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ANTI-INFLAMMATORY AND ANTIDIABETIC ACTIVITY OF *LEUCAS CLARKEI,* A RARE SPECIES OF ODISHA, INDIA

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Abstract

Objective: To evaluate the anti-inflammatory and antidiabetic property of *Leucas clarkei* (whole plant) with preliminary phytochemical profile of the extracts.

Methods: The dried whole plant material (1500gm) was packed in soxhlet apparatus and extracted successively with Pet. Ether (PE) to defat the drug, petroleum ether was removed from the powdered defatted drug which was then extracted with benzene(BE), chloroform(CE) and 95% of Ethanol (EE) as increasing polarity and all extracts screened for anti-inflammatory and antidiabetic activity using carrageenan induced paw edema and streptozotacin induced diabetic respectively. The toxicity and phytochemical screening were done using standard procedure.

Result: The preliminary phytochemical tests revealed the presence of alkaloids flavonoids, phytosterol, terpenoids, and phenolic acid. While carbohydrates and reducing sugars were absent. The acute toxicity study of various extracts of *Leucas clarkei* was conducted and dose of 325 mg/kg is fixed for anti-inflammatory and antidiabetic perperty. The petrolium ether and ethanolic extract of *Leucas clarkei* significantly decreased the paw edema induced by carrageenin in rats at a dose of 325 mg/kg comparable to standard ibuprofen (100 mg/kg). Similarly in case of antidiabetic property , the ethanolic and chloroform extract of *Leucas clarkei* at a dose level 325mg/kg, showed significant reduction in blood sugar level from 2 to 24 hours in progressive manner comparable to glibenclamide. (5mg / kg)

Key words:- *Leucas clarkei*, Phytochemical, carrageenan, anti-inflammatory, streptozotacin and antidiabetic

Introduction

Plants are indispensible sources of medicine since time immemorial. Studies on natural product are aimed to determine medicinal values of plants by exploration of existing scientific knowledge, traditional uses and discovery of potential chemotherapeutic agents. Phytochemicals are used as templates for lead optimization programs, which are intended to make safe and effective drugs [1].

Inflammation is considered as a primary physiologic defense 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 these chronic illnesses [2]. Although it is a defense mechanism, the complex events and mediators involved the inflammatory reaction can induce, maintain or aggravate many diseases [3]. Currently used anti-inflammatory drugs are associated with some severe side effects. Therefore, the development of potent anti-inflammatory drugs with fewer side effects is necessary.

Diabetes mellitus is a group of disorders with different etiologies. It is characterized by derangements in carbohydrate, protein and fat metabolism caused by the complete or relative insufficiency of insulin secretion and / or insulin action [4]. Approximately 140 million people worldwide suffer from diabetes [5]. The disease becomes a real problem for public health in developing countries, where its prevalence is increasing steadily and adequate treatment is often expensive or unavailable[6]. Alternative strategies to the current modern

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pharmacotherapy of diabetes mellitus are urgently needed [7], because of the inability of existing modern therapies to control all the pathological aspects of the disorder, as well as the enormous cost and poor availability of the modern therapies for many rural populations in developing countries.

Plants of genus Leucas (Lamiacae) have been widely employed by the traditional healers to cure many diseased conditions which insinuated that this genus have immense potential for the discovery of new drugs or lead molecules. The genus Leucas comprises of about 80 species.[8] The highest species diversity has been found in East Africa.[9] In India, 43 species are available.[10] Plants of genus Leucas are generally shrubs, subshrubs, annual herbs or perennial herbs with woody root and/or stem-base. Leaves are opposite, entire or with spiky lobes, oval shaped with tapered end, petiolated or sometimes without intervening stalk. The axillary or terminal inflorescence is usually with indeterminate augmentation. Bracteoles are roughly erect. The calyx shape varies within the genus (often tuberlar shape) some times calyx enlarges into fruits. Calyx comprises of five connate sepals (one upper, two lateral and two lower) and 5 - 20 secondary lobes. Whitish hairs are generally present on the outer surface of the upper lip of the corolla, though yellowish cream color or red hair can also be present in some species.[9,11]

