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ANTI-DIABETIC ACTIVITY OF *CURCUMA NEILGHERRENSIS* WT. RHIZOME EXTRACTS ON ALLOXAN INDUCED DIABETIC ALBINO RATS

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ABSTRACT

Prolonged usage of present day drugs like Sulphonylureas and biguanides against diabetes are causing the side effects like micro and macro vascular complications, renal failure, nervous disorders, blindness, obesity and arthritic problems. Hence World Health Organization also recommended for the search of herbal drugs which may reduce the risk of cardiac problems by reducing the lipid peroxidation and further regulates the plasma lipid and lipoprotein contents in the liver. Wild turmeric *Curcuma neilgherrensis* possesses many herbal uses as antiulcer, antidiabetic, anti-inflammatory, anti-arthritis, antimicrobial. Alloxan induced diabetic Albino rats were treated with aqueous and methanol extracts of rhizome and observed effective antidiabetic activity at 250 mg/kg b.wt after 21 days of daily treatment. Blood glucose levels are equal to that of normal and Glibenclamide standard drug treated rats and also no negative effects on body weights, haematological and biochemical parameters when compared to the diabetic rats. There is no any toxic effect upto 5000mg tested acute toxicity as per OECD 425 guidelines. Antidiabetic activity of C.neilgherrensis rhizome extracts also proved equal to that of *C.longa, C.xanthorrhiza, C.raktakanta, Alpinia galanga*. Hence it is proved as effective drug against diabetes as used by the tribals.

KEYWORDS: Blood glucose, Acute toxicity, Glibenclamide, Biochemical, Histopathological.

INTRODUCTION

Diabetes mellitus is a syndrome of disordered metabolism, usually due to combination of hereditary and environmental causes, resulting in abnormally high blood sugar levels. The

incidence of diabetes mellitus is increasing at an alarming rate even in developing countries include India.[1-5] Prolonged diabetes leads to micro and macro vascular complications.[6-7] Atherosclerosis develops rapidly in diabetic patients and the incidence of heart disease and ischemic heart mortality is fairly higher in people with diabetes. Uncontrolled diabetes has been associated with stroke, end-stage renal failure, nervous disorders, blindness and amputations. Insulin resistance, obesity and inflammation are some associated complications.[8-10]

Out of the two types of diabetes, the incidence of Non-Insulin Dependent Diabetes Mellitus (NIDDM) is much higher than the Insulin Dependent Diabetes Mellitus (IDDM). Sulphonylureas and few biguanides are the drugs used in the treatment of hyperglycemia in NIDDM, but they are unable to lower glucose concentrations within normal range and reinstate a normal pattern of glucose homoestatis permanently. Use of these therapies is restricted by their pharmacokinetic properties, secondary failure rates and accompanying side effects.[11] Even insulin therapy does not reinstate a permanent normal pattern of glucose homeostasis, and carries an increased risk of atherogenesis and hypoglycemia. World Health Organization has recommended that medicinal plant research warrant attention.[1] Exposure of liver to elevated glucose levels result in the decreased activities of Superoxide dismutase (SOD), Catalase (CAT), Glutathione-S-transferase(GST) and Glutathione (GSH), which contributed to the increased lipid peroxides in the liver. It has been demonstrated that polyunsaturated fatty acids of mammalian tissues and body fluids undergo lipid peroxidation.[12] Treatment of rats with Streptozotocin (STZ) /Alloxan is an established model for type I or Insulin dependent diabetes. Diabetes is associated with profound alterations in the plasma lipid and lipoprotein profile with an increased risk of coronary heart disease.[13] The liver and some other tissues participate in the uptake, oxidation and metabolic conversion of free fatty acids, synthesis of cholesterol and phospholipids and secretion of specific classes of plasma lipoproteins. Lowering of serum lipid levels through dietary or drug therapy seems to be associated with a decrease in the risk of vascular disease and related complications.[14]

Aldose reductases, a key enzyme in the polyol pathway catalyses the reduction of glucose to sorbitol. Accumulation of sorbitol in the body causes various complications including cataract, neuropathy and nephropathy.[15] Diabetes is projected as one of the world's main disablers and killers within the next 25 years. The diabetic population is likely to increase up

to 300 million or more by the year 2025.[16-17] Type 2 diabetes or non-insulin-dependent diabetes mellitus, is the most common form of the disease, accounting for 90%–95% of cases in which the body does not produce enough insulin or properly use it.[18] The most common endocrine metabolic disorders have caused significant morbidity and mortality due to microvascular (retinopathy, neuropathy, and nephropathy) and macrovascular (heart attack, stroke and peripheral vascular disease) complications.[19]

