



Original Research Article (Experimental)

# Nephroprotective effect of *Curculigo orchiodies* in streptozotocin–nicotinamide induced diabetic nephropathy in wistar rats



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## ABSTRACT

**Background:** Chronic hyperglycemia induced oxidative stress and dyslipidemia in diabetic nephropathy may lead to chronic renal damage. Thus, counteracting oxidative stress might represent an interesting approach in alleviating hyperglycemia-induced renal damage.

**Objective:** The present experimental work was undertaken to explore nephroprotective efficacy of *Curculigo orchiodies* in streptozotocin–nicotinamide induced diabetic nephropathy in laboratory animals.

**Materials and methods:** Single intraperitoneal introduction of freshly prepared STZ (65 mg/kg) was used for induction of diabetic nephropathy in rats, 15 min after NAD administration (230 mg/kg; *i.p.*). The evaluation of nephropathy was done by assessment of serum glucose level, insulin level and renal function test (albumin, urea and creatinine). In addition to this, lipid profile as well as oxidative stress (TBARS, superoxide dismutase, catalase and reduced glutathione) was evaluated. Augmented levels of blood glucose, albumin, urea and creatinine confirmed the development of nephropathic symptoms in rats. After 30 days of STZ administration, different doses (150, 300 mg/kg and 600 mg/kg; *p.o.*) of hydroalcoholic and ethanolic extracts of *C. orchiodies* were administered to rats for 45 days.

**Conclusion:** *Curculigo orchiodies* significantly attenuated hyperglycemia induced increase in lipid profile, oxidative stress and normalized the renal functions (albumin, urea and creatinine); attributing to the efficacy of *C. orchiodies* in diabetic nephropathy. These findings suggest that hydroalcoholic and ethanolic extract of *Curculigo Orchiodes* ameliorated the progression of diabetic nephropathy. The observed nephroprotective effect of *C. orchiodes* is attributed to its hypoglycemic, antioxidant and anti-hyperlipidemic activity.

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## 1. Introduction

*Diabetes mellitus* (DM) is generally considered as multi-factorial and is defined as chronic metabolic disorder which results from damage of pancreatic beta cells (insulin deficiency) or decreased uptake of glucose into cell (dysregulated insulin signaling) [1,2]. DM is widely coupled to long-term microvascular and macrovascular complications *i.e.* retinopathy, neuropathy, nephropathy [3]. Diabetic nephropathy (DN) is a universal micro-vascular impediment in diabetic subjects, illustrated by proteinuria which leads to renal disorder [2,4].

Chronic hyperglycemia induces excessive generation of reactive oxygen species (ROS) leading to high level of oxidative stress and is extensively acknowledged as vital component in diabetes induced renal disorders [5,6]. Chronic hyperglycemia mediated unnecessary generation of ROS is the common factor linking disturbed renal hemodynamics with the dysregulated metabolic pathways [7,8].

In addition, accumulation of advanced glycation end products (AGEs) plays vital role in the establishment of DN [8,9]. Moreover, dyslipidemia and alteration in renal functions (increased levels of blood urea nitrogen, serum creatinine, urea and urine albumin) have been documented in diabetic subjects [2,10,11].

*Curculigo orchiodes* (CO) is widely used traditional medicine with powerful antioxidant, belongs to family Amaryllidaceae [12]. It is used extensively in ayurvedic formulations like *Vidaryadighrta*,

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Vidaryadi lehya, Marmagulika, Musalyadi churna etc. for wide variety of ailments especially as a general tonic and as aphrodisiac.

This *Kali musali* plant has ayurvedic properties like –*Rasa* –*Madhur* (Sweet), *Tikta* (Bitter), *Guna* (Pharmacological Actions) –*Guru* (heavy) *Picchila* (Slimy), *Virya* (Action) – *Ushna*, *Vipaka* (transformed state after digestion) –*Madhur*.

It was used as rasayan in ayurveda and as powder of *krnsa musali* (*Talamuli*) mixed with ghee acts as aphrodisiac. In Ayurveda it is used as *Musali paka*, *Loh rasyana* [13]. The rhizomes of CO are reported to possess anti-diabetic, immunostimulant, aphrodisiac and hepatoprotective activity [14]. Therefore, keeping in view the potent antioxidant and antidiabetic effect of CO; this work was undertaken to assess nephroprotective effect of hydroalcoholic and ethanolic extract of CO rhizome against streptozotocin (STZ)-nicotinamide induced DN.

