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ANTIDIABETIC AND HYPOLIPIDEMIC POTENTIAL OF HYDROALCOHOLIC EXTRACT OF *CANNA INDICA* L. ROOT AND RHIZOMES IN RAT

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ABSTRACT

Background: Tribal groups in India and Bangladesh have been using water extract of *Canna indica* L. (CI) herb since long time to treat diabetes and its other complications. **Objective:** Present study was an evaluation of antidiabetic activity of polar compounds from CI rhizome and roots extract by oral administration in streptozotocin-induced type 1 diabetes in rats. **Material and methods:** Extract was prepared by macerating CI rhizome and roots using mixture of ethanol and water (1:1) and screened phytochemically. Research work was conducted for the period of three weeks on Sprague Dawley (SD) rat groups viz. extract treated normal control group, vehicle treated diabetic control group, and standard drug treated group; and extract treatment groups. Streptozotocin (STZ) (single dose 55 mg/kg, ip) was injected to induce type 1 diabetes, glibenclamide (GB) was given (5 mg/kg, po) once daily as standard drug and CI extract given (100 mg/kg, 200 mg/kg, po) once daily in two test groups. Blood samples were collected weekly and examined for hematological and biochemical parameters. Animals were sacrificed on 21st day for histopathological study. **Results:** CI extract exhibited significant ($p<0.05$) hypoglycemic and hypolipidemic activity comparable to that of standard drug. When 200 mg/kg of extract was administered once daily for three weeks, it reduced 116 mg/dl of mean fasting blood glucose and about 2.5-3 ng/mL of serum insulin; and also elevated pancreatic islets density. Extract significantly elevated HDL cholesterol (20-22 mg/dl) and reduced LDL (25-30 mg/dl) and VLDL (8-10 mg/dl) cholesterol in treatment groups. Histopathological examination demonstrated protective effect of plant extract on pancreas, liver, and kidneys; exhibiting less diabetes-induced degenerative damage. **Conclusion:** Polar compounds from CI rhizome and roots were found effective in controlling type 1 diabetes and its cardiovascular, renal and hepatic complications.

KEYWORDS: Streptozotocin; hypoglycemic; hypolipidemic; serum biochemistry; histopathology; diabetic complications.

INTRODUCTION

Diabetes mellitus is one of the leading disorders across the world, as per the report of recent survey conducted by government and private organizations.[1] Incidences of early diagnosis are rising continuously through last three decades and they are still increasing day by day. Major reasons for this are lack of exercise, bad food habit and an urban life style.[2] There are two different types of diabetes mellitus classified on the basis of etiology. Severe type 1 diabetes (early onset) is symptomatically treated by insulin therapy while type 2 diabetes (late onset) is controlled by antihyperglycemic drug regimen. With the progress of this disorder, typically cardiovascular, cerebrovascular and immunological complications express slowly and result in irreversible damage.[3] Diabetes limits life quality and may reduce life expectancy in most patients if it is not controlled by appropriate treatment on right time [4]. In general, there is no permanent cure for this condition except lifelong treatment.

Some Asian traditional drug therapist have been using a few medicinal plants in diabetes treatment since long time. Considerable volume of Indian Ayurvedic and Chinese herbal medicine is being sold across the world claiming that, 'it is useful in controlling diabetes and it has lesser side-effects than allopathic drugs'.[5] These medicines should be made available in their purest form and should be tested in laboratory animals to confirm their medicinal activity. Present work is inspired from this line of thought and therefore targeted to explore the potential of traditional crude drug in diabetes treatment.

Rahmatullah *et al* (2009) ethnobotanically surveyed Thakurgaon District, Bangladesh and found that tribes residing here suffer from various disorders and use different medicinal plants for treatment.[6] Water extract

of various parts of *Canna indica* L. (CI) plant was being given orally to treat diabetes. Purintrapiban *et al* (2006) described differential activation of glucose transporters in cultured muscle cells by polyphenolic compounds from CI and proposed glucose transporter (GLUT) based mechanism of hypoglycemic activity.[7] These findings encouraged us and supported our intention of proving antidiabetic potential of this plant.

Canna indica is commonly known as 'Indian shot' or 'African arrowroot' and generally found in gardens as flowering ornamental plant. It has been cultivated since ancient times.[8] It is native to South-Central America (Andes), West Indies, Mexico, Europe, Africa and South-East Asia. This plant can be used for the treatment of industrial waste waters for removing organic load, color, and paper mill wastes.[9] Flowers of CI plant are the rich source of anthocyanin color pigments; can be used as antioxidant nutraceuticals in food industry. A purple dye is obtained from the seed.[10] CI yields a fiber from the stem, which is used as substitute to jute while; fiber from the leaves is used in paper making.[8]

Plant taxonomy: Kingdom: Plantae, Sub-kingdom: Tracheobiont, Super-division: Spermatophyta, Division: Magnoliophyta, Class: Liliopsida, Sub-class: Zingibericae, Order: Zingiberales; Family: Cannaceae; Genus: Canna; Species: *Canna indica* L; Common Name: Achira, Canna, Indian Shot, Devkali.[10]

