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Analgesics and Antipyretic Activities of Ethanolic Extract of *Psidium guajava* in Rats

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Abstract

An ethanolic extract of the dried leaves of Psidium guajava was investigated for analgesic and antipyretic activities in rats. Only the higher doses of the extract (100 and 200 mg/kg) produced significant ($p < 0.05$) reduction of pyrexia in the yeast induced hyperpyrexia and hot plate latency assay. However at the doses of 50-200 mg/kg analgesic activities were observed in the early and late phases of formalin induced paw licking test in rats. The latency in the hot plate test was increased from 2.2 + 0.3 to 5.7 + 0.3 second ($P < 0.05$). Likewise, the early and the late phases of formalin test were reduced from 7.4 + 9.4 and 57.8 + 8.4 to 36.6 + 4.4 and 29.2 + 4.4 second respectively. The result confirms the analgesic and antipyretic activities of Psidium guajava leaves.

Key words : Psidium guajava, Antipyretic, Analgesic, Aqueous extract, Activities

Introduction

Psidium guajava L. (Myrtacaea) is a shrub or small tree that can be easily identified by its distinctive thin, smooth copper-coloured bark that flakes off, showing a greenish layer beneath. The leaves bark and flowers of

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P. guajava are employed in the treatment of various ailments by Traditional medical practitioners in Africa and South America (Gill, 1992; Conde Garcia *et al.*, 2003). Some of these Traditional uses include treatment of diarrhoea, dysentery, cough, menstrual pain, tooth ache, fever, sore throat etc (Gill, 1992; Lozoya *et al.*, 2002; Conde Garcia *et al.*, 2003). Many studies revealed that *P. guajava* possess anti-bacterial, antispasmodic, antidiabetic, anticough, antioxidant and a narcotic like activities (Caceres *et al.*, 1990; Lozoya *et al.*, 1994; Lutterodt and Maleque,1998; Jaiarj *et al.*, 1999). Some the active ingredients responsible for the biological activities include; tannins, flavonols flavonoids, saponins, triterpenoids, glycosides (Gill, 1992; Lozoya *et al.*, 1994; Begum *et al.*, 2002; Begum *et al.*, 2004 and Oh *et al.*, 2005).

The present study was undertaken in order to investigate the claim that the plant has analgesic activities since it is used in the treatment of tooth ache and menstrual pain. Study on the antipyretic activity was also undertaken based on the use of the plant in the treatment of fever.

Materials and Methods

Animals: male wistar rats weighing (138.0 ± 3.0 g) were used for the study. They were housed and bred in the animal house of the faculty of Basic Medical Sciences, College of Medicine, University of Ilorin. The animals were housed in groups in cages with free access to food and water. They were fed with mouse cubes (Bendel feed, Ilorin, Nigeria).

The research was conducted in accordance with the ethical rules on animal experimentation, approved by the ethical committee of the College of Medicine, University of Ilorin, Nigeria.

Plants: The *Psidium guajava* leaves, used for the study were collected from guava trees at flower garden, G.R.A., Ilorin in July 2004. A voucher specimen with herbarium file number FHI 106935 was deposited at the forestry research institute of Nigeria, Ibadan (FRIN) after identification by T. K. Odewo, a Staff of the same Forestry Research Institute.

The air dried *Psidium guajava* leaves were reduced to powdery form. 100 g of the powder was extracted with 500 ml of ethanol (analytically grade) the mixture was allowed to stand for 24 hours. The macerated mixture was now filtered and evaporated in a carefully regulated water bath maintained at a temperature of 50 °C to yield a deep green semi-solid extract weighing 7.9 grams. It was stored in a refrigerator at 4 ºC.

Phytochemical screening: The extract was subjected to phytochemical analysis for evaluation of its contents according to the methods described by Trease and Evans, (1983).

Analgesic studies

The hot plate test

This was carried out by slightly modifying the method described by Woolfe and Mac Donald (1944). The rats were placed on a confined hot plate

maintained at 55 ºC + 1 ºC. The time taken for the rats to respond to the thermal stimulus (usually by jumping) was noted as the latency (in seconds).The rats were divided into 5 groups (A to E), each made up of 5 rats. Rats in group B, C, and D were given extracts of *Psidium guajava* orally after 12 hours fast. The doses were 50, 100 and 200 g / kg for the rats in groups B, C and D respectively; representing low medium and high doses. The rats in group A and E were given equivalent doses of normal saline (10 ml / kg) and indomethacin (5 mg / kg) respectively. Each of the rats was placed on the hot plate and the latency was recorded. The mean latency + standard error of mean (S.E.M) was determined for each group.

