Research Article **CODEN: AJPCFF** **ISSN: 2321 – 0915**

Asian Journal of Phytomedicine and Clinical Research

Journal home page: www.ajpcrjournal.com

EVALUATION OF ANTI-INFLAMMATORY AND DIURETIC EFFECTS OF *PSIDIUM GUAJAVA* LEAF EXTRACT ON ALBINO RATS

N. R. Livingston Raja[*1], S. Antony Selvi[1], S. Gopikrishnan[1], N. Venkateshan[2], V. Lava Kumar[2]

[1*]Central Research Laboratory, Rajas Medical Institutions Kavalkinaru 627116, Tamilnadu, India.

[2]Arulmigu Kalasalingam College of Pharmacy, Krishnankoil - 626126, Tamilnadu, India.

ABSTRACT

Diuretics induce negative fluid balance and are useful in the treatment of diseases like edema and hypertension. In the present study methanolic extracts of *P. guajava* was evaluated for anti-inflammatory and diuretic activity of in male *albino rats*. Preliminary phytochemical studies carried out indicated the presence of flavonoids, saponins, carotinoids, glycosides, tannins, phenols and carbohydrates in the extracts of *P. guajava*. Acute toxicity studies of the methanolic extract of the *P. guajava* did not exhibit any signs of toxicity up to 2 g/kg body weight. Since there was no mortality observed at a higher dose, 100 and 200 mg/kg doses were selected for evaluation of anti-inflammatory and diuretic activity. The anti-inflammatory effect was assessed on egg albumin induced paw edema in albino rats. A control having normal saline and a standard containing 25mg/kg Diclofenac sodium was used. The control group of animals showed a high inflammation within the time intervals while the standard animals showed a little inflammation. The extract of the leaves of *P. guajava* (100 mg/kg and 200 mg/kg) exhibited anti-inflammatory effects at different time levels by a dose dependent manner. The diuretic activity of the extract was screened by quantification of urine volume and electrolyte concentration. Different doses of *P. guajava* (100 mg/kg and 200 mg/kg) were administered orally to hydrated rats and the urine output was measured every hour, up to 3 hours. Frusemide (20 mg/kg) was used as standard drug, while normal saline (10ml/kg) was used as control. The treatment of *P. guajava* at varying doses (100 mg/kg and 200 mg/kg) increases the urine output and the potassium-sparing effect at was observed in a dose-dependent manner.

KEYWORDS

Diuretic activity, *Psidium guajava*, Glycosides, Tannins, Carbohydrates and Aspirin.

Author for Correspondence:

Livingston Raja N R,
Central Research Laboratory,
Rajas Medical Institutions,
Kavalkinaru- 627116, Tamilnadu, India.

Email: liviverna2016@gmaiil.com

INTRODUCTION

Diuretics are drugs which enhance the flow of urine. These are generally used for management of secondary hypertension, electrolytic balance and relief of edema. In this connection excretion of sodium ions are used to maintain the volume and composition of body fluids in varies kind of clinical circumstances[1]. Drug-induced diuresis is advantageous in many life- threatening diseases like

congestive cardiac failure, nephritic disorder, cirrhosis, pregnancy toxemia, hypertension, and renal failure[2]. Inflammation is a process in which the body instantly responses to an injurious or any harmful stimulus induced by a wide range of noxious mediators like infections, antibodies, or physical injuries. This response is occurred by the activation of white blood cells, release of immune system chemicals, cytokines, Production and release of inflammatory mediators and PG. In some disease conditions the inflammatory response may lead to severe adverse effects without seeming beneficial effect[3].

Most of the currently available diuretic drugs may produce the adverse effects such as impotence, muscle fatigue, ionic imbalance and weakness. In other hand naturally available diuretics consist of caffeine in coffee, the ophylline from tea which are prevent Na + re absorption and alcohol in beer, wine prevent secretion of ADH level from the posterior pituitary gland[4]. While maximum type of the diuretics showed to be very effective in stimulating sodium excretion through urine, all these cause potassium loss and encouraged the search for potassium sparing diuretic. Hence we search for a new nontoxic diuretic agent that preserves therapeutic efficacy and however lacking of potassium loss in extracellular fluid is justified[5].

