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Experimental Research

Pharmacology Section

Evaluation of Antinociceptive Activity of Aqueous Extract of Bark of *Psidium Guajava* in Albino Rats and Albino Mice

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ABSTRACT

Background: *Psidium guajava* is commonly known as guava. *Psidium guajava* is a medium sized tree belonging to the family Myrtaceae found throughout the tropics. All the parts of the plant, the leaves, followed by the fruits, bark and the roots are used in traditional medicine. The traditional uses of the plant are Antidiarrheal, Antimicrobial Activity, Antimalarial/Antiparasitic Activity, Antitussive and antihyperglycaemic. Leaves are used as Anti-inflammatory, Analgesic and Antinociceptive effects.

Aim: To evaluate the antinociceptive activity of aqueous extract of bark of *Psidium guajava* in albino rats with that of control and standard analgesic drugs aspirin and tramadol.

Materials and Methods: Mechanical (Tail clip method) and thermal (Tail flick method using Analgesiometer), 0.6% solution of acetic acid writhing models of nociception were used to evaluate the extract antinociceptive activity. Six groups of animals, each consists of 10 animals, first one as control, second and third as standard drugs, Aspirin and Tramadol, fourth, fifth and sixth groups as text received the extract (100, 200, and 400 mg/ kg) orally 60 min prior to subjection to the respective test.

Results: The results obtained demonstrated that aqueous extract of bark of *Psidium guajava* produced significant antinociceptive response in all the mechanical and thermal-induced nociception models.

Conclusion: AEPG antinociceptive activity involves activation of the peripheral and central mechanisms.

Keywords: Analgesiometer anti-nociceptive, *Psidium guajava*, Tail flick, Tail clip, Writhing

INTRODUCTION

Pain is a perception, and as such, it is one of the outputs of a system in more highly evolved animals – the nociceptive system – which itself is a component of the overall set of controls responsible for homeostasis [1].

Acute pain is usually managed with medications such as analgesics and anesthetics. Management of chronic pain, however, is much more difficult and may require the coordinated efforts of a pain management team, which typically includes medical practitioners, clinical psychologists, physiotherapists, occupational therapists, and nurse practitioners [2].

Most of the drugs used at present for analgesic effect are synthetic in nature, prolonged use of which cause several adverse and toxic effects like respiratory depression, constipation, kidney damage, physical dependence as well as gastrointestinal irritation [3]. Many of these drugs are not commonly available to the rural folks that constitute the major populace of the world [4]. It is therefore essential that efforts to discover novel effective analgesics with minimum side effects continue. So, studies should be done to introduce new medicinal plants with minimal or no adverse effects.

India being a tropical country has a wide biodiversity and is rich in plant resources. It is expected that screening and scientific evaluation of plant extracts for their analgesic activity may provide new drug molecule that can combat various side effects of the commercially available synthetic drugs, moreover, reducing the cost of medication [5]. Natural medicines derived from plant extracts are being increasingly utilized to treat a wide variety of clinical diseases, though relatively little knowledge about their mode of action is available [6].

So, there is also a need for the development of a potent analgesic without or with least side effects from plant sources. *Psidium guajava* is commonly known as guava. *Psidium guajava* is a medium sized tree belonging to the family *Myrtaceae* found throughout the tropics [7]. *Psidium guajava* is used traditionally for a number of ailments since a long time in history. The part of the plant used maximum is the leaves, followed by the fruits, bark and the roots. The traditional uses of the plant are [7] antidiarrheal, antimicrobial activity, dental plaque removal, antimalarial/antiparasitic activity, antitussive and antihyperglycaemic. Leaves are used as Anti-inflammatory, analgesic and antinociceptive effects. Previous studies are there on anti nociceptive action of leaves [8]. The aim of the study was to evaluate the antinociceptive activity of aqueous extract of bark of *Psidium guajava* in albino rats and mice to elucidate whether the bark of *Psidium guajava* also has anti nociceptive action.

MATERIALS AND METHODS

Animals

All the animals included in the study were procured from animal house of Mamata Medical College, Khammam, India. Laboratory bred albino mice of either sex weighing between 18-22g and albino rats of either sex weighing between 175-250g housed in groups and under strict aseptic conditions were used for the study. The animals were maintained under standard laboratory conditions at 25°C, given commercial pellet diet with water ad libitum, and normal photo period (12h dark/12h light) was strictly followed. Experimental protocol approval was obtained by the Institutional Animal Ethics Committee (IAEC) before the commencement of the study.

