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Evaluation of Anti-Diabetic Effects of *Chrysopogon zizanioides* Linn Root Extracts in Streptozotocin Induced Diabeteic Wistar Rats

G. Rajeswari, V. Rajagopalan

Abstract

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage; dysfunction and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels number of plants have been described in ayurveda and other tradional for the management of diabetes. We are selected *Chrysopogon zizanioides*linn. *Chrysopogon zizanioides*linn extract prepare in sox let methanol extraction 45° c in 48hrs.the phytochemical analysis in qualitative analysis and that active bioactive compound identified on through gc-ms,diabetics are induced on streptotrozin ,the methanolic extract of plant on oral administration. The experimental animal tissue estimation of blood glugose, plasma insulin, hemoglobin, serum lipid profile, enzymatic antioxidant activity and non enzymatic anti oxidants activity, histological observation .the present show that crysopogen zizaniodoslinn was having glycimic control and its having antioxidant hypolipidemic properties in streptozin induced albino rat.

Keywords: *Chrysopogon zizanioides* Linn, Anti-diabetic Activity, Medicinal plant, Streptozotocin, Acute Toxicity Study

Introduction

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. The chronic hyperglycemia of diabetes is associated with long-term damage, dysfunction and failure of various organs, especially the eyes, kidneys, nerves, heart, and blood vessels. Symptoms of marked hyperglycemia include polyuria, polydipsia, weight loss, sometimes with polyphagia, and blurred vision. Impairment of growth and susceptibility to certain infections may also accompany with chronic hyperglycemia. Patients with diabetes have an increased incidence of atherosclerotic cardiovascular, peripheral arterial and cerebrovascular disease. Hypertension and abnormalities of lipoprotein metabolism are often found in people with diabetes. Mortality and morbidity associated with diabetes is mainly due to complications arising from it which include neuropathy, nephropathy, vasculopathy and retinopathy. In the past few decades, type 2 diabetes mellitus has rapidly increased in the world. It has been estimated that the number of diabetic patients will be more than double within 15 years.[1]

The world prevalence of diabetes among adults (aged 20–79 years) will be 6.4%, affecting 285 million adults, in 2010, and will increase to 7.7% and 439 million adults by 2030. Between 2010 and 2030, there will be a 69% increase in numbers of adults with diabetes in developing countries and a 20% increase in developed countries (Shaw et al., 2010). WHO has predicted that India would experience the largest increase (48% increase in total population and 168% increase in population with >65 years of age) in type 2 diabetes and would have the greatest number of diabetic individuals in the world by the year 2030 (31.7 million in 2000 to 79.4 million in 2030). In India, diabetes in adult urban populations varies from a low of 5.4% in a northern state to a high of 12.3–15.5% in Chennai, South India, and 12.3–16.8% in Jaipur, Central India.[2]

Phytochemicals

Natural products from medicinal plants continue to form a common platform for the discovery of new chemical entities in the modern drug discovery programmes. A wide array of plant derived active principles (phytochemicals) for possible use in the treatment of type 2 diabetes mellitus has been reported.

Chrysopogon zizanioides

Chrysopogon zizanioides, also known as khas khas, khas or khus grass, is native to India. It is adensely tufted grass, with long, thin and rigid leaves and can grow up to 1.5 meters high. The grass grows well in rich marshy soil that is found throughout the plains and lower hills of India, especially on the riverbanks. The plant is different from the other grass forms, in that instead of having mat-like root systems, it befits in growing downwards and can grow up to 2-4 meters in depth. The plant is well known for its oil that is used in medicine and perfumery. Along with this, Khas Khas is also used for cooling purposes, flavoring sharbats, and making mats, hand fans etc. Today the plant is cultivated in the North Indian states of Rajasthan, Uttar Pradesh and Punjab and in the South Indian states of Kerala, Tamil Nadu. Its main chemical components are benzoic acid, vetiverol, furfurol, a and b-vetivone,vetivene and vetivenyl vetivenate. The chemical components of the oil obtained from the plant is benzoic acid, furfurol, vetivene, vetivenyl vetivenate, terpinen-4-ol, 5-epiprezizane, khusimene, a-muurolene, khusimone, calacorene, ß-humulene, a-longipinene, , d-selinene,d-cadinene, valencene, calarene,-gurjunene, a-amorphene, epizizanal, 3-epizizanol, khusimol, iso-khusimol, valerenol, ß-vetivone, a-vetivone, vetivazulene.Khasgrass, particularly in North Indian plains, takes a leading role.

Various tribes use the different parts of the grass for many of their ailments such as mouth ulcer, fever, boil, epilepsy,burn, snakebite, scorpion sting, rheuma-tism, fever, headache, etc. The Santhal tribes of Bihar and West Bengal use the paste of fresh roots for burn, snakebite and scorpion sting, and a decoction of the roots as a tonic for weakness; the Lodhas of West Bengal use the root paste for headache, rheumatism and sprain, and a stem decoction for urinary tract infection; the Mandla and Bastar tribes of Madhya Pradesh use the leaf juice as anthelmintic; the tribes of the Varanasi district inhale the root vapour for malarial fever. The root ash is given to patients for acidity by the Oraon tribe. Likewise, there are very many different applications of the plant for different ailments among different ethnic tribes. Apart from the medicinal uses, the culms along with the panicles form a good broom for sweeping. The culms and leaves are also extensively used by the tribes and villagers for thatching their huts, mud walls, etc. Some tribes (in Kerala) use the mats of the roots and leaves as bed for a cooling effect. Since, no work has been carried out so far on methanolic extract for diabetes management therefore the present study was undertaken to evaluate the antidiabetic potential of ethanol extract as of *Chrysopogon zizanioides* in STZ induced diabetic rats.

Materials and methods

Scientific classification of *Chrysopogon zizanioides*

Kingdom	:	Plantae
Class	:	Angiosperms
Sub class	:	Monocots
Order	:	Poales
Family	:	Poaceae
Genus	:	Chrysopogon
Species	:	*C. zizanioides*

Figure1: *Chrysopogon zizanioides* Linn

Chrysopogon zizanioides is commonly known Vetiver, Khas –khas grass in English; Khas in Hindi; Ramacham in Malayalam. A perennial aromatic grass grows up to 2 meter in height. Leaves narrow, linear, erect; spikelets grey in a panicle of numerous racemes, fruits oblong grains. It distributed Throughout India growing wild in planes and lower hills and also cultivated. Plant pacifies vitiated pitta, vata, burning sensation, hyperdipsia, ulcer, skin diseases, vomiting nausea, flatulence, dyspepsia, colic, cough, fever, low back pain, headache and general debility.