Several of the ethnic drugs have been claimed to have anti-inflammatory and diuretic effect in Ayurvedic and Siddha system. Among the several plants, *Psidium guajava* is belongs to Myrtaceae family, In folk medicine extracts of roots, bark, and leaves are used to treat edema, diarrhoea, dysentery, wounds, ulcers, toothache, coughs, sore throat, inflamed gums[6]. However, no scientific anti-inflammatory and diuretic studies carried out with *P. guajava* in order to confirm its expected advantageous properties of the selected plant. Consequently, the present study was assumed to verify the efficacy of the extract of the *P. guajava* as anti-inflammatory diuretic drug in experimental rats' model.

MATERIAL AND METHODS

Drugs and Chemicals

LASIX (Furosemide Sanofi Aventis), Diclofenac Voveran-D (Novaritis), fresh egg white 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, Tamilnadu.

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.

Animals

Adult male albino rat weighing about 200-250g 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 (509/02/C/CPCSEA/2010/AKCP), which follows the guidelines of Committee for the Purpose of Control and Supervision of Experimental Animals (CPSCEA).

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 Wagner test, Braemer's test, Frothing test, Molisch's test and Borntrager's test[7].

DIURETIC ACTIVITY

Male albino rats weighing about 150-250gm were divided in to four groups of five animals each. The

dosages of drugs were administered to the different groups.

Group I: Control (Normal saline 10ml/kg) for 7 days.

Group II: Frusemide (20 mg/kg, p.o.) for 7 days.

Group III: Received *P.guajava* at the dose of 100mg/kg orally for 7 days.

Group IV: Received *P.guajava* at the dose of 200mg/kg orally for 7 days.

Evaluation of diuretic activity

The method of (Kau *et al* 1984)[8,9] with modification was employed for the assessment of diuretic activity. According to this method, the animals were deprived of food and water for 18 hours prior to the experiment and each animal is placed in an individual metabolic cage 24h prior to commencement of the study for adaptation. In this study animals were divided into four groups of five animals each. Group I animals were received normal saline (10 ml/kg, p.o.) for 7 days, Group II animals were received the standard diuretic, Frusemide (20 mg/kg, p.o.) for 7 days and group 3 and 4 animals were received alcoholic extracts of *P. guajava,* 100, 200 mg/kg body weight for 7 days respectively. On seventh day, immediately after administration of the extracts, Frusemide the rats were paired and placed in metabolic cages. Urine was recollected in a graduated cylindrical tube and its volume was recorded at 1-h intervals for 3h. Finally the Electrolytes (Na+, K+) concentrations and pH were estimated from pooled urine sample of each pair of rat at the end of the experiment, 3h after administration[10].

Analytical method

Na+ and K+ concentrations were measured by flame photometer[11]. The instrument was calibrated with standard solution containing different concentrations of Na+ and K+. P^H was directly determined on fresh urine samples using a pH meter, urine volume measured with a micropipette.

Anti-inflammatory Activity

Male albino rats weighing about 150-250gm were divided in to four groups of five animals each. The dosages of drugs were administered to the different groups.

Group I: Control (Normal saline 10ml/kg) for 3 days.

Group II: Diclofenac (25 mg/kg, p.o.) for 3 days.

Group III: Received *P.guajava* at the dose of 100mg/kg orally for 3 days.

Group IV: Received *P. guajava* at the dose of 200mg/kg orally for 3 days.

This method was followed by (Omodamiro O.D 2014)[12], in this study animals were divided into four groups of five animals each. Group I animals were received normal saline (10 ml/kg, p.o.) for 3 days, Group II animals were received the standard diuretic, Diclofenac (25 mg/kg, p.o.) for 3 days and group 3 and 4 animals were received alcoholic extracts of *P. guajava,* 100, 200 mg/kg body weight for 3 days respectively. On third day, Thirty minutes post treatment, inflammation was induced by injecting 0.1 ml of fresh egg albumin into the sub plantar surface of the right hind paw and mean increase in paw edema was measured 0 hour, 1hour, 2 hour, 3 hour and 4 hour after induction of inflammation using a digitalized Vernier caliper. Suppression of paw inflammation of paw edema was assessed.