This short lasting stimulus elicited from the hot plate surface causes little or no damage at all to paw tissues, so it can be followed immediately by the formalin test (Back-Rojecky, 2003).

Formalin induced paw licking test

The formalin induced paw-licking test was carried out in accordance with the method described by Hunskaar and Hole, (1997).

100 µl of 4 % formalin was injected subcutaneously into the plantar surface of the left hind paw of the rats, one hour after oral administration of the extract, indomethacin or saline. The same groups of rats used in the hot plate test and also, the same doses of extract, indomethacin or saline were also used in the test. The time spent by the rats in licking the injected paw as soon as the injection was given (early phase, 0-5 minutes post injection) and in the late phase (20-30 minutes post injection) was recorded. The mean time spent on licking the paw by each group was determined.

Antipyretic study

Brewer's Yeast Induced Hyperpyrexia: The test was carried out using the method described by Adam *et al.* (1968). The animals used for this study were fasted over night before the experiment but water was made available *ad libitum*. The rats were randomly divided into four groups (A to D) containing five rats per groups. Pyrexia was induced by subcutanous injection of 20 % (w/v) brewer's yeast suspension (10 ml / kg) in the dorsum of the rats. 17 hrs after injection, the rectal temperature of each rat was measured, using a clinical thermometer (UNESCO, international, Michigan, USA). Only rats that showed an increase in temperature of at least 0.7 ^{0}C were used for this study. The rats in group A to C were given (orally) 50, 100, and 200 mg / kg of the extract respectively while those in group D were given indomethacin (5 mg / kg). The initial rectal temperature of the rats was measured and this served as the control. The temperatures were subsequently measured at 60, 90 and 120 minutes post extract administration and the mean temperature of each group was recorded.

Statistical analysis

Values were expressed as mean ± standard error of mean (S.E.M). Statistical significance was determined by using student t-test; values with $P < 0.05$ compared with control were considered as significant.

Results

Hotplate anti-nociceptive test

The result from this study shows that oral administration of *Psidium guajava* extract (50-200 mg / kg) significantly($p < 0.05$) increased the reaction time of the animals to thermal stimuli in a dose dependent manner from 2.2 + 0.3 second to 5.9 + 0.3 seconds (Table 1).

Table 1. Effect of Ethanolic extract of *Psidium guajava* leaves on hot plate test in rats.

Groups	Dose (mg / kg)	Reaction time (secs)[a]
Control	10 ml	2.2± 0.3
P. guajava	50	2.7± 0.2
P. guajava	100	3.4± 0.2*
P. guajava	200	5.9± 0.3***
Indomethacin	5	4.6± 0.7*

a Each value is the mean + SEM of 5 rats

* P< 0.05, ***P < 0.001 compares with control; student t-test.

Formalin induced paw licking test

In this model, oral doses (50-200 mg / kg) of the ethanolic extract of *Psidium guajava* inhibited both the early and late phases of the licking response. The licking time was reduced from 77.4 + 9.4 s to 33.6 + 4.4 second in the early phase and from 57.8 + 8.4 to 29.2 + 4.4 s in the late phase (Table 2).

Yeast induced hyperpyrexia test

In this study, the highest dose (200 mg / kg) of the ethanolic extracts of *Psidium guajava* significantly ($p < 0.05$) reduce the rectal temperature of the rats from 38.5 + 0.3 to 36.6 + 0.3 ^{0}C one hour after administration of the extract. Significant ($p < 0.05$) reduction was also observed at 90 minutes and 120 minutes after extract administration.

Table 2. Effect of the ethanolic extract of *Psidium guajava* leaves on formalin-induced paw licking test.

Group	Dose (mg / kg) orally	Licking time (sec) [a]	
		Early phase	Late phase
Control (saline)	-	77.4 ± 9.4	61.8 ± 7.58
P. guajava	50	54.4 ± 3.2*	32.8 ± 4.3 *
P. guajava	100	36.4 ± 2.8**	31.8 ± 7.0*
P. guajava	200	33.6 ± 4.4**	29.2 ± 4.4*
Indomethacin	5	40.4 ± 3.3*	37.5 ± 4.7 *

[a] Each value is the mean ± S.E.M. of 5 rats.
* $P < 0.05$; ** $p < 0.01$, compared with control; student's t-test.