Ethnopharmacological claims: There are numerus ethnopharmacological uses of different parts of CI herb claimed and recommended by traditional medical practitioners in India, Bangladesh, Philippine and Thailand. Rhizome is considered demulcent, diaphoretic, diuretic and antipyretic.[10] Decoction of rhizome is useful in treating dropsy, dyspepsia and hepatitis induced jaundice. Aqueous extract is given orally to control nosebleed. Roots are considered as acrid and stimulant. These medicinal activities are traditional beliefs of the natives; and unfortunately lack pharmacological basis or proper clinical documentation. Therefore, there is need to explore medicinal potential of this herb using technically sound and precise methodology on the scale of modern pharmacology.[11] Recently, few researchers evaluated *C. indica* L. rhizome extract for *in-vitro* anti-HIV.[12] hypoglycemic.[7] and antioxidant.[13] activity. Nirmal *et al*, (2007) studied *in-vitro* anthelmintic activities and *in-vivo* antinociceptive activity of alcoholic extract of this herb in mice.[14]

MATERIAL AND METHODS

Chemicals and reagents

Streptozotocin (Sisco Research Lab, Mumbai, India), Glibenclamide (Emcure Pharma Ltd, Pune, India), Biochemical Estimation Kits – (Biolab Diagnostics (Pvt), Ltd, Mumbai, India). Animal Feed (Nutrivet Life Sciences, Pune, India). All other chemicals of analytical grade were used.

Extract preparation

Fully grown, flower bearing plants 1-2 meter in height were selected from natural habitat; Pavana river shore, at Thergaon, Pune. Rhizome and roots were shade dried in lab for 15 days and powdered later using mortar-pestle and mixer blender. The resulting powder was macerated in ethanol: water (1:1) solvent for a day. Extract was concentrated using rotary evaporator (Evator, Johannesburg, South Africa). Concentrate was then transferred to sterile amber colored glass bottle. One kilogram of powdered crude drug gave 9.67gm of extract (approx. 1%). Extract was then analyzed phytochemically. Later it was administered orally in rats to evaluate anti-diabetic potential.

Phytochemical prospection

Preliminary phytochemical analysis

Extract was tested for the presence of different secondary metabolites (alkaloids, terpene, terpenoids, tannins, and flavonoids) using chemical tests. Test specific for each class of secondary metabolites was based on change in color or formation of precipitate on addition of specific reagent (*Dragendorff's* test, *Shinoda* test, *Libermann-Burchard* test, and $FeCl_3$ test).

Standardization of extract. Indira *et al*, (2015) revealed the presence of *β*-sitosterol in rootstalk of CI.[8] Thus it was used as marker for standardization of extract. Extract was then standardized as per isocratic High – Performance Liquid Chromatography (HPLC) method developed and validated by Jadhav et. al. (2010) with slight modifications using HPLC (Jasco PU-1580) with Purospher, RP-18e, 250 x 4.6, 5 µ column and UV Detector at 215 nm [15]

Quantitative analysis. A separate calibration curve was drawn for gallic acid (GA) and quercetin. In each case extract was treated as unknown sample for estimation by interpolation method. Total phenolic contents in the extract were determined using *Folin-Ciocalteau method* described by Singleton *et al* (1965) with slight modification.[16] Total phenolic contents were measured in ratio with gallic acid and expressed as milligram of gallic acid equivalents in a gram of crude dried extract (mg GAE/g) at 756 nm. Total flavonoid contents were determined using colorimetric method described by Zhishen *et al* (1999) with slight modification [17]. Total flavonoid contents were measured in ratio with quercetin (QC) equivalent and expressed as milligram of quercetin equivalents in a gram of dried extract (mg QC/g) at 415 nm.

Approval and conduct

The present work was conducted as per the protocol approved by the Institutional animal ethics committee (IAEC) with protocol no. 'ICP/IAEC/12-13/07' and authorized by CPCSEA nominee. The plant selected for the work 'CI' was authenticated by the regional government institute '*Botanical Survey of India*, Koregaon Park, Pune' with voucher code (SK01).

Animals used

Sprague Dawley (SD) rats were procured from CPCSEA approved supplier from Mumbai, India; and kept in quarantine for a week to acclimatize with animal house facility. Male and female animals were kept in separate cages and in auto controlled 12 hours light-dark cycles. Male and female rats of age ranging between 12 to 14 weeks and body weight 200 to 300 gm were randomly selected. Throughout the period of this study animals were maintained at room temperature (20-25°C) and 50% (±10%) relative humidity.