Contributory factor in the pathogenesis of diabetes also comprises of oxidative stress.[20,19] Protein glycation and glucose autoxidation generates free radicals in diabetic patients, which in turn catalyses lipid peroxidation.[21] The antioxidant status of the diabetes is compromised and is unable to protect against oxyradical damage.[22] New alternatives, including plant extracts containing antioxidant substances, flavonoids and exogenous antioxidants are under clinical and experimental investigation in an effort to impede the progress of diabetic complications.[23]

C. neilgherrensis which is known to possess tremendous therapeutic potency has been attributed due to the presence of various secondary metabolites such as essential oils, phenols, flavonoids, tannins, saponins and alkaloids which possess anti-inflammatory, Cholagogue, hepatoprotective, blood purifier, antioxidant, antimicrobial, skin diseases, anti-asthmatic, antitumour, stomachic, carminative and regenerator of liver tissue. It is also used for chronic hepatitis, antiarthritis, antidiabetic, antiseptic and menstrual disorders.[24-30] *C. neilgherrensis* produce starchy rhizomes which are used as remedy for infections, antimicrobial, anti -inflammations, anti- diabetic, gastric and skin disorders by the local herbalists but have not been evaluated pharmacognostically.[31] Hence the present study is aimed to evaluate the rhizome extracts against Alloxan induced diabetic rats to prove the herbal remedies.

MATERIALS AND METHODS

Collection and identification of Plant material

Plant material *C. neilgherrensis* was collected from Tirumala and Talakona forests during April – September, 2011 and authenticated the voucher specimens DC 921, DC 922 by Prof. N. Yasodamma; further preserved in the herbarium (SVUTY), Department of Botany, S.V.University, Tirupati as per the standard method. [32] Collected rhizomes were thoroughly washed, cut into pieces and dried under shade at 28 ± 2°C for about 10 days. The dried parts

were ground well in to a fine powder in a mixer grinder and sieved to a particle size of 50-150 mm. The powders were stored in a polythene bags at room temperatures.

Preparation of aqueous and methanol extract

Dried Rhizome powder (70 g) was soaked in water for 72 hrs and filtered extract was dried on water bath. The dried powder (40 g) was extracted in a soxhlet apparatus using 200 ml of methanol; both the filtrates were concentrated on rotavapour. These extracts were stored at 4^0C until further use.

Animal selection

For Antidiabetic activity male Wistar albino rats weighing between 150 - 200 g were selected. The animals were acclimatized to standard laboratory conditions (temperature 25±2^{o}C) and maintained on 12 hours light; 12 hours dark cycle. They were fed with ad libitum. The experiment was conducted according to the ethical norms approved by CPCSEA, Ministry of social justice and empowerment, Government of India and ethical clearance was granted by institutional ethical committee resolution number CPCSEA/IAEC/SVU/NY-BK/dt: 19/11/2011).[33-34]

Acute toxicity study

Acute oral toxicity study was performed as per Organization for Economic Co-operation and Development (OECD-425) guidelines. Wistar rats (n=6) were used for the study. The animals were kept fasting for overnight providing only water, after which the leaf and rhizome aqueous and methanol extracts were administered orally a single dose level of 1000, 2000, 3000 and 5000 mg/kg body weight by intragastric tube and animals were observed individually after dosing at least once during the first 30 min, periodically during the first 24 hrs, with special attention given during the first 4 hrs, and daily thereafter, for a total of 14 days. Observations also made on the changes of skin, fur, eyes, behaviour pattern, body weight, food consumption and fluid intake were recorded daily. If mortality was observed in 2-3 animals, then the dose administered was assigned as toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. Mortality was observed in 50% of the animals and then the LD_{50} was calculated. If no mortality then it is considered of there is no toxicity and the $1/10^{th}$ (500 mg) and $1/5^{th}$ (1000 mg) as LD_{50} value 5000 mg/kg b.wt. If the toxicity is seen it has been considered as the $1/25^{th}$ and $1/100^{th}$ of LD_{50} value has to be considered as recommended safe doses for experimental studies to be

evaluate as the standard drug doses. All the observations were made as per the OECD Guidelines and standard methods.[33-34]