Leucas clarkei [12,13] (Lamiaceae) is a rare species of Odisha which grow as annual herbs in most tropical countries and is widely distributed throughout India. The Tribal of Orissa uses this plant for the treatment of pain caused by obstruction in menstrual flow, epilepsy, sore throat and infectious diseases. It is widely used for healing of wounds [14]. The aim of present study is to evaluate the phytochemical and pharmacological activity (Anti-inflammatory and Antidiabetic activity) of whole plant of *Leucas clarkei.*

2. Materials and methods

2.1 Materials

Streptozotocin (STZ) and Carrageenan was procured from Sigma Chemicals Co (St. Louis, MO, USA), and Himedia laboratories, Mumbai, India respectively. All other chemicals and solvents (Pet.ether, Benzene, Chloroform and Ethanol) used were of analytical grade and obtained commercially from Merck- Limited, India, Mumbai.

2.2. Collection and identification of plant material

The Plant *Leucas clarkei* were collected in the month of October from Bolangir,Odisha, India. The plant material was taxonomically identified and authenticated by Dr. (Mrs.) Uma Devi, Head, Department of Botany, Govt. Women's College, Sambalpur, Odisha. A voucher specimen (GWC/B-315/09) has been deposited in the Herbarium of the Department of School of Pharmaceutical Education & Research, Berhampur University, Berhampur-760007, India for future reference.

2.3. Experimental animals

Swiss albino mice (20-25 g) and Male Wistar rats (150-200 g) were purchased from the animal house of Gosh enterprises, Kolkata and housed in polypropylene cages at room temperature with proper ventilation. Prior to the experiments, mice and rats were fed with standard diet for 1 week in order to adapt to laboratory conditions. They were fasted over night but allowed free access to water before the experiment. The experimental protocols were approved by Institutional Animal Ethics Committee (Reg. No.1339/ac/10/CPCSEA).

2.4. Preparation of plant material

The whole plant was first sun dried for several weeks, crushed by hands and dried again. Then the crushed parts of the plant were ground into coarse powder with the help of a mechanical grinder. By using the concept of the nature of solubility and distribution of the active ingredients, powdered material (1500gm) was packed in soxhlet apparatus [15,16] and extracted successively with Pet. Ether (60-80,34gm) to defat the drug, petroleum ether was removed from the powdered defatted drug which was then extracted with benzene (39gm), chloroform(41gm) and 95% of Ethanol (43gm) as increasing polarity. The whole each mixture then underwent

filtration through Whatman filter paper. The filtrates (Pet. Ether, Benzene, Chloroform and Ethanol filtrate) obtained were evaporated by rotary evaporator at 5 to 6 rpm and at 40ºc temperature. It rendered a gummy concentrates. The gummy concentrate was designated as crude extract which was then freeze dried and preserved at $4^{o}C$.

2.5. Phytochemical Screening

Qualitative phytochemical tests for the identification of alkaloids, flavonoids, steroids, glycosides, saponins, tannins and terpenoids were carried out for all the extracts by the method described by Harborne, Sofowora and Sazada et al. [17-20]

The freshly prepared extracts of L. clarkei were qualitatively tested for the presence of chemical constituents. Phytochemical screening of the extracts was performed using the following reagents and chemicals: alkaloids with Wagner reagent, flavonoids with the use of concentrated hydrochloric acid, tannins with 5% ferric chloride, saponins with ability to produce suds, gum with Molish reagents and concentrated sulfuric acid, steroids with sulfuric acid, reducing sugar with the use α-napthol and sulfuric acid and terpenoids with chloroform and concentrated hydrochloric acid.

2.6 Acute toxicity study (LD_{50})

Acute toxicity study was performed according to Organisation for Economic Co-operation and Development, as per Karber s method. Swiss albino mice of either sex weighing between 20-25 gm were divided into six groups with six animals each and of 90 days were used to determine LD_{50} of various extracts *Leucas clarkei* .The gum acacia solution (2%) was used as a vehicle to suspend various extracts. The extracts were administered orally at different dose levels to group of six mice each, which have been fasted overnight. The LD_{50} values of various extracts are calculated and 1/10 of this i.e. 325mg/kg was considered as dose.

