

ANTI-DIABETIC ACTIVITY OF ONCOCALYXONE A ISOLATED FROM *PRENANTHES SARMENTOSUS*

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ABSTRACT

Objective: Medicinal plants play an important role in the management of diabetes. The main objective of the study was designed to screen the phytochemical constituents of the leaves of *Prenanthes sarmentosus* and also to study its effect on blood glucose of rats.

Methods: The compound oncocalyxone A isolated from *Prenanthes sarmentosus* leaves by using Column chromatography. Structure of the compound was identified by HPLC analysis, UV, IR, NMR spectroscopic methods and tested for anti-diabetic activity in alloxan induced diabetes in albino rats was evaluated. Albino rats weighing about 200-250 g were divided into five groups (n=6). The weights of the animals in each group were recorded daily throughout the duration of the experiment. Biochemical parameters, including glucose, serum urea, creatinine, cholesterol, and protein were also assessed. The blood glucose levels in the different groups were assayed.

Results: The isolated oncocalyxone A increased the body weight and significantly reduced the glucose level at 200 mg/kg was almost equally the reference standard glibenclamide. In our present study, biochemical parameters were unchanged in non-diabetic rats.

Conclusion: The oncocalyxone A has the potential to enhance the glucose dependent insulin release from the pancreatic β cells and there by decrease the blood glucose level in alloxan induced diabetic rats. Further biochemical investigations will clearly elucidate the mechanism of action and will be helpful this compound as a therapeutic target in diabetes research.

Keywords: Alloxan, Biochemical parameters, Blood glucose level, Oncocalyxone A, *Prenanthes sarmentosus*.

INTRODUCTION

Plants have been considered as sources of medicinal agents for the treatment of many diseases [1]. It is important to use the phytochemical methods to screen and analyze bioactive components, not only for the quality control of crude drugs, but also for the elucidation of their therapeutic mechanisms. Modern pharmacological studies indicate that, the binding to receptors or ion channels on cell membrane is the first step of some drug actions [2]. Diabetes Mellitus is a common and very prevalent disease affecting the citizens of both developed and developing countries. Diabetes Mellitus is caused by the abnormality of carbohydrate metabolism, which is linked to low blood insulin level or insensitivity of target organs to insulin [3, 4]. Diabetes exhibits a variety of multisystem complications involving the blood vessels, skin, eye, kidney and the nervous system during the course of disease process. The most common symptoms of Diabetes Mellitus are those of hypoglycemia, loss of weight, ketosis, and arteriosclerosis, pathologic changes in the eye, neuropathy, renal disease and coma [5]. However, available oral hypoglycemic drugs have many side effects such as nausea and vomiting, jaundice and hemolytic anemia, generalized hypersensitivity reactions, dermatological reaction and lactic acidosis [6]. Therefore, searching for effective, low cost and less side effected hypoglycemic agents is important. Medicinal plants play an important role in the management of diabetes mellitus. During the past few years, some of the new bioactive drugs isolated from hypoglycemic agents used in clinical therapy [7]. Many Indian plants have been investigated for their beneficial use in different types of diabetes and report occurs in numerous scientific journals [8]. Based on this aspect, we have selected the plant *Prenanthes sarmentosus* for treatment of diabetes. This study is designed to screen the phytochemical constituents of the leaves of this plant and also to study its effect on blood glucose of rats.

MATERIALS AND METHODS

Plant materials

This is an important medicinal plant commonly called as *Prenanthes sarmentosus* or ehzutaniippundu in Tamil. *Prenanthes sarmentosus* is a genus of plants in the family *asteraceae*, often referred to as

Rattlesnake root. *Prenanthes sarmentosus* was used as the test plant which was collected from rural area around Kumbakonam, Tamilnadu in the month of Feb - March and authenticated by Prof. N. Ramakrishnan, (Department of Botany) and voucher specimens (GACBOT-158) were deposited at the Herbarium of the Department of Botany, Government Arts College (Autonomous), Kumbakonam, Bharathidasan University, India.