Drugs and Chemicals

Tablet Acetyl salicylic acid – Procured from Reckitt Benckiser, Mumbai.

Capsule Tramadol – Procured from Novartis, Mumbai.

0.6% solution of acetic acid – Procured from Finar Chemicals Limited, Ahmadabad.

Instruments

Modified artery clips.

Tail flick Analgesiometer – Inco, Ambala, India.

Animal dose calculation from human dose [9]

Tablet Aspirin (Human dose=600mg).

Group n - 10	Reaction times in seconds at variojus time Intervals			
	0 Minute	30 Minute	60 Minute	90 Minute
Group 1 -Control Normal Saline	5.2±0.36	6.0±0.26	5.2±0.25	5.1±0.31
Group 2 -Aspirin - (54mg/kg)	5.1±0.70	6.0±0.33	5.9±1.14	5.8±0.68
Group 3 - Tramadol - (9mg/kg)	5.7±0.78	9.2±1.21*	21.5±1.42#	25.9±10.85#
Group 4 - AEPG - (100mg/kg)	5.1±0.28	11.6±0.58#	7.8±0.98	5.4±0.31
Group 5 - AEPG 200mg/kg	5.4±0.31	13.6±1.25#	16.3±0.98#	22.6±1.01#
Group 6 - AEPG 400mg/kg	5.2±0.49	7.2±0.49	6.1±0.77	5.8±0.66

[Table/Fig-1]: Effect of aqueous extract of *Psidium guajava* (AEPG) in tail clip method n albino rats

AEPG - Aqueous extract of *Pasidium guajava - Number of animals.* All values are expressed as Mean ± SEM, n = 10, *P<0.05, #P<0.001 when compared to control group

Group n - 10	Reaction times in seconds at variojus time Intervals			
	0 Minute	30 Minute	60 Minute	90 Minute
Group 1 -Control Normal Saline	5.7±0.42	6.1±0.23	5.90±0.35	5.90±0.35
Group 2 -Aspirin - (54mg/kg)	6.10±0.53	6.5±0.72	6.1±0.46	5.5±0.73
Group 3 - Tramadol - (9mg/kg)	6.6±0.79	8.2±0.73	15.9±1.99#	16.50±1.87#
Group 4 - AEPG - (100mg/kg)	5.80±0.33	10.40±3.02	12.9±2.29*	13.10±2.28*
Group 5 - AEPG 200mg/kg	5±0.54	14.71±0.86	15.57±0.98	17.57±1.73#
Group 6 - AEPG 400mg/kg	6±0.39	13.70±0.87#	15.50±0.87	11.9±0.99#

[Table/Fig-2]: Effect of aqueous extract of *Psidium guajava* (AEPG) in tail flick method in albino rats

AEPG - Aqueous extract of *Pasidium guajava - Number of animals.* All values are expressed as Mean ± SEM, n = 10, *P<0.01, #P<0.001 when compared to control group

- For rats=600x0.018=10.8/200gm rat=54mg/kg
- For mice=600x0.0026=1.56/20gm mice=78mg/kg

Capsule Tramadol (Human dose=100mg)

- For rats=100x0.018=1.8/200gm rat=9mg/kg
- For mice=100x0.0026=0.26/20gm mice=13mg/kg

Plant Material and Extraction Procedure

The dried bark of *Psidium guajava* tree were collected and were authenticated by Professor and Head, Department of Botany, Government Degree College, Khammam.

Continuous hot percolation process or Soxhlet extractor [10]

The preparation of crude extract of dried bark of *Psidium guajava* was done in the Department of Pharmacology, Mamata Medical College, Khammam by the Soxhelts extractor. After extraction the solvent is removed, typically by means of a rotary evaporator, yielding the extracted compound. The water soluble extractive value of aqueous extract of *Psidium guajava* is 11.03%w/w.

METHODOLOGY

Acute toxicity study: The median Lethal Dose (LD50) of aqueous stem bark extract of *P. guajava* was carried out according to the method described by Lorke [11]; this method involved two phases of which nine mice were grouped into three groups of three mice each. They received 10, 100 and 1000 mg /kg body weight of the extracts, respectively. In the second phase also nine mice were grouped into three groups of three mice each and they received 1600, 2900 and 5000 mg/kg body weights. The mice were observed daily for any signs of toxicity including death throughout the period of study.

Phytochemical analyses: Quantitative Phytochemicals analyses of stem bark extract of *P. guajava* were carried out according to the following methods: Tannins [12], saponins and alkaloids [13], flavonoids [14].