Plant Material

Fresh root of *Chrysopogon zizanioides* was collected from Pachamalai Hills of Trichy and identified to confirm by the Taxonomist Botanical Survey of India, Tamilnadu, India.

Plant extraction

The roots were cut into pieces and shade dried at room temperature. The dried leaves were subjected to size reduction to a coarse powder by using dry grinder and passed through sieve. 1kg of crushed root was continuously extracted with 95% methanol using soxlet up to 48 hrs. The extract was filtered and concentrated in rotatory evaporator at 35- 40 ºC under reduced pressure to obtain a semisolid material, which was then lyophilized to get a powder.

Phytochemical Screening

Screening of phytochemical constituents of the plant was doneusing standard procedures described by several authors' qualitative analysis and quantitative analysis. Shadow-dried root were used to identify and characterize.

Experimental Animals

Albino wistar male rats 7 – 8 weeks old, weighing 150-200 g, were used for the present study as shown in Figure 4. Animals were housed under standard conditions of temperature (24±2°C) and relative humidity (30-70%) with a 12:12 light: dark cycle. The animals were fed with standard pellet diet (Chakan Oil Mills, Sangli) and water ad libitum. Animal handling was performed according to Good Laboratory Practice (GLP). Ethical clearance was obtained from Institutional Animal Ethics Committee and conducted according to the Indian National Science Academy guidelines for the use and care of experimental animals (CPCSEA/265).

Acute Toxicity Study

Acute toxicity study was performed according to OECD

Group I	:	Control rats given with citrate buffer (pH 4.5) orally.
Group II	:	Rats were made diabetic by a single intraperitoneal injection of streptozotocin (40mg/kg bw) with citrate buffer (pH 4.5).
Group III	:	Diabetic rats treated with (100mg/kg bw) of *Chrysopogon zizanioides* methanolic root extract daily by oral administration for 4 weeks.
Group IV	:	Diabetic rats treated with (200mg/kg bw) of *Chrysopogon zizanioides* methanolic root extract daily by oral administration for 4 weeks.
Group V	:	Diabetic rats treated with (400mg/kg bw) of *Chrysopogon zizanioides* methanolic root extract daily by oral administration for 4 weeks.
Group VI	:	Diabetic rats treated with glibenclamide (5 mg/kg) and served as reference stranded treatment continued for 4 weeks.

guidelines 423. The method used standard procedure.[3]

Experimental Design

Animals were randomized and divided into six groups of six animals each

After the termination of the experiment all the animals were anesthetized using ketamine chloride (24mg/kg bw) and sacrificed by cervical dislocation after an overnight fast. Blood was collected and tissues (pancreas) were immediately removed, blotted and kept at -200C until use. Plasma, serum and tissue homogenates were separated after centrifugation and used for various biochemical estimations.

Processing of blood and tissue samples

Serum preparation

Blood was collected in a dry test tube and allowed to coagulate at ambient temperature for 30 min. Serum was separated by centrifugation at 2000 rpm for 10 min.

Tissue Sampling For Histopathological Study

For histopathological study, rat each group were chosen, the organs removed and perfused with cold physiological saline, followed by formalin (10% formaldehyde). The pancreas were excised immediately and fixed in 10% formalin. Then dehydrated on treatment with a series of different concentrations of ethanol and embedded in paraffin wax. 3-5µm thick sections were cut using a microtome and stained with hematoxylin and eosin. The specimens were evaluated with light microscope. All histopathological changes were examined by a pathologist.

Biochemical Determinations

• Glucose was estimated by the method of Trinder using reagent Kit.

• Plasma insulin was assayed by the solid phase system amplified sensitivity immunoassay using reagent kits obtained from Medgenix-INS-ELISA, Biosource, Europe S.A., Belgium.[4]

• Haemoglobin in the blood was estimated by the method of Drabkin and Austin.[5]

Analisis of Lipid Profile

• Total cholesterol in the plasma and tissues was estimated by the enzymic method described by Allain et al.[6]

• HDL-cholesterol was estimated using the diagnostic kit based on the enzymic method described by Izzo et al.[7]

• Estimation of vldl-and ldl-cholestrol est Friedewald et al.[8]

• Triglycerides in the plasma and tissues were estimated using the diagnostic kit based on the enzymic method described by McGowan et al.[9]

• Phospholipids were estimated by the method of Zilversmit and Davis.[10]

• The concentration of TBARS in the plasma and tissues was estimated by the method of Niehaus and Samuelsson.[11]

Non Enzymatic Antioxidants

• Reduced glutathione in the plasma, erythrocytes and tissues was estimated by the method of Ellman.

• Ascorbic acid in the plasma and tissues was estimated by the method of Roe and Kuether.

• α-tocopherol in the plasma, erythrocytes and tissues was estimated by the method of Baker et al.

Enzymatic Antioxidants

•Superoxide dismutase in the erythrocytes and tissues was assayed by the method of Kakkar et al.

•The activity of catalase in the erythrocytes and tissues was determined by the method of Sinha.

•The activity of GPx in the erythrocytes and tissues was measured by the method of Rotruck et al.

Statistical Analysis

All results are presented as mean ± SEM Data were analyzed by the student's T test. Groups for the pair of observations depended upon each other. Results were considered statistically at $P < 0.001$.

Results

Phytochemicals

The successive extracts of *Chrysopogon zizanioides* have revealed and the presence of alkaloids, flavonoids, saponins and tannins result were presented in Table.1 and the GCMS result were presented in Figure 2.

