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Psidium guajava Linn Confers Analgesic Effects on Mice

N. R. Livingston Raja[1,2] and K. Sundar[2]

[1]*Department of Biotechnology, Kalasalingam University, Krishnankoil - 626 126, Tamilnadu, India*

[2]*Arulmigu Kalasalingam College of Pharmacy, Krishnankoil - 626 126, Tamilnadu, India*

Abstract

Background and objectives: The unrivalled substitute to synthetic medicines, available, for relieving of pain, are the natural products found in plants. They are known to exhibit a variety of activities. The objective of the present study is screening of *P. guajava for its analgesic activity.*

Material and methods: The methanol extracts of the leaves of *P. guajava* were tested three different pain models viz. Hot plate, Tail immersion and Acetic acid induced pain models in mice.

Results and discussion: The treatment of *P. guajava* at varying doses (100 mg/kg and 200 mg/kg) significantly (P>0.001) reduced the pain induced by Hot plate (77.56%), Tail immersion (77.18%) and Acetic acid induced abdominal contraction (56.75%) respectively and the potency was found to be equivalent as compared to the standard drug,Pentazocin,Diclofenacsodium. The presence of flavonoids, tanins and saponins present in the extracts of *P. guajava* may be accountable for the analgesic property manifested.

Conclusion: The results further suggest that *P. guajava* possess analgesic effect which might act through central and peripheral mechanisms.

Keywords: *Psidium guajava*, Pentazocin, Diclofenacsodium, flavonoids, Saponins, Acetic acid.

INTRODUCTION

Pain is defined as an unpleasant sensation that can be either acute or chronic and that is consequences of complex neuro chemical process in the peripheral and central nervous system [1]. Chronic pain and the inflammatory diseases are one of the major health problems in the world [2-3]. Pain and inflammation are the response of living tissues injury. The injured tissue produces a complex enzymatic reaction, inducing mediator like prostaglandin release, extravasations of fluid, cell migration, and tissue repair [4-5].

Management of pain and inflammation are one of clinical medicine's greatest challenges in world. Currently most of the drugs like NSAIDs and narcotic type of analgesics were widely used for the management of pain and inflammation. As a result of adverse effect such as gastric lesions caused by NSAIDs and drug tolerance, drug dependence developed by narcotic type of analgesics. Due to the development of adverse effect most of the analgesic drugs like NSAIDs and opiates have not been successful in all kind of the people's [6]. Therefore new analgesic agents overlapping these adverse effects are being searched all over the world as alternativatives to NSAIDs and opioids [7]. On the other hand, most of the herbal drugs reduce the pain and inflammation, proved to be safe and clinically effective. So, herbal extracts are one of the most attractive sources of new drugs and have been shown to produce a challenging result in the treatment of pain and inflammation [8].

Psidium guajava Linn commonly known as guajava is widely used in folk medicine in India. Extracts of roots, bark, and leaves of this plant are widely used in the treatment of gastroenteritis, vomiting, diarrhoea, dysentery, wounds, ulcers, toothache, coughs, sore throat, inflamed gums, and a number of other conditions [9]. In the present study the leaf extracts of *P. guajava* are evaluated for analgesic effect in various pain models.

MATERIALS AND METHODS

Drugs and Chemicals

Diclofenac sodium and Pentazocin ((Sigma Aldrich, Saint Louis, USA)), methanol were used in this study. All substances were prepared immediately before use and the reagents were used as analytical grade.

Plant Materials

The leaves of *P. guajava* used in this study were collected from Krishnankoil, Srivilliputur (Virudhunagar dist, Tamil Nadu, India.). The plant was authenticated by Dr.Stephen, Department of Botany, American College, Madurai, India.

Extract preparation

P. guajava leaves were shade dried and coarsely powdered. The powdered materials were extracted with methanol. The last traces of the solvent were removed and concentrated to dryness under vacuum using a rotary evaporator. The dried extract was weighed and then kept at -4ºC until ready for use. The yield of the extract was 26.4 % (w/w). In each experiment, the extract was diluted with water to desired concentration.