## 2. Material and methods

### 2.1. Drugs, chemicals and reagents

STZ was procured from Sigma–Aldrich, USA. Gallic acid, Sodium carbonate, Folin–Ciocalteu reagent, and Sodium nitroprusside (10 ml) solution were obtained from Molychem, India and Nicotinamide from Finar, India. Nitro blue tetrazolium, Thiobarbituric acid, 2- DPPH, NADPH oxidase, Glimepiride, DTNB were acquired from Himedia, India. Unless stated, different chemical employed in current work were of analytical rating.

### 2.2. Diagnostic kits

Serum glucose, total cholesterol (TC), LDL, HDL, VLDL, triglycerides (TGs), creatinine, uric acid and urea level was assessed using commercially available kits.

### 2.3. Experimental animals

Wistar rats of age 4–6 months, weighing 200–300 gm were obtained from animal house of Chitkara College of Pharmacy, Rajpura, India. Rats were accommodated in group of six in polypropylene cages crumpled with husk. Rats were provided with 12: 12 h light dark cycle and maintained in standard environmental conditions *i.e.* temperature  $25 \pm 3$  °C and relative humidity  $50 \pm 10\%$ . Rats were nourished with standard chow diet (Adarsh Feed, Chandigarh, India) *ad libitum* and provided open entrée to drinking water. The protocol was evaluated and approved by the Institutional animal ethical committee (IAEC) (IAEC/CCP/18/PR-008) and experimental work was carried out as per guidelines set by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India (Reg. No. 1181/PO/ReBi/S/08/CPCSEA).

#### 2.3.1. Collection and identification of plant

Rhizomes of *Curculigo orchiodes* were collected from local market and authenticated by Dr.K.Madava Chetty Asst.Professor, Dept. of Botany, Sri Venkateshwar University, Tirupati, Andara Pardesh, India wide Voucher no 1623.

### 2.4. Preparation of extract

The rhizomes of *C. orchiodies* were shade dried for 15 days and then powdered. Approximately 1 kg of powdered drug material was extracted using ethanol in the fraction of 1:2 (w/v) and then kept at room temperature for 15 h time interval. Further, suspension was filtered with whatman filter paper no. 4 and serially extracted using chemical solvents in array of their escalating

polarity as alcohol and hydro-alcohol (40%) with the help of Soxhlet apparatus for 72 h. Finally, the extracts were again filtered and then dried under vacuum. Crude extracts were dissolved in water and used for the assessment of *in-vivo* assays. The mass was then weighed and recorded. The percentage of yield was calculated. The weight of dried crude extract obtained was approximately 1.5 g which commemorated with the percentage yield of 15.5% [15].

### 2.5. Phytochemical screening

Phytochemical analysis was carried out to identify various chemical constituents (for *e.g.* alkaloids, carbohydrates, fixed oils and fats, terpenoids, phenols, tannins, glycosides, saponins, proteins, amino-acids and flavonoids) present in the extracts. Phytochemical analysis was carried out in accordance with the methods mentioned in Trease and Evans [16] Harborne with a slight modification.

### 2.6. Induction of diabetic nephropathy

Intraperitoneal administration of freshly prepared solution of STZ (65 mg/kg) in citrate buffer was used for induction of diabetic nephropathy in rats, 15 min after NAD administration (230 mg/kg; *i.p.*). After 72 h of STZ administration, fasting blood glucose (FBG) level was determined to confirm the development of diabetes. The inclusion criterion for rats in the study was FBG level  $\geq 250$  mg/dl. As per previous reports of acute toxicity and pilot studies of *C. orchiodes*, three different doses of the *C. orchiodes* ethanolic and hydroalcoholic extracts (150, 300 and 600 mg/kg) were selected for *in-vivo* study. The symptoms of DN typically develop after 4–5 weeks after STZ administration and therefore level of uric acid, urea, creatinine and BUN was estimated on 30th day. After 30 days of STZ administration, treatment with ethanolic and hydroalcoholic extract and standard drug *i.e.* Glimepiride was continued for next 45 days [2].

### 2.7. Experimental design and groups

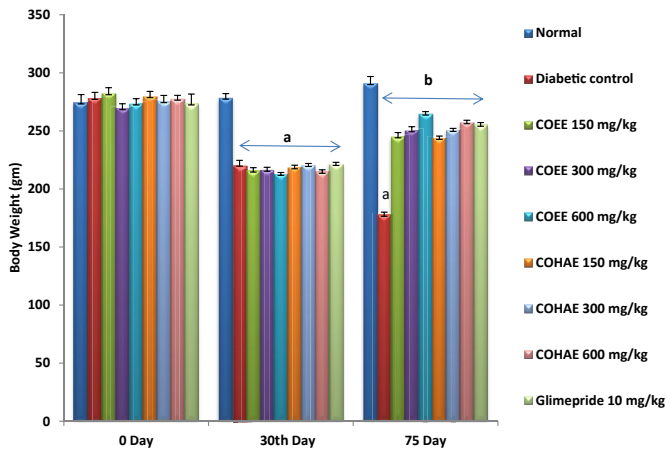
The experimental design is depicted in Fig. 1. The experimental protocol comprises of nine different groups and each group comprises of six animals ( $n = 6$ ) (Table 1).