RESULTS

Preliminary photochemical screening

The phytoconstituents were identified by chemical tests, which showed the presence of various phytoconstituents in 50% methanolic extract of *P.guajava* presented in Table No.1.

Diuretic activity of the alcoholic extract of *P. guajava*

The result of diuretic activity of the alcoholic extract of *P. guajava* at100, 200 mg/kg showed that a dose dependent increase of urinary water excretion slightly modified the urinary pH and electrolytes concentration in normal rats. The results of 200mg/kg treated group showed significant change in electrolytes concentration and urine volume ($P \leq 0.001$) compared with control group. In the present study, alcoholic extract treated groups at different doses (100mg/kg and 200mg/kg) showed significant effect on urinary potassium and

sodium ion concentration. The values were presented in Table No.2 and 3.

The result of diuretic activity of the alcoholic extract of *P. guajava* at 100, 200 mg/kg showed that a dose dependent increase of urinary water excretion.

The result of diuretic activity of the alcoholic extract of *P.guajava* at 100, 200 mg/kg showed that a dose dependent electrolytes concentration in normal rats.

Anti-inflammatory activity

The result of Anti-inflammatory activity of the alcoholic extract of *P. guajava* at 100, 200 mg/kg showed that a dose dependent decrease the inflammation of hind paw (oedema) in diameter (mm) in rats. The results of 200mg/kg treated group showed significant change inflammation ($P \leq 0.001$) compared with control group. In the present study, alcoholic extract treated groups at different doses (100mg/kg and 200mg/kg) showed significant effect anti-inflammatory activity. The values were presented in Table No.4.

DISCUSSION

Diuretics are drugs that increase the rate of urine flow; on the other hand, clinically helpful diuretics also increase the rate of excretion of Na+ (natriuresis) and of an associated anion, generally Cl-. NaCl in the body is the most important determinant of extracellular fluid volume, and most clinical applications of diuretics are intended for toward reducing extracellular fluid volume by decreasing total-body NaCl content[13]. A sustained imbalance between dietary Na+ intake and Na+ loss is incompatible with life. A sustained positive Na+ balance would consequence in volume overload with pulmonary edema, and a sustained negative Na+ balance would result in volume reduction and cardiovascular collapse[14].

Qualitative phytochemical analysis of alcoholic extracts of both the plants showed distinguished principles and can be used as diagnostic values. Preliminary phytochemical screening of the extracts of *P. guajava* revealed the presence of flavonoids, triterpenes, saponins, tannins, carotinoids, alkaloids, glycosides and carbohydrates.

The extract of *P. guajava* showed a significant water excretory effect by a dose-dependent manner at the dose of 100mg and 200mg. It is a notable that increase in the urinary volume confirming its diuretic effects. This might be described by a marked increase in urinary excretion of water for *P. guajava*, which was more significant than the urinary electrolyte excretion[15].

In the present study, alcoholic extract treated groups at different doses (100mg/kg and 200mg/kg) showed significant effect on urinary potassium and sodium ion concentration. Phytochemical screening showed that the presence of carbohydrates, flavonoids, tannins, saponins, phytosterol in *P. guajava.* It is believed that the *P. guajava* diuretic effect to the active principles present in the plant such as essential oils, carbohydrates and flavonoids as the main active components, these compounds may be reliable for the observed diuretic activity. Though at higher concentrations of *P. guajava*, the decrease of urinary volume produced without a parallel reduction of ion excretion in compared with standard, which might be described through a decrease of the glomerular filtration rate (perhaps by renal blood flow decrease) it maybe the presence of essential oils by the plant[16].

Inflammation is caused by the release of chemicals such as prostaglandins, Leukotrienes, Histamine and bradykinin and more recently, platelet – activating factor (PAF) from tissues and migrating cells. Anti-inflammatory drugs which inhibit the cyclo-oxygenase, enzyme and reduce synthesis of both PGs and LTs thereby suppress the inflammation of rheumatoid arthritis and asthma disorder[12]. The aim for the screening of anti-inflammatory activity is to test the new anti-inflammatory agents extent the ability of a compound that reduce local oedema induced by injection of an irritant agent in the hind paw of the rat. Egg albumin induced paw oedema has been generally used as an experimental animal model for inflammation studies[17].

