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ANTI-DIABETIC ACTIVITY OF *Pisonia grandis* R.Br. LEAVES AGAINST STREPTOZOTOCIN (STZ) INDUCED DIABETES MELLITUS IN RATS

S.Vimalavalli[1] and V.Raji Sugumar[2*]

[1]Research Scholar, [2]Associate Professor & Research Guide
Post Graduate & Research Department of Home Science, Bharathidasan Govt. College for Women (Autonomus), Puducherry-605003.

ABSTRACT

Pisonia grandis R. Br. (henceforth Pg) leaves is commonly known as "Nachukottai keerai". The leaf pharmacologically studied for its anti-inflammatory, wound healing, diuretic, analgesic, filariasis, dysentery and rheumatic disorders but the leaves were unfamiliar and underutilized and also used as ornamental plant. The present study was to analyze the anti-diabetic activity of Pg leaves in rats. To study the anti-diabetic activity, ethanolic extract (at the dose level of 250 and 500 mg/kg) were used against Streptozotocin (STZ) induced diabetes mellitus in rats. Results were analyzed statistically by one-way ANOVA followed by Dunnett's multiple comparison tests using Prism software (Version 4). The result of the acute toxicity study shows that the ethanolic extract of *Pg* leaves does not produce any mortality upto 2000mg/kg of body weight. In anti-diabetic activity, both dose levels reduced the blood glucose level from 253 mg% to 239 and 242 mg% (250 mg/kg) and 279 mg% to 233 & 220 mg% (500 mg/kg) at 7^{th} and 14^{th} days respectively which was significant when compared to control drug Glibenclamide, ($p<0.05$). The study reveals that Pg leaves shows significant reduction of blood glucose levels in rats. Hence, this underutilized Pg leaves is an effective option for treating diabetes.

Key words: *Piosnia grandis*, Glibenclamide, Anti-diabetic activity, Acute toxicity.

INTRODUCTION

Diabetes is a chronic disease that occurs either when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces (WHO, 2014). Type 2 diabetes is a major health threat to every nation because nearly 90 percent of the world diabetic population having Type 2 diabetes due to globalization, migration, life style changes, inadequate physical activity and diet faddism (Santos, 2014). Further, it causes some serious complications in vascular, neural, nephro and retino problems in heart, eye, brain and kidney.

Corresponding Author

V.Raji Sugumar
Email: rajis207@gmail.com

Debas *et al.*, 2006 depicted that the WHO is currently working on herbal treatments for HIV/AIDS, malaria, sickle cell anemia, and diabetes to confirm its medical efficacy and safety of therapeutic use. Besides this, it estimates that about three quarters of the world currently uses and other forms of traditional medicines for their ailments.

Chang *et al* 2007 opined that many alternative therapies such as nutritional supplements, herbal medicines, nutritional advice, spiritual healing and relaxation techniques are preferred among the diabetic population. Among the herbal remedies, true cinnamon, bitter gourd, garlic and fenugreeks are used for cooking as well as to treat the diabetes in the many countries such as India, United States and China, **(**Ching *et al.*, 2013). But many medicinal plants are underutilized due to myths and beliefs but now it has been slowly explored scientifically. This clearly indicates that local herbs are underutilized and in addition not much study has been done on useful

local herbs in treating or controlling the diabetes. Thus, this study was ventured upon as a modest attempt to address the issues by utilizing herbal usage for the control of diabetes.

Elumalai *et al.,* 2012 publicized in his review that the different parts of *Pisonia grandis* are extensively used in tribal folk medicines and also used as an Indian traditional medicine for an anti-diabetic (Sunil *et al.*, 2009), anti-inflammatory (Thillaivanan *et al.,* 2013 Radha *et al.,* 2008 and Jayakumari *et al.,* 2012), wound healing (Prabu *et al.,* 2008), diuretic (Anbalagan *et al.,* 2002), analgesic (Anbalagan *et al.,* 2002), filariasis, dysentery (Shanmugam *et al.,* 2013) and rheumatic disorders (Elumalai and Prakash Yoganandam G., 2012). This creates interests towards Pg leaves in giving treatment for diabetes. So, the present study was to highlight the antidiabetic activity of the Pg leaves which is easy to assess, affordable and available in community. The present investigation was set to evaluate the anti-diabetic potential of the ethanolic extract of the Pg leaves.