Acute toxicity study

Acute toxicity study was conducted in two animal groups containing 6 male SD rats in each group, for two weeks. Animals were fasted overnight and treatments were given in the morning. Group I and II were given single dose of 1000mg/kg and 2000mg/kg of CI extract (volume < 2ml) through gastric intubation suspended in 5% acacia solution, respectively. Animals were observed for 8 hours on the day of administration and subsequently twice daily for the period of fourteen days to record possible food- water intake and body weight changes.[18]

Serum insulin assay

Serum insulin assay was conducted using "Rat Insulin ELISA Kit" (CrystalChem, Downers Grove, IL) Small amount (5µL) of separated serum was utilized for assay. Kit was providing assay range between 0.156 – 10 ng/mL in 5µL sample. Procedure was taking 12 hours incubation of samples at 2-8 °C.[19]

Antidiabetic activity

Animals were divided into five groups containing six animals in each group. Animals were fasted overnight and treatments were given in the morning. A soft rubber tube was fitted on 5 ml syringe to administer liquids orally. Group 'Ctrl' was vehicle (non-STZ citrate buffer) and extract treatment control group. Group 'DB-Ctrl', 'GB5', 'CI100' and 'CI200' were injected a single dose 60mg/kg of streptozotocin (STZ, ip) in citrate buffer (pH 4.5) [20]. Group 'Ctrl' was given (200mg/kg, po) extract once daily, group 'DB-Ctrl' was given vehicle (5% acacia, 1ml, po) once daily, group 'GB5' was given glibenclamide (GB) (5mg/kg, po) as standard drug, once daily, group 'CI100', and 'CI200' were administered 100mg/kg and 200mg/kg (in 1ml, po) of test extract once daily through gastric intubation, respectively. All animals were treated for 21 days and sacrificed on the last day. Unwanted placebo effect in case of each control group was blocked by executing appropriate vehicle treatment and same animal handling pattern. Extract and GB doses given orally were suspended in 5% acacia in distilled water. Blood samples were drawn from retro orbital sinus using diethyl-ether anesthesia at weekly intervals for 3 weeks. Blood samples were labeled in such a way that analysis team was kept blind about groups and nature of study design. Body weight measurement and blood sampling were done on 1st, 7th, 14th and 21st day of the study. Animals were sacrificed, dissected and organs were cleaned and collected.[21]

Clinical biochemistry analysis

In OGTT, Strip glucometer (Accu-Check, Mumbai) with direct glucose estimation strip was used to determine glucose content in a drop of a blood. In antidiabetic study, approximately 1ml of blood was collected from each animal into vial containing sodium heparin as an anticoagulant and centrifuged at 3000 rpm for 15 minutes. Separated serums were analyzed using Semi-autoanalyzer (Lablife Chem Master, Ahmadabad, India) and ready-to-use reagents were employed to estimate Glucose, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Urea, Creatinine, Triglyceride, High density lipid cholesterol (HDL), Low density lipid cholesterol (LDL), Very low density lipid cholesterol (VLDL) and Total cholesterol (TC).[22]

Necropsy, organ collection, and histopathology

On last day of the study, all the animals were euthanized and were subjected to a full, detailed gross necropsy. After gross pathology examination, vital organs viz; pancreas, liver, kidneys and injection site of all animals were removed and fixed in 10% neutral buffered formalin for subsequent histopathological examination. Fixed organs after 48 hours were further embedded in paraffin and sectioned approximately 3 to 5 µ thick using microtome (Panchal Sci. corp., Goregaon, Mumbai) and stained with hematoxyllin-eosin stain and mounted for microscopy (Labomed, California, US).

Pancreatic islets density measurement

Pancreatic islets were focused under low power field (LPF i.e. 10X) and number of islets per LPF were counted. In each case, area of highest density was selected for the measurement. Islets of which more than 50% area was covered under a particular LPF were counted while others were excluded. Results were expressed as mean ± SD.

Statistical analysis of data

Each group was containing six animals to establish the minimum level of significance. Results were expressed as Mean ± SEM. Different groups were compared with diabetic control in ANOVA and Dunnett's t test was employed as post-test. Significance was expressed using p value and values of $p<0.05$ were considered sufficient to propose hypothesis. Significance tests were performed using software 'GraphPad InStat 3'.

RESULTS

Extract analysis

Preliminary phytochemical analysis. Phytochemical tests indicated the presence of sterols, flavonoids, tannins and traces of alkaloids in extract while terpenes and terpenoids were found to be absent.

Quantitative analysis. Total phenolic contents were found to be 27.34±0.67 mg/g of GA equivalents (n=3)

while flavonoid contents were found to be 12.05±0.27 mg/g of QC equivalents (n=3).

Acute Toxicity Study

During the fourteen days period of study there were no signs of toxicity observed in all participated animals and no animal died in the study even after administration of higher dose (2000 mg/kg) of CI extract. There was no significant change in food-water intake and body weight of animals.

Serum insulin assay

Serum insulin level was considered as the main direct determinant of type 2 diabetes. In extract treated non-diabetic group and standard drug treated GB5 group there were negligible change in insulin levels. Extract treated CI100 group showed limited recovery in insulin level while CI200 showed considerable recovery of rats form insulinopenia. (Figure 1)

