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STUDY OF ANALGESIC AND ANTIINFLAMATORY ACTIVITY OF MIXTURE OF SOLVENTS EXTRACT OF PSIDIUM -GUAJAVA LEAVES

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ABSTRACT

The present study was designed to evaluate the Analgesic and Anti-inflammatory activity of Mixture of solvents (Chloroform, Methonol, Pet.ether) (1: 1: 1) Leaf extract of Psidiumguajava. This study was planned to examine Phyto chemical studies and Pharmacological activities. Evaluation of analgesic activity was done by Eddy's hot plate method and Acetic acid Induced Writhing movement method. The anti-inflammatory activity was done by Carrageenan Induced rat Paw edema method by using Plethysmometer. The extract of Psidium guajava leaves with mixture of solvents showed very significant activity when compared with control and standard.The presence of flavonoid in the extract was confirmed by doing TLC on silica gel plate. The presenceof Flavonoids in the extract are responsible for Analgesic and Anti inflammatory activity.

Keywords: Psidium guajava, Analgesic, Anti inflammatory, flavonoids, Eddy's hot plate, Plethysmometer.

INTRODUCTION:

India is one of the important centers of origin in crop and plant diversity. The herbal products today symbolize safety in contrast to the synthetics that are regarded as unsafe to human and environment. Phyto Pharmacological studies[1, 2, 3] have confirmed that P. sidium guajava exhibit a broad range of biological effects. However, the crude extract of the plant have been used as a traditional medicine for the treatment of various diseases. Guava is rich in antioxidants compounds and contains a high level of ascorbic acid which are responsible for many medicinal uses**.** It have rich in polyphenolics and flavonoids responsible for amoebicide, analgesic, vermifuge, antimalarial, antibacterial, colic-relief, antispasmodic, astringent, antiulcerous, gastrototonic cough suppressant etc**;** Guava leaf extract contains flavonoids, mainly quercetin derivatives, which are hydrolyzed in the body to give the aglycone quercetin which is responsible for Anti-Inflammatory and analgesic effects of *Psidium guajava* Linn. (Myrtaceae) leaf aqueous extracts in rats and mice were proved[4]**.** The present study was designed to evaluate the Analgesic and Anti-inflammatory activity of Mixture of solvents (Chloroform, Methonol, Pet. ether) (1:1:1) Leaf extract of Psidiumguajava[5, 6].

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MATERIALS AND METHODS:

PLANT COLLECTION:

The leaves of the plant were collected in the month of January and it was authenticated by Prof. Madhavasetty, Head of department of Botany, Sri Venkateswara University, Tirupati, India.

ANIMAL PROCUREMENT:

Animals were purchased from Raghavendra Enterprises,Banglore,India. Animals either sex of Wistar Albino rats having weight of 150-200g were used for Antiinflammatory activity and swiss albino mice having weight of 30-40g were used for analgesic activity. We were maintained animals in laboratory conditions for 10 days before used for experiments, and feed them with pellets and water as needed. The college having institutional ethics committee no: ----------

Standard drugs Used: Tramadol – 10 mg/kg and aspirin - 100mg/kg

METHODOLOGY

Extraction of plant material:

The shade dried leaves are powdered separately to get coarse powder. About 500g of dried and coarsely powdered leaves was extracted by using mixture of solvents of chloroform, methonol and Pet.Ether in 1:1:1 ratio. The extraction was continued for 48 hrs .The extract was filtered and concentrated to a dry mass by using vacuum distillation.

Phytochemical Tests:

The phytochemical studies[7] deals with the phytochemical examination of therapeutic importance of Psidium guajava, an important herbal plant. Phytochemical examinations were carried out for all the extracts as per the standard methods and it was showed the presence of alkaloids, carbohydrates, saponins, phytosterols,diterpens and flavonoids etc.Thin layer chromatography was done and got spots.

IN VIVO METHODS FOR TESTING ANALGESIC ACTIVITY[8, 9]:

Hot plate method:

Groups of 5 mice of either sex with an initial weight of 18 to 22 g used for each dose. Each group received normal saline or plant extract or standard drug (tramadol 10mg/kg).The hot plate, which is commercially available, consists of a electrically heated surface. The temperature is controlled for 55° to 56°C. This can be a copper plate or a heated glass surface. The animals are placed on the hot plate and the time until either licking or jumping occurs is recorded by a stop watch. The latency is recorded before and after 20, 60, and 90 minutes following oral administration of the standard or the test compound.