Group n = 10	Average number of writhes	Percent inhibition %
Group 1 -Control Normal Saline	2.5±1.12	0
Group 2 -Aspirin - (78mg/kg)	6.7±0.92	73.2
Group 3 - Tramadol - (13mg/kg)	11.2±1.88#	55.2
Group 4 - AEPG - (100mg/kg)	16.4±3.01*	34.4
Group 5 - AEPG 200mg/kg	14±3.04	44
Group 6 - AEPG 400mg/kg	18.8±2.75	24.8

[Table/Fig-3]: Effect of aqueous extract of *Psidium guajava* (AEPG) in acetic acid induced writhing test in albino rats

AEPG - Aqueous extract of Pasidium guajava n= Number of animals. Values are Mean ± SEM, n = 10, *P<0.05, #P<0.001 when compared to control group

Experimental design and treatment: A total of 60 rats of either sex weighing 175-250 g were divided into six groups of ten rats each after physical randomization. Group 1 as control received normal saline, 2, and 3 received standard drugs asprin and Tramadol (54mg/kg and 9 mg/kg, respectively), groups 4, 5, and 6 received AEPG (100, 200, and 400 mg/kg, respectively which were obtained from Ist phase of acute toxicity studies, 100mg/kg dose was picked up and doubling of two doses were used), All the drugs were given subcutaneously/orally. For the analgesic evaluation, tail clip method and analgesiometer and 0.6% solution of acetic acid writhing models of nociception was used [15]. Mean values of all data were tabulated and statistical analysis was performed.

Haffner's tail clip method

A metal artery clip was applied to the root of each mouse's tail to induce pain [16]. A sensitivity test was carried out, and animals that did not attempt to dislodge the clip within 10s were discarded. The responsive rats were placed in groups of 6 each containing 10 animals. The tail clip was applied 0, 30, 60 and 90 mins after oral administration of normal saline, aspirin and tramadol to 1st, 2nd and 3rd groups, and the extract (100, 200 and 400 mg/kg) to 4th,5th and 6th groups. The reaction time at various intervals was noted and documented.

Tail Flick Analgesiometer

Effect of test drugs was obtained in terms of tail flick latency period (the time required for flicking of tail, i.e., reaction time), using an analgesiometer at 0, 30, 60, and 90 min. Radiant heat was directed to the proximal third of the tail through a hot wire of the analgesiometer and the reaction time was noted, when the rat tried to pull the tail away. A mean of two pre-drug readings was taken as the basal value (0 min). Rats with a reaction time of more than six seconds were not used in the test. In order to prevent tissue injury, a cut off time of 10s was maintained. The cut off time was considered as the latency period for the animals not responding up to 10s.

Writhing Tests (0.6% Acetic Acid Induced Writhing In Mice)

In this study, 60 albino mice of either sex were taken for the study and were divided into 6 groups of 10 animals each. All the animals were fasted overnight but had free access to water. All the animals in Group 1 were pretreated with 0.2ml of normal saline, Group 2 and 3 with standard drug aspirin-78mg/kg and tramadol-13mg/kg respectively and Group 4, 5 and 6 with aqueous extract of *Psidium guajava* (AEPG) in the dose of 100, 200 and 400mg/kg respectively before injecting acetic acid. A dose of 1ml/100g body weight of 0.6 % acetic acid was injected intraperitoneally which then produced writhing/stretching syndrome. The mice were placed individually under glass beakers and five minutes were allowed to elapse. The mice were then observed for a period of ten minutes and the number of writhes was recorded for each animal. For scoring purposes, a writhe is indicated by stretching of the abdomen with simultaneous

stretching of at least one hind limb. The results were tabulated, percentage inhibition of writhes was calculated and statistical analysis was done.

STATISTICAL ANALYSIS

The data were expressed as the mean ± SEM. Statistical analysis was performed with one-way analysis of variance (ANOVA) for comparison of more than two groups followed by Dunnett's test. $p < 0.05$ was considered statistically significant during the analysis of data.

RESULTS AND OBSERVATION

Acute toxicity study

It was found that aqueous extract of bark of *Psidium guajava* did not produce any mortality up to a dose of 5000 mg/kg., po. All animals were found to be normal after continous observation for 10 d.

Haffner's Tail Clip Method in Rats

The reaction times after application of modified artery clip to all the rats at various time intervals are represented in [Table/Fig-1]. As per statistical analysis, the reaction time in various groups at the baseline or time 0 was not statistically different. In standard groups, at 90 mins post administration of aspirin and tramadol, the mean reaction time was increased to 5.8±0.68sec and 25.9±0.85sec respectively. The mean reaction time also increased in the test groups treated with 100, 200 and 400mg/kg of extract. The maximum reaction time was 22.6±1.01sec seen with 200mg/kg of extract at 90mins post administration within the test groups. The increase in reaction time with 200mg/kg extract is comparable with aspirin and tramadol.