Antidiabetic activity

Fasting blood glucose was determined after depriving food for 16 hrs with free access of drinking water to the selected rats. Hyperglycemia was induced by a single i.p. injection of 120 mg/kg of alloxan in sterile saline. After seven days of alloxan injection, the hyperglycemic rats (glucose level > 250 mg/dL) were separated and divided into different groups comprising of 6 rats each. Group-I control received vehicle (1% Tween 80) at a dose of 216 µl/kg b.wt orally. Group II and III non diabetic received aqueous and methanol extracts at 250 mg/kg of b.wt for 21 days. Group IV the positive control received alloxan 120 mg/kg b.wt in a single dose. Groups V and VI diabetes induced rats received the aqueous and methanol extracts at the dose of 250 mg/kg b.wt. Group-VII the positive control diabetic rats received the standard drug glibenclamide at the dose of 10 mg/kg orally. All treatments continued upto 21 days daily. During this period, animals in all groups had free access to standard diet and water. Body weights and blood glucose levels were estimated on 1^{st}, 7^{th}, 14^{th} and 21^{st} day of the treatment. On the 21^{st} day all the overnight fasted rats were killed by cervical dislocation and blood samples were collected for biochemical studies by cardiac puncture. Liver, pancreas and Kidneys were dissected out, washed in ice cold saline, and immediately stored in deep freeze at -80^0C and processed further for histological studies.[35-37]

Biochemical investigation

Estimation of Blood glucose levels were carried out by using Accu Chek glucometer. Cholesterol; Serum triglycerides; HDL- Cholesterol were carried out by the standard methods.[38-39] By using Friedwald formula the concentrations of VLDL and LDL cholesterol in serum was calculated.

LDL = (Total cholesterol- VLDL- Cholesterol) + (HDL – Cholesterol)

Histopathological studies

A small portion of Pancreas, liver and kidney were fixed in 10% formalin for histopathological studies. Pancreas, liver and kidney 5µm thick microtome cross sections were taken and stained with hematoxylin and eosin. Sections were observed under microscope for histopathological changes.[40]

Statistical Analysis

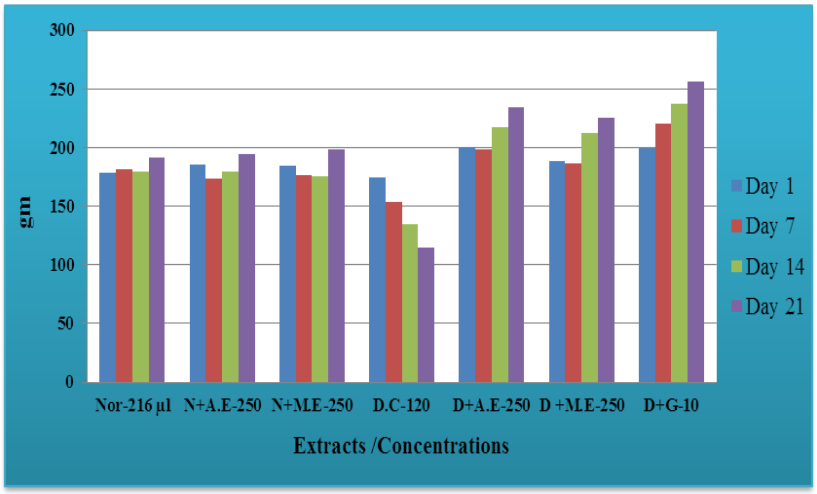
The results were analyzed for statistical significance using one way ANOVA followed by Dunnett's test. The $p< 0.01^{**}$ was considered significant.

RESULTS

Table -1: Effect on body weight changes of diabetic treated rats (g/rat)

Groups	Dose (mg/kg)	Day 1	Day 7	Day 14	Day 21	Mean body weight change (gms)
Normal control (1 % Tween 80)	216 µl	178.3±2.10	181.6±1.66**	179.5±2.07**	191.6±3.07**	13.3
Normal+Aqu	250	185.3±0.47** (+7.0)	173.3±0.47*	179.6±0.47**	194.3±0.47**	9.0
Normal+Met	250	184.0±0.81* (+5.7)	176.3±0.47*	175.3±0.47**	198.3±0.47**	14.3
Diabetic control (Alloxan)	120	175.0±4.47 (-3.3)	153.3±7.71	135.1±4.98	115.0±4.65	-60.0 (-76.6)
Diabetic+Aqu	250	200.3±0.47* (+22.0)	198.6±0.47**	217.3±0.47**	234.3±0.47**	+34.0 (42.7)
Diabetic +Met	250	188.6±0.47** (+10.3)	186.3±0.47**	212.3±0.47**	225.3±0.47**	+36.7 (36.7)
Diabetic+Gliben clamide	10	199.1±3.74** (20.8)	220.8±5.54**	237.8±10.3**	255.8±7.12**	+56.7 (64.2)

All the data are expressed as mean ± SEM, n=6, *p<0.05 and **p<0.01 when compared with control group; One way ANOVA followed by Dunnett's test.



Nor: Normal; **N+A.E:** Normal+Aqueous Extract; **N+M.E:** Normal+Methanol Extract; **D.C:** Diabetic Control (Alloxan); **D+A.E:** Diabetic+Aqueous Extract; **D+M.E:** Diabetic+Methanol Extract; **D+G:** Diabetic+Glibenclamide.