2.7. Experimental design

The animals were divided into following groups comprising of 6 animals in each group.

Group I	:	Control (2% gum acacia solution)
Group II	:	Inflammation induced rats treated with Ibuprofen (100 mg/kg/day) for anti-inflammatory and Glibenclamide (5mg/kg/day) for antidiabetic activity.
Group III	:	Inflammation induced rats treated with Pet. ether extract (325mg/kg/day)
Group IV	:	Inflammation induced rats treated with Benzene extract (325mg/kg/day)
Group V	:	Inflammation induced rats treated with Chloroform extract (325mg/kg/day)
Group VI	:	Inflammation induced rats treated with Ethanolic extract (325mg/kg/day)

3. Anti-inflammatory Activity

The anti-inflammatory activity of *Leucas clarkei* was studied using acute (carrageenan induced paw edema) models of inflammation. The experiment protocols were approved by the Institutional Animal Ethics Committee prior to the conduct of the animal experiments (Reg. No.1339/ac/10/CPCSEA)

Carrageenan-Induced Paw Edema in Rats [21]

This model is based on the principle of release of various inflammatory mediators by carrageenan. Edema formation due to carrageenan in the rat paw is 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 [22,23]. 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, all due to the metabolism of arachidonic acid [24]. 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 five hours.

Method

Anti-inflammatory activity was evaluated using the carrageenan induced rat paw oedema according to the technique of Winter et al. [25-29]. The animals were housed in cages under standard laboratory condition. They had free access to standard diet and water. The animal were divided into 6 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 0.1 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 1, 2, 3, and 4 h after carrageenan injection, and the paw volume was determined. The data were expressed as paw volume (ml), compared with the initial hind paw volume of each rat. Cosolvent (2% gum acacia solution, p.o), various extracts of *Leucas clarkei* as suspension in 2% gum acacia solution (p.o) and ibuprofen (100 mg/kg, p.o) [30] was administered 30 min before carrageenan injection. The group received cosolvent was treated as control.

The hind paw volume was measured plethysmometrically before and after the carrageenan injection, at hourly intervals for 4hr. (Table 1)

$$\% \text{ inhibition of edema} = \left(\frac{Vc - Vt}{Vc} \right) x100$$

Where, V_t = mean paw volume of test group

V_c = mean paw volume of control group

4. Anti diabetic Activities

4.1. Experimental induction of diabetes in rats [31, 32]

Rats were made diabetic with an intraperitoneal injection of Streptozotocin (STZ 60 mg/kg body weight) dissolved in citrate buffer (0.1M, pH 4.5). Diabetes was confirmed in STZ rats by measuring the fasting blood glucose level 48 h after the injection of STZ. Rats with blood glucose level above 250 mg/dl were considered to be diabetic and were used in this experiment.

Table 1. Preliminary Phytochemical analysis of the bark of Leucas clarkei

S/No.	Constituents	PE	BE	CE	EE
1	**Alkaloid**				
	i.Dragendorff's test	+	+	+	-
	ii.Meyer's test	+	+	+	-
2	**Carbohydrates**				
	i.Mohch's test	-	-	-	-
	ii.Burford's test	-	-	-	-
	iii.Fehling (reducing sugar)	-	-	-	-
	iv.Fehling (combined reducing sugar)	-	-	-	-
3	**Cardiac glycosides**				
	Killer-killanis test	-	-	+	+
4	**Flavonoid**				
	i.Shinoda's test	-	-	+	+
	ii.$FeCl_3$ test	-	-	+	+
5	**Saponins**				
	Frothing test	-	+	+	+
6	**Terpene and Steroids**				
	i.Salkowski test	+	+	+	-
	ii.Libarman-Burchard's test	+	+	+	
7	**Tanins**				
	i.$FeCl_3$ test	-	-	+	+
	ii.Lead acetate test	-	-	+	+

PE = Petroleum ether extract, CL = Chloroform extract, BE = Benzene extract, EE = Ethanol extract, (+) = Present & (-) = Absent.

