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ANTIDIABETIC POTENTIAL OF *Costus igneus* LEAF IN STREPTOZOTOCIN INDUCED DIABETIC WISTAR ALBINO RATS

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Streptozotocin

Glibenclamide

Blood urea nitrogen

ABSTRACT

Traditional herbal medicinal plants leaves essentially reduced the fasting and postprandial blood sugar levels and bringing them down towards normal. The present study was aimed to evaluate acute toxicity, *in vivo* anti-diabetic effect of *C. igneus* on streptozotocin induced diabetic wistar albino rats by estimating total cholesterol, triglycerides, high density lipoprotein, low density lipoprotein, very low density lipoprotein cholesterol, serum creatinine and blood urea nitrogen. The result of acute toxicity study revealed that various tested extracts did not show any mortality at all tested concentration. Further, hexane extract of *C. igneus* leaves at 200mg showed significant ($P<0.001$) reduction in blood sugar level (114.8± 7.08) while in case of 400mg hexane leaf extract this reduction in sugar level (91.6± 6.12) was started from day 7^{th} to 28^{th} day. Further, the same treatment also showed highly significant ($P<0.001$) reduction in blood urea nitrogen (30.52±1.42) and it was equipotent as that of Glibenclamide. Similarly significant reduction in serum creatinine level was observed at 400 mg/kg in all the three leaf extracts of *C. igneus* and was similar to the effect produced by that of Glibenclamide. The present study explored the significant anti-diabetic potential of *C. igneus* and from the results of the study it can be concluded that *C. igneus* can be used as safe and cost-effective herbal drug for the treatment of diabetes.

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1 Introduction

Diabetes is a metabolic disorder of endocrine system that participate disturbance in glucose, lipid and protein homoeostasis (Van et al., 2006). Diabetes is generally characterized by hyperglycemia, glucosuria, polyuria and loss of body weight (Tende et al., 2011). Various allopathic medicines are available for the treatment of diabetes but these have various side effects. Traditional herbal medicinal plants have been used widely to effectively treat diabetes (Mukherjee et al., 2006), these herbal products are safer than synthetic formulation (Abou & Ghanema, 2013). Plants which have hypoglycemic action act on blood glucose through several mechanisms. Some of them may inhibit endogenous glucose production (Eddouks et al., 2003) or interfere the gastrointestinal glucose absorption (Musabayane et al., 2006), some may produce insulin like substance (Collier et al., 1987; Gray&Flatt,1999) some may inhibit insulinase activity and some may enhance secretion of insulin from β cells of the pancreas (Khan et al., 1990; Trivedi et al., 2004; Yadav et al.,2008). While some plants may proliferate β cells in pancreas by activating regeneration of these cell (Shanmugasundaram et al., 1990). In diabetes, free radicals are adversely formed through glucose oxidation, non-enzymatic glycation of proteins, and the subsequent oxidative degradation of glycated proteins (Maritim et al., 2003; Mehta et al., 2006). Cancers, diabetes, heart and brain related disease are mainly caused by production of free radicals which alter the structural and functional cell components in human beings (Mclarty, 1997; Yang et al., 2001; Young &Wood, 2001; Sun et al., 2002;Bimal et al., 2011). Large number of plants reputed to possess anti-diabetic properties (Saraswat et al., 2010; Ugwuja et al., 2010; Jafri et al., 2011). Among these very few have received equitable scientific and medical scrutiny in terms of their anti-glycation activities. The currently available anti-diabetic drugs may have some limitations and side effects therefore the growing trend for diabetes treatment was directed to the use of natural agents, such as medicinal herbs (Awanish et al., 2011). Therefore present study was aimed to evaluate acute toxicity, *in-vivo* anti-diabetic effect of *C. igneus* on streptozotocin induced diabetic wistar albino rats and determination of various biochemical parameters.

2 Materials and methods

2.1Collection and identification of plant material

The leaves of *C. igneus* were collected from the outskirts of Hosur, Krishnagiri district of Tamil Nadu and identified on the behalf of taxonomy key developed by Dr. M. Kumar, Assistant Professor, Department of Plant Biology and Biotechnology at Madras Christian College, Chennai Tamil Nadu India. The collected leaf materials were cleaned shade dried and powdered for further extraction.