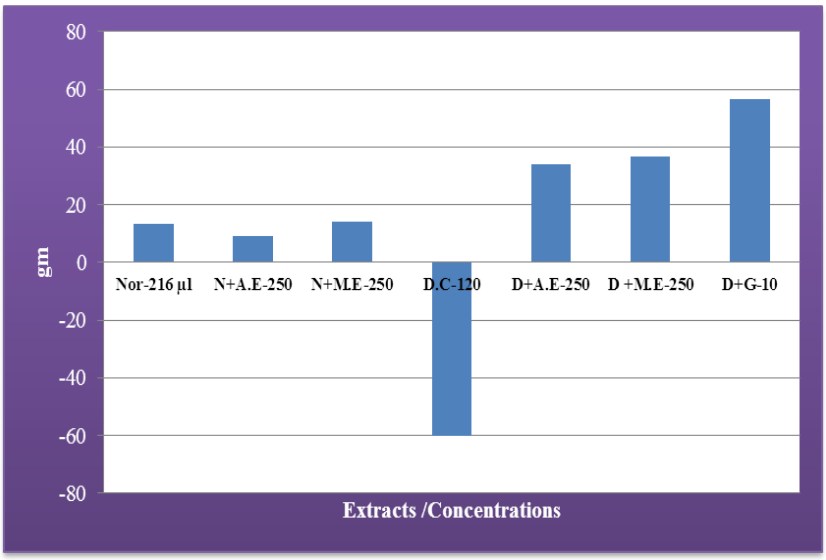


Figure -2: Effect on mean Body weight changes.

Nor: Normal; **N+A.E:** Normal+Aqueous Extract; **N+M.E:** Normal+Methanol Extract; **D.C:** Diabetic Control (Alloxan); **D+A.E:** Diabetic+Aqueous Extract; **D+M.E:** Diabetic+Methanol Extract; **D+G:** Diabetic+Glibenclamide.

Effect on body weight changes of diabetic treated rats (gm/rat)

After 7 days of Diabetic induced rats mean body weights were increased an average of 10.3 to 22.0 gm/rat (288.3) than the normal control rats 178.3 gm/rat but in diabetic control rats reduced body weights by -3.3gm/rat (175.0 gms) were observed. After 21 days of plant extracts treatment at 250 mg/kg b.wt and the standard drug Glibenclamide 10mg/kg b.wt treatment the mean body weights were increased than 1st day of extract treatment as 42.7, 36.7 and 56.7 gm/rat with the aqueous, methanol and Glibenclamide treated rats than the normal control rats with 13.3gm/rat body weight increase. Hence plant extracts treated at 250mg/kg b.wt proved more effective in maintaining the body weights of diabetic rats to that of the normal, diabetic control and standard drug treated rats.

Table -2: Effect on blood glucose levels of diabetic treated rats (mg/ dL).

Groups	Dose (mg/kg)	Day 1	Day 7	Day 14	Day 21	Mean Glucose levels change (mg/dL)
Normal control (1 % Tween 80)	216 µl	92.3±1.40**	92.5±1.33**	90.8±1.57**	96.3±2.15**	+4.0
Normal+Aqu	250	86.6±0.47**	88.3±0.47**	85.3±0.47**	80.3±0.47**	-6.3
Normal+Met	250	85.3±0.47**	90.3±0.47**	83.3±0.47**	81.3±0.47**	-4.0
Diabetic control	120	294.5±4.16	364.0±12.62	288.6±3.80	288.3±2.7	-6.2

		(+202.2)				(+192.0)
Diabetic+Aqu	250	275.6±0.47* (+183.3)	222.4±0.32**	143.6±0.47**	107.3±0.47**	-168.3 (+11.0)
Diabetic +Met	250	286.3±0.47 (+194.0)	242.3±0.47**	161.3±0.47**	110.6±0.94**	-175.7 (14.3)
Diabetic+Glibenclamide	10	295.6±4.97 (+203.3)	216.0±4.08**	166.0±3.45**	115.0±1.79**	-180.6 (18.7)

All the data are expressed as mean ± SEM, n=6, * $p<0.05$ and ** $p<0.01$ when compared with control group; One way ANOVA followed by Dunnett's test.

