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Evaluation of Anti-Inflammatory and Anti-Diabetic Activity of Bark Extracts of *Pajanelia Longifolia* (Willd.) K.Schum

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ABSTRACT

Evaluation of anti-inflammatory and antidiabetic property of Pajanelia longifolia stem bark with preliminary phytochemical profile of the extracts has been carried out in this part of work. The dried plant material was packed in Soxhlet apparatus and extracted successively with pet-ether to de-fat the drug, petroleum-ether was removed from the powdered defatted drug, which was then extracted with chloroform and methanol as increasing polarity. All the extracts were screened for anti-inflammatory and antidiabetic activity using carrageenan induced paw edema and alloxan induced diabetic respectively. The toxicity and phytochemical screening were done using standard procedure. Alkaloids, flavonoids, phytosterols, phenolic compounds, glycosides carbohydrates, proteins, gums and amino acids have been determined by preliminary phytochemical tests. The acute toxicity study of various extracts of Pajanelia longifolia was conducted and dose of 3000 mg kg^{-1} body weight fixed for anti-inflammatory and antidiabetic activity. Among all the extracts, chloroform extract showed a significant decrease in the degree of swelling, after 5 h carrageenan injection as compared with control and exhibited a potent antidiabetic activity at dose dependent manner when compared to diabetic untreated group.

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Keywords: Pajanelia longifolia (Willd.) K.Schum, Anti-inflammatory activity, Anti-diabetic activity, Indomethacin, Alloxan.

INTRODUCTION

Inflammation is considered as a primary physiologic defence mechanism that helps body to protect itself against infection, burn, toxic chemicals, allergens or other noxious stimuli. An uncontrolled and persistent inflammation may act as an etiologic factor for many of this chronic illness. Although it is a defence mechanism, the complex events and mediators involved in the inflammatory reaction can easily be induced [1]. The side effects of the currently available anti-inflammatory drugs pose a major problem during their clinical uses [2]. Therefore, the development of newer and more potent anti-inflammatory drugs with lesser side effects is necessary.

Diabetes mellitus is one of the most common endocrine metabolic disorder has caused significant morbidity and mortality due to microvascular(retinopathy, neuropathy and nephropathy)and macrovascular (heart attack, stroke and peripheral vascular disease) complications [3]. Diabetes mellitus is a complex and a multifarious group of disorder that disturbs the metabolism of carbohydrates, fat and protein. It results from shortage or lack of insulin secretion or reduced sensitivity of the tissue to insulin [4]. Globally diabetes mellitus is the sixth leading cause of death [5]. Several drugs have been used in the management of the disease. These drugs have side effects and thus searching for a new class of compounds is essential to overcome diabetic problems [6]. Traditionally, a number of plants have been used in various herbal preparations in the management of diabetes and only a few of them have been proven scientifically [7]. More than 800 plants have been studied for their antidiabetic potentials [6,8] among thousands of plants used in various regions of the world.

Pajanelia longifolia is a deciduous or evergreen, small to medium sized tree belonging to the family *Bignoniaceae*. The *Pajanelia longifolia* locally known as Bondable in Kannada, the bark is being used to treat skin diseases and mainly eczema and wounds by ethno medical practitioners of Agumbe range of Karnataka. The literature survey revealed that no reports were found on the anti-inflammatory and antidiabetic activity of the bark extracts of *Pajanelia longifolia*. Hence the present study was under taken to investigate the extraction, phytochemical analysis, anti-inflammatory and antidiabetic activity of bark part of *Pajanelia longifolia* plant.

MATERIALS AND METHODS

Collection of Plant material: The bark part of *Pajanelia longifolia* plant material was collected in the month of May-June in Agumbe region of Shimoga district in Karnataka state. The plant was authenticated by Prof. M.S. Pushpalatha, Department of Botany, Sahyadri Science College, Shimoga and voucher label have been maintained in Kuvempu University (Family: Bignoneaceae: 8: 114).

Preparation of Plant Extracts: The collected plant material was then shade dried and coarsely powdered. The powdered plant material (3000g) was subjected to the hot method of extraction using Soxhlet extractor. The extraction process was carried out using various solvents viz., pet-ether, chloroform and methanol with the increasing polarity. The obtained extracts were filtered and evaporated to dryness under reduced pressure in rotary vacuum evaporator.

Animals: Healthy adult Wister rats of either sex weighing 150-180 g were selected for the experimental study. The rats were procured from National College of Pharmacy, Shimoga. They were kept in standard environmental condition (at 26.0±0°C temperature & 55-65% relative humidity and 12 h light/12 h dark cycle) about one week for acclimation. The set of rules followed for animal experiment were approved by the Institutional Animal Ethical Committee [9]. Ref:NCP/IAEC/CL/106/05/2012-13.