2.2 Preparation of extracts

The air dried leaf powdered of *C. igneus* were extracted in Soxhlet extractor for 8-10 hrs, successively with acetone, hexane and hot water and the air dried residues were further extracted with hot water by the method of maceration for 24 hrs and the material was dried in hot air oven at 40°C. The evaporated extracts thus obtained were dissolved in the respective solvents at the concentration of 1 mg/mL and used for further *in vivo* studies.

2.3 Animals and Management

The experiments were carried out using Wistar albino rats (150–200 g) procured from the Animal house, Nandha college of Pharmacy, Erode, Tamilnadu, India. On arrival the animals were placed at random and allocated to treatment groups in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24±2°C and relative humidity of 30 – 70 %. A 12:12 light:dark cycle was followed. All animals were allowed to free access to water and fed with standard commercial rat chaw pallets (M/s. Hindustan Lever Ltd, Mumbai). All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics Committee (Regd. no: 688/PO/Re/S/02/CPCSEA) and were in accordance with the guidelines of the CPCSEA.

2.4 Acute toxicity study

Acute toxicity studies were performed according to OECD-423 (Organization of Economic and Cooperation Development) guidelines. Male Swiss mice were selected by random sampling technique for this study. The animals (n=5) were fasted for 4 hrs with free access to water. The different leaf extracts of *C. igneus* was administered orally at a dose of 5 mg/kg initially and mortality if any was observed for 3 days. If any mortality was observed in two out of three animals, then the dose administered was considered as toxic dose. However, if the mortality was observed in only one animal out of three animals then the same dose was repeated again to confirm the toxic effect. If no mortality was observed, then higher (50, 300, 2000 mg/kg) doses of the different leaf extracts of *C. igneus* were employed for further toxicity studies. The following general behaviour sedative, hypnotics, convulsion, ptosis, analgesia, stupar reaction, motor activity, muscle relaxant, pilo erection, change in skin colour, lacrimal secretion, stool consistency was also observed during the acute toxicity study Ecobichon,(1997).

2.5. Experimental induction of diabetes

For inducing the diabetes, all the experimental group animals were kept fasting for overnight. Diabetes was induced by intra-peritoneal injection of streptozotocin (STZ) dissolved in 0.1 M cold sodium citrate buffer, pH 4.5, at a dose of 55 mg/kg (Aslam

et al., 2007). Group one and two served as untreated healthy, untreated streptozotocin diabetic control and received distilled water. To overcome the drug induced hypoglycaemia the animals were allowed to drink 5% glucose solution overnight. A week later the blood glucose level were checked, the rats with blood glucose range of above 200 mg/dl were considered as diabetic rats and used for the experiment.

2.6 Experimental design

For evaluating the hypoglycemic effect of various extracts of *C. igneus* all the animals were randomly divided in to 9 groups with 5 animals in Each group.

Group I	Normal control (Non – diabetic) received distilled water 1ml/kg
Group II	Diabetic (streptozotocin induced) rats received distilled water 1ml/kg
Group III	Diabetic rats received Standard drug Glibenclamide 5mg/kg
Group IV	Diabetic rats received 200 mg/kg hot water extract of *C. igneus* leaf
Group V	Diabetic rats received 400mg/kg hot water extract of *C. igneus* leaf
Group VI	Diabetic rats received 200 mg/kg of acetone extract of *C. igneus* leaf
Group VII	Diabetic rats received 400mg/kg of acetone extract of *C. igneus* leaf
Group VIII	Diabetic rats received 200 mg/kg of hexane extract of *C. igneus* leaf
Group IX	Diabetic rats received 400mg/kg of hexane extract of *C. igneus* leaf

All the above mentioned experiments were carried out for a period of 28 days. Blood glucose levels were determined on initial (0 day), 4^{th}, 7^{th}, 14^{th} and 28^{th} day. On 29^{th} day the animals under pento-barbitone sodium anesthesia, blood was collected through sinus or retro-orbital puncture and serum was separated for biochemical estimations.

2.7 Determination of serum lipid profile

The blood was collected through sinus or retro-orbital puncture from the animals on the termination day, serum was separated and subjected for analysis of total cholesterol, triglycerides, high density lipoprotein cholesterol, low density lipoprotein, very low density lipoprotein cholesterol, creatinine and blood urea nitrogen.

