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Research Article

Screening of Antidiabetic Activity and Toxicity Studies of *Cephalandra indica* Naud

Anupam Jamwal, Suresh Kumar*

Department of Pharmaceutical Sciences and Drug Research, Punjabi University, Patiala 147 002, Punjab, India.

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ABSTRACT

Cephalandra indica Naud. (Ivy gourd, family Cucurbitaceae) has a long tradition of use in the treatment of diabetes. Therefore, it was envisaged to screen antidiabetic activity of various extracts and fractions of *C. indica*, and to subject bioactive extract for acute and sub-acute toxicity studies. *C. indica* aerial parts were defatted by extracting with petroleum ether (60-80° C). The marc was then subjected to successive extraction using solvents in increasing order of polarity *viz*. chloroform, methanol and water. All extracts were screened for antidiabetic activity using STZ-NAD type -II diabetic model at the doses of 100, 200 or 400 mg/kg, *p.o.* for fifteen days, once daily. The bioactive extract was fractionated with ethyl acetate. The ethyl acetate fraction was also screened for antidiabetic activity at doses of 25 or 50 mg/kg, p.o. for 10 days, once daily. The glucose level in rats was determined using glucometer. Acute and Sub-acute studies were performed on bioactive extract as per OECD guidelines. Methanol extract and its ethyl acetate fraction significantly reduced the glucose level in rats. Acute toxicity study did not reveal any change in behavioral, neurological and autonomic profile. In sub acute toxicity studies, the methanol extract did not affect hematological and biochemical parameters in rats.

Keywords: Acute and Sub-acute toxicity, Antidiabetic, *Cephalandra indica*.

INTRODUCTION

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Type 2 diabetes is the most prevalent form of the disease[1]. Chronic illness requires medical care and patient self-management education to prevent acute complications and to reduce the risk of long-term complications[2]. Diabetes mellitus and obesity are the most frequent endocrine-metabolic diseases and are characterized by insulin resistance, defects in insulin secretion, and hence, a high hepatic production of glucose[3]. Statistical projections about India suggest that the number of diabetics will rise from 31 million in 2000 to 79 million in the year 2030 making the country with the highest number of diabetics in the world followed by China and then USA[4]. *Cephalandra indica* Naud (Synonym- *Coccinia indica* Wight. & Arn, *Coccinia grandis* (L.) Voigt, *Coccinia cordifolia* (L.) Cogn), commonly known as Ivy Gourd, Little gourd and Kovai belongs to family Cucurbitaceae. It is native of Africa and Asia including India, Philippines, China, Indonesia, Malaysia, Thailand, Vietnam, Eastern Papua, New Guinea and Northern territories. It grows in large quantities and widely distributed as weed in all over India[5]. The plant has been used, in Ayurvedic and Unani practice in the Indian subcontinent, as antidiabetic[6]. Other traditional uses are anti-inflammatory, antipyretic, analgesic, antispasmodic, antimicrobial, cathartic, antibacterial and expectorant[7]. Preliminary phytochemical screening studies have shown presence of alkaloids, carbohydrates, glycosides, fixed oils, fats, proteins, amino acids, saponins, tannins, phenolic compound, gums, mucilages, triterpenoids, flavonoids, anthraquinones, and polysaccharides in the plant[8]. In light of its high medicinal value and traditional use in the treatment of various ailments, especially for diabetes mellitus, the present studies were undertaken to evaluate antidiabetic activity of various extracts and fractions of *C. indica*, and also to subject bioactive extract for toxicity studies.

MATERIAL AND METHOD

Plant material- C. indica aerial parts were collected form Patiala and Chandigarh regions in September, 2011. The plant was identified from the Botany Division, Forest Research Institute, Dehradun (Reference no 341/2011-Bot-15-1, dated 12/09/2011). *Extraction method- C. indica* aerial parts were rinsed with normal saline to remove dirt, cut into small pieces, dried under sunlight and powdered in a grinder. Dried and powdered plant material (800g) was successively extracted in a Soxhlet apparatus using solvents in order of increasing polarity *viz.*, petroleum ether (60-80^0 C), chloroform and methanol. The aqueous extract was prepared by boiling the marc of plant material with distilled water for 2 h on a hot plate. The extracts were recovered under vacuum and kept in a desiccator. Ethyl acetate fraction of bioactive extract was prepared as pre standard procedure[9].

