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Phytochemical analysis and anti-diabetic activity of *Cadaba fruticosa* R.Br.

S. Arokiyaraj[1], R. Radha[2*], S. Martin[3] and K. Perinbam[1],
[1] PG & Research Sathyabama University, Chennai-119, India.
[2] Department of Pharmaceutical Sciences, Madras Medical College, Chennai-3.
[3] Jaya College of Engineering, Thiruninravur, Chennai-602024
rradha1970@yahoo.co.in

Abstract: Alcohol and aqueous extract of *Cadaba fruticosa* (leaves) were subjected for hypoglycemic activity in wistar rats (160-200 g). The oral administration of leaf extracts at dose 1000 mg/kg led to a significant blood glucose reduction. Phytochemical analysis of alcohol extract revealed the presence of terpenoids, flavanoids, steroids, proteins, alkaloids, gums, sugars and saponins but negative result was observed in aqueous extract except terpenoids, flavonaids, proteins, furans, gums and sugars. This study brings out the evidence regarding pharmacological and phytochemical activities of *C. fruticosa.*

Keywords: Acute toxicity, anti-diabetic, alloxan-induced diabetes, *Cadaba fruticosa*

Introduction

There are 143 million people worldwide are suffering from diabetes, almost five times more than the estimates ten years ago. This number may probably double by the year 2030. Therefore, the global human population appears to be in the midst of an epidemic of diabetes. Reports from the World Health Organization (WHO) indicate that diabetes mellitus is one of the major killers of our time, with people in Southeast Asia and Western Pacific being most at risk (Maridass & John De Britto, 2008).

Diabetes mellitus (DM) is a metabolic disorder characterized by hyperglycemia. It may be secondary to a deficiency or disturbance in the secretion of insulin or to an abnormal response of peripheral tissues to insulin. The resulting metabolic derangement of the intermediary metabolism of carbohydrate, lipid, and protein affects all organ systems but most prominent in the arteries, arterioles, and capillaries (Damjanov & McCue, 1996). There are two main categories of this disease. Type 1 diabetes mellitus also called insulin-dependent *diabetes mellitus* (IDDM) and Type 2, the non-insulin-dependent diabetes mellitus (NIDDM).

Plants are important not only for the control of type 2DM but also for its prevention, especially for people with elevated levels of blood glucose and blood intolerance who have a greater risk of developing diabetes (Anderson *et al*., 2004). Botanical products can improve glucose metabolism and the overall condition of individuals with diabetes is not only by hypoglycemic effects but also by improving lipid metabolism, antioxidant status, and capillary function (Bailey and Day, 1989). A number of medicinal/culinary herbs have been reported to yield hypoglycemic effects in subjects with diabetes. These include cinnamon, cloves, bay leaves, turmeric (Khan *et al*., 1990), bitter melon (Srivastava, 1996; Raman & Lau, 1996), gurmar (Basakaran *et al*., 1990), Korean ginseng, onions, garlic and holy basil (Koch & Lawson, 1996; Rai & Mani, 1997).

C. fruticosa distributed in Tamil Nadu, Deccan and the Western region. It is commonly known as 'Viludhi" (Tamil) and in English popularly called 'Capper bush'. Leaf juice is used as a remedy for dysentery, stimulant, purgative, fever, cough and lungs problem (Watt & Breyer-Brandwijk, 1962). Moreover, it was reported to possess stachydrine, 3-hydroxystachydrine from the stem, roots and cadabine from leaves (Viqar Uddin *et al*., 1975; Yousif *et al*., 1984). The present study aims at unraveling the hypoglycaemic activity of lead extracts of *C. fruticosa* in the model of alloxan induced diabetes in rats.

Materials and methods

Plant collection

The leaves of *Cadaba fruticosa* were collected in Chengalpattu (Tamil Nadu) in June 2006 and authenticated by a botanist at the Plant Anatomy Research Centre (PARC), Chennai, Tamil Nadu, India. A voucher specimen (69/July/2006) has been deposited at the museum of the department of pharmacognosy, Madras Medical College, Chennai, India.