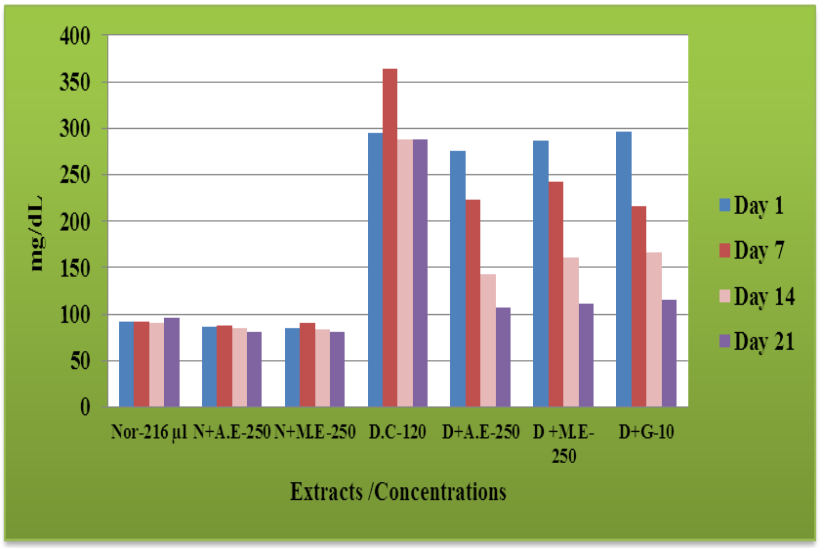


Figure - 3: Effect on blood Glucose levels of Diabetic treated Rats.

Nor: Normal; **N+A.E:** Normal+Aqueous Extract; **N+A.M:** Normal+Methanol Extract; **D.C:** Diabetic Control (Alloxan); **D+A.E:** Diabetic+Aqueous Extract; **D+M.E:** Diabetic+Methanol Extract; **D+G:** Diabetic+Glibenclamide.

Effect on Blood Glucose Levels of Diabetic Treated Rats (mg/dL) (Table- 2; Figure-3)

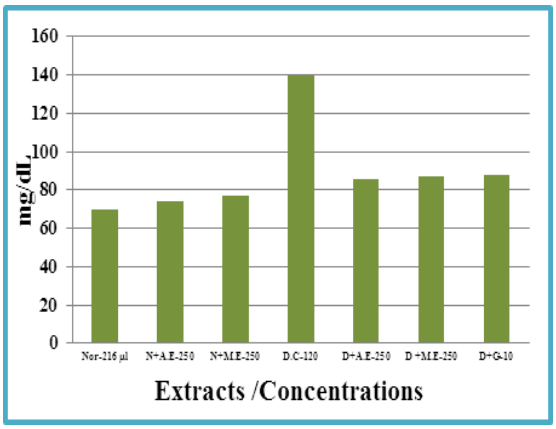
Blood glucose levels of normal rats after 21 days showed 92.3 to 96.3 mg/dL an average of 4.00 mg/dL increase. Whereas diabetic induced control rats after one week showed with hyper glycemic 294.5 mg/dL mean increase by + 202.2 mg/dL than normal rats and after 21 days decreased by -6.2 mg/dL as 288.3 mg/dL. Whereas normal rats treated at 250 mg/kg b.wt with aqueous extracts 86.6 to 80.3 (-6.3) mg/dL and methanol extracts 85.3 to 81.03 (-4.0) showed decreased glucose levels. Diabetic rats treated showed hypoglycemic after 21 days decreased glucose levels by 275.6 to 107.3 (-168.3) with aqueous extract; 286.3 to 110.6 mg/dL (-175) with methanol extract treated rats standard drug Glibenclamide treated rats showed decreased levels by 295.6 to 115.0 mg/dL (-180.6) almost equal to that of normal

rats. Hence aqueous and methanol extracts at 250 mg/kg b.wt proved as effective to that of the present day drugs.

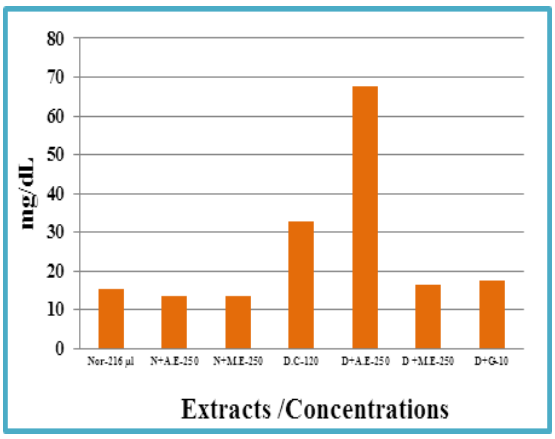
Table- 3: Effect on biochemical parameters of diabetic treated rats (mg/ dL).