2.7.1 Total cholesterol

The analysis of total cholesterol in serum was determined by a colorimetric method described by Roeschlau et al. (1974). This assay principally based on enzymatic hydrolysis and oxidation of cholesterol and the indicator compound, quinoneimine is formed from hydrogen peroxide and 4-aminoantipyrine in the presence of phenol and peroxidase. Composition of reagents involves the mixture of following, 4-aminoantipyrine (0.03 mmol/l), phenol (6 mmol/l), peroxidase (≥0.5 U/ml), cholesterol esterase (> 0.15 U/ml), cholesterol oxidase (> 0.1 U/ml) and pipes buffer (80 mmol/L pH 6.8). The serum sample (10 µl) was mixed with 1 ml of reagent, incubated at 37°C for 5 min, and absorbance measured at 500 nm against the reagent blank.

2.7.2 Triglycerols

The determination of triglycerides (TG) was carried out by colorimetric method given by Tietz, (1990). Principle of the assay involves enzymatic hydrolysis of TG with lipases and the indicator is a quinoneimine formed from hydrogen-peroxide, 4-aminophenazone and 4-chlorophenol under the catalytic activity of peroxidase The enzyme reagent consisted of 4-aminophenazone (0.5 mmol/l), ATP (1.0 m.mol/l), lipases (≥150 U/ml), glycerol-kinase (≥0.4 U/ml), glycerol-3-phosphate oxidase (≥1.5 U/ml), peroxidase (≥0.5 u/ml). The serum sample (10 µl) was mixed with 1000 µl of enzyme reagent, incubated at 37°C for 5 min and absorbance measured at 500 nm against the reagent blank.

2.7.3 HDL Cholesterol

Serum HDL cholesterol was determined using a colorimetric method described by Lopes et al. (1977). Principally the assay is based on quantitatively precipitation of low density lipoproteins (LDL and VLDL) and chylomicron fraction by the addition of phophotungstic acid in the presence of magnesium ions. The cholesterol concentration in the HDL fraction after centrifugation remains in the supernatant is determined. The precipitation reagents consisted of phosphotungstic acid (0.55mmol/l) and magnesium chloride (25mmol/l). The serum sample (200µl) was mixed with 500 µl of precipitation reagent and centrifuged at 4000 rpm for 10 min. The supernatant (100µl) was incubated at 37°C for 5 min and absorbance measured at 500 nm against the reagent blank. The cholesterol standard was 200 mg/dL (5.17 mmol/l). The concentration of cholesterol in the supernatant was calculated by following formula

HDL Cholesterol = ΔA sample / ΔA standard x concentration of standard

2.7.4 LDL and VLDL Cholesterol

The calculation of low density lipoprotein (LDL) and Very low density lipoprotein (VLDL) was done according to Friedwald formula (Friedewald et al., 1972).

LDL = TC – HDL – VLDL,

VLDL cholesterol = Triglycerides / 5

2.7.5 Estimation of Blood Urea

Estimation of blood urea nitrogen was performed according to method given by Natelson et al. (1951). Three test-tubes were taken and labelled as B, T and S. In test tube B 0.02ml of water was pipetted, in test tube T 0.02 ml of blood was taken and in test tube S 0.02ml of standard solution of urea (40 mg urea in 100 ml of water) was taken. To all three testtube0.1 ml of diacetylmonoxime solution and 5 ml of acid regent (Thiosemicarbazide) was added. The reaction mixture in these test tubes was mixed and kept in a boiling water bath for 15 minutes. After cooling, the absorbance was read at 540 nm and concentration of urea in mg/dl was calculated.

2.7.6 Estimation of Serum Creatinine

The estimation of serum creatinine was performed according to the method described by Slot, (1965).Three test-tubes were taken and labelled as B, T and S. 2ml of water was taken in test tube B, 2ml of serum and 4ml of water was taken in test tube T and in test tube S, 3ml of water and 1ml of creatinine was taken.2 ml of ammonium sulphate and 2 ml of sodium tungstate was added in all the three test-tubes and centrifuged. 3 ml of supernatant was removed from each test tube and to the supernatant 1 ml of picric acid and distilled water was added. Absorbance was read at 520 nm and concentration of serum creatinine in mg/dl was calculated.

