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Analgesic activity of *Psidium guajava* (L.) fruit extracts effect in mice

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Abstract

The unrivalled substitutes to synthetic medicines are available, for relieving pain and these substitutes are from natural products of plants. They exhibit variety of activities. The present study evaluated the analgesic activity of the Aqueous fruit extract of the plant. The Aqueous extract of the fruits of *P. guajava* were tested in three pain models viz. to viz. Eddy's Hot plate, Tail immersion and Acetic acid induced pain models in mice. The treatment of *P. guajava* at varying doses (100 mg/kg and 200 mg/kg) of extract was administered orally by gastric gavage. It significantly ($P<0.05$) reduced the pain induced by Hot plate (74.29%) Tail immersion (76.03%) and Acetic acid induced abdominal contraction (73.92%) respectively and potency found was equivalent as compared to standard drug Diclofenac sodium and Pentazocin. The presence of flavonoids, tanins and saponins in the extract of *P. guajava* are responsible for Analgesic effect.

Keywords: *Psidium guajava*, CNS, Analgesic, Diclofenac sodium

Introduction

Herbs are useful for the treatment of different diseases that affect human and animal. Over 1500 herbal products sold are nutraceuticals which are exempt from extensive preclinical efficacy and toxicity testing by the USFDA in 2003[1-2].

According to World Health Organization (WHO), herbal drugs contains active ingredients in crude or processed state and excipients i.e. solvents, diluents or preservatives. Herbal medicine is still the backbone of about 75-80% of world's population, particularly in developing countries, for primary health care. Currently, interest in alternative and plant derived medications that affects the 'mind' is growing [3-4].

Pain is an unpleasant sensation that can be either acute or chronic and that is consequences of complex neurochemical process in the peripheral and central nervous system.[5] Pain is also a disabling accompaniment of many medical conditions and pain control is one of the most important therapeutic priorities[6].

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[7-8].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.

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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[9].Therefore new analgesic agents overlapping these adverse effects are being searched all over the world as alternatives to NSAIDs and opioids[10].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[11].

P. guajava L. commonly known as guajava, widely used in folk medicine in India. Extracts of roots, bark and leaves of the plant are widely used in the treatment of gastroenteritis, vomiting, diarrhea, sore throat, dysentery, ulcers, toothache, coughs, wounds, inflamed gums[12].In present study fruits extracts of *P. guajava* are evaluated for analgesic effect in plethora pain models.

Material and Methods

Drugs and Chemicals

Diclofenac sodium and Pentazocin, methanol was used in this study. All substances were prepared immediately before use and the reagents used were of analytical grade.

Plant Materials

The fruits of *P. guajava* used in the present study were taken from local market of Indore, M.P., and India. The plant was authenticated by Dr. Dipak Birla, Associate Professor, BM college of Pharmaceutical Research Center, Indore, (M.P.) India.

Extract preparation

P. guajava fruits were shade dried and coarsely powdered. Then powdered materials were extracted with water. The last traces of solvent were removed and concentrated to dryness under vacuum using a rotary evaporator. The percentage yield of the extract was 4.12 % (w/w). In each experiment, the extract was diluted with water to desired concentration[13].

Phytochemical screening

A Preliminary phytochemical screening of *P. guajava* fruit Aqoues extract was done to determine the presence or absence of flavonoids, tannins, saponins, phytosterol, steroids, alkaloids, glycosides, terpenoids, Protein, Anthraquinones, reducing sugar and carbohydrates[14].

Experimental Screening

Animals

Adult male albino mice weighing about 20-25 gram 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 (CPCSEA).

The experimental protocol reference No. is **1888/PO/Re/S/16/CPCSEA/2019/03.**

Acute Toxicity Test

Acute Oral Toxicity refers to those adverse effects occurring following oral administration of a single dose of a substance, or multiple doses given within 24 hours. **Acute Oral Toxicity Test:** OECD guideline 423 has been preferred for the activity. The method of Lorke[15] was used for this study. Three animals are used for each step. The dose level to be used as the starting dose is selected from one of four fixed levels 5, 50, 300 and 1000, 2000 mg/kg dosed animals respectively orally by gastric gavage. The starting dose level should be that which is most likely to produce mortality in some of the animals were given free access to feed and water. They were observed over a period of 24 hours for signs of toxicity and mortality.