### 2.8. Estimation of body weight, blood glucose and serum insulin level

The weight of each rat from different experimental group was observed on weekly basis until the end of study. After 3 days of STZ administration, glucose level was measured to authenticate the development of diabetes. In addition, FBG level was measured on 1st day, 30th day and 75th day with the help of commercial available enzymatic kits [17].

### 2.9. Biochemical estimation

Blood samples from fasted animals were withdrawn (under light anesthesia) by using retro-orbital puncture method in the morning and used for assessment of lipid summary (TC, TGs, VLDL, LDL and HDL level), renal function tests (BUN, serum urea, uric acid and creatinine) on day 30 and day 75 after STZ administration. Blood samples were centrifuged at 4000 rpm at 4 °C for 20 min and then serum was separated and finally utilized for biochemical estimation using commercially available kits. For renal tests, kidney was harvested and stored in deep refrigeration at  $-70$  °C until use.



**Fig. 1.** Effect of ethanolic and hydroalcoholic extracts of *Curculigo Orchioides* on body weight. Data are mean  $\pm$  SEM; Data was analyzed by using one-way ANOVA followed by Tukey's multiple test; <sup>a</sup> $p < 0.05$  as compared to Normal control Group; <sup>b</sup> $p < 0.05$  as compared to diabetic control group.

### 2.9.1. Estimation of lipid profiles

Level of TC, TGs, VLDL, LDL and HDL levels were measured with commercially available diagnostics kits.

### 2.9.2. Estimation of renal function

On day 30th and 75th of experimental protocol, blood samples were withdrawn from rats of different experimental groups and subsequently utilized for the assessment of various renal function test (creatinine, uric acid, urea and BUN level).

### 2.9.3. Estimation of SOD, GSH and catalase levels

The estimation of level of anti-oxidant enzymes *i.e.* SOD, GSH and catalase was performed in kidney homogenate as per the previously reported method [18].

### 2.9.4. TBARS estimation

The estimation of TBARS (marker of lipid peroxidation) was done in kidney homogenate as per the procedure explained by Ohkawa et al. [19]. Final concentration was stated as nano moles per mg of protein.

### 2.10. Statistical analysis

The data obtained was analyzed using sigma stat software. Data obtained was expressed as mean  $\pm$  S.E.M. For statistical analysis, one way analysis of variance (ANOVA) was used followed by Tukey's post hoc multiple comparison test.  $p < 0.05$  was well thought-out to be statistically significant.

## 3. Results

### 3.1. Effect of ethanolic and hydroalcoholic extracts of *Curculigo Orchioides* on body weight

During the study, DN control group showed significant ( $p < 0.05$ ) and progressive attenuation in body weight of rats as compared to normal control group. The administration of ethanolic and hydroalcoholic extracts of rhizome of *C. orchioides* at dose of 150, 300 and 600 mg/kg, *p.o.* as well as Glimipride treatment significantly ( $p < 0.05$ ) ameliorated decline in weight in a dose-dependent manner in comparison to DN control group (Fig. 1).

### 3.2. Effect of ethanolic and hydroalcoholic extract of *Curculigo Orchioides* on blood glucose

The administration of ethanolic and hydroalcoholic extracts of *C. orchioides* rhizomes was commenced 30 days after STZ administration. Blood glucose level of each animal was estimated on day 30 and 75. Oral administration of ethanolic and hydroalcoholic extracts *C. orchioides* (150, 300 and 600 mg/kg) and glimepiride (10 mg/kg) for 45 days, produced significant ( $p < 0.05$ ) attenuation in elevated blood glucose level in comparison to DN control rats (Fig. 2).

### 3.3. Effect of ethanolic and hydroalcoholic extract of *Curculigo Orchioides* on renal function

A marked augmentation in albumin, urea and creatinine levels was observed in diabetic nephropathy control rats in comparison to normal control rats. Administrations of ethanolic and hydroalcoholic extracts of *C. orchioides* (150, 300 and 600 mg/kg) and glimepiride (10 mg/kg) resulted in marked ( $p < 0.05$ ) attenuation of elevated albumin, urea and serum creatinine level in comparison to DN control rats (Figs. 3–5).