2.8 Statistical Analysis

The values of results were expressed as mean ± SEM. The statistical analysis of data was carried out by one way analysis of variance (ANOVA) followed by Dunnet's 't' - test. *P* values <0.05 were considered significant.

3 Results

3.1 Oral acute toxicity study of various leaf extracts of *C. igneus*

The results of acute toxicity study of hot water, acetone and hexane extracts of *C. igneus* leaf were shown in Table 1. All tested leaf extracts of *C. igneus* has not shown any mortality even after 72 hours at 2000mg/kg. The acetone and hexane extracts of *C. igneus* leaves showed mile sedation and hexane extract showed muscle relaxant and CNS depressant activity at 2000mg/kg. While hot water extract of *C. igneus* leaves didn't alter any of the general behaviour. There was no lethality or toxic reported during and after the study period with all the three different leaf extract of *C. igneus.*

3.2 Hypoglycemic effect of *C. igneus* leaf extract

The effect of different leaf extracts of *C. igneus* was studied for its hypoglycemic effect against Streptozotocin induced diabetic rats and the results are shown in Table 2. Glibenclamide was used as reference control and it significantly reduced the blood sugar levels from 4th day on wards and on 28th day the blood sugar was

Table 1 Oral acute toxicity study of different leaf extracts of *C. igneus* (2000mg/kg) in mice

S. No.	General Behaviour	Hot Water Extracts	Acetone Extracts	Hexane Extracts
1	Sedation	-	+	+
2	Hypnosis	-	-	-
3	Convulsion	-	-	-
4	Ptosis	-	-	-
5	Analgesia	-	-	-
6	Stupar Reaction	-	-	-
7	Motor activity	-	-	-
8	Muscle Relaxant	-	-	+
9	CNS Stimulant	-	-	-
10	CNS Depressant	-	-	+
11	Pilo Erection	-	-	-
12	Skin Colour	-	-	-
13	Lacrimation	-	-	-
14	Stool Consistancy	-	-	-

'+' Present & '-' Absent

Table 2 Effect of different extracts of *C. ignus* leaf on blood sugar level in Streptozotocin induced wistar albino rats

Group	Blood Sugar Level (mg/dl)					
	Initial	*After STZ*	*4th day*	*7th day*	*14th day*	*28th day*
Normal control	98±4.66	98.2±3.12	101.2±7.15	91.0±6.37	98.2±3.94	94.6±3.56
Diabetic control	100.6±8.12	213.0±9.62	220.2±8.15	215.2±9.06	215.4±8.92	222±9.12
Standard Glibenclamide (5mg/kg)	88.0±3.41	217.6±3.72	175.8±3.69*	151.6±4.03**	129.2±2.92***	119.8±3.16***
Hot water extract 200mg/kg	100.6±9.96	212.2±8.12	196.2±8.06	173.6±7.84**	169.2±7.92**	142.8±8.02**
Hot water extract 400 mg/kg	97.2±6.12	213.8±8.31	184.2±7.39*	164.8±7.82**	141.8±6.94**	120.0±7.09***
Acetone extract 200 mg/kg	98.0±6.51	211.8±7.36	185.6±7.81*	178.0±8.01*	143.8±6.94**	117.0±7.01***
Acetone extract 400 mg/kg	96.8±7.02	217.0±8.12	183.4±7.62*	145.4±8.12**	111.6±8.22***	105.8±7.88***
Hexane extract 200 mg/kg	106.2±8.02	212.6±7.09	172.6±6.92**	162.4±6.86**	120.8±7.12***	114.4±7.08***
Hexane extract 400 mg/kg	95.0±6.12	212.6±7.32	165.4±7.16**	132.8±6.82***	108.8±7.09***	91.6±6.12***

Values are in mean ± SEM (n=6), *P<0.05 , **P<0.01, ***P<0.00Vs Diabetic Control

Table 3 Effect of different extracts of *C. ignus* leaf on lipid profiles in Streptozotocin induced wistar albino rats