Groups	Dose (mg/kg)	Cholesterol	VLDL	HDL	LDL	TGL
Normal control (1 % Tween 80)	216 µl	70.0±0.74**	15.4±0.35**	85.4±2.37**	-30.8±2.37	77.3±1.75**
Normal+Aqu	250	74.3±0.47**	13.5±0.08**	86.6±0.30**	-25.8±0.04	75.6±0.20**
Normal+Met	250	77.3±0.47**	13.6±0.09**	82.7±0.12**	-19.0±0.04	76.0±0.12**
Diabetic control (Alloxan)	120	140.0±4.43	32.6±1.37	60.9±2.3	48.6±4.90	163.1±6.87
Diabetic+Aqu	250	85.6±0.08**	16.8±71.7**	64.8±0.11	-46.7±0.09	84.8±0.12
Diabetic +Met	250	87.1±0.08**	16.4±0.16**	90.6±0.35	-12.9±0.16**	86.6±0.09**
Diabetic+Glibenclamide	10	88.2±1.45**	17.5±0.45**	107.0±1.52	-36.2±2.80**	87.5±2.27**

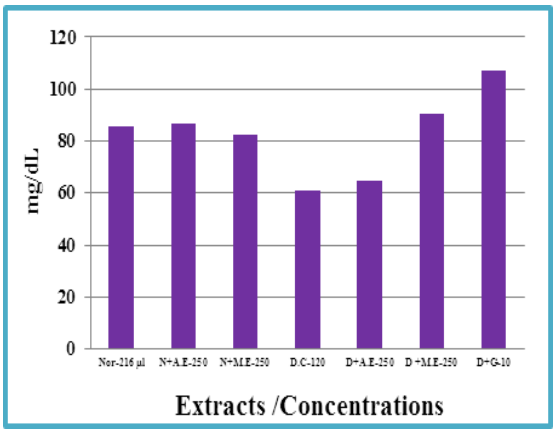
All the data are expressed as mean ± SEM, n=6, * p<0.05 and **p<0.01 when compared with control group; One way ANOVA followed by Dunnett's test.



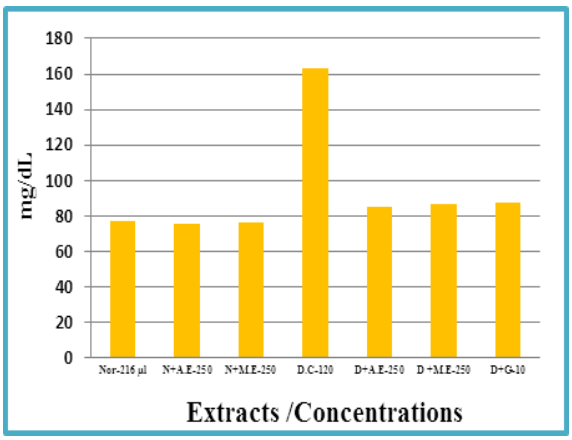
CHOLESTEROL



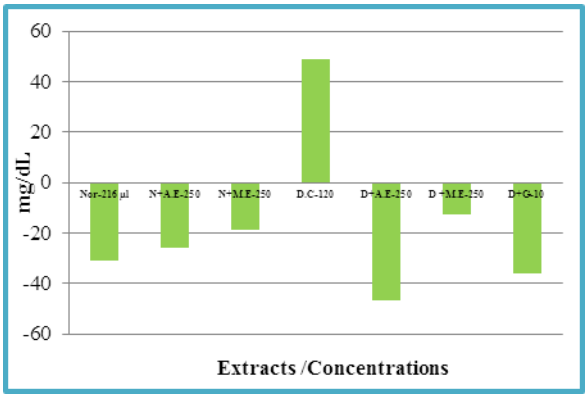
VLDL



HDL



TGL



LDL

Figure-4: Effect on biochemical Parameters of Diabetic treated Rats

VLDL: Very Low Density Lipoprotein; **HDL:** High Density Lipoprotein; **LDL:** Low Density Lipoprotein; **TGL:** Tryglycerides.

Effect of Rhizome Extracts on Biochemical Parameters of Diabetic Treated Rats (Table-3; Figure-4)

In diabetic rats blood cholesterol, VLDL and TGL were increased and HDL was decreased than the normal rats. Plant extracts treated rats after 21 days cholesterol levels were decreased drastically with aqueous extracts 85.6 mg/dL; methanol extracts 87.1 mg/ml than the diabetic rats 140.0 mg/dL; equal to the normal rats 70.0 mg/dL; and with standard drug Glibenclamide treated rats 88.2 mg/dL. The normal rats treated with plant extracts shown as 74.3 and 77.3 mg/dL respectively. VLDL levels increased in diabetic rats upto 32.6 mg/dL than in normal rats 15.4 mg/dL, diabetic treated with aqueous and methanol extracts showed 16.8 mg/dL and 16.4 mg/dl, and in Glibenclamide treated rats 17.5 mg/dL, where as normal rats treated with plant extracts it was observed as 13.5 and 13.6 mg/dL respectively.