Discussion and Conclusions

The analgesic and antipyretic properties of the ethanolic extract of *Psidium guajava* leaves were investigated in this study using three laboratory models. The hot plate test, the formalin induced paw licking test and brewer's yeast induced pyrexia.

The models chosen for the analgesic test were carefully selected based on the advantages and the disadvantages of each of the models. The hot plate thermal test is a form of acute (phasic) test which is important in determining the fast type of pain. It is mainly sensitive to strong analgesics and causes limited tissue damage (Prado *et al.,* 1990; Tjølsen *et al.,* 1992, Hunskaar and Hole, 1997). However, this model has a short coming, because it last for a short time and it is difficult to access modulatory mechanism that may be triggered by the stimulus itself (Tjolsen *et al.,* 1992).The formalin test differ from the hot plate test in that it mimic human clinical pain condition in which the pain last for longer period of time and tonic in nature due to the inflammation accompanying the formalin injection (Tjølsen *et al.,* 1992; Back-Rojecky, 2003). It is sensitive to non-steroidal anti-inflammatory agents and other mild analgesic (Hunskaar and Hole, 1997).

In the present study, administration of the extract led to significant increase in the latency to thermal (hot plate) stimulus and also a significant reduction in the licking time in both the first and second phase of formalin induced paw licking tests.

The observed analgesic activities of *P. guajava* may be due mainly to quercetin, a flavonoid contained in this plant (Morales *et al.,* 1994). Previous

Table 3. Effect of ethanolic extract of *P. guajava* on yeast induced hyperpyrexia in rats[a]

Groups	Dose (mg / kg)	Pre-Drug Temp (^{0}c)	Post Drug Temp (60min)	Post Drug Temp (90min)	Post Drug Temp (120min)
P.guajava	50	38.4± 0.3	38.3± 0.3	38.2± 0.3	37.9± 0.3
P.guajava	100	38.2± 0.4	37.8± 0.4	37.6± 0.5	37.0± 0.2*
P.guajava	200	38.5± 0.3	37.6± 0.2*	37.1± 0.1**	36.6± 0.3**
Indomethacin	5	38.5± 0.3	37.5± 0.4*	37.1± 0.4*	36.9± 0.4*

[a] Each value is the mean ± S.E.M. of 5 rats.
* $P < 0.05$; **$p < 0.01$ compared with control; student's t-test.

report (Lozoya *et al.,* 1994) has demonstrated that the leaves of *Psidium guajava* are rich in flavonoids; in particular, quercetin. Much of its therapeutic activity is attributed to this flavonoid. Quercetin has demonstrated antibacterial, antidiarheoal, antispasmodic and antioxidant properties (Morales *et al.,* 1994; Yamashiro *et al.,* 2003). Typical narcotic-like effect has also been observed with the alcoholic leaf extract of *P. guajava* (Lutterodot and Maleque, 1998). Quercetin is also known for its calcium-antagonistic effect and it may inhibit pain sensation by reducing calcium influx in nerves, which in turn reduce pain transmission by the receptors (Morales *et al.,* 1994). There are other quercetin-like substances present in the form quercetin aglycone together with glycosides (Lozoya *et al.,* 1994). The results of the phytochemical screening conducted in this present study also confirm the presence of cardiac glycosides, tannins, alkaloids, saponins and flavonoids.

In the brewer's yeast induced hyperpyrexia model, artificial hyperthermia was induced by administration of exogenous pyrogens in the form of yeast. General reduction of the rectal temperature was observed 60 minutes, 90 minutes and 120 minutes after oral administration of the highest dose (200 mg / kg) of the extract. The observed antipyretic effect of the extract may be due to the flavonoids and alkaloids contents of the leaves. These flavonoids and alkaloids may act by blockage of the synthesis of prostagladins E_2 (– a peripheral fever mediator) through the inhibition of prostaglandins synthetase (Ramaswamy *et al.,* 1985). Therefore the extract could be mediating it analgesic and antipyretic effects like the non steroidal anti-inflammatory drugs (Vane, 1971 Zeil and Krupp, 1975).

In conclusion this study has confirmed the analgesic and antipyretic activities of *P. guajava* leaves extract. It also show that the analgesic activity is more pronounced than the antipyretic activity since lower doses of the extract produced analgesia while the lower doses fail to reduce pyrexia.

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