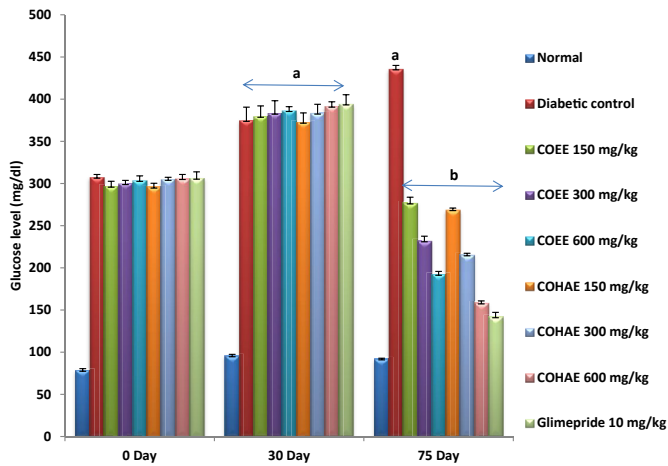
### 3.4. Effect of ethanolic and hydroalcoholic extract of *Curculigo Orchioides* on lipid profile

Serum concentration of TC, TGs, VLDL and LDL was found to be significantly ( $p < 0.05$ ) elevated while HDL concentration was found to be significantly decreased in DN control rats when compared to normal control group. Treatment with ethanolic and hydroalcoholic extracts of *C. orchioides* (150, 300 and 600 mg/kg) and glimepiride (10 mg/kg) for 45 days significantly and dose dependently ( $p < 0.05$ ) ameliorated the level of aforementioned lipoproteins as compared to DN control rats. Moreover, administration of ethanolic and hydroalcoholic *C. orchioides* extract (150, 300 and 600 mg/kg) and glimepiride (10 mg/kg) significantly ( $p < 0.05$ ) increased in level of serum HDL-cholesterol when compared to DN control rats (Table 2).

**Table 1**  
Experimental groups.

S. No.	Experimental Groups	Treatment
1	Group I	Normal Control (Saline + DDW treated; <i>p.o.</i> )
2	Group II	Diabetic nephropathy (DN) control [STZ-NAD treated (65 mg/kg; <i>i.p.</i> )]
3	Group III	STZ (65 mg/kg; <i>i.p.</i> ) + NAD (230 mg/kg; <i>i.p.</i> ) + COEE (150 mg/kg; <i>p.o.</i> )
4	Group IV	STZ (65 mg/kg; <i>i.p.</i> ) + NAD (230 mg/kg; <i>i.p.</i> ) + COEE (300 mg/kg; <i>p.o.</i> )
5	Group V	STZ (65 mg/kg; <i>i.p.</i> ) + NAD (230 mg/kg; <i>i.p.</i> ) + COEE (600 mg/kg; <i>p.o.</i> )
6	Group VI	STZ (65 mg/kg; <i>i.p.</i> ) + NAD (230 mg/kg; <i>i.p.</i> ) + COHAE (150 mg/kg; <i>p.o.</i> )
7	Group VII	STZ (65 mg/kg; <i>i.p.</i> ) + NAD (230 mg/kg; <i>i.p.</i> ) + COHAE (300 mg/kg; <i>p.o.</i> )
8	Group VIII	STZ (65 mg/kg; <i>i.p.</i> ) + NAD (230 mg/kg; <i>i.p.</i> ) + COHAE (600 mg/kg; <i>p.o.</i> )
9	Group IX	STZ (65 mg/kg; <i>i.p.</i> ) + NAD (230 mg/kg; <i>i.p.</i> ) + Glimipride (10 mg/kg; <i>i.p.</i> )

**Note:** DN: Diabetic Nephropathy; STZ: Streptozotocin; NAD: Nicotinamide; EECO: Ethanolic extract of *Curculigo orchioides*; HACO: Hydroalcoholic extract of *Curculigo orchioides*.



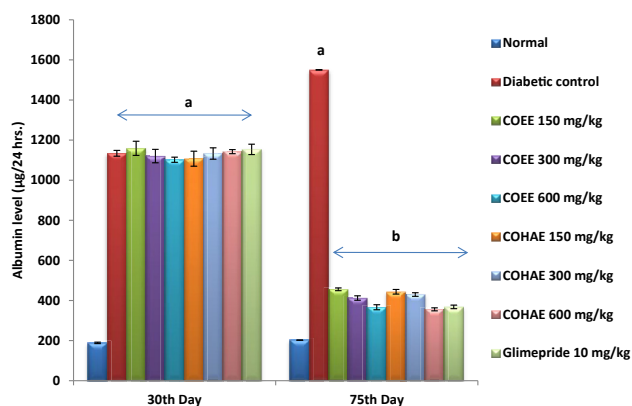
**Fig. 2.** Effect of ethanolic and hydroalcoholic extract of *Corchiodies* on glucose level. Data are mean  $\pm$  SEM; Data was analyzed by using one-way ANOVA followed by Tukey's multiple test; <sup>a</sup> $p < 0.05$  as compared to Normal control Group; <sup>b</sup> $p < 0.05$  as compared to diabetic control group.