Animals

*Author for Correspondence: thakur_pu@yahoo.com

Table 1: Effect of various extracts/fractions of *C. indica* on blood glucose level in STZ-NAD diabetic (type-II) SD rats.

Groups	Dose (mg/kg)	Blood glucose concentration (mg/dL)			
		0 day	5th day	10th day	15th day
Vehicle control	-	84.33 ± 10.42*	86.33 ± 8.75*	86.33 ± 8.75*	91.33 ± 11.29*
STZ-NAD control	50/100	172.50 ± 15.79	191.33 ± 16.64	201.16 ± 14.59	215.16 ± 14.58
Metformin	150	181.13 ± 19.34	146.33 ± 15.37*	114.83 ± 15.38*	87.50 ± 9.05*
Methanol	100	190.33 ± 13.58	158.50 ± 13.72*	118.80 ± 24.64*	90.83 ±6.79*
Methanol	200	190.66 ± 17.95	155.66 ± 24.35*	107.83 ±18.26*	94.83 ±7.41*
Methanol	400	203.50 ± 25.44	159.83 ± 25.68*	108.50 ±11.64*	86.33 ± 10.50*
Chloroform	100	174.83 ± 18.28	181.83 ± 15.28	195.66 ± 17.94	212.83 ± 14.90
Chloroform	200	169.00 ± 18.41	185.50 ± 10.21	206.16 ± 17.37	213.67 ± 20.41
Chloroform	400	166.83 ± 16.64	179.83 ± 9.10	201.16 ± 15.44	208.16 ± 15.33
Aqueous	100	165.00 ± 17.56	176.50 ± 20.00	187.33 ± 23.09	196.83 ± 20.54
Aqueous	200	176.83 ± 26.54	190.00 ± 17.40	199.83 ± 14.02	217.66 ± 15.37
Aqueous	400	180.33 ± 15.34	191.16 ± 15.86	199.50 ± 18.73	214.16 ± 16.35
Ethyl acetate	25	183.36 ± 19.36	167.00 ± 16.22*	93.33 ± 7.68*	--------
Ethyl acetate	50	180.21 ± 21.61	91.53 ± 9.83*	88.50 ± 6.95*	--------
REM	200	167.49 ± 33.68	194.51 ± 12.11	208.82 ± 9.95	--------

n = 6; The data is expressed as Mean ± S.D.; **P<0.05* vs. STZ-NAD control; Two-way ANOVA followed by Student-Newman-Keul's test.

Table 2: Effect of methanol extract of *C. indica* on body weight.

Groups	Initial weight	Final weight
Control	237 ± 9.57	240 ± 3.69
Methanol	267 ± 6.45	262 ± 7.58

n = 10; The data is expressed as Mean ± S.D.

Sprague Dawley (SD) rats, either sex, of body weight 250-350 g purchased from Indian Veterinary Research Institute, Izatnagar, Bareilly (U.P) were used. The animals were fed with normal laboratory pellet diet and water *ad libitum*. The approval was taken from Institutional Animal Ethics Committee of Department of Pharmaceutical Sciences and Drug Research, Punjabi University, Patiala before carrying out animal studies (107/99/CPCSEA-2012-47, dated 02/10/12).

Solvents, Chemicals and instruments

Solvents, *viz.*, petroleum ether (60-80^0 C), chloroform, methanol, and ethyl acetate (E, Merck, India, LR grade), were employed in present investigations. Streptozotocin (Sigma chemicals, USA), sodium citrate (Loba, India), citric acid (S.D. Fine, Chemicals, Mumbai), nicotinamide (Himedia; Mumbai), metformin (Cipla, India), diagonstic kits (Erba, India) were used in present studies. Rotary vacuum evaporator (Buchi, Switzerland) was used for recovery of solvent under reduced pressure.

Induction of diabetes mellitus (type-II)

A freshly prepared solution of streptozotocin (STZ, 50 mg/kg, *i.p.*) in 0.1 M citrate buffer (pH 4.5) was administered intraperitoneally to rats. The nicotinamide (NAD; 100 mg/kg) was given intraperitoneally 15 min before STZ administration[10]. After 72 h of administration, rats with moderate diabetes having glycosuria and hyperglycemia (>145 mg/dL) were taken for experimental studies[11]. Fasting glucose level was estimated by using a glucose oxidase–peroxidase reactive strips and a glucometer (Contour, Bayer- Healthcare, Japan).

