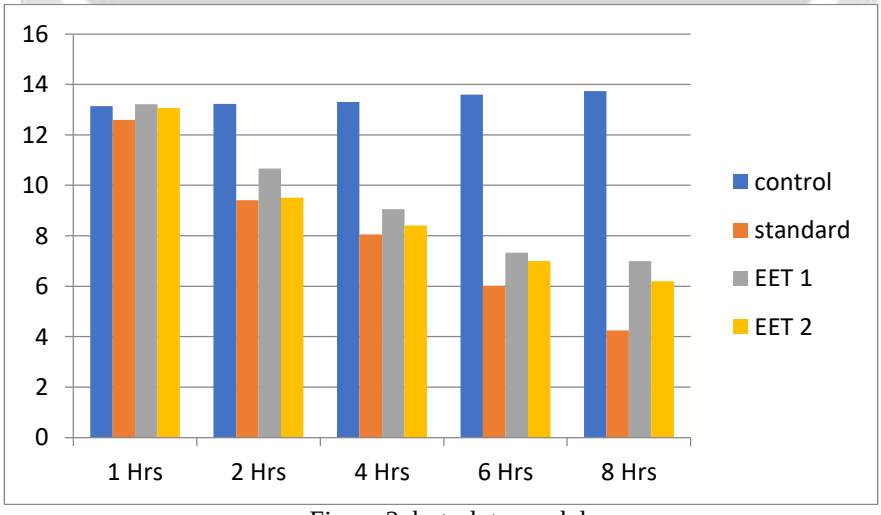

Figure 2. hot plate model

There was no significant difference in tail flick latency among groups . The paw withdrawal latency of fibromyalgia control group was significantly decreased *(p< 0.001)* on 1,4,6 &,8,Hr. as compared to controll groups. This paw withdrawal latency significantly increase*)* from the day 4 onwards by the treatment of extract as compare to fibromyalgia control rats.Ibuprofen treated rats showed significantly increased *(p< 0.01)* in paw withdrawal latency

on 8 Hr as compared to fibromyalgia control group Whereas treatment with Extract (200 mg/kg p.o) & 400 mg/kg showed significant increase (P< 0.001) respectively in paw withdrawal latency only on 8 th Hr respectively, as compared to fibromyalgia control group.

3.3 Inclined Plane / Randall Sellito apparatus

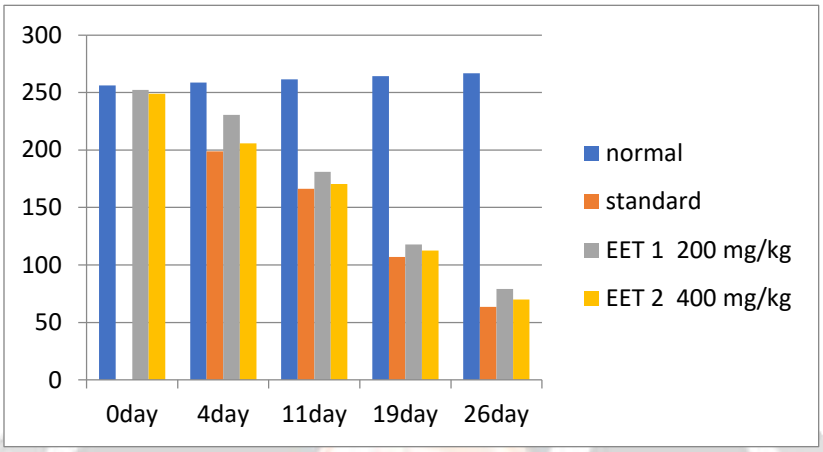

Figure 3. Inclined Plane

The paw withdrawal threshold of all the group of animals on the day 0 were found to be in range 256-264gram. There was no significant difference in paw withdrawal threshold among groups on 0 day. The paw withdrawal threshold reduced after reserpine administration induced fibromyalgia. Fibromyalgia control group showed significant reduction *(p<0.001)* in paw withdrawal threshold from the day 11 onwards when compared to normal control rats. Oral administration of extract significantly increased *(p<0.001)* paw withdrawal threshold on 11, 19 and 26 days when compared with fibromyalgia control group. Diazepam (30mg/kg) was found to significantly elevate *(p< 0.01)* the paw threshold in post-treatment period on day 26 when compared to fibromyalgia control group. Treatment with extract (200 mg/kg) significantly elevated *(p<0.001, p<0.001)* paw withdrawal threshold on day 19 and 26 respectively when compared to fibromyalgia control group. Oral administration of extract (400 mg/kg) significantly increased *(p<0.05, p<0.001)* paw withdrawal latency on 11,19,26thday, respectively, when compared with fibromyalgia control group.