### 3.5. Effect of ethanolic and hydroalcoholic extract of *Curculigo Orchiodies* on TBARS and anti-oxidant enzymes level

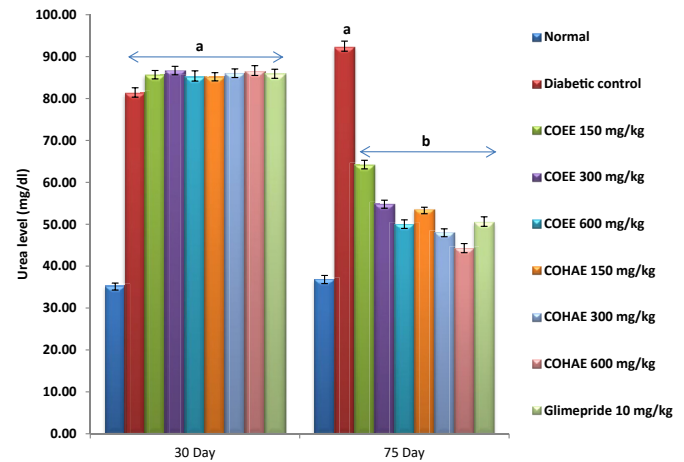
TBARS content was found to be significantly elevated whereas level of antioxidant enzymes viz. SOD, GSH and catalase was significantly decreased ( $p < 0.05$ ) in the kidney of DN control rats as compared to normal control rats. Treatment with the ethanolic and hydroalcoholic extracts of *C. orchiodies* (150, 300 and 600 mg/kg) and glimepiride (10 mg/kg) for 45 days significantly ( $p < 0.05$ ) attenuated the increase in the level of TBARS and significantly ( $p < 0.05$ ) increased the level of SOD, GSH and catalase as compared to DN control rats (Table 3).

### 3.6. Effect of alcholic and hydroalcholic extract of *C. orchiodies* on histopathological changes in renal tissue of kidney of rats

The kidney of diabetic nephropathy rats showed mesangial expansion and thickening of glomerular capillaries. Glomeruli infiltrated by inflammation cells along with infiltration seen in cortex and medulla area. Administration of maximum dose of



**Fig. 3.** Effect of ethanolic and hydroalcoholic extract of *C. orchiodies* on albumin level. Data are mean  $\pm$  SEM; Data was analyzed by using one-way ANOVA followed by Tukey's multiple test; <sup>a</sup> $p < 0.05$  as compared to Normal control Group; <sup>b</sup> $p < 0.05$  as compared to diabetic control group.



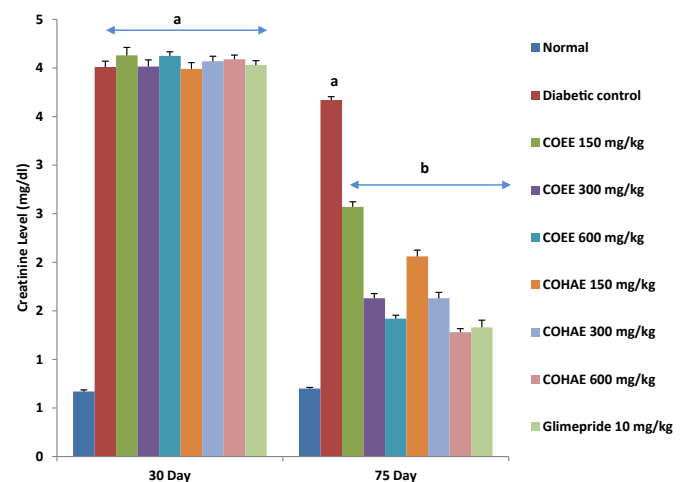
**Fig. 4.** Effect of ethanolic and hydroalcoholic extract of *C. orchiodies* on urea level. Data are mean  $\pm$  SEM; Data was analyzed by using one-way ANOVA followed by Tukey's multiple test; <sup>a</sup> $p < 0.05$  as compared to Normal control Group; <sup>b</sup> $p < 0.05$  as compared to diabetic control group.

600 mg/kg of alcoholic and hydroalcoholic extract of *Curculigo Orchiodies* and glimepiride treatment group attenuated the nephritic changes in renal tissue (Fig. 6).