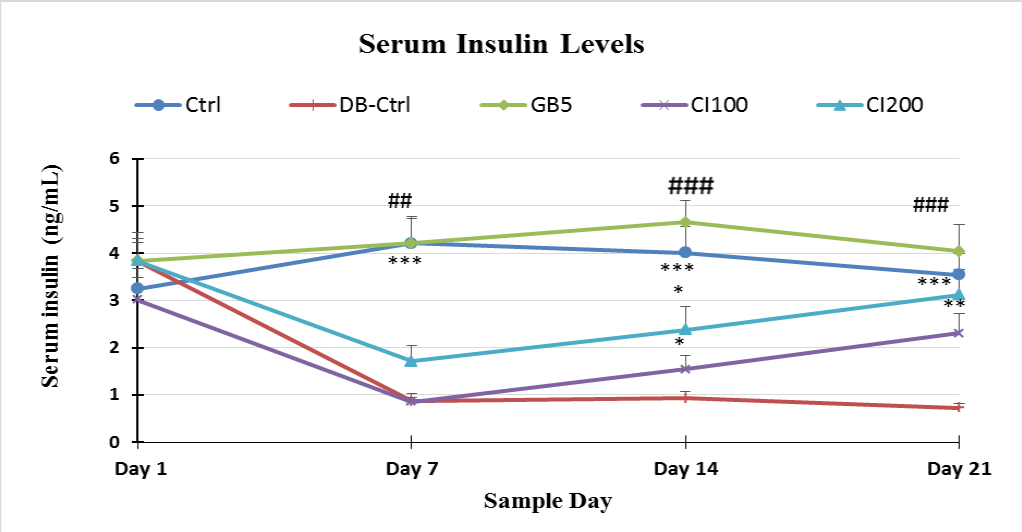


Figure 1: Serum insulin assay at different time points

Ctrl: Vehicle treated control; GB5: Standard drug glibenclamide group dose 5 mg/kg; CI100: CI Extract group dose 100mg/kg; CI200: CI Extract group dose 200mg/kg; Results are expressed as Mean ± SEM (n=6). Difference analyzed amongst groups using one-way ANOVA and Dunnett's t test as post-test. *Difference in insulin levels when compared with diabetic control significant at p<0.05. **Difference in insulin levels when compared with diabetic control significant at p<0.01. ###Difference in insulin levels of GB5 when compared with diabetic control significant at p<0.001.

Body weight loss (g)

Polyuria and progressive weight loss is generally considered as early manifestation of diabetes induction. There were progressive weight losses in diabetic control group and glibenclamide treated groups. There was negligible weight loss in extract treated groups. Particularly, 200mg/kg dose of extract could restore approximately 30 to 40 gram of weight in diabetic animals observed against diabetic control. (Figure 2)

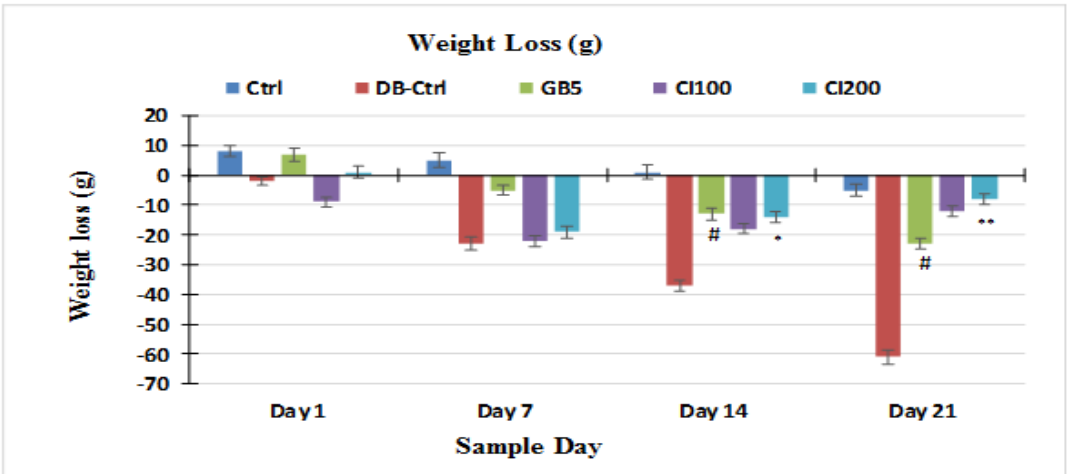


Figure 2: Body weight loss at different time points

Ctrl: Extract treated control; DB-Ctrl: Vehicle treated diabetic control; GB5: Standard drug glibenclamide treated group, dose 5 mg/kg; CI100: CI Extract treated group, dose 100mg/kg; CI200: CI Extract treated group, dose 200mg/kg; Results are expressed as Mean ± SEM (n=6). Difference analyzed amongst groups using one-way ANOVA and Dunnett's t test as post-test. *Difference in body weight of a group when compared with diabetic control significant at p<0.05. **Difference in body weight of a group when compared with diabetic control significant at p<0.01. #Difference in body weight of GB5 group when compared with diabetic control significant at p<0.05.

Serum biochemistry

Blood glucose level was considered important indirect determinant in Type 1 diabetes. Early morning fasting glucose levels were determined in this study for all groups. STZ initially produced hypoglycemia in SD rats for 2 to 3 days and later it slowly converted it to hyperglycemia in next 3 to 4 days. It takes approximately a week, to get constant hyperglycemia for each rat group. Severe hyperglycemia was observed in diabetic control group but no death was reported during the study. Extract did not induce hypoglycemia in extract treated non-diabetic rats. Extract dose of 200mg/kg demonstrated reasonably good control on glucose levels ($p<0.05$) by reducing mean to 116mg/dl which is comparable to standard drug ($p<0.01$).