Drug Treatment	Lipid Profiles (mg/dl)				
	Total Cholesterol	Triglycerols	HDL - Cholesterol	LDL - Cholesterol	VLDL - Cholesterol
Control	111.32±5.87	71.37±5.91	34.83±3.28	42.11±2.63	18.63±1.08
Diabetic Control	142.80±6.90	113.62±6.31	21.63±1.01	76.25±4.89	31.54±1.76
Glibenclamide	119.01±7.62***	72.55±4.82***	33.57±1.16***	45.16±2.60***	21.14±1.70**
Hot Water Extract 200 mg	123.72±6.03*	91.14±6.24**	26.63±0.87*	58.18±3.42**	23.59±1.62*
Hot Water Extract 400 mg	117.74±4.42**	72.93±3.76***	32.17±1.73***	45.05±2.97***	22.68±1.71**
Acetone Extract 200 mg	123.90±5.20*	88.42±3.61**	25.63±1.92*	61.35±4.42*	24.50±1.62*
Acetone Extract 400 mg	116.22±4.40**	73.50±3.17***	31.47±2.46***	43.06±1.97***	21.67±1.71**
Hexane Extract 200 mg	127.15±5.21*	88.14±3.24**	25.82±1.02*	57.32±2.42**	24.51±1.62*
Hexane Extract 400 mg	118.62±3.19**	75.36±5.27***	31.61±1.94***	45.60±3.97***	20.66±1.71**

Values are in mean ± SEM (n=6), *P<0.05, **P<0.01, ***P<0.001 Vs Diabetic Control

found 119.8± 3.16 mg/dl. Among various tested extract, 200 mg/kg of hot water extract of *C. igneus* leaves moderately decreased the blood sugar level (142.8±8.02) from 4th day onwards and the effect was maintained until the end of the treatment against the reference control 119.8± 3.16mg/dl. As compared to other extracts, hexane extracts *C. igneus* at 400mg/kg has shown high significant (P<0.001)decrease in blood sugar levels (91.6± 6.12) form 7th day to 28thday of treatment as that of the reference control 119.8± 3.16 mg/dl.

3.3 Effect of leaf extracts of *C. igneus* on Lipid Profile

The results of analysis of Total cholesterol (TC), Triglycerides (TG), High Density Lipoprotein Cholesterol (HDL-C), Low Density Lipoprotein Cholesterol (LDL-C) and Very Low Density Lipoprotein Cholesterol (VLDL-C) are given in Table 3.

3.3.1 Total cholesterol

The animals treated with streptozotocin (STZ) have higher value of total cholesterol as compared to normal control. Glibenclamide (5 mg/kg) treatment significantly (P<0.001) reduced the level of total cholesterol (119.01±7.62) which enhanced by STZ treatment (142.80±6.90). The low dose (200 mg/kg) of hot water, acetone and hexane extracts of *C. igneus* were observed less significant (P<0.05) in reducing cholesterol level as compared to diabetic control. High dose (400 mg/kg) of hot water (117.74±4.42) and acetone leaf (116.22±4.40) extract of *C. igneus* was found more significant (P<0.01) to decrease cholesterol as compared to diabetic control (142.80±6.90). Similarly high dose (400mg/kg) of hexane extracts of *C. igneus*found more significant (P<0.01) to reduce cholesterol level (118.62±3.19) as compared to diabetic control (142.80±6.90).

3.3.2 Triglycerols

The animals treated with streptozotocin (STZ) enhanced the triglycerides compared to normal control. Treatment with glibenclamide (5 mg/kg) significantly (P<0.001) decreased the level of triglycerides (113.62±6.31) enhanced by streptozotocin (STZ) (113.62±6.31). The low dose (200 mg/kg) of hot water, acetone and hexane extracts of *C. igneus* were find less significant (P<0.05) to decrease triglycerides. Whereas hot water (72.93±3.76), acetone (73.50±3.17) and hexane (75.36±5.27) extract at 400 mg/kg has shown high significance (P<0.01) reduction in triglycerides level (75.36±5.27) as compared to diabetic control (113.62±6.31).