4.3. Estimation of blood glucose

Blood samples were collected from the tail tips at 12, 24, 36 and 48 hour after streptozotacin administration and found that stable hyperglycemia produced after 48 hours. The blood sugar level was measured by digital display glucometer (One touch – Johnson & Johnson Ltd.). Initial blood sample were taken before the oral administration of the standard drug (Glibenclamide) and extracts. The blood glucose levels were measured at 2, 4, 8, 12 and 24 hours after oral administration of Glibenclamide and various extracts of *Leucas clarkei* (Table2)

TABLE-2 Effect of various extracts of *Leucas clarkei* (whole plant) on carrageenan-induce Rat Paw Edema

Treatment	Dose (mg/kg)	Mean paw volume (m L) ± SEM			
		Time in minutes			
		60	120	180	240
Control(2% gum acacia)	-	.....	0.78±0.09	0.85±0.12	0.89±0.14
Ibuprofen	100	0.29±0.07* (39.6)	0.28±0.07* (64.10)	0.24±0.06* (71.80)	0.23±0.13* (74.2)
Pet. Ether. Ext	325	0.43±0.09 (10.4)	0.36±0.07* (53.8)	0.32±0.09* (62.4)	0.30±0.12* (66.29)
Benzene. Ext	325	0.44±0.07 (8.3)	0.71±0.09 (8.9)	0.77±0.07 (9.4)	0.79±0.11 (11.2)
Chloroform. Ext	325	0.40±0.09 (16.66)	0.49±0.12 (37.2)	0.58±0.15 (31.8)	0.56±0.11 (37)
Ethanol. Ext	325	0.27±0.02* (43.8)	0.30±0.01* (61.5)	0.29±0.12* (65.8)	0.28±0.13* (68.5)

4.4. Statistical Analysis

Statistical Analysis

The experimental results were expressed as mean ± SEM. Data were assessed by ANOVA

method followed by student's t-test. $p<0.05$ was considered as statistically significant in case of anti-inflammatory activity and the data was analyzed statistically using ANOVA followed by Dunnett's Multiple Comparison Test using SPSS 10.0 statistical software for antidiabetic activity. The level of significance was fixed at 5%.

Table- 3: Antidiabetic activity of various extracts of *Leucas clarkei* (Whole Plant)

Group		Blood Sugar level in mg/dl					
		Time (hr) → / Dose ↓	2hr	4hr	8hr	12hr	24hr
I	Normal control	--	88.4±2.07	87.4±1.12	87.1±0.6	87.6±1.7	87.2±1.03
II	Diabetic control	2% Gum acacia solution	288.4±8.06	286.7±9.14	287.1±10.4	289.6±7.6	293.2±5.23
III	Glibenclamide	5mg / kg per Day	227.0±4.04*	171.0±4.57*	139.4±2.64*	122.2±2.3*	114.0±3.7*
IV	Pet.Ether Ext	325mg/kg	284.3±8.2	283.1±10.4	280.6±9.6	279.3±5.9	281.3±8.3
V	Benzene. Ext	325mg/kg	278.1±7.2	276.4±5.8	279.6±4.8	287.4±8.7	289.8±9.2
VI	Ethanol. Ext	325mg/kg	250.4±5.9*	243.2±3.6*	200.4±8.2*	167.7±2.8*	171.4±8.7*
VII	Chloroform. Ext	325mg/kg	272.4±7.5*	247.0±3.8*	211.3±8.6*	208.7±6.3*	218.4±8.2*

Each value is Mean ± SEM (n= 6), * Denote significant difference compared to control value at P<0.05

5. Result

5.1. Phytochemical constituents of the plant

The preliminary phytochemical tests revealed the presence of alkaloids flavonoids, phytosterol, terpenoids, and glycoside. While carbohydrates and reducing sugars were absent. (Table 1)

5.2. Acute toxicity study

The acute toxicity study of various extracts of *Leucas clarkei* was conducted and the LD_{50} of all extracts found to lay between 3000-4000mg/kg body weight. The LD_{50} is in a higher dose range so the extracts are safe and 1/10 average of all extracts i.e. 325mg/kg was taken as dose for pharmacological screening procedure.