Radiant Heat Method in Rats (Tail Flick Analgesiometer)

The reaction times after exposing the rat's tail to Radiant heat using analgesiometer at various time intervals are represented in [Table/Fig-2]. As per statistical analysis, the reaction time in various groups at the baseline or time 0 was not statistically different. In standard groups, the rats treated with aspirin showed no significant increase in the mean reaction time. But, the rats treated with tramadol showed increase in the mean reaction time at 30, 60 and 90 mins post administration and the maximum reaction time was 16.50±1.87sec seen at 90mins post administration. The mean reaction time also increased in the test groups treated with 100, 200 and 400mg/kg of extract. The maximum reaction time in the test group was 17.57±1.73sec seen with 200mg/kg of extract at 90 mins post administration.

Writhing Test (0.6% Acetic Acid Induced Writhing in Mice)

In this test, the average number of writhes in control group was 2.5±1.12. The mice pretreated with 100, 200 and 400mg/kg of AEPG produced 16.4±3.01, 14±3.04, and 18.8±2.75 writhes respectively. The reduction in the number of writhes was significant ($p<0.05$) with the doses of 100 and 200mg/kg of AEPG respectively when compared to the control group. It was observed that there was a dose dependent reduction in the number of writhes in the test animals pretreated with AEPG. The percentage inhibition of abdominal constrictions (percentage of inhibitory level) using the following formula: (mean of (control-test group)/control group × 100%). The percentage inhibition in number of writhes was 34.4%, 44% and 24.8% in the animals pretreated with 100, 200 and 400mg/kg of AEPG respectively. Reduced number of writhes, 6.7±0.92 and 11.2±1.88, was seen in mice treated with aspirin and tramadol respectively. This is highly significant ($p<0.001$) when compared to AEPG. The percentage inhibition was maximal with aspirin (73.2%) in comparison to tramadol (55.2%). According to the literature acetic acid induced writhing test identifies centrally as well as peripherally acting analgesic compounds [17]. Hence, it can be concluded that AEPG at the doses of 100 and 200mg/kg ($p<0.05$) has both peripheral and central mechanisms of antinociception.

DISCUSSION

Any injury or tissue damage is associated with pain and inflammation. Analgesics can act on peripheral or central nervous system. Peripherally acting analgesics act by blocking the generation of impulses at chemoreceptor site of pain, while centrally acting analgesics not only raise the threshold for pain, but also alter the physiological response to pain [17]. The current study was done to evaluate the antinociceptive activity of aqueous extract of bark of *Psidium guajava* (AEPG) using various methods like Haffner's tail clip method in albino rats, radiant heat method in albino rats and acetic acid induced writhing test in albino mice.

Haffner's Tail Clip Method in Rats

The test animals treated with AEPG have shown an increase in the reaction time at 30, 60 and 90mins post administration compared to pretreatment levels. It was highly significant ($p<0.001$) with the dose of 200mg/kg body weight when compared to the control group indicating antinociceptive activity of AEPG. In one of the studies, the stem bark extract of *Psidium guajava* shows a significant suppression of nociceptive response when compared to the control [18]. In the standard Group 2 (aspirin) the increase in reaction time is insignificant. The maximum increase in the reaction time ($p<0.001$) in the Group 3 (tramadol) was seen at 90 min. According to the results obtained increase in reaction time with tramadol is greater when compared to aspirin. As already quoted previously the tail flick method is used primarily to evaluate analgesics acting through central mechanism (tramadol) [17]. The test animals treated with AEPG in this test have shown positive response. It can be thus inferred that the AEPG has central mechanism of antinociceptive activity analogous to tramadol as represented in the [Table/Fig-2].

The Radiant Heat Method in Rats (Tail Flick Analgesiometer)

As per statistical analysis, the reaction time in various groups at the baseline or time 0 was not statistically different. The rats treated with AEPG have shown a highly significant ($p<0.001$) increase in the reaction time compared to the control group. Maximum increase in the reaction time in these animals treated with AEPG was at 200mg/kg dose. The effect was insignificant in aspirin treated animals. Tramadol treated animals exhibited maximum increase in reaction time at 90mins post administration of the drug. Radiant heat method is known to evaluate centrally acting analgesics [17]. So, according to the values obtained it can be concluded that AEPG has an antinociceptive activity similar to tramadol i.e. central mechanism as shown in [Table/Fig-3]. Leaf extracts of *Psidium guajava* were evaluated on several experimental models for its anti-inflammatory and analgesic properties [19]. The same bioactive compounds present in the bark might have produced the same antinociceptive action.