Extraction and Isolation

The important stage in the experimental work includes first the isolation of chemical substances from the chosen plant and secondly, the characterization of those isolated compounds. A dried and powdered *Prenanthes sarmentosus* (3 Kg) leaves were exhaustively extracted with 95% methanol (MeOH, 2.5 L \times 3) at room temperature. The isolation of the methanol extract was dried under reduced pressure to yield (129 g) and then subjected to column chromatography over silica gel (230-400 mesh, Sigma Aldrich, India) as the stationary phase. The charged column was then eluted with CHCl_3 , EtOAc and re-chromatographed on silica gel eluted with acetone- CHCl_3 , to yield oncocalyxone A. The fractions were collected such as CHCl_3 (66.0 g), EtOAc (22.5 g) and MeOH (27.4 g) and the solvent recovered by simple distillation. Structural elucidation of the compound isolated from CHCl_3 extract of *Prenanthes sarmentosus* leaves was accomplished by HPLC, UV, IR, and NMR spectroscopic methods. The hydro soluble components contained greater than 98 % of oncocalyxone A (Figure 1) estimated from the ^1H NMR spectrum and HPLC analysis.

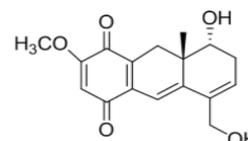


Fig. 1: Structure of oncocalyxone A

Oncocalyxone A Wine red color solid; mp 210 - 211°C; UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm 208 - 320; IR (KBr) cm^{-1} 3440, 3375, 1650, 1620, 1580, 1490, 975, 850. Analysis of ^1H and ^{13}C NMR data's were

predicted in Table 1. HPLC retention time 3.861 min, and this compound was isolated and its chemical structure determined according to the literature [9, 10].

Table 1: ^{13}C NMR and ^1H NMR spectroscopic data for Oncocalyxone A

^{13}C NMR (100 MHz, CDCl_3 , δ in ppm, J in Hz)		^1H NMR (500 MHz, CDCl_3 , δ in ppm, J in Hz)	
Carbon	Oncocalyxone A	H	δ_{H}
1	181.0	3	6.10 (1H, s)
2	158.4	6	5.98 (1H, d, J=3.9)
3	105.4	7	2.56 (1H, d, J=17.2)
4	184.2	7	2.62(1H,dd,J=17.2,3.9)
4a	134.0	8	3.32 (1H, s)
5	145.8	9	2.26 (1H, d, J=18.4)
6	127.6	9	2.88 (d, J=18.4)
7	30.8	10	6.20 (1H, s)
8	69.2	11	4.10 (2H, s)
8a	38.0	12	0.74 (3H, s)
9	28.2	CH_3O	3.65 (3H, s)
10	110.8	OH	4.61 (1H, t, J=5.2)
10a	135.4	OH	4.78 (1H, d, J=4.5)
11	61.0		
12	21.4		
CH_3O -	55.9		

A Shimadzu (Columbia, MD) HPLC system, was used with UV detection at 280nm. HPLC utilizes a column that holds chromatographic packing materials (stationary phase), a pump that moves the mobile phase(s) through the column and a detector that shows the retention times of the molecules. A chromatographic system comprising a Spectra Physics (Fremont, CA, USA) P-200 series gradient pump, a rheodyne injector fitted with a 20-FL loop, the C18 column (250 x 4.6 mm i.e., symmetry) equipped with a phenomenex was used. Elution was with a linear gradient of water: acetonitrile from 95:5 to 70:30 in 20 min at a flow rate of 1.0 ml/ min. The detector was operated at 200 nm were carried out in triplicate. All samples and solvents were filtered through nylon membrane filters (0.5 F) prior to analysis. UV spectral analyses were recorded using UV- Visible Spectrophotometer Lambda 35 from Perkin Elmer. IR spectra were recorded on a Perkin - Elmer spectrum one spectrometer using a KBr disc given in cm^{-1} . Supporting evidence for the structure of the compound is provided by the ^1H (CDCl_3 , 500 MHz) and ^{13}C -NMR (100 MHz, CDCl_3) spectra were recorded on a Bruker AMX 500 NMR spectrometer. Chemical shifts were reference to the respective residual solvent peaks and the values were recorded in δ .