3.3.4 HDL Cholesterol

The animals treated with streptozotocin (STZ) have elevated level of HDL cholesterol compared to normal control. Glibenclamide (5 mg/kg) significantly (P<0.001) decreased the level of HDL cholesterol (33.57±1.16). Low dose (200 mg/kg) of hot water (26.63±0.87), acetone (25.63±1.92) and hexane (25.82±1.02) extracts of *C. igneus* were found less significant (P<0.05) to decrease the level of HDL cholesterol as compared to diabetic control (21.63±1.01). Whereas high dose (400 mg/kg) of hot water (32.17±1.73), acetone (31.47±2.46) and hexane (31.61±1.94) extract of *C. igneus* were found more significant (P<0.01) in reducing the level of HDL cholesterol as compared to diabetic control (21.63±1.01).

3.3.5 LDL and VLDL Cholesterol

Induction of streptozotocin (STZ) in animals increased the LDL cholesterol compared to normal control. Treatment with glibenclamide (5 mg/kg) significantly (P<0.001) reduced the LDL cholesterol level (45.16±2.60) enhanced by streptozoztocin (STZ) (76.26±4.89). It was observed that low dose (200 mg/kg) of hot water (58.18±3.42), acetone (61.35±4.42) and hexane (57.32±2.42) extracts of *C.igneus* were less significant (P<0.05) in reducing the LDL cholesterol level as compared to diabetic control (76.26±4.89). The high dose (400 mg/kg) of hot water extracts of *C. igneus* was found more significant (P<0.01) to reduce LDL cholesterol level (45.05±2.97). Similarly high dose (400mg/kg) of acetone and hexane extracts of *C. igneus* more significantly (P<0.01) reduced the LDL cholesterol level (43.06±1.97), (45.60±3.97) respectively as compared to diabetic control (76.26±4.89). The results of VLDL cholesterol analysis revealed that there was less significant (P<0.05) reduction of VLDL cholesterol levels on treatment with hot water, acetone and hexane leaf extracts of *C. igneus* as compared to diabetic control. High dose (400 mg/kg) of hot water leaf extract *C. igneus* showed more significant (P<0.01) potential in reduction of VLDL cholesterol (22.68±1.71) as compared to diabetic control (31.54±1.76). Similarly high dose of acetone and hexane extracts of *C. igneus* leaves also showed more significant (P<0.01) reduction in VLDL cholesterol (21.67±1.71), (20.66±1.71) respectively as compared to diabetic control (31.54±1.76).

3.3.6 Effect of different leaf extracts on serum blood urea nitrogen (BUN)

The effects of various leaf extracts of *C. igneus* on serum blood urea nitrogen (BUN) in STZ induced diabetes rats and the results were shown in the Table 4.The blood urea nitrogen was increased in the STZ induced diabetes animals as compared to normal control animals. Hot water and acetone leaf extracts of *C. igneus* at dose of 200 mg/kg showed less significance (P<0.05) in bringing down blood urea nitrogen level as compared to diabetic control. Whereas hexane extract at 200 mg/kg found significant (P<0.01) in lower down the blood urea nitrogen (38.17± 2.22). At high dose 400 mg/kg among all the three leaf extracts of *C. igneus*, hexane extract showed high significance (P<0.001) in reduction of blood urea nitrogen level (30.52±1.42) as compared to diabetic control (51.82 ± 2.67). It was observed the effect of hexane extract on blood urea nitrogen was equipotent as that of the reference control Glibenclamide.

Table 4 Effect of various leaf extracts of *C. igneus* on Blood urea nitrogen and Creatinine in Streptozotocin induced wistar albino rats

Drug Treatment	Kidney Function Test	
	BUN (mg/dl)	Creatinine (mg/dl)
Control	22.32 ± 1.72	0.48 ±0.06
Diabetic Control	51.82 ± 2.67	0.16 ±0.01
Glibenclamide	32.56 ± 1.63***	0.37 ±0.04***
Hot Water Extract 200 mg	48.73± 2.22*	0.29 ±0.01*
Hot Water Extract 400 mg	31.62 ± 5.86***	0.34 ±0.01***
Acetone Extract 200 mg	45.61± 2.09*	0.24±0.01*
Acetone Extract 400 mg	32.50±1.62***	0.35±0.02***
Hexane Extract 200 mg	38.17± 2.22**	0.29±0.01*
Hexane Extract 400 mg	30.52±1.42***	0.36±0.01***