The effect of various treatment groups and its mean hind paw diameter was showed in (Table No.4) and the extract suppressed inflammation, as the various time intervals increased were showed in Figure No.1 to 5). The results of the leaf extract of *Pisidium guajava* specified reduction in the induced edema which was significant (P<0.001). It clearly shows that the leaves extract administered at different dose (100mg, 200mg) concentrations revealed a good anti-inflammatory activity that was dose dependent. The anti-inflammatory effect of the extract perceived may be due to its phytochemical constituents such as tannins and flavonoids. This result was supported that the selected plant contains chemical constituents such as tannins, flavonoids, saponins were recognized to have anti-inflammatory effects[18].

The investigation of the present study confirmed that the leaf extract of *P. guajava* exhibited diuretic and anti-inflammatory effects against experimentally induced animal models. Further studies will be required to get more information about the potential diuretic, anti-inflammatory value of this *P. guajava plant*, and to assess the effects of long-term administration on diuretic and anti-inflammatory activity.

Table No.1: Phytochemical screening of the extracts of *P. guajava*

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

Table No.2: Effects of oral administration of *P.guajava* on Urinary volume excretion

S.No	Groups	Dose	Volume of urine (ml/3h)	Diuretic index
1	Normal saline	10ml/kg	01.8±0.12	-
2	Frusemide	20 mg/kg	14.5±0.53	8.055
3	*P.guajava*	100 mg/kg	2.32±0.32	1.288
4	*P. guajava*	200mg/kg	7.34±0.48	4.077

Results are expressed as mean ± SEM from five observations as compared to Control group the two-tailed paired *t* test. Graph Pad's software method, (**P< 0.001) by conventional criteria; this difference is considered to be statistically significant.

Table No.3: Effects of oral administration of *P. guajava* on urinary electrolytic excretion in rats

S.No	Groups	Dose	Electrolyte Concentration in PPM		pH	Saluretic index	
			Na+	K+		Na+	K+
1	Normal saline	10ml/kg	17.31±0.53	3.35±0.06	6.34	-	-
2	Frusemide	20 mg/kg	49.21±0.74**	1.28±0.02**	7.36	2.842	0.382
3	*P. guajava*	100 mg/kg	19.47±0.47	1.76±0.32	6.42	1.124	0.525
4	*P. guajava*	200mg/kg	36.87±0.94**	2.47±0.23**	7.56	2.129	0.737

Results are expressed as mean ± SEM from five observations as compared to Control group the two-tailed paired *t* test. Graph Pad's software method, (**P< 0.05) this difference is considered to be statistically significant.

Table No.4: Effects of oral administration of *P. guajava* on inflammation of hind paw in rats

S.No	Group	Dose	Inflammation of hind paw (oedema) in diameter (mm)				
			0 hour	1hour	2hour	3hour	4hour
1	Normal saline	10ml/kg	7.45±0.12	7.48±0.23	7.43±0.14	7.32±0.12	7.28±0.23
2	Frusemide	20 mg/kg	7.34±0.12	7.23±0.21	6.24±0.23	5.43±0.35	4.83±0.32
3	*P. guajava*	100 mg/kg	7.16±0.13	7.12±0.12	6.92±0.31	6.73±0.23	6.43±0.37
4	*P. guajava*	200mg/kg	7.25±0.21	7.24±0.21	6.72±0.23	5.82±0.34	5.12±0.42

Results are expressed as mean ± SEM from five observations as compared to Control group the two-tailed paired *t* test. Graph Pad's software method, (**P< 0.001) this difference is considered to be statistically significant.