Analgesic activity

Thermal Model: Eddy's Hot plate test: [16-19]

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

Group I: Received Normal saline10ml/kg, *p.o., control group*

Group II: Received Pentazocin 10mg/kg, *i.p., standard group*

Group III: Received *P. guajava* Aqueous extract at the dose of 100mg/kg *p.o., test group*

Group IV: Received *P. guajava* Aqueous extract at the dose of 200mg/kg *p.o., test group*

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 Aqueous extract of *Psidium guajava 1*00 mg/kg & 200 mg/kg body weight respectively. The animals were placed on Eddy's hot plate kept at a temperature of 55+0.5°C. A cut of period of 15 sec. was observed to avoid injury 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. The average basal reaction time and the % increased in basal reaction time were calculated using student t-test.

Thermal Model: Tail Immersion test[16,20-21]

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 Normal saline10ml/kg, *p.o., control group*

Group II: Received Pentazocin 10mg/kg, *i.p., standard group*

Group III: Received *P. guajava* Aqueous extract at the dose of 100mg/kg *p.o., test group*

Group IV: Received *P. guajava* Aqueous extract at the dose of 200mg/kg *p.o., test group*

The basal reaction time of 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 saline, extracts and standard drug.

Chemical Model: Acetic acid induced abdominal contraction test[22-24]

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 Normal saline10ml/kg, *p.o., control group*

Group II: Received Diclofenac sodium 50mg/kg, *i.p., standard group*

Group III: Received *P. guajava* Aqoues extract at the dose of 100mg/kg *p.o., test group*

Group III: Received *P. guajava* Aqoues extract at the dose of 200mg/kg *p.o., test group*

Male albino mice 20-25 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, intra peritoneal) served as control, second group served as positive control were received Diclofenac sodium (50mg/kg) while third and fourth group were received Aqueous extracts of *P. guajava* 100,200 mg/kg body weight respectively. All the extracts were administered orally by using intra gastric tube 15 minutes 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 10 minutes and percentage of protection is calculated for analgesic activity.

Statistical analysis

The results presented as mean ± SD. The data were also analyzed by one-way ANNOVA dunnets t-test. $^{**}p < 0.05$ this difference is considered to be extremely statistically significant.

Results and Discussion

Preliminary phytochemical screening of the extracts of *P. guajava* revealed the presence of flavonoids, tannins, saponins, phytosterol, steroids, terpenoids alkaloids, glycosides, reducing sugar and carbohydrates. Acute toxicity studies of the Aqueous extract of the *P. guajava* fruits has not been exhibits any signs of toxicity up to 2 g/kg body weight. Since there has been no any mortality of the animals found at highest dose. Hence 100, 200 mg/kg dose of the extract selected for evaluation of analgesic activity.

Table 1: Morphological Parameters of *P. guajava L.*

S.No.	Parameters	Fruit
1.	Size	Normal
2.	Shape	Cylindrical
3.	Color	Light green
4.	Odor	Normal
5.	Taste	Sweet & Acrid

Table 2: Percentage yield of Aqueous Extracts *P. guajava L.*

S.no	Extract	Estimated Percentage	Color of Extract	Nature of Extract	pH
1.	Fruit extract (Aqueous)	4.12	Brown	Semi Solid	7.00

Table 3: Phytochemical screening of *P. guajava L.* extract

S. No.	Tests	*P. guajava L. (Aqueous)*
1.	Glycoside	+
2.	Carbohydrates	+
3.	Phytosterol	+
4.	Flavonoids	+
5.	Protein	-
6.	Alkaloids	+
7.	Tannins	+
8.	Saponin	+
9.	Terpenoids	+
10.	Steroids	+
11.	Reducing sugar	+
12.	Anthraquinones	+

+ = Presence, - = Absence

Table 4: Acute Toxicity Study for *P. guajava L.*

Plant Name	LD_{50}	ED_{50}
P. guajava L.	2000 mg/kg	200 mg/kg

Eddy's Hot plate test

Results of the *P. guajava* on Eddy's Hot plate test are presented in **table 6**. The Aqoues extract of the plant is found to exhibits dose dependent increase in reaction time when compared with control. At 120 minutes the percent inhibition of two different doses (100 and 200mg /kg) was 66.70 & 74.29%.