5.3. Anti-inflammatory activity

The pet ether and ethanolic extract of *Leucas clarkei* significantly decreased the paw edema induced by carrageenin in rats at a dose of 325 mg/kg comparable to standard ibuprofen (100 mg/kg) shown in Table 2

Fig. 1: Effect of various extracts of *Leucas clarkei* (whole plant) on carrageenan-induce Rat

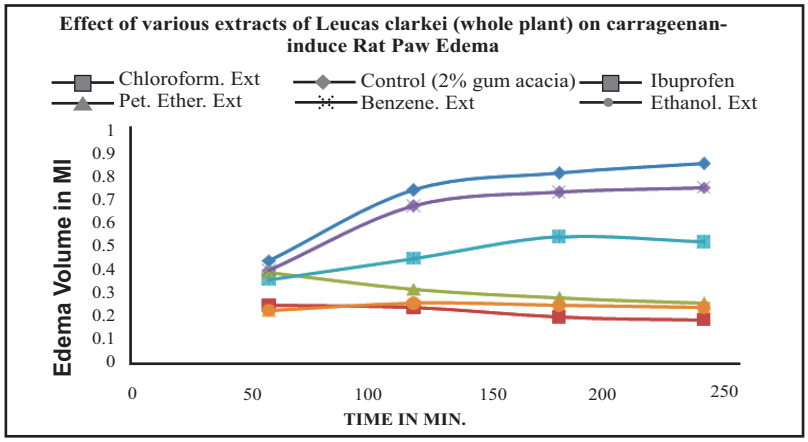
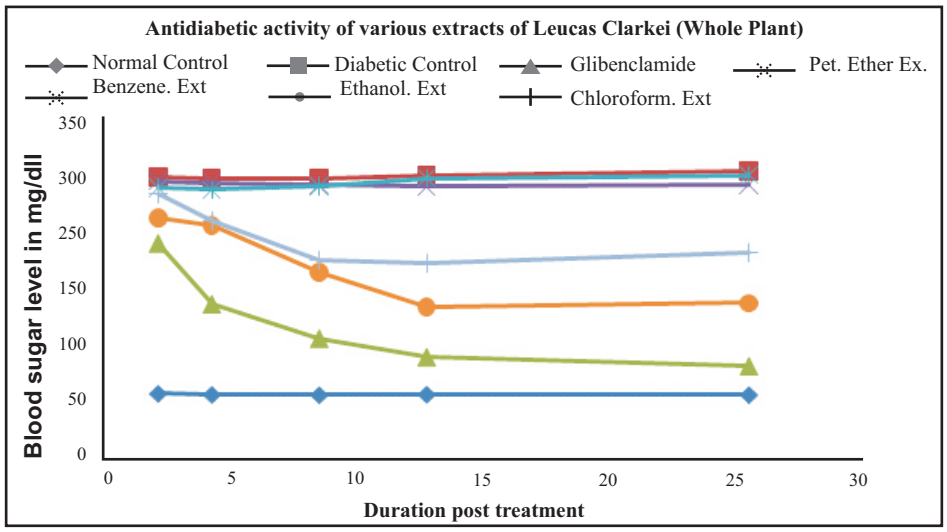


Fig 2: Antidiabetic activity of various extracts of *Leucas clarkei* (Whole Plant)



5.4. Antidiabetic activity

The result of antidiabetic effect depicted in Table 3. From the table it is revealed that the ethanolic and chloroform extract of *Leucas clarkei* at a dose level 325mg/kg, showed significant reduction in blood sugar level from 2 to 24 hours in progressive manner comparable to standard glibenclamide,.

5. Discussion

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 including diabetes [33] and several species of plants have been described as having a hypoglycemic activity [34-36]. This study was undertaken to evaluate the antidiabetic in streptozotocin- induced diabetic rats. Oral administration of Pet.ether, Benzene, Chloroform Ethanol extracts of *Leucas clarkei* for 24 hours and it was found Chloroform and Ethanol extracts cause a significant decrease in blood glucose level as compared to standard drug Glibenclamide ($P<0.05$).In case of anti-inflammatory activity in carrageenan-induce Rat Paw Edema,
Pet.ether and Ethanol extract shows significant reduction of inflammation as compared to Ibuprofen ($P<0.05$).

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