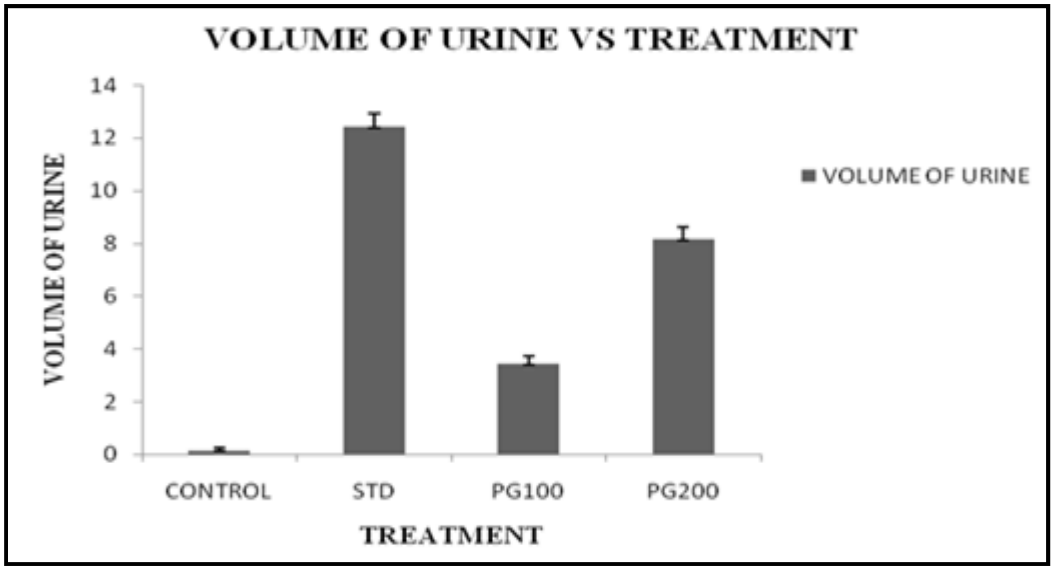

Figure No.1: Treatment Vs volume of Urine excretion in *P. guajava* treated rats

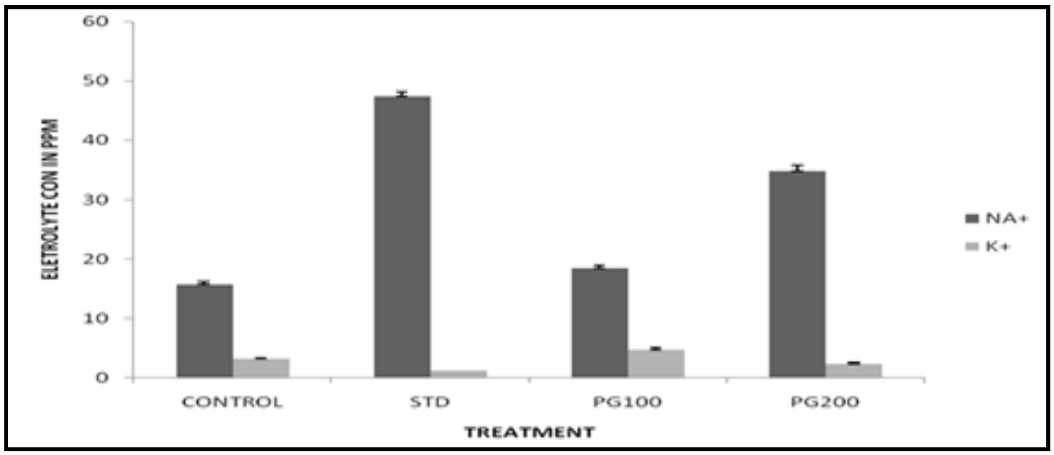

Figure No.2: Treatment Vs Electrolyte Concentration in PPM in *P. guajava* treated rats