Table 5: Effect of Aqueous Extract of *Psidium guajava* on Eddy's Hot Plate Model

Treatment Dose mg/kg, *p.o.*	Mean of jumping response after Drug administration (seconds)					% Reaction time
	0 mint.	30 mint.	60 mint.	90 mint.	120 mint.	120 mint.
Normal saline 10ml/kg	7.24 ± 0.13	8.35 ± 0.13	8.34 ± 0.11	8.52 ± 0.10	8.56 ± 0.12	---
Diclofenac sodium 5 mg/kg	4.43 ±0.11*	2.16 ±0.18**	1.70 ±0.16**	1.33 ± 1.0**	1.20 ± 1.25**	85.98
P. guajava 100 mg/kg	2.41 ± 0.12*	3.42 ± 0.14**	3.52 ± 0.18**	2.87 ± 0.11**	2.85 ± 0.15**	66.70
P. guajava 200 mg/kg	2.19 ± 0.12*	2.52 ± 0.13**	2.22 ± 0.14**	2.02 ± 0.12**	2.20 ± 0.14**	74.29

Results are expressed as mean ± SEM, compared to Control group by one-way ANNOVA dunnets t-test, **$p < 0.05$, is found

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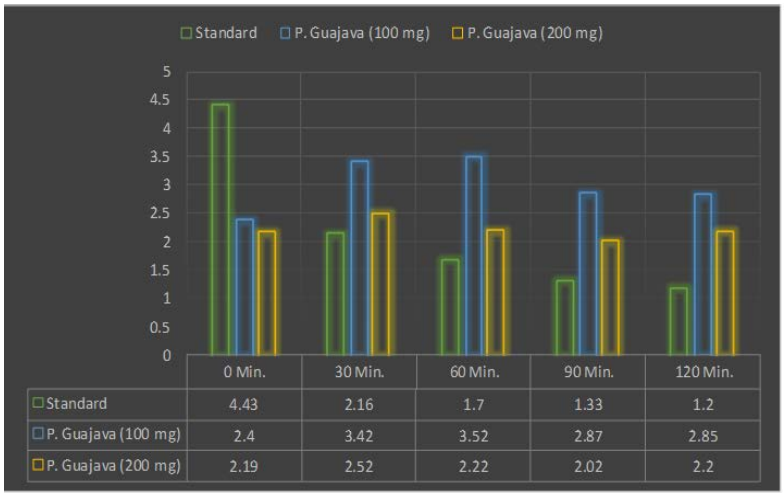


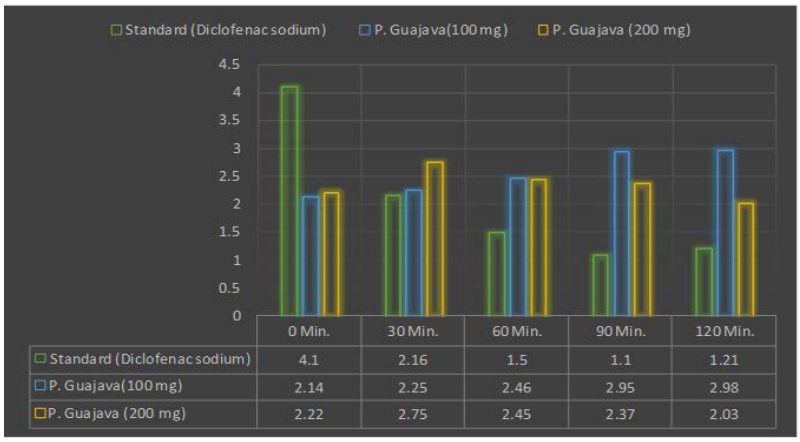
Tail Immersion test

The data of the *P. guajava* on Tail immersion test is presented in **table 7**. In this method, the Aqoues extract of the plant showed a dose dependent increase in latency time when compared with control. At 120 minutes. The percent inhibition of two different doses (100 and 200 mg/kg) was 64.81 & 76.0%.