Elevated ALT, AST level is an indicator of impaired protein metabolism in liver. These levels may rise many times higher than normal in liver disease, depending on the intensity of damage caused by the underlying condition. There was 4 to 5 times and 5 to 6 times rise in ALT and AST levels in diabetic control, respectively; whereas there was negligible change in ALT, AST levels in case of extract treated control. Extract dose of 200mg/kg provided significant control on the diabetes induced rise in ALT ($p<0.01$) and AST ($p<0.001$) levels by reducing them at least 4 to 5 times, respectively.

Diabetes induces polyuria, which further leads to hypovolemia and increases blood urea. With dehydration, diabetic nephropathy caused by persistent hyperglycemia is also equally responsible for this increase in blood urea. Extract treated non-diabetic animals did not show any change in blood urea levels. Standard drug very significantly reduced urea levels in blood ($p<0.001$), while 200mg/kg of extract could reduce about 40mg/dl of urea ($p<0.01$) in respective group.

There was no change in creatinine levels in extract treated non-diabetic rats. Standard drug very efficiently controls the elevating creatinine levels in GB-Ctrl group ($p<0.001$). CI extract dose of 200 mg/kg reduced about 0.2 to 0.3 mg/dl creatinine levels ($p<0.01$). This effect was lower as compared to standard drug, but quite significant when compared to diabetic rats in control group.

Table 1: Serum biochemistry

	Sampling time points during study period				
	Groups	**Day 1**	**Day 7**	**Day 14**	**Day 21**
Serum Glucose Levels (mg/dl)	Ctrl	89±18	103±16	92±19	99±19
	DB-Ctrl	97±16	254±27	282±27	269±28
	GB5	93±16	133±17##	135±19##	131±18##
	CI100	101±16	217±20*	222±19*	228±18*
	CI200	96±16	171±18*	166±18*	178±19*
Serum ALT Levels (U/L)	Ctrl	33±2.7	37±2.9	29±3.3	34±2.8
	DB-Ctrl	36±2.9	167±6.5	188±6.0	187±5.8
	GB5	47±3.3	067±3.6##	089±4.1##	079±3.6##
	CI100	34±2.9	135±5.3**	129±4.9**	117±4.9**
	CI200	36±2.4	073±3.7**	076±3.8**	076±3.9**
Serum AST Levels (U/L)	Ctrl	115±6.3	106±5.4	108±5.1	111±6.4
	DB-Ctrl	117±5.9	503±30.2	581±30.1	603±31.2
	GB5	102±5.1	147±07.4###	149±08.3###	164±07.2###
	CI100	107±5.3	425±21.3*	436±21.8**	429±22.5**
	CI200	117±5.9	232±10.2**	228±12.4***	212±09.3***
Serum Urea Levels (mg/dl)	Ctrl	15±4.8	18±5.2	19±4.4	17±5.1
	DB-Ctrl	15±4.7	89±4.5	93±4.6	98±4.0
	GB5	16±4.6	38±4.4###	37±4.2###	36±4.4###
	CI100	18±3.7	72±4.1	79±4.4	84±4.3
	CI200	17±4.2	63±5.3**	61±4.2**	57±3.9**
Serum Creatinine Levels (mg/dl)	Ctrl	0.34±0.06	0.37±0.07	0.33±0.07	0.32±0.09
	DB-Ctrl	0.31±0.08	0.86±0.04	0.77±0.04	0.73±0.06
	GB5	0.29±0.08	0.35±0.06###	0.36±0.06###	0.39±0.07###
	CI100	0.34±0.08	0.59±0.06	0.58±0.03	0.56±0.06
	CI200	0.29±0.07	0.53±0.04**	0.48±0.08**	0.48±0.07**

Ctrl: Extract treated control; DB-Ctrl: Vehicle treated diabetic control; GB5: Standard drug glibenclamide treated group, dose 5 mg/kg; CI100: CI Extract treated group, dose 100mg/kg; CI200: CI Extract treated group, dose 200mg/kg; Results are expressed as Mean ± SEM (n=6). Difference analyzed amongst groups using one-way ANOVA and Dunnett's t test as post-test. *Difference in blood parameter of a group, when compared with diabetic control significant at p<0.05. **Difference in blood parameter of a group, when compared with diabetic control significant at p<0.01. ***Difference in blood parameter of a group, when compared with diabetic control significant at p<0.001. #Difference in blood parameter of a GB5 group, when compared with diabetic control significant at p<0.05. ##Difference in blood parameter of a GB5 group, when compared with diabetic control significant at p<0.01. ###Difference in blood parameter of a GB5 group, when compared with diabetic control significant at p<0.001.

Diabetic lipid profile

In the present study, serum triglycerides, LDL cholesterol, VLDL cholesterol and total cholesterol (TC) were clearly elevated while HDL cholesterol was decreased in vehicle treated diabetic rats. There was insignificant change in these parameters in case of extract treated non-diabetic control and significant change in standard drug treated group. When these parameters were tested in CI extract treated groups, unexpectedly we got significant hypolipidemic activity of extract in CI200 diabetic rats, along with hypoglycemic activity. Extract dose of 200mg/kg could increase about 24 to 28mg/dl of HDL cholesterol, and reduced about 25 to 30mg/dl of triglyceride, 45 to 50mg/dl of LDL cholesterol, 08 to 12 mg/dl of VLDL cholesterol, and 70 mg/dl of total cholesterol.