3.4 Evaluation of anti-depression property

3.4.1 Forced swim test

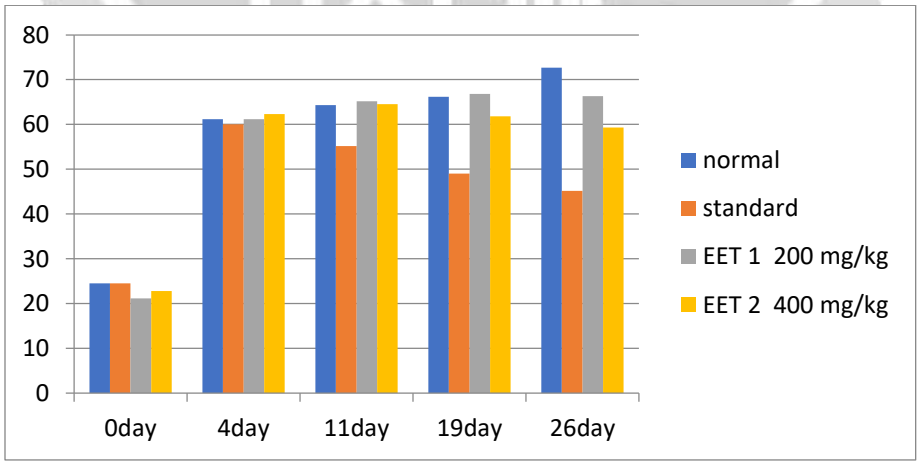

Figure 4. forced swim test

The immobility period of the all the group of animals on the day 0 were found to be in range 21-24sec.There was no significant difference in immobility period among groups on 0 day. The mean immobility period of reserpine treated animals was significantly increased *(p<0.001)* on 4,11,19 and 26th day as compared to normal control group.

Whereas treatment fluoxetine (20 mg/kg p.o) showed significantly decreased *(p< 0.01,p< 0.001)* in immobility time on 19,26th day, respectively, as compared to fibromyalgia control group. Treatment with the extract 1 (200 mg/kg p.o) and extract 2 (400mg/kg) showed the significant decrease *(p<0.001)* immobility time on 26th day as compared to fibromyalgia control group.

3.4.2 Elevated plus -maze model

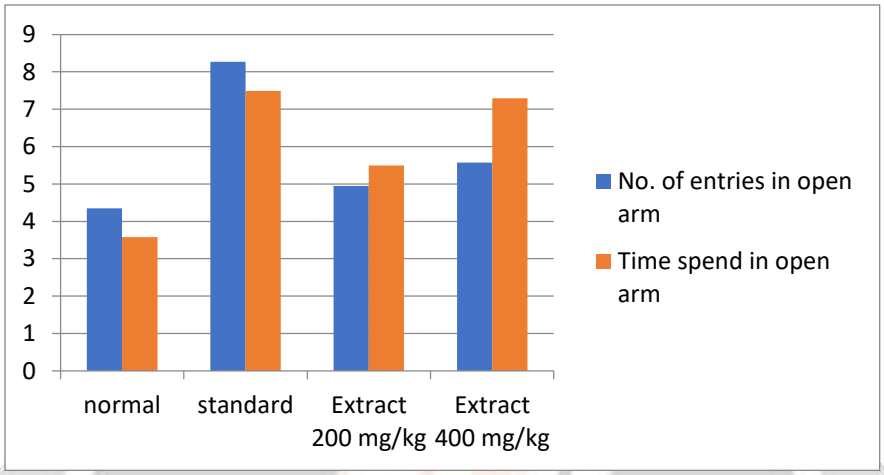

Figure 5. Elevated plus -maze model

The mobility period of the all the group of animals in the open arm gradualy increased in standard group . The mean mobility period of reserpine treated animals was significantly increased *(p<0.001)* as compared to normal standard group. And as well as extract 1 (200 mg/kg), with extract 2 (400 mg/kg). Whereas treatment with diazepam (2 mg/kg p.o) showed significantly encreased *(p< 0.01, p< 0.001)* in mmobility time and also time spend in open arm., respectively, as compared to fibromyalgia control group. Treatment with the ethanolic extract of *passiflora* morein 400 mg/kg as compare with lesser dose.

4. SUMMARY AND CONCLUSION

The present study is to explain the effect of the ethanolic extract of leaves of Passiflora edulis sims on fibromyalgia. It's not a single word but combined effect of various effect like muscle fatigue, muscular pain, their strength as well as mood behaviour like depression and anxiety. In this project we took the ethanolic extract of leaves of Passiflora edulis sims.

On the basis of literature survey, it is found that plant showed high yield with ethanol and since extract will be given orally so it's also minimized possible toxic effect in case of using methanol.