Phytochemical screening

A Preliminary phytochemical screening of *P. guajava* was conducted to determine the presence or absence of alkaloids, tannins, phenols, saponins, volatile oil, ascorbic acid, carbohydrates and glycosides by suitable methods [10].

Animals

Adult male albino mice weighing about 20-25g were used in this study. They were maintained in clean, sterile, polypropylene cages and fed with commercial pellet rat chow (M/S Hindustan lever limited, Bangalore, India) and water ad libitum. The study was approved by the Institutional Ethical Committee, which follows the guidelines of Committee for the Purpose of Control and Supervision of Experimental Animals (CPSCEA).

Analgesic activity of *Psidium guajava*

Eddy's Hot plate test:

Male albino mice weighing about 20-25gm were divided in to four groups of five animals each. The dosage of drugs was administered to the different groups.

Group I : Received Control (Normal saline10ml/kg)
Group II : Received Pentazocin 10mg/kg.
Group III : Received *Psidium guajava* at the dose of 100mg/kg orally.
Group IV : Received *Psidium guajava* at the dose of 200mg/kg orally.

Healthy male albino mice weighing 20-25gm were divided in to four groups each consists of five animals. Group-1 were considered as control, Group-2 were received Pentazocin (10mg/kg) served as positive control while group 3&4 were received alcoholic extracts of *Psidium guajava*200 mg/kg body weight respectively. The animals were placed on Eddy's hot plate kept at a temperature of 55+0.5C. A cut of period of 15 sec (Franzotti et al., 2000) was observed to avoid damage to the paw. Reaction time was recorded when animals licked their fore or hind paws, or jumped prior to and 0, 30, 60, 90 and 120 min after oral administration of the samples [11-12]. The average basal reaction time and the % increased in basal reaction time were calculated using student t-test.

Tail Immersion test [11].

Male albino mice weighing about 20-25gm were divided in to four groups of five animals each. The dosage of extract was administered to the different groups.

Group I : Received Control (Normal saline10ml/kg)
Group II : Received Pentazocin 10mg/kg.
Group III: Received *Psidium guajava* at the dose of 100mg/kg orally.
Group IV: Received *Psidium guajava* at the dose of 200mg/kg orally.

The basal reaction time of the each animal was determined by using tail withdrawal response, when one third of tail was immersed in the hot water maintained at 55°c.The reaction time was evaluated 30, 60, 90 and 120 minutes ,after oral administration of extracts, control and standard drug.

Acetic acid induced abdominal contraction test [13]

Male albino mice weighing about 25 – 30 gm were divided in to four groups of five animals each. The dosages of extract were administered to the different groups.

Group I: Received Control (Normal saline10ml/kg)
Group II : Received Diclofenac sodium 50mg/kg
Group III : Received *Psidium guajava* at the dose of 100mg/kg orally.
Group IV : Received *Psidium guajava* at the dose of 200mg/kg orally.

Male albino mice20-25 gm body weight was divided in to four groups of five animals each. First group of the animal were received acetic acid (0.1ml of 0.6% v/v, intraperitoneal)served as control, second group served as positive control were received Diclofenac sodium (50mg/kg) while third and fourth group were received Alcoholic extracts of *Psidium guajava* 100,200 mg/kg body weight respectively. All the extracts were administered orally by using intra gastric tube 15mins prior to the administration of acetic acid injection. The writhing effect indicator by the stretching of abdomen with simultaneous stretching of at least one hind limb, observed for about 10mins and % of protection is calculated for analgesic activity.

Statistical analysis

The results are presented as mean ±SD. The data were also analyzed by one way ANNOVA Tukey's Multiple Comparison Test.

RESULTS

Preliminary phytochemical screening of the extracts of *P. guajava* revealed the presence of flavonoids, triterpenes, saponins, carotinoids, alkaloids, glycosides and carbohydrates Table1. Acute toxicity studies of the alcoholic extract of the *P. guajava* did not exhibit any signs of toxicity up to 2 g/kg body weight. Since there was no mortality of the animals found at high dose. Hence 100, 200 mg/kg dose of the extract selected for evaluation of analgesic activity.