Table 1: Phytochemical analysis of *Chrysopogon zizanioides* root particle

S. No	Phytochemical Test	Status of the compound
1.	Alkaloid	Presence
2.	Flavanoid	Presence
3.	Saponins	Presence
4.	Tannis	Presence

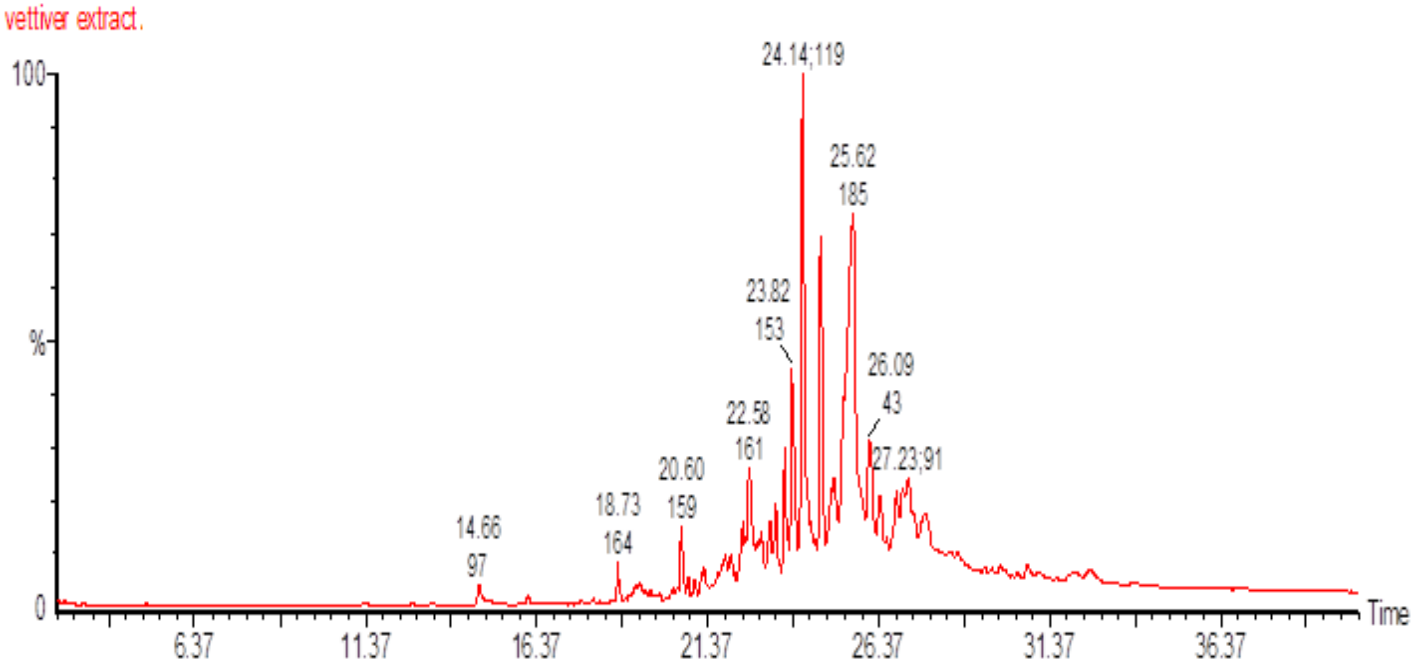

Figure 2: GC-MS chromatogram of methanol root extract of *Chrysopogen zizanaidis* Linn

Estimation of Body Weight

The body weight changes in control and experimental groups were illustrated in Table 2. The body weight of diabetic rats significantly decreased when compared with control group. Supplementation of methanolic extracts of *Chrysopogon zizanioides* showed a significant improvement in the body weight of diabetic rats. There were no significant changes observed between control treated group animals.

Table 2: Effect of *Chrysopogon zizanioides* methanolic extract on the changes of body weight of control and experimental rats

S. No	Groups	Body weight (g)	
		Initial (0 day)	Final (28 days)
1	Control	205.15 ± 19.12	230.21 ± 19.14
2	Diabetic	182.04± 1.2	149.11 ± 11.4*
3	Diabetic + *C. zizanioides* methanolic extract (100 mg/kg bw)	185.05± 3.02	188.19± 2.45
4	Diabetic + *C. zizanioides* methanolic extract (200 mg/kg bw)	190.13 ± 18.11*	195.14± 12.21*
5	Diabetic + *C. zizanioides* methanolic extract (400 mg/kg bw)	198.06 ± 19.43*	229.02 ± 11.41**
6	Glibenclamide (5mg/Kg)	202 ± 11.25 **	225 ±12.35 **

Values are given as mean ± S.D (n=6 rats)

*P<0.01 Vs control

**P<0.001Vs control by students't' test

Estimation of Blood Glucose, Plasma Insulin and Heamoglobulin

Table 3 shows the blood glucose plasma insulin and total hemoglobulin levels of normal and experimental rats. There was a significant increased level of blood glucose and plasma insulin and decreased hemoglobulin was observed in diabetes animals compared to the corresponding control group. Treatment with *Chrysopogon zizanioides* methanolic root extract decreased the levels of blood glucose and plasma insulin and increase hemoglobulin level in diabetic group of rats.

Table 3: Effect of *Chrysopogon zizanioides* methanolic extract on the levels of blood glucose, plasma insulin and Hemoglobulin in control and experimental rats

S.No	Groups	Blood glucsoe (mg/dL)	Plasma insulin(µg/mL) increase	Total hemoglobin (g/dL)
1	Control	86.44 ± 8.81	16.27 ±0.13	12.47 ± 1.35
2	Diabetic	285.11± 19.33	7.28 ± 1.54	8.97 ± 0.49
3	Diabetic + *C. zizanioides* methanolic extract (100 mg/kg bw)	108.14 ± 4.57*	11.06 ± 1.21*	11.88 ± 0.45*
4	Diabetic + *C. zizanioides* methanolic extract (200 mg/kg bw)	103.11 ± 4.57*	14.05 ± 1.21*	13.07 ± 1.58*
5	Diabetic + *C. zizanioides* methanolic extract (400 mg/kg bw)	90.04 ± 3.12**	17.26 ± 1.05**	13.99 ± 0.45**
6	Glibenclamide (5mg/Kg)	84.27 ±6.23 **	15.16 ±2.43 **	14.88±3.12 **

Values are given as mean ± S.D (n=6 rats)

* P<0.01 Vs control

**P<0.001Vs control by student's' test.

Etmatison Serum Lipid Profile

As shown in Table 4 methanolic extracts of *Chrysopogon zizanioides* administration in diabetic rats serum lipids levels. The Triglycerides, VLDL, LDL, and the Total cholesterol levels were significantly decreased and HDL level improved in oral administration of *Chrysopogon zizanioides* root methanolic extract in 400mg/kg of body weight, which was compared to that of glibenclamide group. However, no significant changes were observed control treated groups.