LDL levels increased very much in diabetic control rats as 48.6 mg/dL than in normal rats as -30.8 mg/dL and in extracts treated rats decreased very much as -46 and -12 mg/dL respectively to that of Glibenclamide -36.2 mg/dL and in normal rats treated plant extracts also shown decreased LDL levels than the control rats as -25.8 and -19 mg/dL. TGL levels also increased very much in diabetic control rats as 163.1 mg/dL than normal control rats 77.3 mg/dL; and in plant extracts treated decreased TGL levels as 84.8 and 86.6 mg/dL respectively equally to that of Glibenclamide 87.5 mg/dL; normal rats treated showed equal levels of TGL to that of normal control rats as 75.6 and 76.0 mg/dL.

But HDL levels were decreased in diabetic rats as 60.9 mg/dL than the normal rats as 85.4 mg/dL; and in normal rats treated showed increased levels as 86.6 and 82.7 mg/dL; and in

diabetic rats treated showed 64.8 and 90.6 mg/dL with aqueous and methanol extracts respectively, and in Glibenclamide treated rats also it was increased to 107.0mg/dL.

Histopathological Studies

Kidney

Normal rats treated with 1% tween 80 (216µl) shown kidney with normal glomeruli, tubular epithelial cells and bowmans capsule. Diabetes induced intravenal with 120 mg/kg b.wt alloxan shows complete damage and degeneration of glomeruli, enlarged tubules and intratubular vacuolization. Normal rats treated with 250mg of aqueous and methanol rhizome extracts showed normal kidney conditions without any damage. The diabetic rats treated with 250mg of both the rhizome extracts shows atropic glomeruli, regeneration of tubular epithelial cells and picnotic nuclei equally to that of the diabetic rats treated with glibenclamide (10mg) the standard diabetic drug.

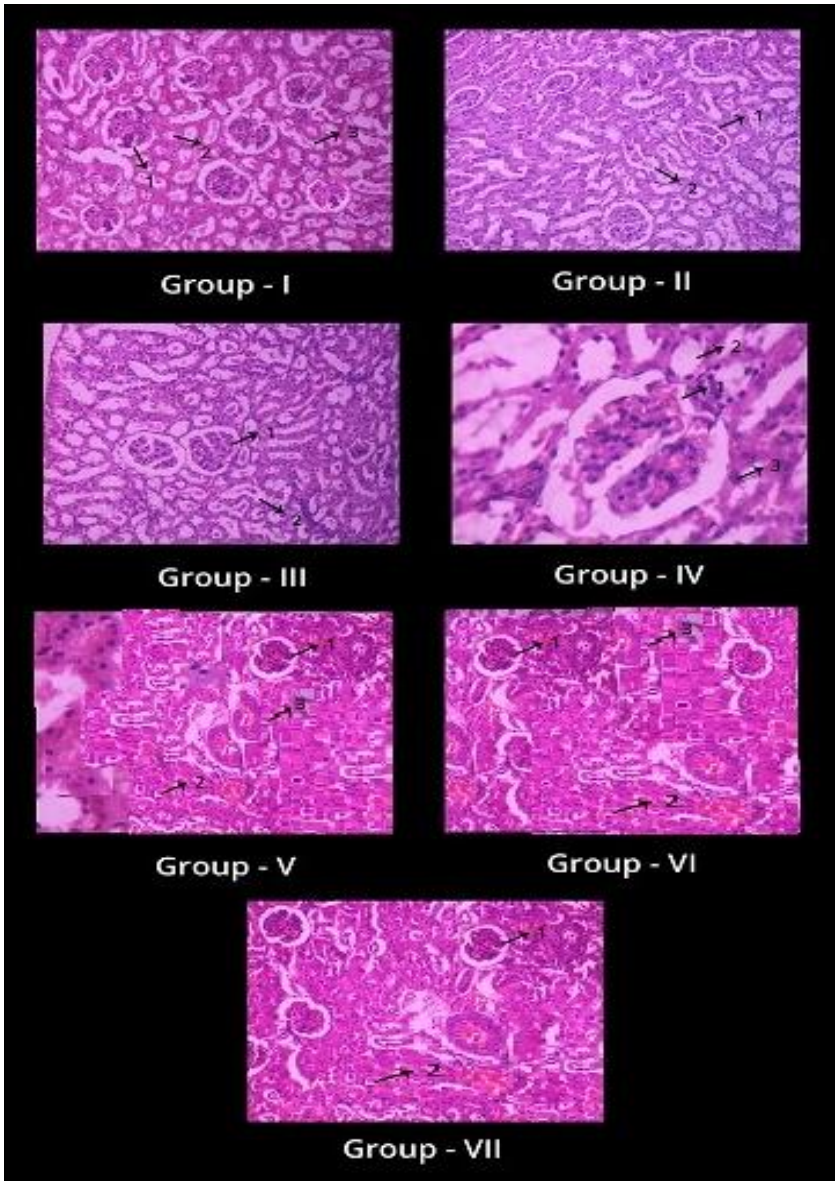
Liver

Normal rats shows the liver with normal central vein and liver cells and also observed the same with normal rats treated with plant aqueous and methanol rhizome extracts at 250 mg/kg b.wt. The rats induced diabetic with 120mg alloxan shows central vein with high hemorrhages along with dense kupper cells. But rats treated with aqueous and methanol rhizome extracts on diabetic rats observed normal central vein with mild degeneration and vacuolization as that of the diabetic rats treated with glibenclamide.