Table 1: Phytochemical screening of the extracts of *P. guajava*

S. No.	Test	*P. guajava*
1	Glycoside	+
2	Carbohydrates	+
3	Phytosterol	+
4	Flavanoids	+
5	Protein	+
6	Alkaloids	-
7	Tannins	+
8	Saponin	+

Hot plate test

Results of the *P. guajava* on Hot plate test are presented in table 2. The extract of the plant is found to exhibit dose dependent increase in reaction time when compared with control. At 120mts the percent inhibition of two different doses (100 and 200mg /kg) was 62.50%, 77.56%. Results are expressed as mean ± SEM from five observation as compared to Control group by One way ANNOVA Tukey's Multiple Comparison Test. **p < 0.05. This difference is considered to be extremely statistically significant.

Tail Immersion test

The data of the *P. guajava* on Tail immersion test is presented in table 3. In this method, the extract of the plant showed a dose dependent increase in latency time when compared with control. At 120mts the percent inhibition of two different doses (100 and 200 mg/kg) was 64.39%, 77.18 %. Results are expressed as mean ± SEM from five observation as compared to Control group by One way ANNOVA Tukey's Multiple Comparison Test. **p < 0.05.

Table 2: Effect of *Psidium guajava* on heat (Hot plate) induced pain in mice

Treatment Dose mg/kg	Mean increased reaction time (sec) Before and after Drug administration.					% Reaction time
	0mts	30mts	60mts	90mts	120mts	120mts
Normalsaline10ml/kg	2.43 ±0.23	2.54 ±0.19	2.16 ±0.18	2.40 ±0.21	2.34 ±0.15	--
Pentazocine10mg/kg	2.74 ±0.21	5.56 ±0.48	9.82 ±0.86	12.43 ±1.1	14.52±1.45**	83.88**
P. guajava 100mg/kg	2.36 ±0.16	3.74 ±0.53	4.82 ±0.61	5.34 ±0.61	6.24±0.56	62.50
P. guajava 200mg/kg	2.14 ±0.17	4.82 ±0.63	7.36 ±0.54	8.25 ±0.67	10.43±0.74**	77.56**

Results are expressed as mean ± SEM from five observation as compared to Control group by One way ANNOVA Tukey's Multiple Comparison Test. **p < 0.05.

Table 3: Effect of *Psidium guajava* on hot water (Tail immersion) induced pain in mice

Treatment Dose mg/kg	Mean increased reaction time (sec) Before and after Drug administration.					% Reaction time
	0mts	30mts	60mts	90mts	120mts	120mts
Normalsaline10ml/kg	2.16 ±0.31	2.36 ±0.24	2.53 ±0.19	2.28 ±0.19	2.25±0.61	--
Pentazocine 10mg/kg	2.43 ±0.14	5.48 ±0.45	7.68± 0.58	11.36± 1.15	12.48±1.41**	81.97**
P. guajava 100mg/kg	2.62 ±0.32	3.64 ±0.18	4.60 ±0.23	5.12 ±0.43	6.32±0.47	64.39
P. guajava 200mg/kg	2.18 ±0.15	4.32 ±0.42	6.10 ±0.26	8.76 ±0.61	9.86±0.73**	77.18**

Results are expressed as mean ± SEM from five observation as compared to Control group by One way ANNOVA Tukey's Multiple Comparison Test. **p < 0.05.

Table 4: Effect of *Psidium guajava* on Acetic acid induced abdominal constrictions in mice

Groups	Dose mg/kg	Number of abdominal constrictions in 10mts.	% inhibition of abdominal constriction in 10mts.
I	Normal saline 10ml/kg	42.36 ±1.73	--
II	Diclofenac sodium50mg/kg	11.20 ± 0.78**	73.55**
III	*P. guajava* 100mg/kg	26.54 ±1.64	37.34
IV	*P. guajava* 200mg/kg	18.32±1.40**	56.75**

Results are expressed as mean ± SEM from five observation as compared to Control group by One way ANNOVA Tukey's Multiple Comparison Test. **p < 0.05.