Experimental design

Group I served as a normal control. Group II treated with STZ/NAD (50/100 mg/kg, *i.p.*), served as diabetic control and group III, treated with metformin (150 mg/kg, *p.o.*), was standard. Group IV, V and VI were treated with chloroform extract of *C. indica* at three dose levels - 100, 200 and 400 mg/kg p.o. respectively. Group VII, VIII and IX were treated with methanol extract of *C. indica* at three doses of 100, 200 and 400 mg/kg *p.o*. respectively. Group X, XI and XII were treated with aqueous extract of *C. indica* at three doses level 100, 200 and 400 mg/kg, *p.o.* respectively. Group XIII and XIV were treated with ethyl acetate fraction at doses of 25 and 50 mg/kg, *p.o.* respectively. Group XV was treated with remaining methanol extract at the dose 200 mg/kg, *p.o.* The standard drug and test extracts were administered orally once daily at 9:00 AM for 15 days to diabetic rats. The blood glucose concentration was determined in the groups on 0 day, 5th day, 10th day and 15th day. The ethyl acetate fraction and remaining methanol extract were administered orally once daily at 9:00 AM for 10 days to diabetic rats, and blood glucose concentration was determined in the groups on 0 day, 5th day and 10th day. Each group comprises six rats.

Acute toxicity studies

Test was performed as per Organization for Economic Cooperation and Development (OECD) guidelines-423[12,13].

Sub-acute toxicity

Test was performed as per Organization for Economic Cooperation and Development (OECD) guidelines-407[14]. Half of the dose used in acute toxicity studies, i.e., 1000 mg/kg/b.w/*p.o.* of bioactive extract was selected for sub-acute toxicity studies. The rats were divided into 2 groups of 6 rats in each group. Group I, served as control group received vehicle. Group-II, served as test group received bioactive extarct. Hematological and biochemical estimations were also made.

Table 3: Effect of methanol extract of *C. indica* on serum parameters.

Parameter	Control	Methanol extract
Total protein (g dL^{-1})	5.95 ± 0.20	6.14 ± 0.29
Serum proteins (g dL^{-1})	6.23 ± 0.41	5.92 ± 0.36
Albumin (g dL^{-1})	3.51 ± 0.21	3.21 ± 0.03
Globulin (g dL^{-1})	2.11 ± 0.16	2.19 ± 0.02
Sodium (mmol/l)	145.16 ± 3.76	153.42 ± 2.93
Potassium (mmol/l)	5.42 ± 0.34	6.42 ± 0.29
Bicarbonate (mmol/l)	23.04 ± 0.65	24.46 ± 0.38
Total bilirubin (mg dL^{-1})	0.56 ± 0.04	0.54 ± 0.03
Direct bilirubin (mg dL^{-1})	0.15 ± 0.22	0.17 ± 0.02
Unconjugated biliurbin (mg dL^{-1})	0.42 ± 0.03	0.44 ± 0.02
Glucose (mg dL^{-1})	101.34 ± 5.11	65.87 ± 2.23
Cholesterol (mg dL^{-1})	75.34 ± 2.01	73.14 ± 1.07
Triglycerides (mg dL^{-1})	66.00 ± 2.36	65.40 ± 1.51
HDL (mg dL^{-1})	110.26 ± 5.61	108.44 ± 2.31
LDL (mg dL^{-1})	94.11 ± 2.61	96.69 ± 1.91
VLDL (mg dL^{-1})	53.98 ± 1.13	54.18 ± 1.01
Urea (mg dL^{-1})	32.44 ± 0.21	33.89 ± 0.35
Creatinine (mg dL^{-1})	0.49 ± 0.01	0.53 ± 0.01
Uric acid (mg dL^{-1})	2.47 ± 0.01	2.69 ± 0.05
Blood urea nitrogen (mg dL^{-1})	40.71 ± 0.12	43.11 ± 0.35
SGOT (u/L)	399.00 ± 5.65	346.20 ± 3.56
SGPT (u/L)	137.50 ± 2.58	125.66 ± 2.50
ALP (u/L)	522.75 ± 2.75	494.87 ± 1.48
ACP (u/L)	6.88 ± 0.26	5.27 ± 0.26
LDH (u/L)	2006 ± 10.04	1810 ± 9.43

n = 6; The data is expressed as Mean ± S.D.