METHODOLOGY

Authentication of plant material and extraction procedure:

The leaves of *Pisonia grandis* R.Br. were collected from Puduchery district. The plant was authenticated by Dr. K. Althaf Ahamed Kabeer, Scientist of Botanical Survey of India, Coimbatore, Tamilnadu, India. The leaves of *Pisonia grandis* were washed thoroughly in tap water, shade dried and pulverised to coarse powder. These coarse powders of the Pg leaves were used for further studies. The powder (300 gm) was extracted with 3000 ml of ethanol (90%) with constant stirring. The residue was removed by filtration and the filtrate was evaporated to dryness at 40ºC under reduced pressure in a rotary evaporator. The yield of the ethanol extract was found to be 25 gm. The extract was dried in a desiccator and it was referred to as PAEE. It was suspended in 0.5 % carboxy methyl cellulose and used for the experiments.

Protocol

(i) Animals: Male Wistar rats weighing 180–240 g and female Wistar rats weighing 134-166g were used in this study. The animal husbandry details are given below;

(ii) Conditions: Animals were housed and maintained under laboratory conditions of temperature of 18-24 ^{0}C, relative humidity of 55-75%, with a 12 h light and 12 h dark cycles.

(iii) Housing: The rats were housed in groups of 6 per cage in suspended polypropylene rat cages (size approximately: L 410 x W 220 x H 140 mm) with stainless steel mesh bottom and stainless steel top grill having facilities for holding pellet food and drinking water in bottles fitted with stainless steel sipper tubes.

(iv) Diet *ad libitum*: Extruded rat feed manufactured by M/s. Amruth labs Private Ltd. Bangalore, India was provided.

(v) Water *ad libitum*: Deep bore-well water passed through activated charcoal filter and exposed to UV rays in Aquaguard on line water filter cum purifier manufactured by Eureka Forbes Ltd., Mumbai, India, was provided.

Test item information and CPCSEA approval information:

Name to be used in the report : Ethanol extract
Code by test facility : Ethanol extract
Storage conditions : 2-8 ° C for long term storage
CPCSEA approval no: SSCP/IAEC/CADRAT/01 /2014-15

Acute oral toxicity study

Preparation of test item

The test item was prepared immediately prior to administration on respective treatment days. A quantity of 2g of the test item was suspended in 0.5% carboxymethyl cellulose and the volume made up to 10ml to get a test item concentration of 200mg/ml. Homogeneity of the test item in the vehicle was maintained during treatment by constant stirring and mixing. The test substance was administered soon after preparation.

Experimental procedure

Limit test at a dose of 2000mg/kg, b.wt. was carried out with six female rats (three animals per step). The test item was administered as a single oral dose to overnight fasted (16-18 hours) rats at the dose volume of 10ml/kg b.wt. Mortality, clinical observations, body weights and gross necropsy findings were evaluated. Food was offered about 3-4h after dosing. Water was not withheld. At the end of the observation period the rats were sacrificed using diethyl ether anaesthesia and subjected to detailed gross necropsy studies.

Anti-diabetic activity

(i) Preparation of STZ in 0.1M Citrate Buffer pH 4.5: The citrate buffer was prepared by mixing 21g citric acid in 1L distilled water, and 29.4g sodium citrate in 1 L distilled water. The pH was adjusted to 4.5 with 1M sodium hydroxide. Streptozotocin (STZ) (225mg) was Dissolved in 50ml of 0.1M citrate buffer pH 4.5 and immediately injected to male Wistar rats.

(ii) Procedure for inducing Diabetes Mellitus in Rats: 40 male Wistar rats were injected with STZ at dose of 45 mg/kg, i.p. to induce diabetes mellitus. The induction was confirmed by measuring the blood glucose after a week. The blood was collected from retro-orbital plexus under light both anaesthesia and the serum separated at 3000rpm was used for glucose estimation by using glucose kit (Merck ltd., Mumbai). The animals which show blood glucose levels above 200 mg/dl was used in the study

(iii) Preparation of test item: The test item/standard was prepared immediately prior to administration on respective treatment days. A suspension of 25 and 50 mg/ml of the test item and 1.0 mg/ml of the standard, glibenclimide were prepared in 0.5% carboxymethyl cellulose. Homogeneity of the test item in the vehicle was maintained during treatment by constant stirring and mixing. The test substance was administered soon after preparation.

(iv) Experimental procedure: 30 male Wistar rats (which are previously treated with STZ and selected) were divided into 5 groups of 6 each.

Group 1: Vehicle (10 ml/kg, p.o.)
Group 2: Control (10 ml/kg, p.o.)
Group 3: Ethanol extracts (250 mg/kg, p.o.)
Group 4: Ethanol extracts (500 mg/kg, p.o.)
Group 5: Glibenclamide(10 mg, kg, p.o.).