Table 4: Effect of *Chrysopogon zizanioides* methanolic root extract on lipid profile of control and experimental rats

S.No	Treatment	TGL Mg/dl	HDL Mg/dl	VLDL Mg/dl	LDL Mg/dl	Total C Cholesterol	Serum Phospho-lipid(mg/dL)
1	Control	77.15±6.68	39.6±2.71	17.03±1.51	43.9±4.25	98.16±8.76	106.61 ± 5.09
2	Diabetic	133.32±10.05	25.31±1.64	32.14±2.86	96.47±8.12	215.2±7.23	67.18 ± 4.27
3	Diabetic + C. zizanioides methanolic extract (100 mg/kg bw)	104.45±11.14	27.56±2.62	31.24±1.64	94.19±6.77	182.86±10.05	71.12 ± 5.17
4	Diabetic + C. zizanioides methanolic extract (200 mg/kg bw)	94.18 ± 8.76*	37.23 ± 3.06*	30.19±2.8*	88.41±2.34	172.3±5.12*	87.51 ± 4.19*
5	Diabetic + C. zizanioides methanolic extract (400 mg/kg bw)	89.49±7.45**	38.27±2.56**	21.52±3.4**	71.27±5.28**	115.6±6.90**	93.48 ± 5.36**
6	Glibenclamide (5mg/Kg)	82.04±6.71**	38.91±5.14**	16.92±1.34**	33.9±2.66**	96.2±4.8**	99.73 ± 5.44**

Values are given as mean ± S.D (n=6 rats)

* P<0.01 Vs control, **P<0.001Vs control by students't' test

Estimation of Enzymatic, Non- Enzymatic Antioxidant and Lipid Peroxidation

Table 5 shows the inhibition of antioxidant activity during STZ induced toxicity may be due to the improved generation of reactive free radicals, which can create an oxidative stress in the cells. The administration *Chrysopogon zizanioides* 100, 200 and 400mg/kg doses inversed the SOD, CAT, GPx, GSH, Vitamin C and E activity as well as TBARS level in the blood plasma, which protected from the free radical induced oxidative stress. This results supports that, the antioxidant properties of the *Chrysopogon zizanioides* was excellent as compared with the standard drug glibenclamide.

Table 5: Effect of *Chrysopogon zizanioides* methanolic extract on enzymatic and non-enzymatic level in control and experimental rats

S.No	Treatment	SOD (mg %)	CAT (mg %)	GPX (mg %)	GSH (mg %)	Vitamin C	Vitamin E	Plasma TBARS (nmol/ml)
1	Control	3.86 ± 0.16	14.98±0.79	6.08±0.21	26.31±1.35	1.64± 0.09	1.96+ 0.08	3.07±0.15
2	Diabetic	2.08 ±0.07	7.35± 0.27	3.02±0.17	18.67±0.92	0.91± 0.05	3.28 + 0.15	7.14±0.25
3	Diabetic + ***C. zizanioides*** methanolic extract (100 mg/kg bw)	2.19±0.09	10.17±2.31	3.30±8.47	20.11±1.12	1.45±0.08	3.01±0.12	4.12±0.21
4	Diabetic + ***C. zizanioides*** methanolic extract (200 mg/kg bw)	3.05±0.21*	10.85±5.12*	4.11±1.44*	22.14±3.21*	1.81±0.07*	2.99±1.24*	4.07±1.27*
5	Diabetic + ***C. zizanioides*** methanolic extract (400 mg/kg bw)	3.68±0.12 **	12.96±0.52**	5.89±0.24**	24.12±1.38**	2.97± 0.11**	2.78± 0.13**	3.14±0.17**
6	Glibenclamide (5mg/Kg)	3.54±0.15 **	12.91±0.55**	5.71±0.25**	23.78±1.58**	2.81± 0.15**	2.54± 0.12**	3.55± 0.11**

Values are given as mean ± S.D (n=6 rats)

* P<0.01 Vs control, **P<0.001Vs control by students‘t’ test

Histologial Observation

Histological findings of pancreas in the extract-administered group and control group were similar. The orally administered *Chrysopogon zizanioides* to STZ-induced diabetic rats elicited a significant anti-diabetic activity and significantly ($p < 0.001$) increased the plasma insulin levels. Methanol extract of *Chrysopogon zizanioides* treated groups IV and V (Figure 8 and 9) rats showed a significant ($p < 0.00\ 1$) increase in plasma insulin level when compared with group II (Figure 7). Since there was no significant different was identified in the plasma insulin levels between groups I (Figure 6) and VI (Figure 10).

Figure 6: Histology of islet of langerhans of rat (Control)

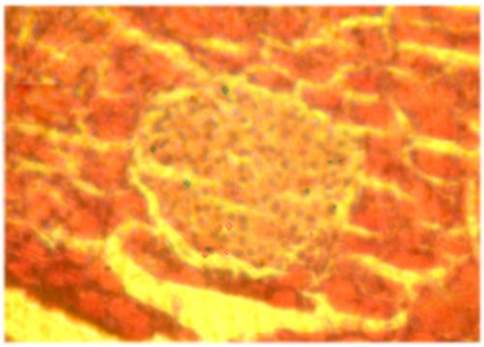

Figure 8: Histology of islet of langerhans of rat treated with *Chrysopogon zizanioides* (200 mg/kg)

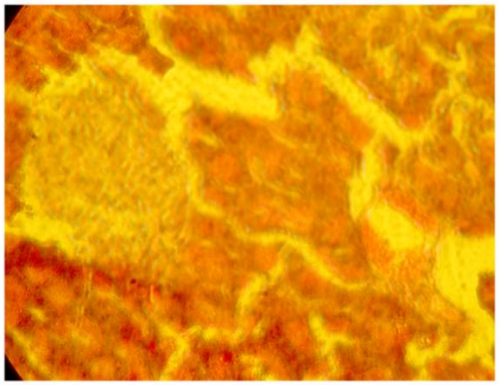

Figure 7: Histology of islet of langerhans of Diabetic rat (STZ- induced)