The ethanolic extract of leaves of *Passiflora edulis sims are* tested orally at the doses of 200 mg/kg and 400 mg/kg for treating for analgesic and anti-depressions that induced by Hot plate,Tail flick , Incliend plane, Forced Swim stress, Y maje model.

The ethanolic extract of leaves of *Passiflora edulis sims* exhibited a dose dependent inhibition of analgesic & depressions in all the models. And these models designed to evaluate neuroprotective, nociceptive, muscle relaxant effect of Passiflora edulis sims in reserpine induced fibromyalgia in wistar rats.

In the present study, reserpine rats exhibited increased pain sensitivity in tail flick latency (thermal hyperalgesia), immobility time and decreased paw-withdrawal threshold in Randall-sellito test (mechanical hyperalgesia) , Rats treated with ethanolic extract (200 and 400 mg/kg) showed significant decrease in duration of immobility and tail withdrawal latencyand significant increase in thermal hyperalgesia , mechanical hyperalgesial

In hot plate method the paws of mice and rats are very sensitive to heat at temperatures which are not damaging the skin. The responses are jumping, withdrawal of the paws and licking of the paws. The abdominal constriction response induced by reserpine. It is a sensitive procedure to evaluate peripherally acting analgesics. In general, reserpine causes pain by liberating endogenous substances such as serotonin histamine, prostaglandins (PGs), bradykinins, endings. Local peritoneal receptors are postulated to be involved in the abdominal constrictions response.The method has also been associated with prostanoids in general that is, increased The significant decreases pain threshold produced by tests and standard.

These models suggest involvement of central pain pathways. Pain is centrally modulated via a number of complex processes including opiate, dopaminergic descending noradrenergic and serotonergic systems.

The analgesic effect produced by the tests and standards may be via central mechanisms involving these receptor systems or via peripheral mechanisms involved in the inhibition of prostaglandins, leukotrienes, and other endogenous substances that are key players in pain.

Unpleasant sensory and emotional experience associated with acute or potential tissue damage leads to pain. Analgesic effect against thermal noxious stimuli may be elicited through opioid receptors or through modulation of several neurotransmitters involved in relevant phenomena. From the above results we can conclude that the plant *Passiflora edulis sims* having analgesic activity and better results are obtained from ethanol extract. The analgesic activity of the *Passiflora edulis sims* extract may be due to the interference of their active principles with the release of pain mediators. Thus, further study is needed to identify the chemical constituents present in the extract that may have analgesic activity.

In Inclined Plane method it has been proven to be a simple assay for muscle relaxant activity. Although the muscle relaxant tests satisfy the criteria of sensitivity and relative potency compared with standard doses. The study showed significantly decreases falling from incline plane (79.13 & 241.00) as compared with control group the effects of extract with dose 400 mg/kg are significantly ($p < 0.001$) not clearly differentiated from neuroleptics and even from neurotoxic compounds.

In the present study, the antidepressant activity of ethanolic extract of leaves of *Passiflora edulis sims* were studied in two screening animal models for depression, & anxity. the forced swim test and elevated plus maje model. In depression treatment is required for a prolonged period to get an optimal response; hence it is important to perform not only acute but chronic administration of the drugs in animal models. The results of the present study indicate that acute and chronic administration of ethanolic extract of *Passiflora edulis sims* at a dose of 200 mg/kg & 400 mg/kg has significant antidepressant activity compared to normal control. This antidepressant effect is comparable with standard Fluoxetin(20 mg/kg) has shown significant antidepressant activity..Result shows that extract was effective in restoring the altered monoamine level and to overcome oxidative stress state.

The result of this study confirms the use of the ethanolic extract of leaves of *Passiflora edulis sims* in traditional management of pain and enhance behaviour able activity

This study revealed significant anti-inflammatory, inhibitory activity on both gastrointestinal and locomotors activity. The study, however, revealed no skeletal muscle activity. It may, therefore, be concluded from this study that the plant possessed constituents that revealed above pharmacological properties which may generate lead molecules for development of newer drugs. However, to reach any conclusive decision a detailed phytochemical study for isolation, purification, identification, and characterization of the compound and biological studies with exact mechanism of action responsible for the particular activity, is necessary. Hence, further scientific investigation and specific studies are highly recommended for better evaluation of the potential effectiveness of the plant.

Chemical substances derived from plants have been used to treat human diseases since the dawn of medicine. Roughly 60% of new chemical entities introduced during the past two decades are from natural products. Therefore, efforts should be directed towards isolation and characterization of the active principles and elucidation of the relationship between structure and activity. Furthermore, detailed analysis of the active constituents of natural drugs should be directed towards clinical relevance.

Further research is required to isolate the active phytochemical constituents present in the extract and pharmacological studies on the healing action of drug on muscular pain as well as on the possible side effects. The investigation on mode of action may pave way for establishment of new fibromyalgia therapy regimen.

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