Phytochemical Screening: The extracts were subjected to preliminary phytochemical investigation to identify various phytoconstituents i.e., alkaloids, steroids, carbohydrates, flavonoids, phenolics, tannins, saponins, glycosides, amino acids etc. present in bark part of plant by using standard tests [10,11].

Acute toxicity studies: The acute oral toxicity studies were performed to study the acute toxic effects and to determine minimum lethal dose of the plant extracts. Swiss albino mice of either sex weighing 18-25 g were used for the study. The pet-ether, chloroform and methanol extracts were administered orally to different groups of overnight fasted mice at the doses of 1000, 2000, and 3000 mg kg^{-1} body weight. After the administration of extracts, animals were observed continuously for the first 3 h for any toxic manifestation. Thereafter, observations were made at regular intervals for 24 h. Further the animals were under investigation up to a period of one week [12]. This study showed that, drug is safe up to the dose of 3000 mg kg^{-1} for all the extracts.

Experimental design: Thirty experimental animals were randomly selected and divided into five groups denoted as Group I, Group II, Group III, Group IV and Group V, consisting of 6 Wister rats in each group. Each group received a particular treatment i.e. control, standard and the three doses of the extract. Prior to any treatment, each rat was weighed properly and the dose of the test samples and control material was adjusted accordingly. Group III to Group V received the crude extracts orally at the dose of 300 mg kg^{-1} body weight. Group II received intraperitoneal administration of Indomethacin at a dose of 10 mg kg^{-1} - body weight as standard for anti-inflammatory study, while Group I was kept as control giving 2% gum acacia solution in normal saline water. Similarly thirty-six animals were selected and divided into six groups denoted as Group I, Group II, Group III, Group IV, Group V and Group VI consisting of 6 Wister rats in each group. Each group received a particular treatment i.e. normal control, diabetic control, standard and the three doses of extracts. Prior to any treatment, each rat was weighed properly and the doses of the test samples and control materials were adjusted accordingly. Group IV to Group VI received the crude extracts orally at the dose of 300 mg kg^{-1}- body weight. Group III received intraperitoneal administration of Glibenclamide at the dose of 5 mg kg^{-1} -body weight as standard drug for anti-diabetic study, while Group II was kept as diabetic control giving 2% gum acacia solution in normal saline water.

Anti-inflammatory activity: The anti-inflammatory activity of *Pajanelia longifolia* was studied using acute (Carrageenan induced paw edema) model of inflammation. This model is based on the principle of release of various inflammatory mediators by carrageenan. Edema is due to carrageenan in the rat paw as biphasic event. The initial phase is attributed to the release of histamine and serotonin. The second phase of edema is due to the release of prostaglandins, protease and lysosome [13,14]. Subcutaneous injection of carrageenan into the rat paw produces inflammation resulting from plasma extravasations, increased tissue water and plasma protein exudation along with neutrophil extravasations, these results due to the reaction of arachidonic acid [15]. The first phase begins immediately after injection of carrageenan and diminishes in two hours. The second phase begins at the end of first phase and remains through third hour up to 5 h.

Method: Carrageenan-Induced Paw Edema in Rats: Anti-inflammatory activity was evaluated using the carrageenan induced rat paw edema by known technique [15-20]. The animals were housed in cages under standard laboratory conditions. They had free access to standard diet and water. The animals were divided into 5 groups of six animals each and fasted for 12 h before the experiment. The initial right hind paw volume of the rats were measured using a plethysmometer and then 0.5 ml of 1% w/v carrageenan solution in normal saline was subcutaneously injected into the sub plantar region of the right hind paw. The volume of right hind paw was measured at 0, 1, 2, 3 and 5 h after carrageenan injection and the paw volume was determined. The data were expressed as paw volume (ml) and compared with the initial hind paw volume of each rat. Co solvent (2% gum acacia solution, p.o), various extracts of *Pajanelia longifolia* as suspension in 2% gum acacia solution (p.o) and indomethacin (10 mg kg^{-1}, p.o) [14] was administered 30 min before carrageenan injection. The group received co solvent was treated as control. The hind paw volume was measured plethysmometrically before and after the carrageenan injection, at hourly intervals for 5 h.

$$\% \text{ inhibition of edema} = \frac{Vc-Vt}{} \text{ X } 100\ Vc$$

Where, Vt = mean paw volume of test group

Vc = mean paw volume of control group

Evaluation of Anti-diabetic Activity [21, 22, 23]: Before starting the experiment, animals were separated according to their body weight. The animals were injected intraperitoneally (i.p.) at a dose of 150 mg kg^{-1} - body weight alloxan freshly prepared in normal saline solution. After 1h of alloxan administration, animals were given feed *ad libitum* and 1mL of (100 mg mL^{-1}) glucose i.p. to combat severe hypoglycaemia after 72 h of alloxan injection; the animals were tested for evidence of diabetes by estimating their blood glucose level using glucometer (Pulsatum, Pulsatum Health Care Pvt. Ltd., Bangalore).