Table 2 Diabetic lipid profile

	Sampling time points during study period				
	Groups	**Day 1**	**Day 7**	**Day 14**	**Day 21**
Serum Triglyceride Levels (mg/dl)	Ctrl	61±5	59±6	53±5	57±4
	DB-Ctrl	63±4	83±7	91±6	97±7
	GB5	58±6	73±5#	78±5#	73±9#
	CI100	52±7	83±8	87±5	86±6
	CI200	59±5	74±4	73±8*	69±7**
Serum HDL Cholesterol Levels (mg/dl)	Ctrl	48±3	46±4	49±3	51±5
	DB-Ctrl	55±4	49±3	41±4	39±3
	GB5	48±3	56±4	58±4#	51±2
	CI100	49±3	57±2	59±3**	53±4*
	CI200	49±3	53±3	50±2*	63±3**
Serum LDL Cholesterol Levels (mg/dl)	Ctrl	71±8	70±9	67±11	79±8
	DB-Ctrl	77±9	139±12	143±11	149±10
	GB5	68±6	076±08###	073±09###	071±06###
	CI100	72±8	117±05**	129±07*	131±07**
	CI200	92±6	092±05**	097±07**	101±08**
Serum VLDL Cholesterol Levels (mg/dl)	Ctrl	16±1.2	19±1.1	23±0.9	17±1.3
	DB-Ctrl	17±0.9	35±1.3	39±1.4	37±1.1
	GB5	22±1.0	25±0.9###	23±0.9###	26±1.3###
	CI100	19±1.5	28±1.2**	32±1.6**	33±0.9**
	CI200	18±1.3	27±1.4***	28±1.0***	26±1.5***
Serum Total Cholesterol Levels (mg/dl)	Ctrl	51±5	57±6	49±7	61±8
	DB-Ctrl	48±4	126±6	145±3	147±7
	GB5	58±9	084±8###	082±9###	071±6###
	CI100	69±8	103±7**	111±6**	109±4**
	CI200	63±5	083±4***	089±6***	081±7***

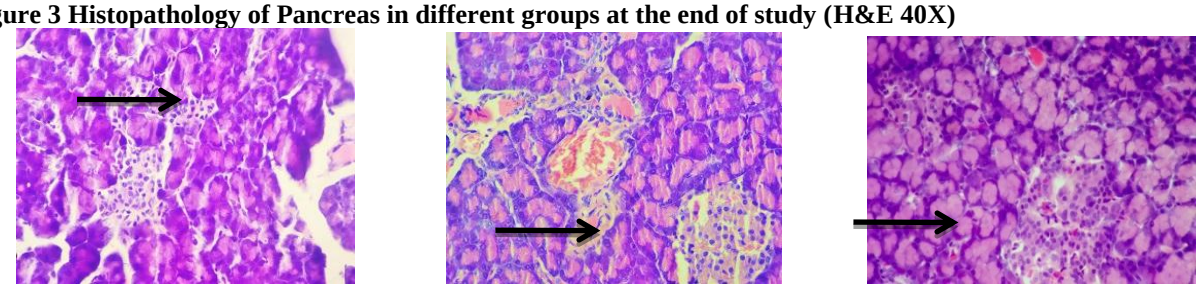
Ctrl: Extract treated control; DB-Ctrl: Vehicle treated diabetic control; GB5: Standard drug glibenclamide treated group, dose 5 mg/kg; CI100: CI Extract treated group, dose 100mg/kg; CI200: CI Extract treated group, dose 200mg/kg; Results are expressed as Mean ± SEM (n=6). Difference analyzed amongst groups using one-way ANOVA and Dunnett's t test as post-test. *Difference in blood parameter of a group, when compared with diabetic control significant at p<0.05. **Difference in blood parameter of a group, when compared with diabetic control significant at p<0.01. ***Difference in blood parameter of a group, when compared with diabetic control significant at p<0.001. #Difference in blood parameter of a GB5 group, when compared with diabetic control significant at p<0.05. ##Difference in blood parameter of a GB5 group, when compared with diabetic control significant at p<0.01. ###Difference in blood parameter of a GB5 group, when compared with diabetic control significant at p<0.001.