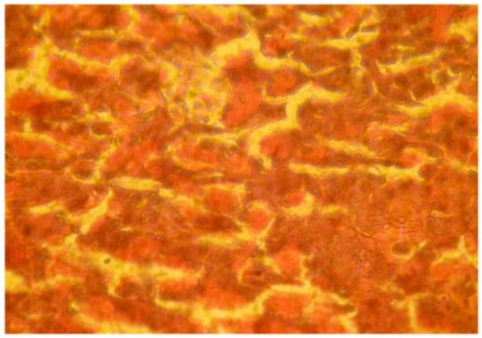

Figure 9: Histology of islet of langerhans of rat treated with *Chrysopogon zizanioides* (400 mg/kg)

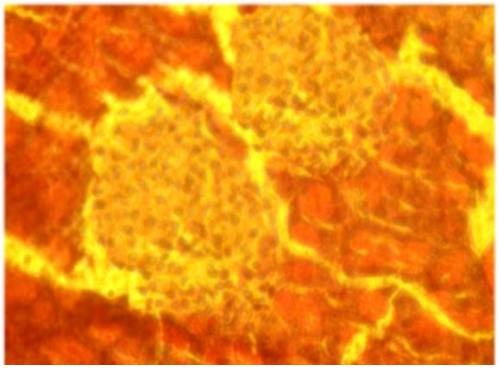

Figure 10: Histology of islet of langerhans of rat treated with Glibenclaminde (5 mg/kg)

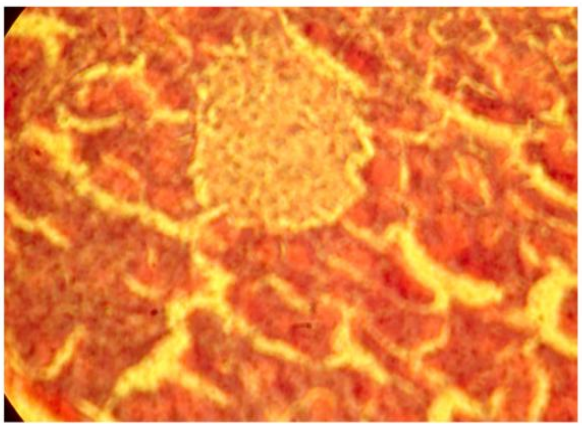

Figure 4 of: Figure: 6, 7, 8, 9, and 10 "Histologial Observation"

Gc-Ms Analysis

The phytochemical compounds present in the methanol extracts of *Chrysopogon zizanioides* was identified by GC-MS analysis. The active principles with their retention time (RT), molecular formula (MF) concentration (%) in the extract was presented. Totally forty four compounds identified from the methanol extract of the *Chrysopogon zizanioides* are presented in Table 6.

Table 6: Qualitative and quantitative determination of biochemical constituents in *Chrysopogon zizanioides* by GC-MS

S.No.	Peak Name	Retention time	Peak area	%Peak area
1.	Name: 2-Butanone, 4,4-diethoxy- Formula: $C_8H_{16}O_3$ MW: 160	4.96	3865589	0.0736
2.	Name: 3,5-Octadien-2-one Formula: $C_8H_{12}O$ MW: 124	11.33	24058104	0.4580
3.	Name: Glycerin Formula: $C_3H_8O_3$ MW: 92	12.71	8608481	0.1639
4.	Name: 2-Methoxy-4-vinylphenol Formula: $C_9H_{10}O_2$ MW: 150	16.10	19417574	0.3697
5.	Name: Biphenylene, 1,2,3,6,7,8,8a,8b-octahydro-4,5-dimethyl- Formula: $C_{14}H_{20}$ MW: 188	16.50	6225470	0.1185
6.	Name: Phenol, 2-methoxy-4-(1-propenyl)-, (E)- Formula: $C_{10}H_{12}O_2$ MW: 164 .(E)-Isoeugenol	17.86	1688392	0.0321
7.	Isovanillin	17.98	8225777	0.1566

	Formula: $C_8H_8O_3$ MW: 152			
8.	Name: Phenol, 2-methoxy-4-(1-propenyl)-, (E)- Formula: $C_{10}H_{12}O_2$ MW: 164	18.73	77665104	1.4786
9.	Name: 2-Butanone, 4-(2,6,6-trimethyl-2-cyclohexen-1-ylidene)- Formula: $C_{13}H_{20}O$ MW: 192	18.96	1631735	0.0311
10.	Name: à-Muurolene Formula: $C_{15}H_{24}$ MW: 204	19.09	457725	0.0087
11.	ç-Gurjunene Formula: $C_{15}H_{24}$ MW: 204	19.26	5691463	0.1084
12.	.à-Curcumene Formula: $C_{15}H_{22}$ MW: 202	19.69	8243792	0.1569
13.	Name: 17-Norkaur-15-ene, 13-methyl-, (8á,13á)- Formula: $C_{20}H_{32}$ MW: 272 .(+)-Beyerene	19.79	533831	0.0102
14.	Name: á-Vatirenene Formula: $C_{15}H_{22}$ MW: 202	19.97	11397908	0.2170
15.	Name: 4-Pentenoic acid, 4-(4-methylphenyl)-, ethyl ester Formula: $C_{14}H_{18}O_2$	20.14	2617508	0.0498

	MW: 218			
16.	Name: à-Calacorene Formula: $C_{15}H_{20}$ MW: 200	20.31	8467667	0.1612
17.	Name: 9-Methyl-S-octahydrophenanathracene Formula: $C_{15}H_{20}$ MW: 200	20.49	3620166	0.0689
18.	Name: á-Vatirenene Formula: $C_{15}H_{22}$ MW: 202	20.60	116183280	2.2118
19.	Name: 2-(4a,8-Dimethyl-2,3,4,4a,5,6,7,8-octahydro-2-naphthalenyl)-2-propanol Formula: $C_{15}H_{26}O$ MW: 222	20.80	25066514	0.4772
20.	Name: Benzene, 1-(1,2-dimethyl-3-methylenecyclopentyl)-4-methyl-, cis- Formula: $C_{15}H_{20}$ MW: 200 Laurene	21.00	24209180	0.4609
21.	Name: Hinesol Formula: $C_{15}H_{26}O$ MW: 222	21.27	82400352	1.5687
22.	.(+)-Valencene Formula: $C_{15}H_{24}$ MW: 204	21.65	3921236	0.0747
23.	Juniper camphor Formula: $C_{15}H_{26}O$ MW: 222	21.88	40318052	0.7676
24.	(ñ)-Cadinene	22.05	38146376	0.7262