## 4. Discussion

STZ-NAD induced diabetes in rodents serves as a gold standard experimental tool to study diabetes and associated abnormalities including DN [20]. The massive and selective demolition of  $\beta$ -cells in pancreas by STZ results in the reduced compassion of insulin for glucose utilization by body cells [2,20]. NAD is a powerful antioxidant which when administered along with STZ; neutralizes to some extent the excessive lethal effect of STZ by scavenging ROS; subsequently decreases injury to  $\beta$ -cell and thereby producing type II diabetes [2,21]. This harmful act of STZ on pancreatic  $\beta$ -cells in rodents produces chronic hyperglycemic state in blood followed by excessive ROS production.

Chronic hyperglycemic environment prevailing in vascular system for longer duration stimulate the over activation of



**Fig. 5.** Effect of ethanolic and hydroalcoholic extract of *C. orchiodies* on creatinine level. Data are mean  $\pm$  SEM; Data was analyzed by using one-way ANOVA followed by Tukey's multiple test; <sup>a</sup> $p < 0.05$  as compared to Normal control Group; <sup>b</sup> $p < 0.05$  as compared to diabetic control group.

**Table 2**  
Effect of Ethanolic and Hydroalcoholic extract of *Curculigo orchioides* on Lipid profile.

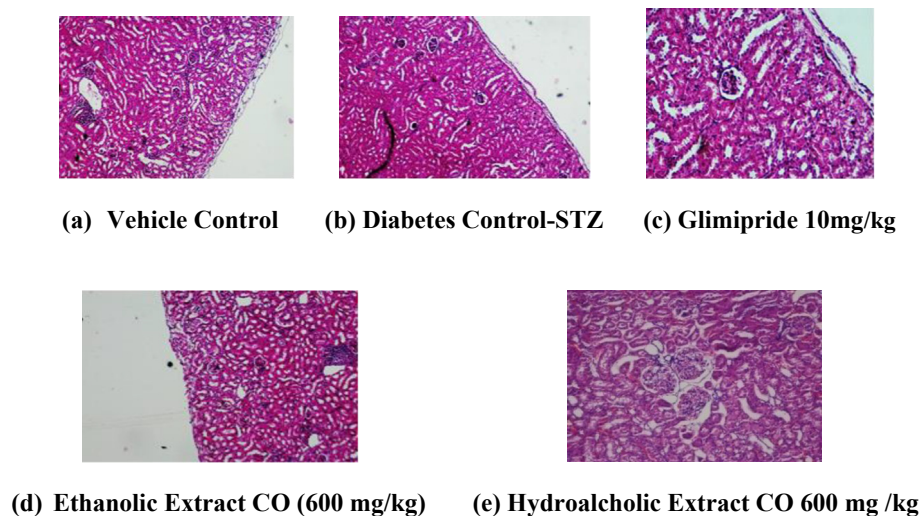
S. No.	Groups	Total Cholesterol (mg/dL)		HDL (mg/dL)		LDL (mg/dL)		TGL (mg/dl)		VLDL (mg/dl)	
		30th Day	75th Day	30th Day	75th Day	30th Day	75th Day	30th Day	75th Day	30th Day	75th Day
1.	Group 1	98.33 ± 3.52	104.30 ± 2.04	59.50 ± 1.12	61.50 ± 0.96	32.90 ± 3.91	33.50 ± 1.64	80.70 ± 2.40	82.70 ± 1.41	16.40 ± 0.48	15.30 ± 0.28
2.	Group 2	245.50 ± 2.70 <sup>a</sup>	299.9 ± 2.10 <sup>a</sup>	24 ± 0.97 <sup>a</sup>	21 ± 0.77 <sup>a</sup>	182.10 ± 2.94 <sup>a</sup>	219.33 ± 2.21 <sup>a</sup>	171 ± 2.14 <sup>a</sup>	212.5 ± 1.61 <sup>a</sup>	35.5 ± 0.43 <sup>a</sup>	42.5 ± 0.32 <sup>a</sup>
3.	Group 3	250.67 ± 1.53 <sup>b</sup>	183.83 ± 1.91 <sup>b</sup>	26.83 ± 0.8 <sup>b</sup>	33.33 ± 0.56 <sup>b</sup>	201.8 ± 1.36 <sup>b</sup>	123.90 ± 1.61 <sup>b</sup>	171.2 ± 2.71 <sup>b</sup>	143 ± 1.27 <sup>b</sup>	34.5 ± 0.54 <sup>b</sup>	28.6 ± 0.25 <sup>b</sup>
4.	Group 4	252.50 ± 3.48 <sup>b</sup>	160.33 ± 2.44 <sup>b</sup>	26.17 ± 1.02 <sup>b</sup>	38.33 ± 0.85 <sup>b</sup>	194.70 ± 3.29 <sup>b</sup>	100.33 ± 3.06 <sup>b</sup>	168.5 ± 2.59 <sup>b</sup>	125.8 ± 1.17 <sup>b</sup>	33.6 ± 0.52 <sup>b</sup>	25.7 ± 0.28 <sup>b</sup>
5.	Group 5	258.33 ± 3.69 <sup>b</sup>	144.33 ± 2.33 <sup>b</sup>	26.50 ± 0.77 <sup>b</sup>	49.70 ± 0.67 <sup>b</sup>	189.76 ± 4.03 <sup>b</sup>	83.40 ± 2.21 <sup>b</sup>	167.3 ± 1.23 <sup>b</sup>	109.33 ± 1.21 <sup>b</sup>	33.67 ± 0.25 <sup>b</sup>	23.67 ± 0.24 <sup>b</sup>
6.	Group 6	270.67 ± 2.24 <sup>b</sup>	177.30 ± 2.12 <sup>b</sup>	28 ± 0.69 <sup>b</sup>	38 ± 0.69 <sup>b</sup>	206.30 ± 2.53 <sup>b</sup>	112.26 ± 2.20 <sup>b</sup>	169.70 ± 2.57 <sup>b</sup>	128.17 ± 1.23 <sup>b</sup>	33.87 ± 0.45 <sup>b</sup>	25.7 ± 0.27 <sup>b</sup>
7.	Group 7	268.30 ± 3.78 <sup>b</sup>	157.70 ± 1.91 <sup>b</sup>	27.17 ± 0.98 <sup>b</sup>	45.33 ± 1.06 <sup>b</sup>	200.80 ± 4.26 <sup>b</sup>	94.66 ± 1.96 <sup>b</sup>	171.33 ± 2.24 <sup>b</sup>	104.83 ± 0.80 <sup>b</sup>	33.5 ± 0.15 <sup>b</sup>	24.67 ± 0.28 <sup>b</sup>
8.	Group 8	265.18 ± 2.65 <sup>b</sup>	136.50 ± 1.79 <sup>b</sup>	27 ± 0.82 <sup>b</sup>	53.83 ± 0.71 <sup>b</sup>	206.16 ± 3.00 <sup>b</sup>	72.26 ± 1.47 <sup>b</sup>	169.50 ± 0.73 <sup>b</sup>	91.67 ± 1.85 <sup>b</sup>	33.5 ± 0.15 <sup>b</sup>	21.1 ± 0.21 <sup>b</sup>
9.	Group 9	271.50 ± 2.63 <sup>b</sup>	139.30 ± 2.19 <sup>b</sup>	28.50 ± 0.77 <sup>b</sup>	39.17 ± 0.80 <sup>b</sup>	196.83 ± 2.99 <sup>b</sup>	84.90 ± 1.59 <sup>b</sup>	169.83 ± 1.43 <sup>b</sup>	93.83 ± 1.80 <sup>b</sup>	33.17 ± 0.29 <sup>b</sup>	20.47 ± 0.36 <sup>b</sup>