Acetic Acid Induced Writhing Method:

This study was carried out using acetic acid induced abdominal writhing reflex pain model. Fifteen mature mice were randomly divided into 3 groups of 5 mice per group, fasted for 12 hours and treated as normal saline or plant extract or standard drug(aspirin 100mg/kg) respectively using gastric gavage. One hour after drug and extract administration, 0.6% glacial acetic acid (10ml/kg) was administered intraperitoneally (I.P) to all the mice to induce abdominal contortions or writhings. The analgesic effect was assessed in each mouse for 30 minutes and recorded.

IN VIVO SCREENING METHODS OF ANTI INFLAMMATORY ACTIVITY[10, 11, 12.]

Paw edema

Male or female rats with a body weight between 150 to 200 g are used. The animals are starved overnight. To insure uniform hydration, the rats receive 5 ml of water by stomach tube (controls) or the test drug dissolved or suspended in the same volume. Thirty minutes later, the rats are challenged by a subcutaneous injection of 0.05 ml of 1% solution of carrageenan into the plantar side of the left hind paw. The paw is marked with ink at the level of the lateral malleolus and immersed in mercury up to this mark. The paw volume is measured plethysmographically immediately after injection, again 3 and 6 h, and eventually 24 h after challenge.

Statistical data:

Statistacal Significance data of analgesic activity of PsidiumGuajava leaf Extract [PGE] by Eddy's Hot Plate Method

Data of control and standard:

S. No	Group	Control	Standard
1	Mean	4.325	7.950
2	SD	0.399	1.953
3	SEM	0.141	0.690
4	N	8	8

Paired *t* test results of control and standard:

1	Mean of control minus standard	-3.625
2	**Statistical significance**	**very statistically significant**
3	95% confidence interval of this difference	-5.220 to -2.030
4	t	5.3746
5	df	7
6	Standard error of difference	0.674

Data of control and PGE (200mg):

S. No	Group	Control	Standard
1	Mean	4.325	6.000
2	SD	0.399	0.980
3	SEM	0.141	0.346
4	N	8	8

Paired *t* test results of control and PGE (200mg):

Two-tailed P value	0.0019
Statistical significance	**very statistically significant**
Mean of control minus standard	-1.675
95% confidence interval of this difference	-2.499 to -0.851
t	4.8085
df	7
Standard error of difference	0.348

Data of control and PGE (400mg):

S. No	Group	Control	Standard
1	Mean	4.325	8.100
2	SD	0.399	2.280
3	SEM	0.141	0.806
4	N	8	8

Paired *t* test results of control and PGE (400mg):

Two-tailed P value	0.0023
Statistical significance	**very statistically significant**
Mean of control minus standard	-3.775
95% confidence interval of this difference	-5.689 to -1.861
t	4.6647
df	7
Standard error of difference	0.809

Statistical significance data of analgesic activity of Psidiumguajava Leaf extract [PGE] by acetic acid induced writhing movement

Data of control and standard:

S. No	Group	Control	Standard
1	Mean	120.427	85.760
2	SD	18.901	12.593
3	SEM	4.880	3.252
4	N	15	15

Paired *t* test results of control and standard:

Two-tailed P value	0.0001
Statistical significance	**Extremely statistically significant.**
Mean of control minus standard	34.667
95% confidence interval of this difference	27.290 to 62.043
t	5.5131
df	14
Standard error of difference	8.102

Data of control and PGE (200mg):

S. No	Group	control	standard
1	Mean	120.427	88.560
2	SD	18.901	13.666
3	SEM	4.880	3.529
4	N	15	15

Paired *t* test results of control and PGE (200mg):

Two-tailed P value	0.0019
Statistical significance	**Very statistically significant.**
Mean of control minus standard	31.867
95% confidence interval of this difference	13.969 to 49.764
t	3.8189
df	14
Standard error of difference	8.345

Data of control and PGE (400mg):

S. No	Group	Control	Standard
1	Mean	120.427	78.920
2	SD	18.901	11.936
3	SEM	4.880	3.082
4	N	15	15

Paired *t* test results of control and PGE (400mg):

Two-tailed P value	0.0001
Statistical significance	**Extremely statistically significant.**
Mean of control minus standard	41.507
95% confidence interval of this difference	24.479 to 58.534
t	5.2282
df	14
Standard error of difference	7.939

Statistical significance data of Antiinflammatory activity of Psidiumguajava Leaf extract [PGE] by Carrageenan induced rat paw edema method

Data of control and standard:

S. No		Group	Control	Standard
1		Mean	2.9000	2.6720
2		SD	0.3391	0.1254
3		SEM	0.1517	0.0561
4		N	5	5

Paired *t* test results of control and standard:

Two-tailed P value	0.0032
Statistical significance	**very statistically significant**
Mean of control minus standard	0.228
95% confidence interval of this difference	0.4073 to 1.0487
t	6.3021
df	4
Standard error of difference	0.116

Data of control and PGE (200mg):

S. no	Group	Control	Standard
1	Mean	2.9000	2.3320
2	SD	0.3391	0.0540
3	SEM	0.1517	0.0242
4	N	5	5

Paired *t* test results of control and PGE (200mg):

Two-tailed P value	0.0268
Statistical significance	**statistically significant**
Mean of control minus standard	0.5680
95% confidence interval of this difference	0.1069 to 1.0291
t	3.4199
df	4
Standard error of difference	0.166

Data of control and PGE (400mg):

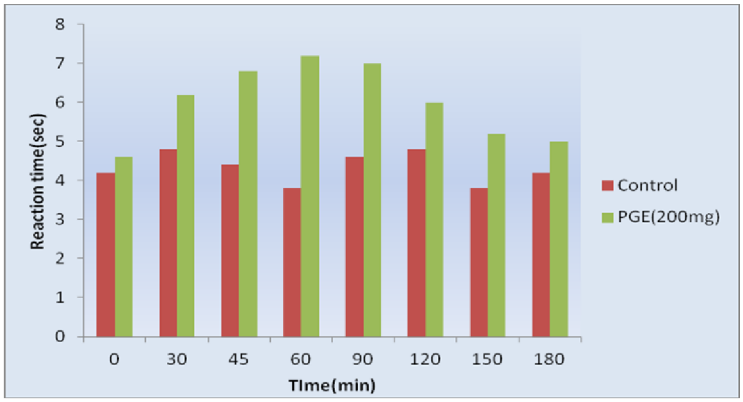
S. No	Group	Control	Standard
1	Mean	2.9000	2.3920
2	SD	0.3391	0.1188
3	SEM	0.1517	0.0531
4	N	5	5

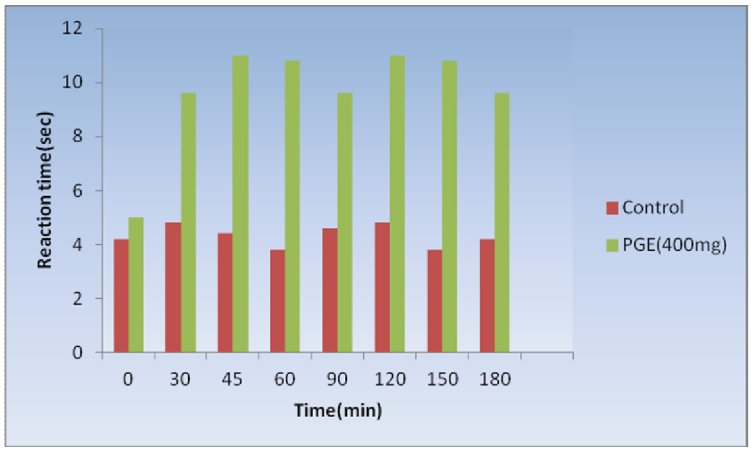
Paired *t* test results of control and standard:

Two-tailed P value	0.0345
Statistical significance	**statistically significant**
Mean of control minus standard	0.5080
95% confidence interval of this difference	0.0601 to 0.9559
t	3.1490
df	4
Standard error of difference	0.161

Graph.1

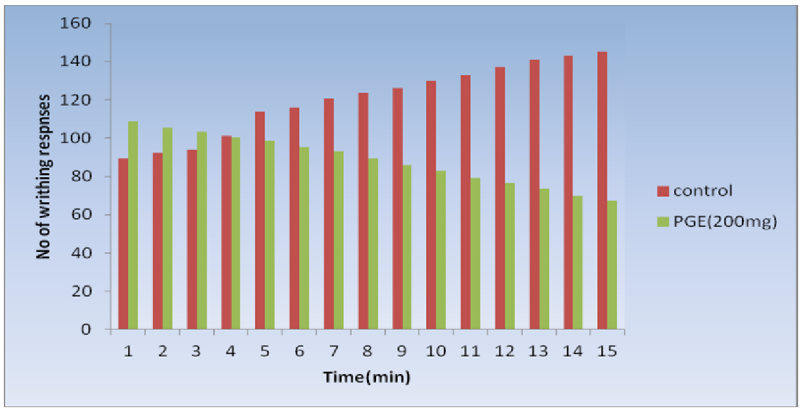
Graphical data of Analgesic activity of Psidium guajava leaf extract [PGE] by Eddy's hot plate method