	Formula: $C_{15}H_{24}$ MW: 204			
25.	Name: à-Cadinol Formula: $C_{15}H_{26}O$ MW: 222	22.38	42059976	0.8007
26.	Name: Longifolene-(V4) Formula: $C_{15}H_{24}$ MW: 204	22.58	186343632	3.5475
27.	Name: 6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol Formula: $C_{15}H_{24}O$ MW: 220	22.94	41527828	0.7906
28.	Name: [5,5-Dimethyl-6-(3-methyl-buta-1,3-dienyl)-7-oxa-bicyclo[4.1.0]hept-1-yl]-methanol Formula: $C_{14}H_{22}O_2$ MW: 222	23.18	70620832	1.3444
29.	Name: ç-Himachalene Formula: $C_{15}H_{24}$ MW: 204	23.36	119292784	2.2710
30.	à-Gurjunene Formula: $C_{15}H_{24}$ MW: 204	23.61	198876016	3.7861
31.	Name: 7-Acetyl-2-hydroxy-2-methyl-5-isopropylbicyclo[4.3.0]nonane Formula: $C_{15}H_{26}O_2$ MW: 238	23.82	464353472	8.8401
32.	Name: cis-à-Copaene-8-ol	24.14	1107401984	21.0822

	Formula: $C_{15}H_{24}O$ MW: 220			
33.	Name: Azulene, 1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1R-(1à,3aá,4à,7á)]- Formula: $C_{15}H_{24}$ MW: 204 ç-Gurjunene	24.67	629248256	11.9793
34.	Name: 2(3H)-Naphthalenone, 4,4a,5,6,7,8-hexahydro-4,4a-dimethyl-6-(1-methylethenyl)-, [4R-(4à,4aà,6á)]- Formula: $C_{15}H_{22}O$ MW: 218	24.98	64122004	1.2207
35.	Name: 7-Isopropenyl-1,4a-dimethyl-4,4a,5,6,7,8-hexahydro-3H-naphthalen-2-one Formula: $C_{15}H_{22}O$ MW: 218	25.08	30933148	0.5889
36.	Isokhusenic acid Formula: $C_{15}H_{22}O_2$ MW: 234	25.64	1177313792	22.4131
37.	Name: Cyclohexene, 1,3-diisopropenyl-6-methyl- Formula: $C_{13}H_{20}$ MW: 176	26.09	253305584	4.8223
38.	Name: 3,5,7-Nonatrien-2-one, 8-methyl-7-(1-methylethyl)-, (E,E)- Formula: $C_{13}H_{20}O$ MW: 192	26.40	107588672	2.0482
39.	Name: 5(1H)-Azulenone, 2,4,6,7,8,8a-hexahydro-3,8-dimethyl-4-(1-methylethylidene)-, (8S-cis)-	26.58	14559006	0.2772

	Formula: $C_{15}H_{22}O$ MW: 218			
40.	Name: ç-Himachalene Formula: $C_{15}H_{24}$ MW: 204	26.89	90707752	1.7269
41.	Name: 2'-Hydroxy-5'-methylacetoacetophenone Formula: $C_{11}H_{12}O_3$ MW: 192	27.24	55777856	1.0619
42.	Elixene Formula: $C_{15}H_{24}$ MW: 204	29.45	11204467	0.2133
43.	Name: Eudesma-5,11(13)-dien-8,12-olide Formula: $C_{15}H_{20}O_2$ MW: 232	29.92	17771598	0.3383
44.	Name: 6-(1-Hydroxymethylvinyl)-4,8a-dimethyl-3,5,6,7,8,8a-hexahydro-1H-naphthalen-2-one Formula: $C_{15}H_{22}O_2$ MW: 234	32.52	47114620	0.8969

The plant sample relived the synthesis of2-Butanone 4,4-diethoxy; 3,5-Octadien-2-one; Glycerin; 2-Methoxy-4-vinylphenol; Biphenylene, 1,2,3,6,7,8,8a,8b-octahydro-4,5-dimethyl; Phenol, 2-methoxy-4-(1-propenyl)-, (E); Isovanillin; Phenol, 2-methoxy-4-(1-propenyl)-, (E); 2-Butanone, 4-(2,6,6-trimethyl-2-cyclohexen-1-ylidene); α-Muurolene; α –Gurjunene; α –Curcumene; 17-Norkaur-15-ene, 13-methyl-, (8á,13á); α-Vatirenene; 4-Pentenoic acid, 4-(4-methylphenyl)-, ethyl ester; α-Calacorene; 9-Methyl-S-octahydrophenanathracene; α-Vatirenene; 2-(4a,8-Dimethyl-2,3,4,4a,5,6,7,8-octahydro-2-naphthalenyl)-2-propanol; Benzene, 1-(1,2-dimethyl-3-methylenecyclopentyl)-4-methyl-, cis; Hinesol; (+)-Valencene; Juniper camphor; (ñ)-Cadinene; α –Cadinol; Longifolene-(V4); 6-Isopropenyl-4,8a-dimethyl-1,2,3,5,6,7,8,8a-octahydro-naphthalen-2-ol; 5,5-Dimethyl-6-(3-methyl-buta-1,3-dienyl)-7-oxa-bicyclo[4.1.0]hept-1-yl]-methanol; ç-Himachalene; à-Gurjunene; 7-Acetyl-2-hydroxy-2-methyl-5-isopropylbicyclo[4.3.0]nonane; cis-à-Copaene-8-ol; Azulene, 1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7-(1-methylethenyl)-, [1R-(1à,3aá,4à,7á)]-; 2(3H)-Naphthalenone, 4,4a,5,6,7,8-hexahydro-4,4a-dimethyl-6-(1-methylethenyl)-, [4R-(4à,4aà,6á)]-; 7-Isopropenyl-1,4a-dimethyl-4,4a,5,6,7,8-hexahydro-3H-naphthalen-2-one; Isokhusenic acid; Cyclohexene, 1,3-diisopropenyl-6-methyl; 3,5,7-Nonatrien-2-one, 8-methyl-7-(1-methylethyl)-, (E,E); 5(1H)-Azulenone, 2,4,6,7,8,8a-hexahydro-3,8-dimethyl-4-(1-methylethylidene)-, (8S-cis);