Anti-diabetic study

Male albino rats weighing about 200-250 g were maintained under standard experimental conditions (Temperature $27 \pm 2^\circ\text{C}$, relative humidity 60 ± 5 and 12 hours light/dark cycle) and they were fed with standard rate feed. Before starting the experiment on animals, the experimental protocol was subjected to the scrutiny of the Institutional Animal Ethics Committee (IAEC), Bharathidasan University, Trichirappalli, Tamilnadu, India (Approval No. BDU/IAEC/2011/31/29.03.2011).

Experimental design

The animals were divided into five groups each containing six animals.

Group-I: Served as normal control. Control rats received only normal saline.

Group-II: The Second group of rats with diabetes was induced by intraperitoneal injection of alloxan for 2 days.

Group-III: Alloxan treated rats were administered the Glibenclamide (10mg/kg) and served as standard.

Group-IV: Alloxan treated rats were administered the Oncocalyxone A from extract of *Prenanthes sarmentosus* (100mg/kg)

Group-V: Alloxan treated rats were administered the Oncocalyxone A from extract of *Prenanthes sarmentosus* (200mg/kg)

After the treatment period all the groups of rats were euthanized by anesthesia using chloroform vapor and the rats were sacrificed by decapitation. Then the blood was collected in a tube for analysis.

Acute toxicity studies

Acute toxicity studies were carried out according to the literature [11]. Animals of either sex were fasted for eighteen hours and used. A dose of 200 mg/kg of Oncocalyxone A from extract of *Prenanthes sarmentosus* were administered orally to 12 mice, additionally three mice were kept as control. The control group received distilled water. Then they were absorbed for 72 hours. Since no mortality was absorbed and the behavioral pattern was unaffected. No death was absorbed at the end of the study.

Biochemical analysis

Serum glucose level test was done on the normal, diabetic, and treated diabetic rats on days 4, 8, and 12 of the experimental period to determine blood glucose levels in the animals. The blood samples collected were centrifuged at 3500 rpm for 20 minutes and serum glucose levels were determined using the GOD / POD method [12]. Serum urea [13] and creatinine [14] were determined using diagnostic kits from Biosystems, Barcelona, Spain. The total protein present in serum was estimated by the Biuret method [15] using an Autopak kit. Serum cholesterol [16] was determined using reagent kits purchased from Piramel Health care, Mumbai, India.

Statistical analysis

The experimental results were expressed as statistical comparisons of Mean \pm SEM were carried out by one way analysis of variance (ANOVA) followed by Dunnet Multiple Comparisons Test. P values less than 0.05 was considered as statistically significant.

RESULTS AND DISCUSSION

Chemical constituent

The isolation of organic compound from leaves of *Prenanthes sarmentosus* was subjected to column chromatographic separation analysis. Structure of the isolated compound was identified by HPLC and UV, IR, NMR spectroscopic methods. HPLC is a novel method of screening herbal composition for pharmaceutically active component in medicinal plants. The oncocalyxone A was well determined and quantified by HPLC in isocratic conditions and short time analyses (10 min). A typical chromatogram of chloroform extract is shown in the Figure 2, which illustrates the separation of the phytochemical compound. The peak in the chromatogram was identified based on the retention time of standards injected separately and by addition of standard solutions. The analysis time is an important factor in analytical work and the run time should be reduced to a minimum in order to optimize equipment use and reduce solvent consumption. The identified peak was considered to represent only one compound each because the UV spectra at the up slope and down slope inflection points were indistinguishable. The peak with the retention time of 3.861 min corresponds to oncocalyxone A from the literature [17, 18].