Extraction Procedure

The freshly collected leaves of *Cadaba fruticosa* were shade dried and then coarsely powdered in a blender. The coarse powder was successively extracted in an aspirated bottle with ethanol (yield 3%), water (2.64%) by cold maceration for 3-7 days. After decantation and filtering through Whatmann filter paper no.41, nearly 81% of the solvent was removed by distillation over boiling water bath and remaining under reduced pressure. The extracts so obtained were further dried in vacuum desiccators. The residue obtained from various extracts was used for further studies by preserving it in refrigerator.

Phytochemical studies

The presence of phytochemicals, triterpenoids (Noller's test), flavones (Shinadow's test), steroids (Libermann-Burchard test), proteins (Biuret test),

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furans (Ehrlich's test), alkaloids (Dragendroff's reagent), anthraquinones (Borntrager's test), gums, tannins (5% ferric chloride), saponins (Frothing test), phenols and sugars were evaluated according to the method described by Edeoga *et al*., (2005).

Drugs

Alloxan monohydrate was purchased from sigma chemicals (St. Louis, USA). All other chemicals used for this study were of analytical grade.

Animals

Inbred male and female Wistar albino rats (160-200 g) were procured from the animal experimental laboratory of Madras Medical College and used throughout the study. The study was conducted after obtaining Institutional animal ethical committee's clearance (20/236/Aug' 2006). The animals were maintained in colony cages at 25±2°C, relative humidity of 45-55% maintained under 12 h light and 12 h dark cycles. The animals were fed with standard animals feed (Hindustan Lever Ltd.) and water *ad libitum*. All the animals were acclimatized for a week before use and they were maintained in hygienic environment in our animal house.

Acute toxicity study (Ecobian, 1997)

The procedure was followed by using OECD (Organization of Economic Cooperation and Development) guidelines 423 (Acute toxic class method). Male wistar rats weighing 160-200 gm were used for the study. In this study, a dose of (5, 50, 300, 2000 mg/kg) of dried extracts were orally administered to 6 mice; additionally 2 mice were kept as control. Then they were observed for 48 h. Since no mortality was observed and the behavior pattern was unaffected, further studies were carried out (Table 1).

Induction of diabetes in rats

Diabetes mellitus was induced by single intraperitoneal injection of freshly prepared alloxan monohydrate (120 mg/kg) body weight (Cooper Stein & Walkin, 1981). Rats were supplied with 5% glucose solution for 48 h after alloxan injection in order to prevent hypoglycemia. The control animals were treated with only citrate buffer (pH 4.5). Diabetes was developed and stabilized in the alloxan treated rats over a period of 3 days. After 3 days of alloxan administration, serum glucose levels of each rat were determined. Rats with serum glucose range of >200 mg/dl were considered diabetic and included in this study (Santoni *et al*., 1996). Blood was collected from the tail vein.

Experimental design & treatment schedule

In the present experiment, the rats were divided into 7 groups as follows:

Group	Animals
I	Normal – Control rats (Normal saline)
II	Diabetic – Control rats (Saline + alloxan 10 ml/kg b.w)
III	Diabetic rats treated with aqueous extract of *Cadaba fruticosa* 200 mg/kg b.w
IV	Diabetic rats treated with aqueous extract of *Cadaba fruticosa* 1000 mg/kg b.w
V	Diabetic rats treated with alcoholic extract of *Cadaba fruticosa* 200 mg/kg b.w
VI	Diabetic rats treated with alcoholic extract of *Cadaba fruticosa* 1000 mg/kg b.w
VII	Diabetic rats treated with glibenclamide 10 mg/kg b.w

Each group consisted of six rats. *C. fruticosa* extracts (alcohol and aqueous) was dissolved in DMSO 0.5% and 1 ml/kg b.w was administed orally daily by intragastric tube at different dosage (200, 1000 mg/kg b.w). Control animals were treated with vehicle alone. After 15 days of treatment, the blood was collected and measured using glucometer. The glucose levels of all the animals were measured on 3, 6, 9, 12,15th day respectively.