Table 4: Effect of methanol extract of *C. indica* on hematological parameters.

Parameter	Control	Methanol
Hb (g dL^{-1})	13.58 ± 0.11	14.23 ± 0.13
Platelet count ($x10^3$/µl)	4.51 ± 0.72	4.92 ± 0.65
Erythrocytes ($x10^6$/µl)	6.87 ± 0.11	6.71 ± 0.13
Lymphocytes (10^3/µl)	4.11± 0.21	4.63± 0.67
TLC ($x10^3$/µl)	11600 ± 6.33	12100 ± 5.94
PCV (%)	36.47 ± 0.11	34.79 ± 0.26
MCV (fl)	55.41 ± 2.23	54.59 ± 3.34
MCHC (%)	35.11 ± 1.01	36.74 ± 1.41
RDW-CV (%)	14.37 ± 0.15	14.54 ± 0.33
MCH (Pg)	20.61± 1.10	20.11± 1.86

n = 6; The data is expressed as Mean ± S.D.

Effect on general behavior, food intake and body weight

Animals were observed for toxic effects and general behavioral changes at an interval of 1, 2.5 and 4 h after administration of bioactive extract of *C. indica* and then at least once in a day during the whole study period of 28 days. Mortality of animals was recorded during the entire study period. Food intake and body weight of individual animal was recorded on 0, 7th, 14th, 21st and 28th day prior to administration of test substance.

Biochemical studies

Total protein, serum proteins, albumin, globulin, cholesterol, triglycerides, serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP), Acid phosphatase (ACP), Lactate dehydrogenase (LDH), creatinine, bilirubin (total, direct and unconjugated), glucose, cholesterol, triglycerides high-density lipoprotein (HDL), low-density lipoprotein (LDL), very low density lipoprotein (VLDL), serum electrolyte levels (sodium, potassium and bicarbonates), creatinine, uric acid, blood urea nitrogen (BUN) and urea were assayed at the end of the study in all groups of animals. The tests were carried out with the help commercial diagnostic kits using semi auto chemical analyzer (RA-50, BAYAR).

Hematological studies

Diethyl ether was used to anaesthetize animals before blood samples were collected through cardiac puncture into ethylene diamine tetraacetic acid (EDTA) tubes. The blood samples were analyzed for red blood cells (RBC), white blood cells (WBC), platelet count, erythrocyte, lymphocytes and hemoglobin (Hb) content using standard techniques[15]. The packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), relative distribution width of red blood cells by volume, coefficient of variation (RDW-CV), mean content of hemoglobin (MCH) and total leukocyte count (TLC) were also estimated[16,17].

Statistical analysis

The data were expressed as mean ± S.D. The statistical analysis was carried out by using Sigma stat version 3.5. The obtained results were analyzed by two-way ANOVA followed by Student- Newman-Keul's test.

RESULTS

Various extracts of *C. indica* aerial parts viz., chloroform, methanol and aqueous were evaluated for antidiabetic activity in rats using Streptozotocin-NAD induced type-2 diabetes model. Metformin was used as a standard drug. Table 1 shows effect of methanol, chloroform and aqueous extract, ethyl acetate fraction, and remaining methanol extract on blood glucose levels in diabetic rats. Amongst various extracts tested, only methanol extract exhibited significant antidiabetic activity at all dose levels, i.e, 100, 200 and 400 mg/kg, p.o. Ethyl acetate fraction exhibited significant activity at 25 or 50 mg/kg. Both methanol extract and its ethyl acetate fraction significantly reduced the blood glucose level ($P<0.05$) when compared with diabetic control. Acute toxicity studies revealed the non-toxic nature of the methanol extract of *C. indica* aerial parts at the dose of 2000 mg/kg/b.w/*p.o.* as it did not show any mortality or change in behavioral, neurological and autonomic profile in rats. There was no lethality or any toxic reactions observed until the end of the study period. As per OECD-423