Values are in mean ± SEM (n=6),*P<0.05, **P<0.01, ***P<0.001 Vs Diabetic Control

3.3.7 Effect of different leaf extracts on Serum Creatinine

Serum creatinine level was enhanced by the STZ administration and it was reversed by the Glibenclamide. Treatment containing 200 mg/kg dose of *C. igneus* hot water has less significant (P<0.05) effect on reducing creatinine level (0.29 ±0.01) similar effect has been reported for 200 mg/kg dose of acetone (0.24±0.01) and hexane (0.29±0.01extract. Further a significant reduction was reported in serum creatinine level at 400 mg/kg in

all the three, hot water (0.34 ±0.01), acetone (0.35±0.02) and hexane (0.36±0.01) leaf extracts of *C. igneus* as compared to normal control (0.48 ±0.06). The effect produced by hot water, acetone and hexane leaf extracts of *C. igneus*at dose of 400 mg/kg was similar to effect produced by that of Glibenclamide (0.37 ±0.04).

4 Discussion

The results of the study revealed that all the extracts of *C. igneus* did not have any mortality at 2000mg/kg even after 72 hours. Although acetone and hexane extracts showed mile sedation while hexane extract showed muscle relaxant and CNS depressant activity at 2000mg/kg. Further hot water extract didn't alter the general behaviour of experimental animals. No lethality or toxic reactions found during and after the study period with all the three different extract of *C. igneus*. Substances with 50%lethal dose of 1000 mg/kg body weight/oral route are regarded as being safe or of low toxicity as per (Clarke & Clarke, 1977).

Plant derivatives with hypoglycemic properties have been used in folk medicine and traditional healing systems around the world from ancient times. Despite, the introduction of hypoglycemic agents from natural and synthetic sources, diabetes and its secondary complications continue to be a major medical problem to people (Ravi et al., 2005). Medicinal plants used to treat hypoglycemic and hyperglycemic conditions are of considerable interest to ethno-botanical community as the plants contain valuable medicinal properties in its different parts. Study carried by Palanivel et al. (2013) observed that diabetic rats treated with ethanolic extract of *C. igneus* 250mg/kg decreased blood glucose levels at the end treatment which was carried for 14 days indicating good hypoglycemic activity. Treatment with ethanolic and aqueous extracts of *C. igneus* at a dose of 500mg/kg showed significant reduction in increased blood glucose (Kumudhavalli & Jaykar, 2012).

Present study on hypoglycemic effect of *C. igneus* in streptozotocin induced diabetic rats revealed that experimental animals with blood glucose range of above 200 mg/dl were considered as diabetic rats after the administration of STZ. Glibenclamide was used as reference control and it significantly reduced the blood sugar levels. 200mg/kg dose of hexane extract of *C. igneus* showed significant (P<0.001) decline in blood sugar level. Among the higher doses of 400 mg/kg hexane extract of *C. igneus* showed high significant (P<0.001) reduction in blood sugar level form 7^{th} day to 28^{th}day of treatment.

Reports of clinical trials have demonstrated that the increase in plasma low density lipoprotein cholesterol (LDL-C) levels is implicated in the early development and progression of atherosclerosis. West et al. (1983) revealed that in diabetes triglycerides are also a risk and high density lipoprotein cholesterol (HDL-C) is an anti-atherogenic fraction. In normal condition it is well known that lipoprotein lipase enzyme is activated by insulin and hydrolyzes the triglycerides. The reports of previous studies on evaluation of anti-diabetic effect of ethanolic and aqueous extracts of *C.ignues* in streptozotocin induced diabetes. The treatment with ethanolic and aqueous extracts of *C.ignues* at a dose of 500mg/kg for the period of 15 days showed significant reduction in cholesterol, triglycerides, LDL and elevated the decreased HDL level as that of standard (Kumudhavalli & Jaykar, 2012).