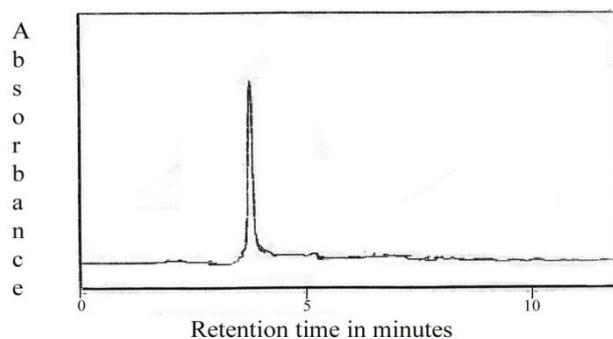


Fig. 2: HPLC spectrum of oncocalyxone A from *Prenanthes sarmentosus*

The UV spectrum of the compound obtained from the chloroform fraction exhibited two major peaks at 320 nm (band I) and 208 nm

(band II) which showed a quinone skeleton. IR spectroscopy can also usefully contribute to structural elucidation, a quinone with one α -OH shows two bands at 1650cm^{-1} and at 1620cm^{-1} . Analysis of ^1H and ^{13}C NMR data revealed that the aromatic signals are close to those reported for oncocalyxone A [9, 10].

Anti-diabetic study

Anti-diabetic study was designed to find out the correlation of alloxan induced diabetes mellitus and also the effect of oncocalyxone A isolated from *Prenanthes sarmentosus* in experimental animals. In the present study, alloxan monohydrate (120mg/kg) was used as a diabetogen. It induces diabetes by destroying β -cells of the pancreas partially, through production of reactive oxygen species [19]. The cytotoxic action of alloxan is mediated by reactive oxygen species, with simultaneous massive increase in cytosolic calcium

concentration, leading to a rapid destruction of β cells [20]. The insulin deficiency, results in improved glucose, causing hyperglycemia. A significant reduction in the body weight was observed in the alloxan induced diabetic rats. The decrease in the weight in diabetics is due to continuous excretion of glucose and decrease in peripheral uptake of glucose and glycogen [21].

Effects on blood glucose

Experimental studies reveals that the compound oncocalyxone A (100 and 200mg/kg) isolated from *Prenanthes sarmentosus* leaves orally administered for 12 days produced a significant decreases in the blood glucose level when comparison was done with the diabetic control group and reference standard glibenclamide. A significant increase was observed in the level of glucose in alloxan induced rats when compared to normal rats (Table 2).

Table 2: Effect of Oncocalyxone A from *Prenanthes sarmentosus* on fasting blood glucose level in alloxan induced diabetic rats

Group	Treatment	Fasting blood glucose level (mg/dl)			
		Initial	4 th day	8 th day	12 th day
I	Normal Control	91.73±0.25	93.33±0.21	92.60±0.20	89.43±0.21
II	Diabetic Control	245.60±0.20	243.50±0.10	239.47±0.31	237.50±0.10
III	Standard (Alloxan + glibenclamide 10 mg/kg)	236.37±0.42**	218.87±0.21***	201.30±0.36***	193.40±0.46***
IV	Alloxan + Oncocalyxone A (100 mg/kg)	240.43±0.35	229.20±0.26***	218.17±0.25***	211.50±0.30***
V	Alloxan + Oncocalyxone A (200 mg/kg)	238.53±0.45	217.70±0.46***	205.67±0.21***	195.27±0.50***

Values are expressed in Mean \pm Standard Deviation (n=6)

Superscript letters represent $P < 0.05$ (Duncan test). Group II compared with Group III, IV and V. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

A significant decrease was observed in the level of glucose in the isolated oncocalyxone A treated rats. The 100 mg/kg dose of oncocalyxone A reduced in blood glucose level but the results found less effective than standard glibenclamide mean time the dose of 200 mg/kg was found almost equally the reference standard glibenclamide. Decrease in serum glucose may be regeneration of β cell of the pancreas, which was destroyed by alloxan. The oncocalyxone A has the potential to enhance the glucose dependent

insulin release from the pancreatic β cells and there by decrease the blood glucose level in alloxan induced diabetic rats. The basal blood sugar level of normal albino rats correspond with the findings of previous workers [22]. Normal control group was found to be stable in their body weight but the significant reduction in the body weight was observed in the alloxan induced diabetic rats. Weight gain was observed statistically significant with standard glibenclamide and oncocalyxone A are shown in Table 3.