Statistical analysis

Table 1.Acute toxicity study of alcohol and aqueous extract of C. fruticosa

Behaviors	Dose				
	5 mg/kg	50 mg/kg	300 mg/kg	2000 mg/kg	Control
Alertness	+	+	+	+	+
Grooming	-	-	-	-	-
Restlessness	-	-	-	-	-
Touch response	+	+	+	+	+
Pain response	+	+	+	+	+
Tremors	-	-	-	-	-
Convulsions	-	-	-	-	-
Righting reflex	+	+	+	+	+
Pupils	N	N	N	N	N
Skin color	N	N	N	N	N
Respiration	N	N	N	N	N

(+)-Positive; (-)-Negative; (N)- Normal

All treated groups were compared with the control group (Group II) and the results were analyzed statistically using ANOVA and followed by Dunnet's test to identify the difference between treated groups and control. The data were considered significant at $p < 0.05$.

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Table 3. Anti-diabetic activity of alcohol and aqueous extract of Cadaba fruticosa

Groups	Blood sugar level in mg/dl (days)				
	3rd	6th	9th	12th	15th
I	75±4	72±4	77±2	72±5	74±3
II	480±3	381±4	347±5	299±6	252±6
III	298±9	289±7	256±5	201±3	191±3
IV	237±4	194±4	168±2	143±4	124±3
V	280±3	201±3	131±3	146±5	112±3
VI	233±4	188±5	142±2	124±3	103±3
VII	274±2	232±5	163±4	153±3	91±2

Values are mean ± S.D. p<0.001 statistical evaluation done by ANOVA followed by Dunnet's test.

Table 2. Phytochemical constitutes of Cadaba fruticosa

Chemical constituents	Aqueous extract	Alcohol extract
Terpenoids	+	+
Flavones	+	+
Steroids	-	+
Proteins	+	+
Furan	+	-
Alkaloids	-	+
Anthraquinone	-	-
Gum	+	+
Tannins	-	-
Saponins	-	+
Phenols	-	-
Sugars	+	+

(+) - Positive; (-) Negative

Results and discussion

The results of the phytochemical analysis are presented in Table 2.

Acute toxicity

The findings indicated that *C. fruticosa* was safe when tested in wistar rats at 2000 mg/kg b.w. The results showed no clinical signs and mortality of the animal (Table 1).

Anti-diabetic activity

Cadaba fruticosa alcohol extract showed significant plasma glucose lowering effect when compared to aqueous extracts. Plasma glucose levels in normal, experimental rats basal and 15th day treatment are given in Table 3. The present study indicates that alloxan induced tissue injury is reversed by continuous administration of *C. fruticosa* extract with subsequent decrease in blood sugar. Alloxan treated diabetic rats showed significant increase in the levels of fasting plasma glucose levels when compared to normal rats. Alloxan generate free radicals in the body, leads to tissue damage (including pancreases) and would be responsible for increase blood sugar seen in the animals. Oral administration of *Cadaba fruticosa* alcohol extract at 1000 mg/kg showed significant ($p<0.05$) plasma glucose lowering effect.

The antihyperglycemic activity of *Cadaba fruticosa* may be due to the stimulatory effect of insulin secretion from pancreatic β cells (Chakravarthy & Gode, 1985) and insulin like activity or by converting proisulin to insulin (Ahmad *et al*., 1998). Durgacharan *et al*., (2008) have shown that the oral administration of leaf extracts at doses of 200 mg/kg lead to significant blood glucose reduction.

Sulphonylureas such as glibenclamide have been used for many years to treat diabetes, to stimulate insulin secretion from pancreatic β cells (Tians *et al*., 1998). Sulphonylureas potentate insulin's action on target tissues and increase glucose disposal from blood. Sulphonylureas increase the binding of insulin to peripheral tissue insulin receptors, thereby enhancing glucose uptake and utilization by peripheral tissues. Also administration of sulphonyureas may reduce the blood glucagons concentrations. The present study provides evidence of the anti-diabetic property of *C. fruticosa.* The effects may due to flavonoids and steroids observed in the extracts. From the results of the present study, it may be suggested that the mechanism of action of *C. fruticosa* alcohol extract is similar to glibenclamide. However, further studies are necessary to identify and isolate the active constituents and thereby enabling to elucidate its mechanism of anti-diabetic action.

Conclusion

The present study indicates that the plant contains potential anti-diabetic components such as flavonoids, terpenoids and steroids that may be of use for development of phytomedicine for the therapy of diabetic. Our results prompt for further research to establish the exact anti-diabetic mechanism of action of alcoholic extract of *Cadaba fruticosa*.

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