To the animals, the test extracts (300 mg kg^{-1} b.w. orally) and standard drug glibenclamide tablet (10 mg kg^{-1} b.w. orally) were administered by dissolving in 2% Tween-80 and normal saline respectively. For acute study, 0.2 mL of blood sample was withdrawn through the tail vein puncture technique using hypodermic needle at the interval of 0^{th}, 1^{st}, 3^{rd}, 5^{th} and 7^{th} h of administration of single oral dose. The animals were segregated into six groups of six rats each for each extract. For all extracts, groups of normal, diabetic control and standard glibenclamide were kept same and compared with pet-ether, chloroform and methanol extracts of drugs. The mean ± SEM were statistically calculated for each parameter [24].

RESULTS AND DISCUSSION

The results obtained in the carrageenan-induced edema test are shown in table 1. The bark extracts of *Pajanelia longifolia* (300 mg kg^{-1}; p.o) administered 30 min before the injection of carrageenan caused a significant($P<0.01$) and dose dependent inhibition of increase in paw edema. In the carrageenan test, the maximum inhibition elicited by the chloroform extract (45.28%) was comparable to that of indomethacin (10 mg kg^{-1}; p.o; 67.92%). Pet-ether extract showed a poor inhibition while methanol extract showed a moderate inhibition of increase in paw edema in rats when compared to standard Indomethacin.

Table 1: Anti-inflammatory activity of *Pajanelia longifolia* bark extracts by Carrageenan induced paw edema in rats

Groups	Dose (mg kg^{-1} b.w)	Increase in paw edema(cm) at time T(h)					
		0h	1h	2h	3h	5h	% inhibition
Control		0.56±0.01	0.56±0.02	0.54±0.05	0.53±0.02	0.53±0.03	-------
Indomethacin	10	0.55±0.04	0.46±0.01	0.41±0.04	0.33±0.05	0.18±0.02	67.92
Pet-ether	300	0.55±0.01	0.55±0.03	0.51±0.02	0.47±0.07	0.42±0.05	20.75
Chloroform	300	0.55±0.03	0.52±0.01	0.46±0.04	0.38±0.02	0.29±0.01	45.28
Methanol	300	0.55±0.06	0.54±0.05	0.46±0.01	0.41±0.04	0.35±0.03	33.96

There were observable changes in the body weight of treated and untreated diabetic rats. Treated group of diabetic rats with the bark extracts of *Pajanelia longifolia* or glibenclamide improved the weight gain as compared to untreated diabetic rats and are shown in table 2.

A dose-dependent reduction in blood glucose level was observed in alloxan-induced diabetic rats treated with various extracts of *Pajanelia longifolia*. After a single dose of the extract given to the alloxan-induced diabetic rats, there was a significant ($P<0.01$-0.001) reduction in blood glucose level of the diabetic rats within the period of acute study as compared to control. The maximum effect was observed at 7h with the various extracts exerting comparable effect. Among the extracts, pet-ether extract exhibited a potent anti-diabetic activity and are shown in table 3.

During prolonged study (7days), the various bark extracts produced a sustained significant ($P<0.001$) reduction in blood glucose level of the diabetic rats as compared to control and are tabulated in table 4. The effect of pet-ether extract were more potent than that of the chloroform and methanol extracts when compared with standard drug glibenclamide.(10 mg kg^{-1}, on day 7).