Data is expressed as mean ± SEM; Data was analyzed by using one-way ANOVA followed by Tukey's multiple test; <sup>a</sup>*p* < 0.05 as compared to Normal control Group; <sup>b</sup>*p* < 0.05 as compared to diabetic control group.

**Table 3**  
Effect of Ethanolic and Hydroalcoholic extract of *C. orchioides* on anti-oxidant enzymes level and TBARS level.

S. No.	Groups	Catalase (μMole of H <sub>2</sub> O <sub>2</sub> /min)	GSH (μM/mg protein)	SOD (μ/mg protein)	TBARS (nano moles per mg protein)
1.	Group 1	59.5 ± 0.32	74.5 ± 0.47	4.16 ± 0.08	0.48 ± 0.02
2.	Group 2	30.5 ± 0.85 <sup>a</sup>	38.23 ± 0.44 <sup>a</sup>	1.79 ± 0.03 <sup>a</sup>	2.75 ± 0.03 <sup>a</sup>
3.	Group 3	35.87 ± 0.49 <sup>b</sup>	43.97 ± 0.97 <sup>b</sup>	1.92 ± 0.02 <sup>b</sup>	2.01 ± 0.02 <sup>b</sup>
4.	Group 4	44.7 ± 0.46 <sup>b</sup>	58.08 ± 1.41 <sup>b</sup>	2.05 ± 0.03 <sup>b</sup>	1.85 ± 0.01 <sup>b</sup>
5.	Group 5	55.7 ± 0.45 <sup>b</sup>	65.55 ± 0.56 <sup>b</sup>	3.17 ± 0.02 <sup>b</sup>	1.55 ± 0.02 <sup>b</sup>
6.	Group 6	38.03 ± 0.78 <sup>b</sup>	42.9 ± 0.97 <sup>b</sup>	1.97 ± 0.01 <sup>b</sup>	1.99 ± 0.03 <sup>b</sup>
7.	Group 7	54.5 ± 0.68 <sup>b</sup>	59.83 ± 1.41 <sup>b</sup>	2.30 ± 0.03 <sup>b</sup>	1.75 ± 0.01 <sup>b</sup>
8.	Group 8	60.5 ± 0.59 <sup>b</sup>	69.5 ± 0.56 <sup>b</sup>	3.70 ± 0.02 <sup>b</sup>	1.23 ± 0.02 <sup>b</sup>
9.	Group 9	59.7 ± 0.45 <sup>b</sup>	68.51 ± 0.48 <sup>b</sup>	3.67 ± 0.03 <sup>b</sup>	1.17 ± 0.03 <sup>b</sup>