Table 3: Effect of oncocalyxone A from *Prenanthes sarmentosus* on body weight in alloxan induced diabetic rats

Group	Treatment	Body weight of the animal (g)			
		Initial	4 th day	8 th day	12 th day
I	Normal Control	197.80±0.20	198.67±0.41	199.40±0.43	201.37±0.15
II	Diabetic Control	190.07±0.30	189.60±0.52	186.37±0.25	185.17±0.35
III	Standard (Alloxan + glibenclamide 10 mg/kg)	188.17±0.25	190.17±0.25*	193.87±0.15***	197.77±0.25***
IV	Alloxan + Oncocalyxone A (100 mg/kg)	190.57±0.40*	191.17±0.32**	190.83±0.45*	192.00±0.20***
V	Alloxan + Oncocalyxone A (200 mg/kg)	191.00±0.40**	191.73±0.41**	192.37±0.45***	193.83±0.15***

Values are expressed in Mean \pm Standard Deviation (n=6)

Superscript letters represent $P < 0.05$ (Duncan test). Group II compared with Group III, IV and V. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Table 4: Effect of oncocalyxone A from *Prenanthes sarmentosus* on 12th day on biochemical parameters in alloxan induced diabetic rats (mg/dl)

Group	Treatment	Serum Urea	Serum Creatinine	Serum Cholesterol	Serum Protein
I	Normal Control	35.14±0.25	0.76.00±0.03	91.91±0.11	5.17±0.02
II	Diabetic Control	67.73±0.43	1.53±0.07	183.74±0.32	3.75±0.04
III	Standard (Alloxan + glibenclamide 10 mg/kg)	22.13±0.20***	1.02±0.01***	97.31±0.33***	5.12±0.10***
IV	Alloxan + Oncocalyxone A (100 mg/kg)	34.22±0.37***	1.08±0.02***	104.42±0.49***	4.60±0.15***
V	Alloxan + Oncocalyxone A (200 mg/kg)	24.83±0.14***	1.04±0.02***	98.70±0.47***	4.86±0.04***

Values are expressed in Mean \pm Standard Deviation (n=6)

Superscript letters represent $P < 0.05$ (Duncan test). Group II compared with Group III, IV and V. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Biochemical analysis

In our present study, biochemical parameters were unchanged in non-diabetic rats. The level of serum urea, creatinine and lipid profiles (total protein, total cholesterol) of different experimental groups is also represented in Table 4. Our results also showed diabetic control rat significant increase in creatinine and urea levels as compared with control animals.

The diabetic hyperglycemia induced by alloxan produces elevation of plasma levels of urea and creatinine, which are considered as significant markers of renal dysfunction [23]. There was no significant difference in the activity of 100 and 200 mg/kg. The activity of 200 mg/kg was more active and the activity was comparable with that of the standard drug, glibenclamide. The serum lipid profile reached a peak in diabetic control rats, whereas the level of total protein decreased. Total protein levels are significantly decreased in rats with alloxan-induced diabetes due to increased protein catabolism [24]. Alloxan-induced diabetic rats showed higher cholesterol as compared with control. After continuous treatment with the oncocalyxone A and standard drug for 12 days, rats with alloxan-induced diabetes showed a marked decrease in cholesterol levels with a significant increase in lipoprotein. The present study found that daily administration of an oncocalyxone A sample from *Prenanthes sarmantosus* to diabetic rats gradually increased their protein levels, and that the oncocalyxone A had a pronounced effect on protein content, with results for total protein being in accordance with those of previous report [25]. There was a significant increase in serum total protein content in the treated diabetic rats compared with the diabetic controls. The mean total protein content in rats treated with oncocalyxone A (200 mg) was 4.86 ± 0.04 mg, which was slightly lower than the total protein content in rats treated with standard glibenclamide (5.12 ± 0.10 mg). In our study, diabetic rats showed higher cholesterol and the treatment with oncocalyxone A and standard drug significantly decreased cholesterol levels.

CONCLUSION

The compound oncocalyxone A isolated from *Prenanthes sarmantosus* leaves tested for anti-diabetic activity as it significantly lowered the serum glucose levels and increased the body weight of diabetic rats. Further biochemical investigations will clearly elucidate the mechanism of action and will be helpful this compound as a therapeutic target in diabetes research. A dose of 200mg/kg significantly brought the blood sugar level of the alloxan induced diabetic rats to normal condition.

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