Table 2: Anti-diabetic effect of bark extracts of *Pajanelia longifolia* on body weights of alloxan-induced diabetic rats (mean±SEM) (n=6)

Group	Serum Glucose (mg/dl) level in Alternative day-Change in Body weight				
	1^{st} day	3^{rd} day	5^{th} day	7^{th} day	Total Change in B.wt
Normal	174.50±3.78	**180.50**±4.68	**184.50**±2.17	196.33±3.83	21.83±4.70
Diabetic control	177.67±4.64	165.33±4.70	158.67±2.88	152.00±2.28	-25.67±5.19
Glibenclamide	178.50±6.00	170.33±4.41	178.17±4.29	195.33±5.48	16.83±5.43
Pet-ether	180.00±6.32	153.67±9.79	161.17±4.32	189.50±7.75	9.5±2.70
Chloroform	182.67±7.29	166.00±9.79	176.50±5.47	187.17±5.37	4.50±1.23
Methanol	178.00±6.01	171.33±10.14	178.67±2.03	184.33±6.32	6.33±1.51

Table 3: Antidiabetic effect of bark extracts of *Pajanelia longifolia* on blood glucose level of alloxan-induced diabetic rats during acute study (mean±SEM) (n=6)

Group	Serum Glucose (mg/dl) Time				
	0h	1h	3h	5h	7h
Normal	108.83±3.09	117.67±3.19	114.33±4.49	109.67±4.46	100.33±2.50
Diabetic control	310.83±1.35	317.17±1.25	324.50±1.61	334.50±2.29	342.17±2.30
Glibenclamide	308.67±1.84	302.00±3.39	287.00±4.16**	262.83±7.71**	204.50±6.67**
Pet-ether	309.83±1.49	303.83±2.43*	296.50±3.68*	274.17±1.94**	218.00±7.18**
Chloroform	309.67±2.25	317.67±5.61	324.50±7.64	311.50±22.48	286.00±3.42
Methanol	309.50±2.23	316.33±6.12	312.33±1.43	290.67±7.75*	239.50±4.63*

Table 4: Anti-diabetic effect of bark extracts of *Pajanelia longifolia* on blood glucose level of alloxan-induced diabetic rats during prolonged treatment (mean±SEM) (n=6).

Group	Serum Glucose (mg/dl) level in Alternative day				
	0 h	1st day	3rd day	5th day	7th day
Normal	108.83±3.09	100.33±2.50	97.67±1.74	100.17±2.55	102.00±1.26
Diabetic control	310.83±1.35	332.17±2.30	343.17±7.41	356.67±6.46	359.17±10.79
Glibenclamide	308.67±1.84	204.50±6.67**	181.67±12.67	133.67±6.96**	106.67±5.24**
Pet-ether	309.83±1.49	218.00±7.18**	206.67±3.56	149.17±2.27*	121.50±0.85**
Chloroform	309.67±2.25	286.00±3.42	260.33±7.09	230.50±7.03	176.00±7.35*
Methanol	309.50±2.23	239.50±4.63*	231.17±5.04	199.50±2.83*	147.83±5.10**

Inflammation is a common phenomenon and it is a reaction of living tissues towards injury. It is the body response to inactivate or destroy the invading organisms, to remove the irritants and set the stage for tissue repair [25]. Steroidal anti-inflammatory agents will lyse and possibly induce the redistribution of lymphocytes, which cause rapid and transient decrease in peripheral blood lymphocyte counts to affect longer term response. Carrageenan has been widely used as a noxious agent; it can able to induce experimental inflammation for the screening of compounds possessing anti-inflammatory activity [26]. This phlogistic agent, when injected locally into the rat paw, produced a severe inflammatory reaction, which was discernible within 30 min [27]. The development of edema induced by carrageenan corresponds to the events in the acute phase of inflammation, mediated by histamine, bradykinin and prostaglandins produced under an effect of cyclooxygenase [28]. Stem bark extracts of *Pajanelia longifolia* showed significant anti-inflammatory activity. This significant anti-inflammatory effect may be due to the inhibition of any inflammatory mediators by the glycosides or steroids [29] present in the extract.

Similarly the treatment of diabetes with medicines of plant origin that proved much safer than synthetic drugs is an integral part of many cultures throughout the world and gained importance in recent years. India has a rich history of using various potent herbs and herbal components for treating various diseases diabetes [30] and several species of plants have been described as having a hypoglycaemic activity [31-33].

From evaluation, it has been concluded that the Pet-ether, chloroform and methanol extracts showed significant reduction of inflammation as compared to Indomethacin. Pet-ether extract cause a significant decrease in blood glucose level as compared to standard drug Glibenclamide. The present result indicated the efficacy of bark part of *Pajanelia longifolia* as an effective therapeutic agent in the treatment of acute inflammations and diabetic and also the present study authentified the folk lore information on the anti-inflammatory and antidiabetic property of the plant *Pajanelia longifolia*. Further and detailed studies are in progress for the isolation of active constituent responsible for this property and in the identification of the possible mechanism for the said properties.

APPLICATIONS

Pajanelia longifolia stem bark extracts applied successfully for the studies on anti-inflammatory and antidiabetic properties.

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