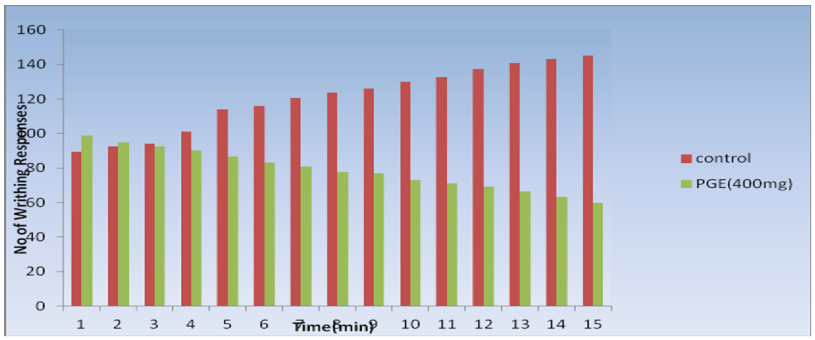




Graph.2

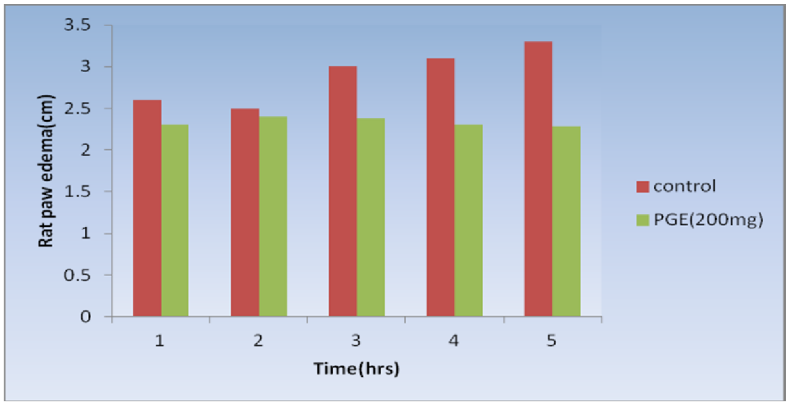
Graphical data of Analgesic activity of Psidium guajava leaf extract[PGE] by Aceticacid induced Writhing movement method

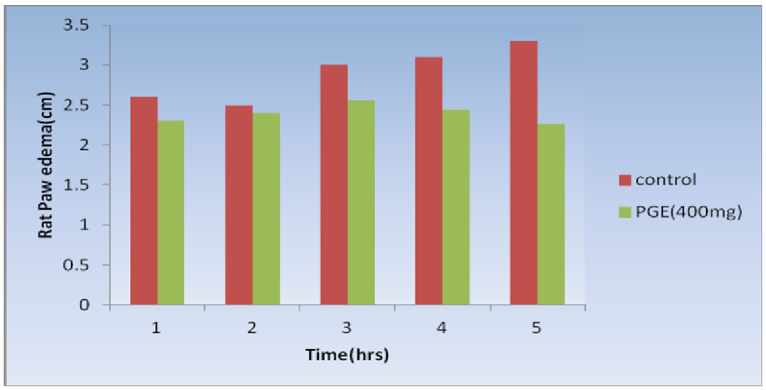




Graph.3

Graphical data of Antiinflammatory activity of Psidium guajava leaf extract[PGE] by Carrageenan Induced Rat paw edema method





DISCUSSION:

Paired *t* test results were done by using Quick Calcs software where PGE extract was compared with control and the result came as very statistically significant. Preliminary phytochemical studies revealed that the presence of flavonoids, phytosterols, saponins, tannins, terpinoids and alkaloids. Treatment with mixture of solvent extract of *Psidiumguajava leaf* at the doses of 200mg/kg and 400mg/kg showed a significant analgesic activity by eddy's hot plate method and Acetic acid induced writhing method. It also having Anti inflammatory activity screened by Carrageenan induced rat paw edema method by using Plethysmometer. At a dose of 400mg showing good activity when compared with standard and 200mg.The Analgesic activity results shows Very Statistically significant effect when compare with control but whereas Anti inflammatory activity results shows Significant effect when compare with control. Thus the presence of flavonoids in leaf extract of *Psidiumguajava* might offer the Analgesic and Anti-inflammatory effect.

CONCLUSION:

We may conclude that Psidium guajava leaf extract having Analgesic and Anti inflammatory activity.Present studies will be used for the further extending the research over the Analgesic and Anti inflammatory effect of Psidium Guajava leaves.

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