The effect of different leaf extracts of *C. igneus* on blood serum sample were subjected to lipid analysis and biochemical parameters like total cholesterol (TC), triglycerides (TG), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C) and very low density lipoprotein cholesterol (VLDL-C) in the present study. The animals treated with streptozotocin (STZ) have higher value of total cholesterol as compared to normal control. Glibenclamide (5 mg/kg) treatment significantly (P<0.001) reduced the level of total cholesterol which enhanced by STZ treatment. The low dose (200 mg/kg) of hot water, acetone and hexane extracts of *C. igneus* were observed less significant (P<0.05) in reducing cholesterol level as compared to diabetic control. The high dose (400 mg/kg) of hot water leaf extract and acetone extract of *C. igneus* was found more significant (P<0.01) to decrease cholesterol respectively as compared to diabetic control. Similarly high dose (400mg/kg) of hexane extracts of *C. igneus* found more significant (P<0.01) to reduce cholesterol level as compared to diabetic control.

The animals treated with streptozotocin (STZ) enhanced the triglycerides compared to normal control. Treatment with glibenclamide (5 mg/kg) significantly (P<0.001) decreased the triglycerides enhanced by streptozotocin (STZ). The low dose (200 mg/kg) of hot water, acetone and hexane extracts of *C. igneus* were find less significant (P<0.05) to decrease triglycerides. Whereas higher dose (400 mg/kg) of hot water extract found significant (P<0.01) in reducing triglyceride level. Similarly high dose (400 mg/kg) of acetone extract was found more significant (P<0.01) to decrease triglycerides. The high dose (400 mg/kg) of hexane extract of *C. igneus* also found more significantly (P<0.01) in reducing triglycerides level as compared to diabetic control.

The animals treated with streptozotocin (STZ) have elevated level of HDL cholesterol compared to normal control. Glibenclamide (5 mg/kg) significantly (P<0.001) decreased the level of HDL cholesterol. Lower dose (200 mg/kg) extracts of *C.igneus* were found less significant (P<0.05) to decrease the level of HDL cholesterol. Whereas higher dose (400 mg/kg) extracts were found

more significant in reducing HDL cholesterol level as compared to diabetic control (P<0.01).

Induction of streptozotocin (STZ) in animals increased the LDL cholesterol and VLDL cholesterol compared to normal control. Treatment with glibenclamide (5 mg/kg) significantly (P<0.001) reduced the LDL cholesterol level enhanced by streptozoztocin (STZ). It was observed that all three extracts of *C. igneus* (200 mg/kg) were less significant (P<0.05) in reducing the LDL cholesterol and VLDL cholesterol levels. However high dose (400 mg/kg) of *C. igneus* extracts was found more significant (P<0.01) to reduce LDL cholesterol and VLDL cholesterol level.

Pervious study by Palanivel et al. (2013) reported that *C. igneus* ethanolic extract (Whole plant) 250mg/kg also showed statistically significant decrease (p<0.01) in blood urea nitrogen and creatinine levels as compared to diabetic control. The reduction in BUN in animals receiving various plant extracts interpreted as mechanism responsible for reabsorption of urea in nephrons. Creatinine, on other hand is organic base formed of degradation outcome of creatinine phosphate produced in muscle protein metabolism Mayes, (1988).

In the present study the effects of various extracts of *C. igneus* leaves on serum blood urea nitrogen (BUN) and creatinine in STZ induced diabetes in rats showed that the blood urea nitrogen and serum creatinine was increased in the STZ induced diabetes animals as compared to normal control animals. Treatment with *C. igneus* hexane extracts have highly significance (P<0.001) effect in reduction of blood urea nitrogen level at both concentrations and its effect on blood urea nitrogen was equipotent as glibenclamide. Similarly the high dose (400 mg/kg) of all the extracts of *C. igneus* has shown a significant reduction in serum creatinine level(P<0.001).

Conclusion

Acute toxicity studies of *C. igneus* extracts revealed notoxic reactions and did not have any mortality at 2000mg/kg even after 72 hours. Hexane extract of *C. igneus* shown high significant hypoglycaemic activity in streptozotocin induced diabetic rats and at 400 mg/kg extracts of *C. igneus* has shown high significance in reduction of serum lipid profile. The effect of hexane extract at 400 mg/kg on blood urea nitrogen and serum creatinine was similar to the effect produced by glibenclamide. The present study explored that *C. igneus* has potential to reduce the blood glucose level and other factors associated with diabetes. Hence *C. igneus* can act as natural, safe and cost-effective for the treatment of diabetes.

Conflict of interest

The corresponding author declares that there is no conflict of interest.

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