α-Himachalene; 2'-Hydroxy-5'-methylacetoacetophenone; Elixene; Eudesma-5,11(13)-dien-8,12-olide; 6-(1-Hydroxymethylvinyl)-4,8a-dimethyl-3,5,6,7,8,8a-hexahydro-1H-naphthalen-2-one. The GC-MS extracts of *Chrysopogon zizanioides* these compounds are of chromatogram of methanol extracts of *Chrysopogon zizanioides* is shown. All these compounds are of pharmacological importance as they possess the properties such as anti-diabetic, analgesic, antibacterial, and antifungal activity.

Discussion

Diabetes mellitus is a chronic metabolic disorder of carbohydrates, proteins and fat due to absolute or relative deficiency of insulin secretion with/without varying degree of insulin resistance. It is characterized with chronic high blood glucose that could lead to morbidity and mortality. The number of people suffering from diabetes worldwide is increasing at an alarming rate. Diabetes mellitus is the most common serious metabolic disorder and it is considered to be one of the five leading causes of death in the world. A recent study predicting the worldwide prevalence of diabetes will increase from 2.8% in 2000 to 4.4% in 2030, resulting in 366 million affected people. [12] A number of investigations, of oral antihyperglycemic agents from plants used in traditional medicine, have been conducted and many of the plants were found with good activity.

In the present study, Streptozotocin-induced diabetes is characterized by severe loss in body weight due to the degradation of structural proteins, which are responsible for the changes in body weight. However, treatment with *Chrysopogon zizanioides* methanolic was significantly increased the body weight suggested its recover activity against diabetes. Because the extract treatment in STZ rat led to marked increase in body weight due to increased adipose tissue mass, an observation also seen in humans treated.

On the other hand, our results showed that a STZ results in significant increase in plasma glucose, insulin level and decrease in blood Hb level. Increased insulin has been found to be a consequence of diabetic complications. Several studies documented that insulin resistance most often precedes the onset of overt type 2 diabetes and is compensated initially by hyperinsulinemia.[13] This hyperinsulinemia is due to hyper secretion and by reduced hepatic extraction of insulin. But this chronic secretion of large amounts of insulin to overcome tissue insensitivity can itself finally lead to pancreatic beta cell failure and occurrence of hyperglycemia, decreased tissue sensitivity of insulin may be responsible for decreased Hb synthesis. In the present study, oral administration of *Chrysopogon zizanioides* produces a significant decrease in plasma glucose, insulin level and increase in blood Hb level which might be due to improved glycemic control.

Diabetes is associated with profound alterations in the plasma lipid, TC and lipoprotein profile and with an increased risk of coronary heart disease. Lowering the plasma lipid levels through dietary or drug therapy appears to be associated with a decrease in the risk of vascular disease.[14] In the present study, we observed higher levels of cholesterol in the plasma and tissues of STZ rat. Administration of *Chrysopogon zizanioides* to STZ rat decreased the levels of cholesterol. Normally, circulating LDL-C undergoes reuptake in the liver via specific receptors and gets cleared from the circulation. This increased LDL concentration in the plasma of STZ rat might be due to the defect in LDL-C receptor either through failure in its production (or) function. HDL-C is protective by reversing cholesterol transport, inhibiting the oxidation of LDL-C and by neutralizing the atherogenic effect of oxidized LDL-C. A greater increase of LDL-C and VLDL-C may also cause a greater decrease of HDL-C as there is a reciprocal relationship between the concentration of VLDL-C and HDL-C. Decreased HDL-C may also be due to diminished lecithin cholesterol acyl transferase activity. HFD treated with BT showed a significant elevation in HDL-C and reduction in LDL-C and VLDL-C. Thus, BT could alleviate the risk of cardiovascular diseases.

Hypertriglyceridemia is a common finding in patients with DM and is responsible for vascular complications. Braun and Severson have reported that deficiency of LPL activity may contribute significantly to the elevation of TG in diabetes.[15] The abnormal high concentration of serum lipids in STZ rat subjects is mainly due to the increase in the mobilization of FFA from fat depots. PL are vital components of biomembranes rich in polyunsaturated fatty acids, which are susceptible substrate for free radicals, such as $O_2^{\bullet -}$ and •OH radicals. These PL are important for the maintenance of cellular integrity, microviscosity and survival. The level of PL increased in STZ rat, which on treatment with BT decreased. Thus, our findings demonstrate that *Chrysopogon zizanioides* has hypolipidemic effect, which is evidenced by the decreased levels of TC, and TG in plasma and tissues and decreased

LDL-C, VLDL-C and elevated levels of HDL-C in the plasma of STZ rat.

Numerous studies have demonstrated that oxidative stress is a key pathogenic factor in the development of diabetic complications. Oxidative stress induces the production of highly reactive oxygen species that are toxic to the cell, particularly the cell membrane in which these radicals interact with the lipid bilayer and produce lipid peroxides. However, endogenous antioxidant enzymes (SOD, CAT, and GPx) are responsible for the detoxification of the deleterious oxygen species. SOD and CAT are the two major scavenging enzymes that remove radicals in vivo. SOD can catalyze dismutation of O2•− into H2O2, which is then deactivated to H2O by CAT or GPx. A decrease in the activity of these enzymatic antioxidants can lead to an excess availability of superoxide anion (O2•−) and hydrogen peroxide (H2O2), which, in turn, generate •OH, resulting in initiation and propagation of lipid peroxidation. GPx has a key role in enzymatic defense systems and reduces organic peroxides (H2O2, lipid or organic peroxides) into their corresponding alcohols. The decrease may be due to the decreased availability of its substrate, GSH, which has been shown to be depleted during diabetes. GSH metabolizing enzymes, GPx and GST work in concert with glutathione in the decomposition of hydrogen peroxide and other organic hydroperoxides to non-toxic products, respectively, at the expense of GSH. Reduced activities of GPx and GST were due to inactivation of these enzymes by reactive oxygen species. *Chrysopogon zizanioides* augmented the activities of antioxidant enzymes in STZ-treated rats by inhibiting lipid peroxidation. The ability of *Chrysopogon zizanioides* to enhance the levels of antioxidants along with its antilipid peroxidative activity suggest that this compound might be potentially useful in counteracting free radical mediated injuries involved in the development of tissue damage caused by STZ- induced diabetic in rat.