All the rats was given the above assigned treatment for a period of 14 days and fasting blood glucose was measured on day 0, 7 and 14. The antidiabetic activity was assessed by comparing the blood glucose values of treated groups with that of control.

Statistical analysis

The data was represented as mean ± S.D. Results were analyzed statistically by one-way ANOVA followed by Dunnett's multiple comparison tests using Prism software (Version 4). The minimum level of significance was set at $p<0.05$.

RESULTS AND DISCUSSIONS

The following paragraphs deals with results of acute toxicity and anti-diabetic activity of ethonolic extract of Pg leaves.

Acute oral toxicity study

The selected wistar female rats were injected with a daily dose of 2000 mg/kg of *Pisonia grandis R. Br.* leaves. The results of acute oral toxicity study are given in Table 1 and 2. There were no toxic signs and pre-terminal deaths observed in any of the treated animals. All the rats gained weight through the observation period and the gross necropsy studies revealed no abnormal findings. Based on the results, the acute oral toxicity (Acute Toxic Class Method) of the test item was considered greater than 2000mg/kg in Wistar female rats, the LD 50 of the test item may be classified as GHS category 5 (LD50 >2000mg/kg) as per OECD Guideline No. 423. To reiterate the results the average initial weight of the female wistar rats was 158.5 and the average weight gain on 8^{th} and 15^{th} day was 8.8 g and 2.7 g respectively. Added to these all the rats survived the feed without any case of mortality. The results show the absence of toxic elements in the Pg leaves extract.

Similarly, in the present study it was identified scientifically that the ethanolic extract of *Pisonia grandis* leaves does not produce any mortality upto 2000 mg/kg of body weight. The findings by Balakrishnan *et al* 2007 and Jayakumari *et al* 2012 found that *Pisonia grandis R. Br*. does not produce any toxicity upto the dose level of 2000 mg/kg and also it does not produce any morbidity upto the above mentioned dose level (Christudas *et al.,* 2009). Therefore, the *Pisonia grandis R. Br*. leaves can be used as a leafy vegetable in day to day cooking as like drumstick leaves.

Anti-diabetic study

The results of anti-diabetic study are given in Table 3 and Figure 1.

The induction of experimental diabetes in the rat using chemicals such as Streptozotocin (STZ) which selectively destroy pancreatic β cells is very convenient and simple to use. Streptozotocin is synthesized by Strettomyces Achrmogenes and is used to induce both Insulin Dependent Diabetic Mellitus (IDDM) & Non Insulin Diabetic Mellitus (NIDDM). The STZ is taken up by pancreatic β cells via Glucose Transporter (GLUT2). This action results in changes of DNA in pancreatic β cells comprising its fragmentation. Since STZ is a nitric oxide donor, it was found to bring about the destruction of pancreatic islet cells, thereby induction of diabetes mellitus. The same was confirmed in the present study. The STZ treated animal's shows the increased blood glucose level of 271 mg% on Day 0 which was further increased to 289 mg% on Day 14. The same is depicted in Fig 1.

The ethanolic extract of the *Pisonia grandis R.Br.* leaves made into a suspension of Carbon Methoxy Cellulose (CMC) and administered at the dose level of 250 & 500 mg/kg per oral against STZ induced diabetic rats. The 250 mg/kg of ethanolic extract reduced the blood glucose level from 253 mg% to 239 and 242 mg% at 7^{th} and 14^{th} days respectively. The 500 mg/kg of ethanolic extract reduced the blood glucose level from 279 mg% to 233 & 220 mg% at 7^{th} and 14^{th} days respectively which was significant when compared to control ($p<0.05$). The results of the extract was comparable to that of 10 mg/kg of Glibenclimide ($p<0.05$). The probable mechanism of action may due to

either regeneration of pancreatic β cells or by its antioxidant potentials. Shanmugam *et al.,* 2013 reported the Hepato-protective activity of these extracts against carbon tetra chloride and paracetomol induced hepacto-toxicity which is also due to its antioxidant potential.