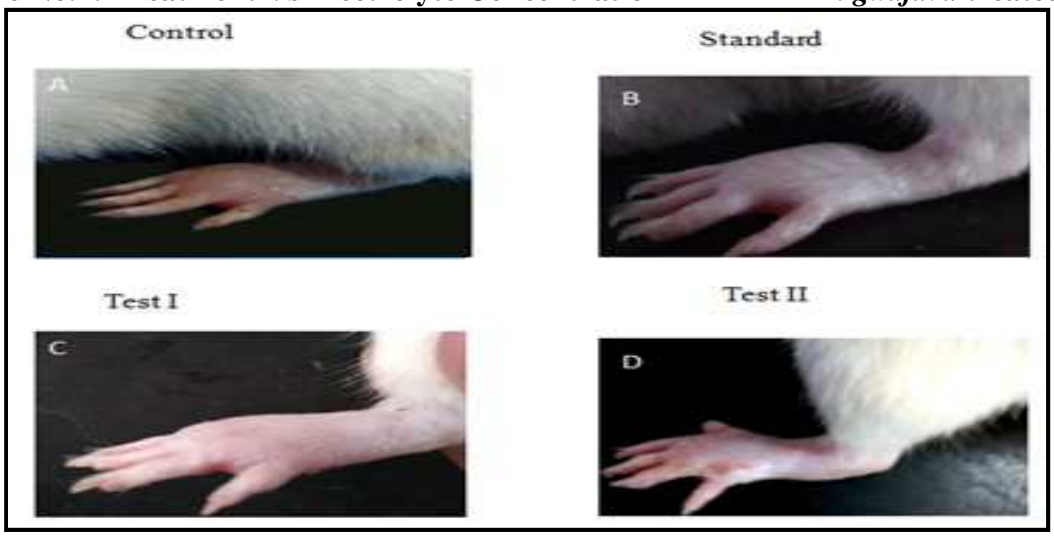

Figure No.1: Represents the anti-inflamatory activity of plant extract of A. Control, B. Standard, C. Test I and D. Test II on Initial hours

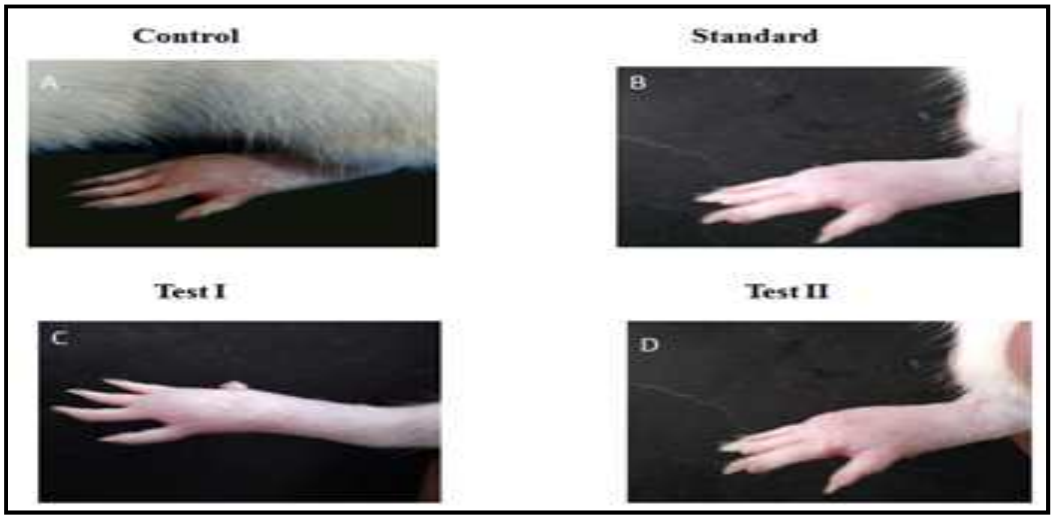

Figure No.2: Represents the anti-inflamatory activity of plant extract of A. Control, B. Standard, C. Test I and D. Test II on 1 hours