Data is expressed as ± SEM; Data was analyzed by using one-way ANOVA followed by Tukey's multiple test; <sup>a</sup>*p* < 0.05 as compared to Normal control Group; <sup>b</sup>*p* < 0.05 as compared to diabetic control group.

**Fig. 6.** Histological studies of Effect of ethanolic & hydroalcoholic extract of *C. orchioides* on renal tissue of wistar rat by haematoxylin-eosin staining of transverse section of kidney (X = 100).

multiple biochemical pathways resulting in extreme oxidative stress, flawed insulin gene expression, and augmented cell death of  $\beta$ -cells [20]. The association between oxidative stress, dyslipidemia and hyperglycemia is documented in preclinical and clinical studies [7]. Moreover, chronic hyperglycemia induced polyol pathway activation may contribute to osmotic and oxidative stress; an explanation provided for micro-vascular diseases associated with diabetes including DN [22]. Previous studies have shown that oxidative stress contributes to onset of cellular and vascular inflammation, altered  $\beta$ -cell secretion as well as glucose utilization in peripheral muscles and tissues, and thereby leading to secondary complications like DN [7,17,23].

Unrelenting and chronic hyperglycemia is a crucial aspect in growth and succession of DN [17,24]. It has been documented that level of creatinine, uric acid, urea and BUN increases during DN [25]. Apart from increase in metabolic wastes products, renal damage is also facilitated by extreme ROS production under unceasing hyperglycemic circumstances. Augmented boost in the standard indicators of oxidative strain, including increased TBARS, malondialdehyde (MDA), and abridged levels of antioxidant enzymes viz. SOD, GSH have been documented in diabetic subjects [7,26].

In the current study, STZ-NAD administration led to noteworthy rise in TBARS level and decline in antioxidant enzymes level i.e. SOD

and GSH in the kidney tissue. Administration of ethanolic and hydroalcoholic extracts of *C. orchiodies* for 45 days; restricted free radical mediated injury in DN rats through improvement in altitude of antioxidant status and reduction in formation of TBARS. The results obtained are in queue with earlier reported studies. In addition to this, in the present study, renal injury is augmented by decreased clearance of urea, uric acid, and BUN from kidney. Furthermore, DN control rats showed signs of DN as evidenced by increase in polydypsia (urine volume) as well as reduced clearance of creatinine in urine. Administration of ethanolic and hydroalcoholic extracts of *Curculigo Orchiodies* effectively attenuated increased level of renal functional markers viz. uric acid, creatinine, urea and BUN level; Administration of ethanolic and hydroalcoholic extracts of *Curculigo Orchiodies* significantly restored the altered level of renal performance parameters.

*C. orchiodies* is an exceptional traditional medicine and rasayana herb universally known as *Kali Musli* [27]. CO comprises of numerous chemical components like glycosides, phenols, phenolic glycosides, saponins, mucilages and other aliphatic compounds [28] which might have contributed to the observed nephroprotective effect of CO. Previously documented studies regarding phytochemical investigations on the rhizomes of CO have revealed the presence of curculigoside glycosides, benzoic acid, curculiginines, curculigol, and curculigosaponins [29]. It has been documented that phenolic glycosides of CO exhibit anti-diabetic potential by increasing the peripheral utilization of glucose [29].

## 5. Limitations and future scope of study

Mechanistic exploration of the active constituents may provide an insight into the mechanism of action as well as can pave the way to use *C. orchiodies* as a remedy in the treatment of diabetic nephropathy.

## 6. Conclusion

The data obtained suggests that high dose administration of ethanolic and hydroalcoholic extract of rhizomes of *C. orchiodies* (600 mg/kg) produced significant effect in ameliorating the progression and development of STZ induced DN. The present study confirms that alcoholic and hydroalcoholic extract of *C. orchiodies* rhizome possesses anti-diabetic and anti-oxidant activity as well as nephroprotective properties.

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## Conflict of interest

None declared.

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