Table 1. Body weight, body weight changes and pre-terminal deaths before and after feeding for 15 days

Dose (mg/kg b.wt.)	Rat No.	Sex	Body weight (g)					No. dead/ No. tested
			Initial	Day 8	Weight change (day 8–Initial)	Day 15	Weight change (day 15 -Initial)	
2000	R-001	Female	166	177	11	192	26	0/6
	R-002	Female	163	166	03	178	15	
	R-003	Female	155	160	05	174	19	
	R-004	Female	155	171	16	181	26	
	R-005	Female	139	155	16	161	22	
	R-006	Female	134	136	02	150	16	

Table 2. Individual toxic signs and necropsy findings observed every one hour on day 1 for 15 days
Dose: 2000mg/kg, body weight Sex: Female

Rat No.	Body Weight (in gms)	Dose volume (ml)	Day 1					Day of observation														Necropsy
			30 Min	1hr	2 hr	3 hr	4 hr	2	3	4	5	6	7	8	9	10	11	12	13	14	15	
R-01	151	1.5	001	001	001	001	001	001	001	001	001	001	001	001	001	001	001	001	001	001	001	NAD
R-02	148	1.5	001	001	001	001	001	001	001	001	001	001	001	001	001	001	001	001	001	001	001	NAD
R-03	137	1.4	001	001	001	001	001	001	001	001	001	001	001	001	001	001	001	001	001	001	001	NAD
R-04	144	1.4	001	001	001	001	001	001	001	001	001	001	001	001	001	001	001	001	001	001	001	NAD
R-05	125	1.3	001	001	001	001	001	001	001	001	001	001	001	001	001	001	001	001	001	001	001	NAD
R-06	118	1.2	001	001	001	001	001	001	001	001	001	001	001	001	001	001	001	001	001	001	001	NAD

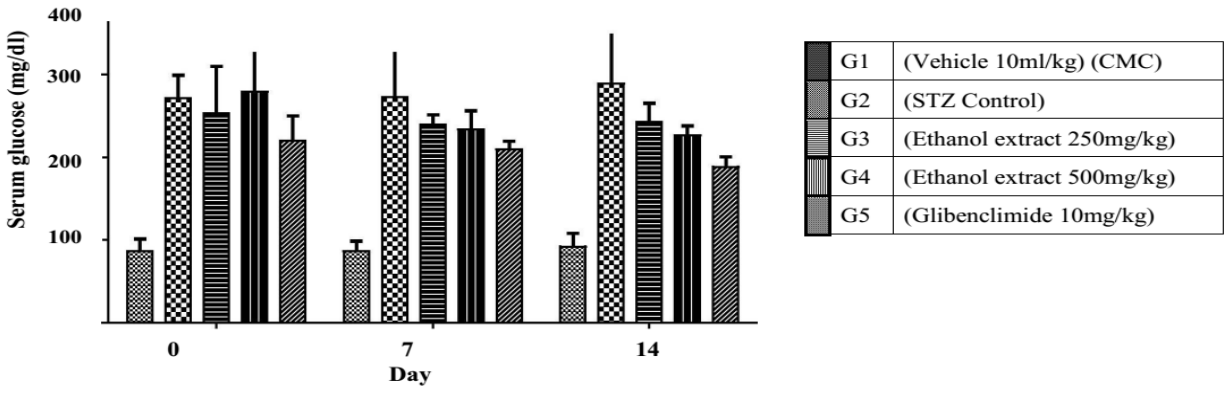
Abbreviations: F-Female; Min-Minute; hr-Hour; NAD-No abnormality detected; 001-No specific findings.

Table 3. Effect of various dose of ethanol extract of leaves of *Pisonia grandis R.Br.* leaves on Streptozotocin (STZ) induced changes in blood glucose in rats (mg %)

Days	G1	G2	G3	G4	G5
	Vehicle 10ml/kg	STZ Control	Ethanol extract 250mg/kg	Ethanol extract 500mg/kg	Glibenclimide 10mg/kg
Day-0	86.5±14.6	271.5±27.4$^{\#}$	253.0±56.6	279.2±47.7	220.0±30.0
Day-7	86.7±11.8	272.5±55.0$^{\#}$	239.7±11.69	233.5±22.5	209.3±9.8*
Day-14	92.0±15.9	289.0±60.2$^{\#}$	242.5±22.8*	226.2±11.7*	188.3±11.8*

Values are mean ± S.D; n=6; $^{\#}$p<0.05 when compared to G1; *p<0.05 when compared to G2. Standard hypoglycemic drugs

Figure 1. Effect of ethanol extract of leaves of *Pisonia grandis* against Streptozotocin (STZ) induced changes in blood glucose in rats



CONCLUSION

From these results, we observed that the ethanolic extract of Pg leaves didn't produce any toxicity and it shows the significant reduction in glycaemic levels of diabetic male rats.

The significance reduction may be due to the presence of phyto-constituents reported in the Pg leaves. Hence, the future research is aimed to highlight the anti-diabetic activity of Pg leaf powder in human subjects using day to day food consumption.In this backdrop and our country having abundance of herbs, it is high time to highlight the medicinal herb *Pisonia grandis R.Br.* leaves as food and as medicine in curing high prevalent disease like diabetics which causes huge burden on national and individual's expenditure.

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