Acetic and induced abdominal contraction test

The analgesic effect of *P. guajava* on acetic acid induced abdominal contraction test is presented in table 4. In this method, *P. guajava* reduced the abdominal constriction induced by the acetic acid in a dose dependent manner. The percent inhibition of both the doses (100, 200 mg/kg) was 37.34, 56.75. The standard drug diclofenac sodium was found more potent than the plant extract at all of the dose levels. Results are expressed as mean ± SEM from five observation as compared to Control group by One way ANNOVA Tukey's Multiple Comparison Test. **p < 0.05. This difference is considered to be extremely statistically significant.

DISCUSSION

Pain and inflammations are linked with various clinical symptoms like cancer, rheumatic arthritis and cardiovascular diseases [14]. In various time-honored medicinal systems a many more numbers of natural substances are used to counter act the symptoms of pain. In this present study, the analgesic effects of *Psidium guajava i*s tested by involving thermal (Hot plate, Tail immersion) and chemical (Acetic acid) induced pain models on mice. These models symbolize some of the most common causes of pain in humans [15]. The results were unveiled that, the animals treated with *Psidium guajava* showed a significant activity in different animal models of pain.

The Hot plate method is found to be suitable model for evaluation of centrally acting analgesics. This method is one of the acute nociceptive models generally used for testing the central nociceptive activity [16]. The extract of *Psidium guajava* described an increased reaction time when compared to control and standard in a dose dependent manner. These reports are suggested that the extract of *Psidium guajava* must have a property of centrally acting like drugs.

Tail immersion induced pain model also considered for the drugs acting on central nervous system. The *Psidium guajava* extract showed a significant (P<0.001) analgesics activity as compared to control group in a dose dependent manner. A result obtained by this method is confirmed the extract of *Psidium guajava* having a central activity against thermal induced noxious stimuli [17].

Acetic acid induced abdominal contraction method has been used to evaluate peripherally acting analgesics. Acetic acid induced abdominal contraction in mice is attributed for

visceral pain hence finds much important for investigation of analgesic drugs[18]. Pain sensation in acetic acid induced abdominal contraction method is evoked by initiating a localized inflammatory response resulting by the release of free arachidonic acid from the tissue phospholipids through cyclooxygenace enzyme and prostaglandin biosynthesis [19]. In other hand, the acetic acid induced abdominal contraction method has been connected with increased level of PGE2 and PGF2α in peritoneal fluids as well as lipogenase enzyme [20]. The increase in prostaglandin level with in the peritoneal cavity leads to enhances inflammatory pain by increasing the capillary permeability [21].

The *Psidium guajava* extract exhibited a significant analgesic activity in acetic acid induced abdominal contraction (writhing) test in mice. The *Psidium guajava* extract significant reduced the abdominal constriction response in mice with acetic acid. The abdominal contraction response is generated indirectly through endogenous mediator like prostaglandin and part of local peritoneal receptors [22]. So, it can be suggested that the *Psidium guajava* extract may interfered with prostaglandin synthesis and peritoneal receptor to bring about analgesia. The results are indicated that the *Psidium guajava* extract possessed peripheral mediated analgesic activity.

Phytochemical screening showed that the presence of carbohydrates, flavanoids, tannins, saponins, phytosterol in *Psidium guajava*. It is believed that these compounds may be reliable for the observed analgesic activity. Flavanoids were reported to have an analgesic activity by acting on prostaglandin pathway [23]. There is also having a report on the role of tannins in analgesic activity [24]

The investigation of the present study confirmed that the leaf extract of *Psidium guajava* exhibited central peripheral types of pain inhibition against experimentally induced pain models. These data corroborate with earlier observation of *Pergularia daemia*[*25*] *Lantana trifolia* [26] relieved the experimental induced pain models. There fore the analgesic effects of the leaf extract of *Psidium guajava* may be acting through central and peripheral mechanism. However further study in essential in order to understand the exact mechanism.

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