Apart from the enzymatic antioxidants, nonenzymatic antioxidants such as vitamin C, E and GSH play an excellent role in preventing the cells from oxidative threats. Vitamin E is the earliest antioxidant in the lipid phase. In our study, vitamin E increased in STZ induced rat, which could be due to increased membrane damage by ROS. Treatment with *Chrysopogon zizanioides* methanol extract modulate vitamin E to near normal levels which could be as a result of decreased membrane damage as evidenced by decreased lipid peroxidation.

Vitamin C and vitamin E are interrelated by recycling process. Recycling of tocopheroxyl radical's vitamin E to is achieved with vitamin C. However, vitamin C decreased in diabetic rats as reported earlier. The decreased level of ascorbic acid in diabetic rats may be due to either increased utilization as an antioxidant defense against increased reactive oxygen species or to a decrease in glutathione level, since glutathione is required for the recycling of vitamin C. Treatment with *Chrysopogon zizanioides* methanol extract significantly improved the vitamin C to near normal levels which could be due to decreased utilization.

Glutathione is a major non-protein thiol in living organisms which play a central role in co-ordinating the antioxidant defense process in our body. It is involved in the maintenance of normal cell structure and function, probably through its redox and detoxification reaction. GSH functions as a free radical scavenger and in the repair of free radical caused biological damage. GSH is required for the recycling of vitamin C and acts as a substrate for GPx and GST that are involved in preventing the deleterious effect of oxygen radicals. STZ exhibited a decreased level of GSH which might be due to increased utilization for scavenging free radicals and increased consumption by GPx and GST, treatment with *Chrysopogon zizanioides* methanol extract significantly improved GSH level in the plasma of STZ induced diabetic rat which could be due to decreased utilization as lipid peroxidation is low. Thus, *Chrysopogon zizanioides* is having a good antioxidant propty, as evidenced by increased antioxidants status and decreased lipid peroxidation, which reflects the protective effect of *Chrysopogon zizanioides* methanol extract from the risk of diabetic complications.

In particular of our study, determined the serum glucose, lipid profile enzymatic and non enzymatic antioxidant activity, lipid peroxidation level and also noted the damage to pancreas in STZ treated diabetic control, and comparable regeneration was shown by methanolic extract of roots of *Chrysopogon zizanioides* photomicrographical dated in our studies confine healing of pancreas by *Chrysopogon zizanioides* as a plausible mechanism of their anti diabetic activity and regeneration of β-cell by glibenclamide was also observed. Our results show that the analyzed plant can be considered as a potential source of required elements other than diet for patients with chronic diabetes.

Moreover, preliminary phytochemical screening of root samples of *Chrysopogon zizanioides* showed the presence of alkaloids, flavonoids, saponins, and tannins, phenols and carbohydrates. All these compounds were identified from methanol extract. Hence, an attempt has been made to qualitatively and quantitatively (GC-MS) determine the chemical constituents present in these medicinally important plants. It was concluded from this study that the presence of these phytochemical in, *Chrysopogon zizanioides* might be the reason for its larvicidal activity. The result of this experiment indicates that these medicinal plants could be studied further in detail and its beneficial effect in controlling the diabetes and could be utilized to create a healthy environment. Our study also showed that methanol extract of *Chrysopogon zizanioides* has antioxidant activity. Therefore methanol extract of *Chrysopogon zizanioides* is suggested to be effective for reducing oxidative stress and free radical-related diseases including diabetes.[16]

Summary and Conclusion

Chrysopogon zizanioides has a long history as a medicinal plant. Since ancient times, the root decoction of the plant was used in analgesic and inflammation, rheumatism, anthelmintic, antipyretic and antioxidant. Different parts of plant including roots are used for the treatment of aliments, such as mouth ulcer, acidity relief, headache, tooth-ache, sprain, malarial fever and urinary tract infection, various fungal and bacterial infections. As no report was available on the invivo antidiabetic effect of *Chrysopogon zizanioides*, in this study we have investigated the effect of *Chrysopogon zizanioides* on glycaemic control, oxidative stress, Hyperlipidemia, and also Histopathological alterations in STZ induced albino rat.

The body weight, plasma glucose and plasma insulin increased and blood hemoglobin decreased significantly in STZ diabetic rat on treatment with *Chrysopogon zizanioides* in body weight lowered the plasma glucose, plasma insulin and elevated body weight, and blood hemoglobin significantly.

STZ rat had elevated levels of total cholesterol, TG, in the plasma Decreased level of HDL-C and increased level of LDL-C, and VLDL-C were observed in the plasma. Treatment with *Chrysopogon zizanioides* prevented the above changes in diabetic rat and improved towards normal levels.

Diabetic rat had elevated levels of TBARS and decreased activities of enzymatic antioxidants (SOD, CAT, GPx) and the levels of non-enzymatic antioxidants (vitamin C and reduced glutathione) in the plasma. Vitamin E level was increased in the plasma. Treatment with *Chrysopogon zizanioides* prevented the above changes and improved towards normal levels, showing its antioxidant and antiperoxidative property.

Histopathological examination of diabetic pancreas showed the eosinophilic amorphous deposits within islets, which results in cellular necrosis. Administration of *Chrysopogon zizanioides* reduced fatty infiltration in normal pancreatic cells.

The present study shows that *Chrysopogon zizanioides* is having good glycemic control, and is having antioxidant, Hypolipidemic properties in STZ-induced diabetic rat. It also possesses protective effect against liver and kidney injury associated with diabetic rat. The biochemical studies were supported by Histopathological studies.

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