Histopathology difference analysis

Pancreas

Extract treated non-diabetic rats showed normal histological architecture of pancreas (group not in figure). Normal prominent sections of islets of Langerhans were found in the tail region of the pancreas. STZ treated diabetic group shows asymmetrically scattered round texture of damaged islets of Langerhans in several clusters with irregular shape. Oxidative damage caused by STZ to pancreatic acini and islets result in vacuolations in the tissue. This bursting of islets in extract treated groups is lesser than that in glibenclamide treated group. (Figure 3)

Figure 3 Histopathology of Pancreas in different groups at the end of study (H&E 40X)



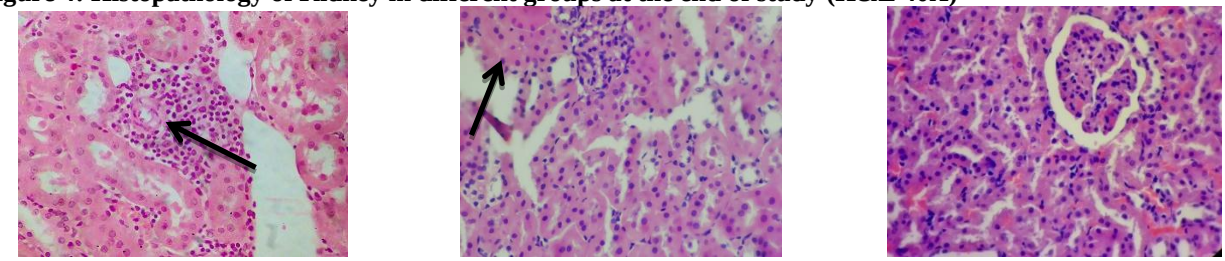
3A: DB-Ctrl Great reduction in the size of pancreatic islets.
3B: GB5 Minimal reduction in the size of pancreatic islets
3C: CI200 Normal size pancreatic islets.

DB-Ctrl: Vehicle treated diabetic control; GB5: Standard drug glibenclamide treated group, dose 5 mg/kg; CI200: CI Extract treated group, dose 200mg/kg.

Kidney

Kidney sections were taken in such a way that they will show both glomerular portions and tubular portion on the same slide. Extract treated non-diabetic group (group not in figure) showed normal histological architecture at glomerular and tubular regions of the kidney. There was brushing of the epithelial tubular layer observed in case of diabetic control rats, indicated progressive kidney damage. There were prominent vacuolations in the tissue as well as inflammatory cell infiltration were observed. Extract treated group exhibited minimal vacuolations or infiltration of inflammatory cells. (Figure 4)

Figure 4: Histopathology of Kidney in different groups at the end of study (H&E 40X)



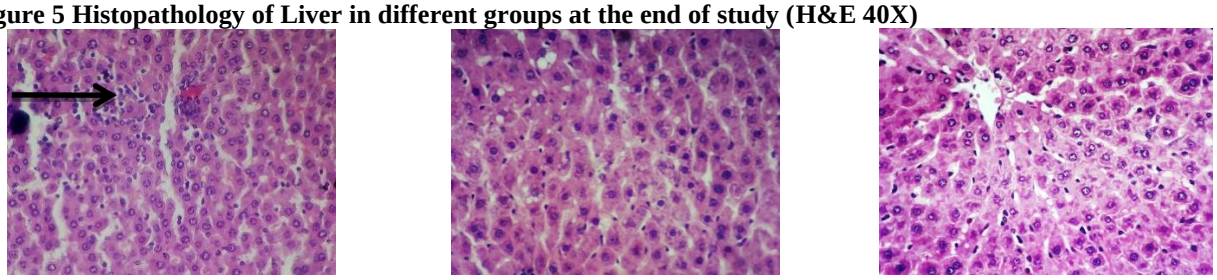
4A: DB-Ctrl Moderate to high tubular and glomerular damage with loss of integrity and disruption of tubules, infiltration of MNC (arrow).
4B: GB5 Minimal glomerular and tubular damage and moderate infiltration of MNC (arrow)
4C: CI200 Normal renal parenchyma with normal histomorphology of glomeruli and renal tubules. (Nil)

DB-Ctrl: Vehicle treated diabetic control; GB5: Standard drug glibenclamide treated group, dose 5 mg/kg; CI200: CI Extract treated group, dose 200mg/kg.

Liver

Extract treated non-diabetic group showed eminent hepatocytes with central vein. Diabetic control group clearly showed damage to hepatocytes and central vein architecture in the form of vacuolations and focal degeneration. Extract treated liver showed normal architecture of hepatocytes and central vein without any kind of focal degeneration. (Figure 5)

Figure 5 Histopathology of Liver in different groups at the end of study (H&E 40X)



5A: DB-Ctrl Vacuolations in hepatocytes, focal necrosis, infiltration of inflammatory cells (arrow).
5B: GB5 Vacuolations in hepatocytes, focal Degeneration.
5C: CI200 Minimal focal degeneration of hepatocytes.

DB-Ctrl: Vehicle treated diabetic control; GB5: Standard drug glibenclamide treated group, dose 5 mg/kg; CI200: CI Extract treated group, dose 200mg/kg.

Pancreatic islets density

Ctrl group pancreas showed 3.74 (±0.69) structurally normal islets per LPF while DB-Ctrl showed 1.68 (±0.43) distorted islets per LPF possibly due to STZ induced damage. GB5 group pancreas showed about 50% reduction in the islets count like other groups due to STZ administration but there were complementary rise in drug induced insulin secretion which did not allow glucose level to rise. Extract treated groups also showed approximately 30-40% islets count showing that these animals were relatively more resistant to oxidative free radicle damage caused by STZ. CI100 and CI200 group pancreas were showing 2.13 (±0.63) and 2.96 (±0.57) islets per LPF and structural loss of islets was relatively lesser as compared to DB-Ctrl group.