guidelines, the dose is said to be "unclassified" under the toxicity scale. Hence further study with lower doses was not executed. Sub-acute toxicity studies revealed no significant difference in the body weight of all the rats after administration of methanol extract of *C. indica* for 28 days, once daily (Table 2)**.** Toxicity signs such as piloerection, salivation and lacrimation were not observed in rats. Biochemical studies showed that there was no significant increase in the levels of serum proteins in rats after sub-acute treatment of methanol extract. Levels of total protein, serum protein, albumin and globulin were found to be normal in methanol treated rats compared with rat treated with vehicle (Table 3)**.** Serum electrolyte (sodium, potassium and bicarbonate), total bilirubin, direct bilirubin and unconjugated bilirubin, lipid profile (cholesterol, triglycerides, HDL, LDL and VLDL) were found with in limits. Levels of urea, creatinine, uric acid and blood urea nitrogen in methanol treated rats were similar to the animals of control group suggesting normal functioning of kidney of treated rats. A marked decrease in level of glucose was observed in methanol treated rats, inferring hypoglycemic effect of bioactive methanol extract of *C. indica*. Liver enzyme, concentration (SGOT, SGPT, ALP, ACP and LDH) was also found to be in limits. No significant changes were observed in hemoglobin (Hb), red blood cell (RBC), white blood cell (WBC), packed cell volume (PCV), and red cell indices (MCV, MCHC, RDW-CV, MCH and TLC) in test group (Table 4).

DISCUSSION

Streptozotocin-nicotinamide exhibit a mild decline of glucose tolerance in rats due to loss of early-phase insulin secretion. STZ-NAD induced diabetic rats are sensitive to the hypoglycaemic effects of insulinotropic agents, and have many pathological features resembling type 2 diabetes, which may be useful in the pharmacological investigation of numerous antidiabetic drugs[10]. Methanolic extract of *C. indica* and its ethyl acetate fraction showed significance decrease in glucose level at all doses in STZ-NAD induced type-II diabetes in animal model, when compared with diabetic control. Behavioral, neurological and autonomic profile of animals during acute and sub-acute toxicity studies provide the information regarding short and long term effects, level of toxicity, dose regimens and safe use of herbal products in animals for further studies. The changes in general behavior, body weight and food intake are critical for the evaluation of any product on test animals as first sign of toxicity[18]. Acute toxicity studies did not report any lethality, change in behavioral pattern or any kind of bizarre reaction in rats treated with methanol extract. Sub-acute studies also did not show mortality and any signs of toxicity at any stage of study during 28 days period in methanol extract treated group of animals. After sub-acute treatment with test substances, various general, hematological / biochemical parameters, marked increase and decrease in body weight of animals is indicator of adverse effect and improvement in general health[19]. *C. indica* methanol extract did not show significant change in body weights in experimental rats. Alteration in protein profile is sign of metabolic disorder or more specific hepatic injury[20]. Liver function tests (SGOT, SGPT, ALP, ACP and LDH) act as indicator of liver damage along with increase level in bilirubin (direct and unconjugated). Their elevated levels show sign of liver toxicity or hepatic injury[21]. Bioactive methanol extract did not increase level of liver enzymes, total proteins and serum proteins and serum electrolytes (sodium, potassium and bicarbonate, total biliurbin, direct biliurbin and unconjugated bilirubin) and lipid components in rats. Any deformities in glomerular results in high level of creatinine or increase production of protein metabolites by liver (increase metabolism of amino acid cause rise in urea level in the urine). Thus, impairment of renal function results in elevation of end products of protein metabolism[21]. No adverse effect has been observed in renal function test as creatinine, uric acid and blood urea level remained unaltered after treatment, inferring no kidney toxicity exists in animals treated with methanol extract. Abnormalities in hemoglobin, erythrocytes counts, red cell indices (PCV, MCV, MCHC, RDW-CV, MCH and TLC levels) and platelet / lymphocyte counts due to lysis of blood cells and/or inhibition of blood cell synthesis are associated with anemia[22]. No such abnormalities were observed in animals treated with methanol extract.

CONCLUSION

It is finally concluded from preclinical studies that *C. indica* can be developed as a safe antidiabetic drug.

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