Writhing Test (0.6% Acetic Acid Induced Writhing In Mice)

In this method the number of writhes a mouse develops after injecting acetic acid i.p was counted in all the groups and is shown in [Table/Fig-3].The average number of writhes decreased significantly after administration of standard drugs asprin and tramadol. Even the mice treated with test extract also showed decreased number of writhes but the maximum decrease was seen with 200mg/kg. The acetic-acid-induced writhing test described as a typical model for inflammatory pain, has long been widely used as a tool to screen for analgesic or anti-inflammatory properties of new agents [20] and, in most cases, used as a model to study the peripheral antinociceptive effect of extracts/compound. The antinociceptive effects were probably due to essential oil present in the guava plant [21] and could also be due to the high flavonoid content [22].

The aqueous extract was evaluated in the acetic acid-induced writhing test for its analgesic activity. Acetic acid-induced abdominal writhing model represents pain sensation by releasing arachidonic acid via cyclooxygenase and prostaglandin biosynthesis which plays a role in the nociceptive mechanism [23] Prostanoids such as PGE2 and PGF2a as well as lipoxygenase products have been found at higher level in the peritoneal fluid after intraperitonial injection of acetic acid. The analgesic effect occurs due to its action on visceral receptors that is sensitive to acetic acid by inhibiting the production of algogenic substances or inhibiting the transmission of painful

messages at the central level [24]. The acetic acid induced writhing response is a sensitive method to evaluate peripherally acting analgesics. The response is mediated by either peritoneal mast cells or acid sensing ion channels or the prostaglandin pathways [25]. Thus, the reduction in the number of writhing indicates that AEPG might exert antinociceptive activity by inhibition of prostaglandin synthesis or action of prostaglandins.

The techniques used in this study to evaluate antinociceptive activity of AEPG were Haffner's Tail Clip Method, Radiant Heat Method (Tail Flick Analgesiometer) and 0.6% Acetic acid induced writhing in mice. The results obtained during this study suggest antinociceptive activity of AEPG with all the three pain models used.

Psidium guajava is rich in vitamin C, vitamin A, iron, calcium, and phosphorus. Manganese is also present in the plant in combination with phosphoric, oxalic and malic acid. Bark contains Tannins, Resin, and Crystals of Calcium oxalate [26]. Twigs contain Calcium, Magnesium, Phosphorus, Potassium, Sodium, Fluoride, Copper, Iron, Zinc, Manganese and Lead, Flavonoids, Sesqiterpenes alcohols and Acid triterpenoids [27]. It also contains saponin combined with oleanolic acid. Saponins are known to have analgesic, anti-inflammatory and anti-rheumatic effect. Various studies on leaves of *Psidium guajava* documented the presence of Cytokinins like Zeatin [28], Zeatinriboside, Zeatin nucleotide, Flavonoids, Saponins, Oleanolic acid, Nerolidiol, Ursolic acid, Crategolic acid, Guayavolic acid, Essential oils like beta-Caryophyllene, alfa-pinene, 1,8-cineole, Tannins, Guavanoic acid, 2-alfa-hydroxy ursolic acid, lleletifol, Isoneriucoumaric acid, Guajadial, 2alfa-hydroxyoleanolic acid, Morin-3-O-alfa-L-arabopyranoside, Quercetin, and Hyperin [29]. It is therefore probable that the saponin component of the extract may contribute in part for the observed antinociceptive activity. Flavonoids such as quercetin are very much effective in acute inflammation. There are also reports on the role of flavonoids in analgesic activity primarily by targeting prostaglandins. Tannins are also claimed to possess analgesic activity [30]. The AEPG may be effective in pain and acute inflammatory disorders but further studies are needed to prove its anti nociceptive activity.

CONCLUSION

The study shows some biological activity (antinociceptive activity) of aqueous extract of *Psidium guajava* (AEPG) after oral administration. Further studies are needed to authenticate these results using other experimental models. Pharmacodynamic studies are needed to understand the mode of action of aqueous extract of *Psidium guajava* (AEPG). Further phytochemical analysis, isolation and purification of crude aqueous extract of *Psidium guajava* (AEPG) is needed to establish the active principles having significant antinociceptive activity and its mode of action.

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