Table 6: Effect of Aqueous Extract of *Psidium guajava* by Tail Immersion Model

Treatment Dose mg/kg, *p.o.*	Mean increased reaction time before and after Drug administration (seconds)					% Reaction time
	0 mint.	30 mint.	60 mint.	90 mint.	120 mint.	120 mint.
Normal saline 10 ml/kg	7.13±0.11	8.11±0.11	8.13±0.10	8.42±0.10	8.47 ± 0.13	---
Diclofenac sodium 5 mg/kg	4.10±0.10*	2.06±0.15**	1.50±0.13**	1.10±1.1**	1.21 ± 1.10**	85.71
P. guajava L. 100 mg/kg	2.14±0.08*	2.25±0.18**	2.46±0.05**	2.95±0.10**	2.98 ± 0.15**	64.81
P. guajava L. 200 mg/kg	2.22±0.14*	2.75±0.12**	2.45±0.14**	2.37±0.17**	2.03 ± 0.10**	76.03

Results are expressed as mean ± SEM, compared to Control group by one way ANNOVA dunnets t-test. **p < 0.05, is found significant.



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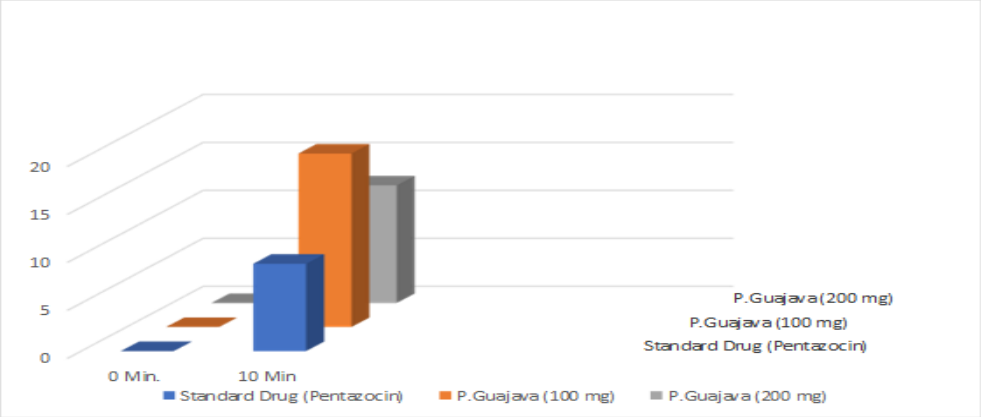
Acetic and induced abdominal contraction test

The results of acetic acid induced abdominal contraction are present in **table 8**. 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 62.45 & 73.92%. The standard drug diclofenac sodium was found more potent than the plant Aqoues extract at all of the dose levels.

Table 7: Effect of Aqueous extract of *Psidium guajava* on Acetic acid induced (Chemical) Model

Treatment Dose mg/kg, *p.o.*	Number of abdominal constrictions in 10 mints.	% inhibition of abdominal constriction in 10 mint.
Normal saline10ml/kg	48.16 ±1.22	---
Pentazocine 3 mg/kg	09.10 ± 0.25*	81.10
*P. guajava L.*100mg/kg	18.08 ± 2.01**	62.45
*P. guajava L.*200mg/kg	12.26 ± 2.04**	73.92

Results are expressed as mean ± SEM, compared to Control group by one-way ANNOVA dunnets t-test. **p < 0.05, is found significant



Conclusion

Pain and inflammations are connected with allied clinical symptoms like rheumatic arthritis, cancer and cardiovascular diseases. 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 *P. 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. The results were unveiled that, the animals treated with *P. 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. The extract of *P. guajava* described an increased reaction time when compared to control and standard in a dose dependent manner. These reports are suggested that the Aqoues extract of *P. 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 *P. 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 Aqoues extract of *P. guajava* having a central activity against thermal induced noxious stimuli.

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. 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. In other hand, the acetic acid induced abdominal contraction method has related to increased level of PGE2 and PGF2α in peritoneal fluids as well as lipogeneses enzyme. The increase in prostaglandin level within the peritoneal cavity leads to enhances inflammatory pain by increasing the capillary permeability.

The *P. guajava* Aqoues extract exhibited a significant analgesic activity in acetic acid induced abdominal contraction (writhing) test in mice. The *P. guajava* Aqoues extract significantly 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. So, it can be suggested that the *P. guajava* Aqoues extract may interfered with prostaglandin synthesis and peritoneal receptor to bring about analgesia. The results are indicated that the *P. guajava* extract possessed peripheral mediated analgesic activity.

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

The investigation of the present study confirmed that the fruit extract of *P. guajava* exhibited central peripheral types of pain inhibition against experimentally induced pain models.

Therefore, the analgesic effects of the fruit extract of *P. 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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