DISCUSSION

This study was focused on evaluation of antidiabetic potential of CI rhizome and roots extract. Preliminary phytochemical analysis on extract showed the presence of polyphenols, flavonoids and few sterols. Some compounds from each of these category have significant anti-diabetic potential viz. *β*-sitosterol has reported antidiabetic activity [23]. These compounds also have antioxidant activity demonstrated using diphenyl picrylhydrazyle (DPPH) free radical scavenging model.[24, 25, 26] Bassirat *et al* (2000) concluded that free radicals play a role in altering microvascular function in diabetes.[27]

Extract treated control group did not exhibit any significant hypoglycemic effect during three weeks of antidiabetic study. This is important because most diabetic patients face hypoglycemia as frequent side effect when taking antidiabetic drug therapy and they need to monitor it much carefully. Also, hypoglycemic effect in diabetes was dose-dependent, therefore, dose titration will be possible in case of CI extract therapy. Elevation in blood levels of ALT, AST, urea and creatinine were efficiently controlled by 200mg/kg daily dose of an extract. This finding is in agreement with that concluded by Bassirat *et al* (2000).[27] This is an important attribute of an extract treatment that, it not only control the blood glucose level but also block diabetes-induced progressive damage of liver and kidneys in diabetic rat.

Hyperlipidemia is a known complication of type 1 diabetes and it is characterized by increased levels of triglycerides, cholesterol, and phospholipids. Lipid profile was studied as cholesterol is associated with diabetes-induced atherosclerosis and heart disease.[28] Control of hyperlipidemia is prerequisite for prevention of diabetic microvascular complications (retinopathy, nephropathy and neuropathy) and macrovascular complications (ischemic heart disease), cerebral vascular disease (CVD) and arteriosclerosis in diabetes.[29]

The most typical lipid abnormality is hypertriglyceridemia with increase in plasma cholesterol.[30] Diabetic ketoacidosis contributes to hypertriglyceridemia, which is a major toxic complication observed in type 1 patients. Both of these conditions produce acute pancreatic damage and worsen diabetes.[31] Studies have strongly suggested an inverse relationship of HDL cholesterol with atherosclerosis. There is inverse relationship between HDL levels and coronary heart disease (CHD). Low HDL levels are considered as a tool to predict CHD. HDL level decreases due to its enhanced glycation and faster clearance of glycated complex.[32] Extract treatment significantly increases HDL and reduces triglycerides in the diabetic rats.

An increase in LDL cholesterol, as a lipid metabolic complication is usually observed in diabetic people. LDL cholesterol concentrations are positively related to atherosclerosis in these individuals.[33] Glycated LDL interacts with platelets and alters platelet aggregation, platelet nitric oxide production, intracellular Ca^{2+} concentration, activities of Na^{+}-K^{+} and Ca^{2+} ATPcases [34]. More VLDL is synthesized in liver due to influx of increased amounts of free fatty acids and converted to LDL ultimately. On one side there is more production of VLDL cholesterol however, on other hand, there is drainage of it from the blood due to diabetes.

Like standard drug, extract treatment significantly reduced both LDL and VLDL levels in diabetic rats.[35] Free radicle attack by streptozotocin on the pancratic islets create considerable inflammatory damage in inducing diabetes. CI extract is reported to have inhibitory action on inflammatory cytokines that may contribute to the antidiabetic activity at pancreas level.[36]

Peripheral antidiabetic activity may be via increasing adipose tissue peroxisome proliferator-activated receptor (PPAR) γ expression,[37] or expression of GLUT and/or induction of GLUT4 translocation with subsequent stimulation of glucose uptake.[38]

Hypolipidemic acitvity of the extract may be attributed to the reduction of HMG CoA reductase activity, or promoting anbolic disposition of cholesterol in periferal tissues. This essentially minimizes serum cholesterol level and play vital role in preventing cardiovascular diseases like coronary atheroscelrosis in diabetic animals. Extract treatment reduces chances of hypertriglycetidemia possily by promoting fatty acid absorption and metabilism in periferal tissues.[39]

Complete mechanism of antidiabetic and antilipidemic activity of extract is still unclear. There may be multiple mechanisms involved; one at pancreas and other at tissue or peripheral site. There is future scope to investigate unknown mechanism of antidiabetic and hypolipidemic activity of CI extract.

CONCLUSION

Results showed that CI extract has significant antidiabetic and hypolipidemic activity comparable to standard Glibenclamide. Hypolipidemic activity is potential added advantage with antidiabetic activity. Extract was also found efficient in controlling diabetic micro- and macro-vascular complications. Extract efficiently controlled STZ-induced insulinopenia at pancreatic level and diabetes-induced pathological deterioration of peripheral vital organs. This may be attributed to ionic compounds like polyphenols and flavonoids in the extract. Therefore, CI plant could be a good candidate for bio-assay guided fractionation and isolation of antidiabetic lead molecules. Hypolipidemic activity of CI extract with special focus will also be an area of interest for future research. This study supports the use of this plant extract in treatment of diabetes by traditional medical practitioners.

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