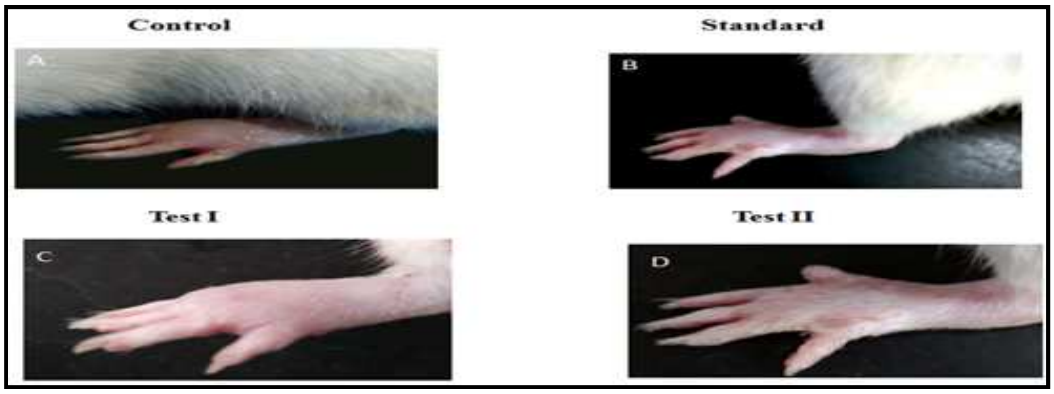

Figure No.3: Represents the anti-inflamatory activity of plant extract of A. Control, B. Standard, C. Test I and D. Test II on 2 hours

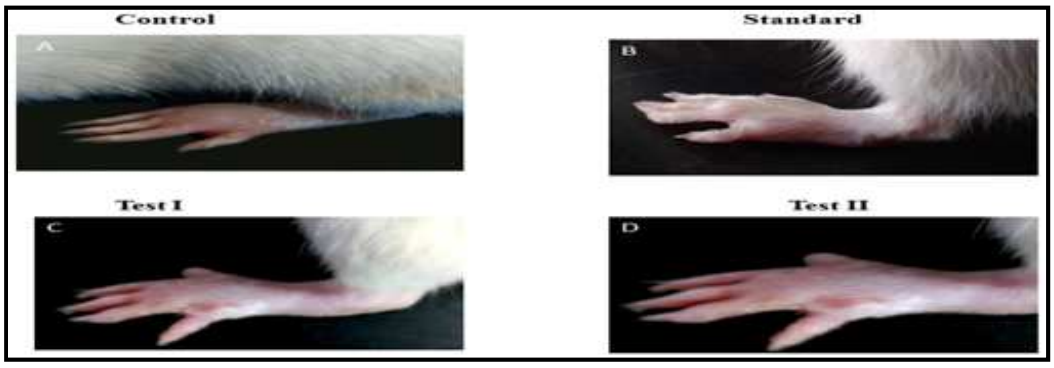

Figure No.4: Represents the anti-inflamatory activity of plant extract of A. Control, B. Standard, C. Test I and D. Test II on 3 hours

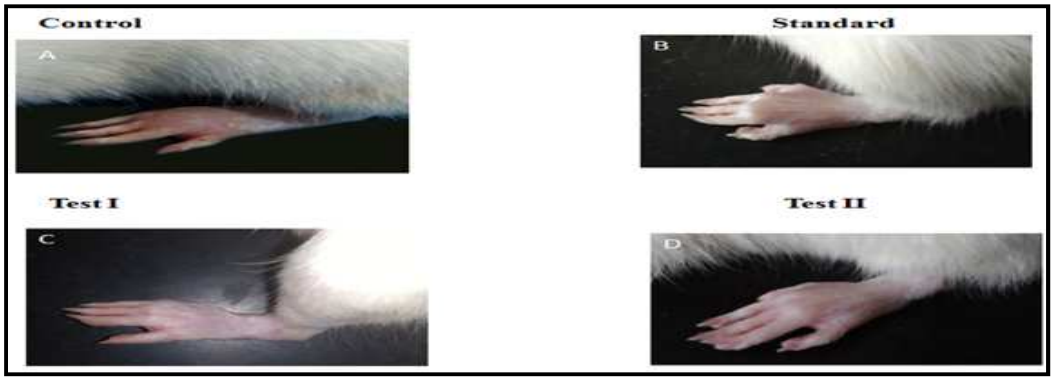

Figure No.5: Represents the anti-inflamatory activity of plant extract of A. Control, B. Standard, C. Test I and D. Test II on 4 hours

CONCLUSION

Subsequently the results elucidated above, it can be concluded that the methanolic extract of *P. guajava* having significant diuretic and anti-inflammatory activity by increasing the total urine volume output, increased excretion of sodium with sparing of potassium levels and reduction in inflammation when compared with control group animals.

ACKNOWLEDGEMENT

The authors wish to express their sincere gratitude to Central Research Laboratory, Rajas Medical Institutions Kavalkinaru 627116, Tamilnadu, India for providing necessary facilities to carry out this research work.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

BIBLIOGRAPHY

1. Umesh Kumar S, Uma Shankar S, Abhishek S, Vijit A. Diuretic activity of *Kigeliapinnata* bark extract, *Journal of Pharmacology Research,* 1(2), 2010, 17-20.
2. Agunu A, Abdurahman E M, Andrew G O, Muhammed Z. Diuretic activity of the stem-bark extracts of Steganotaenia araliacea hochst [Apiaceae], *J Ethnopharmcol,* 96(3), 2005, 471-475.
3. Johnny O O, Olubukola T A, *et al*. "Studies on the Anti-inflammatory and Analgesic properties of *Jatropha curcas* leaf extract" *Acta Vet. Brno,* 80(3), 2011, 259-262.
4. Naik D, Kalugonda Murali Krishna, Jayasri P. Evaluation of diuretic activity of*Sidaspinosa*linn leaves extract, *J. Chem. Pharm. Res,* 3(6), 2011, 1004-1008.
5. Rang H P, Dale M M, Ritter J M. In: Text book of Pharmacology, *Churchill Livingstone,* 3rd Edition, 2004, 428-38.
6. Morton J. Guava. In: J.F. Morton, Fruits of warm climates, *Julia F. Morton, Miami F L,* 1987, 356- 363.
7. Kokate C K, Gokhale S B. Phytochemica test, In: Pharmacognonsy, *Nirali Prakashan, Pune, India,* 1996, 510-512.
8. Kau S T, Keddie J R and Andrews D. A method for screening diuretic agents in the rat, *J. Pharmacol Meth,* 11(1), 1984, 67-75.
9. Lipschitz W L, Haddian Z and Kerpscar A. Bioassay of diuretics, *J. Pharmcol. Exp. Ther,* 79(2), 1943, 97-110.
10. Rejitha Gopinath *et al*. Diuretic activity of *Eupatorium odoratum* Linn, *Journal of Pharmacy Research,* 2(5), 2009, 844-846.
11. Jeffery G H, Bassett J, Mendham J, and Denny R C. Vogel's Text book of quantitative Chemical analysis, *Addision Werley Longman Ltd, England,* 5th Edition, 1989, 801.
12. Omodamiro O D and Jimoh M. A Evaluation of Anti-inflammatory and Diuretic Effects of Ethanol Leaf Extract of Piper guineense on Wistar Albino Rats, *American Journal of Ethno medicine,* 1(4), 2014, 250-259.
13. Robert W. Schrie Diagnostic Value of Urinary Sodium, Chloride, Urea and Flow, *J Am Soc Nephrol,* 22(9), 2011, 1610-1613.
14. Robert F. Reilly, Edwin K. Jackson Regulation of Renal Function and Vascular Volume Goodman and Gilman's Manual of Pharmacology and Therapeutics, Chapter 25, 12th Edition.
15. Jouad H, Lacaille-Dubois M A, Eddouks M. Chronic diuretic effect of the water extract of *Spergulariapurpurea* in normal rats, *Journal of Ethno pharmacology,* 75(2-3), 2001, 219-223.
16. Perfumi M, Paparelli F, Cingolani M L. Spasmolytic activity of essential oil of *Artemisia thuscula Cav*. from the Canary Islands, *Journal of Essential Oil Research,* 7(4), 1995, 387-392.
17. Winter C A and Risley E A. Egg albumin induced oedema in the hind paw of rat as an assay for anti-inflammatory drug", *Procsoc. Expel Biol Med,* 111(3), 2002, 544-547.
18. Menthey J A, Grohamanu K, Guthric N. Biological properties of citrus flavonoids pertaining to cancer and inflammation, *Curr Med Chem,* 8(2), 2001, 135-153.

Please cite this article in press as: Livingston Raja N R *et al*. Evaluation of anti-inflammatory and diuretic effects of *psidium guajava* leaf extract on albino rats, *Asian Journal of Phytomedicine and Clinical Research,* 6(2), 2018, 62-70.