Pancreas

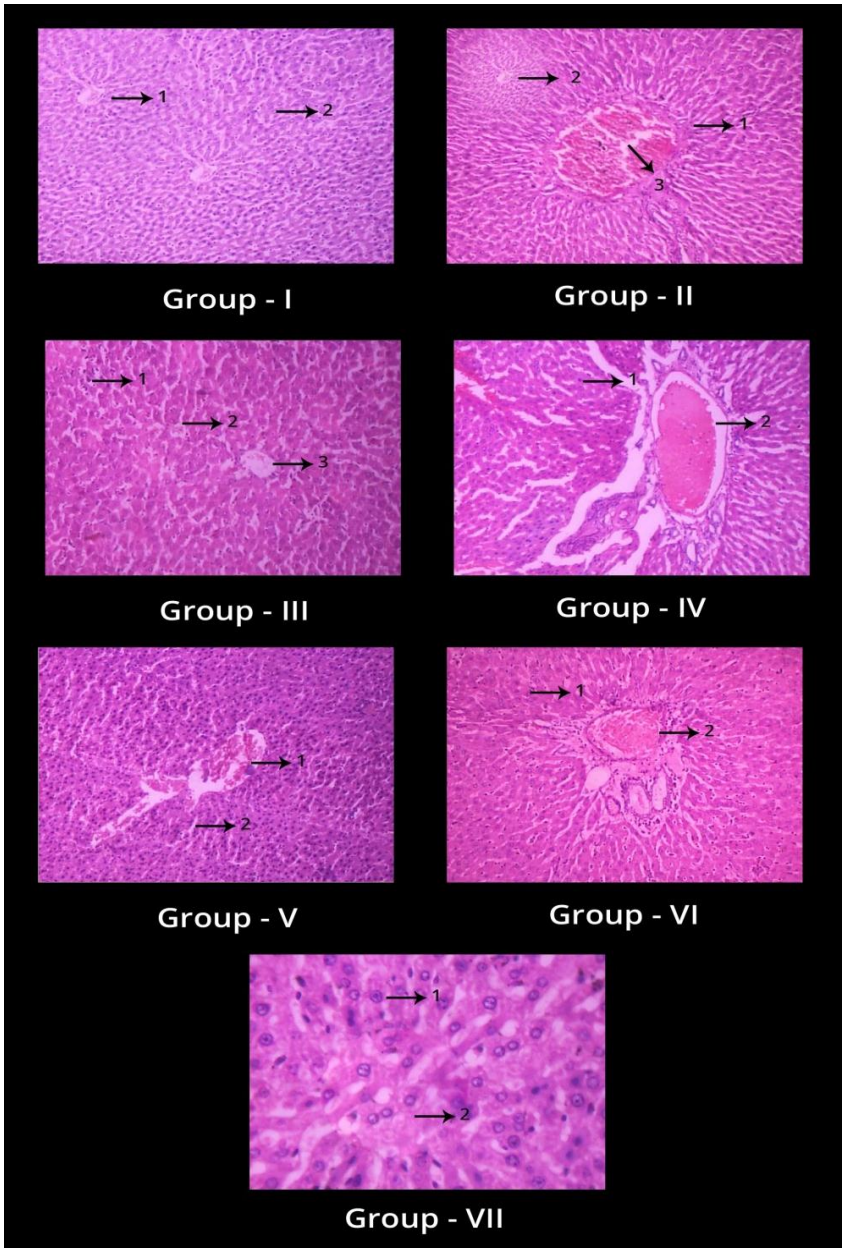
In the control rats pancreas observed with normal Islets of langerhans and β-cells and the same was observed in the normal rats treated with aqueous and methanol rhizome extracts whereas diabetic rats shows the destruction of islets of langerhans and β-cells with shrunken islets. Diabetic rats treated with aqueous and methanol rhizome extracts at 250mg/kg b.wt shows the normal islets of langerhans with mild recovery of β-cells as that of the diabetic rats treated with glibenclamide the standard drug at 10mg/kg b.wt.

HISTOPATHOLOGICAL STUDIES OF KIDNEY



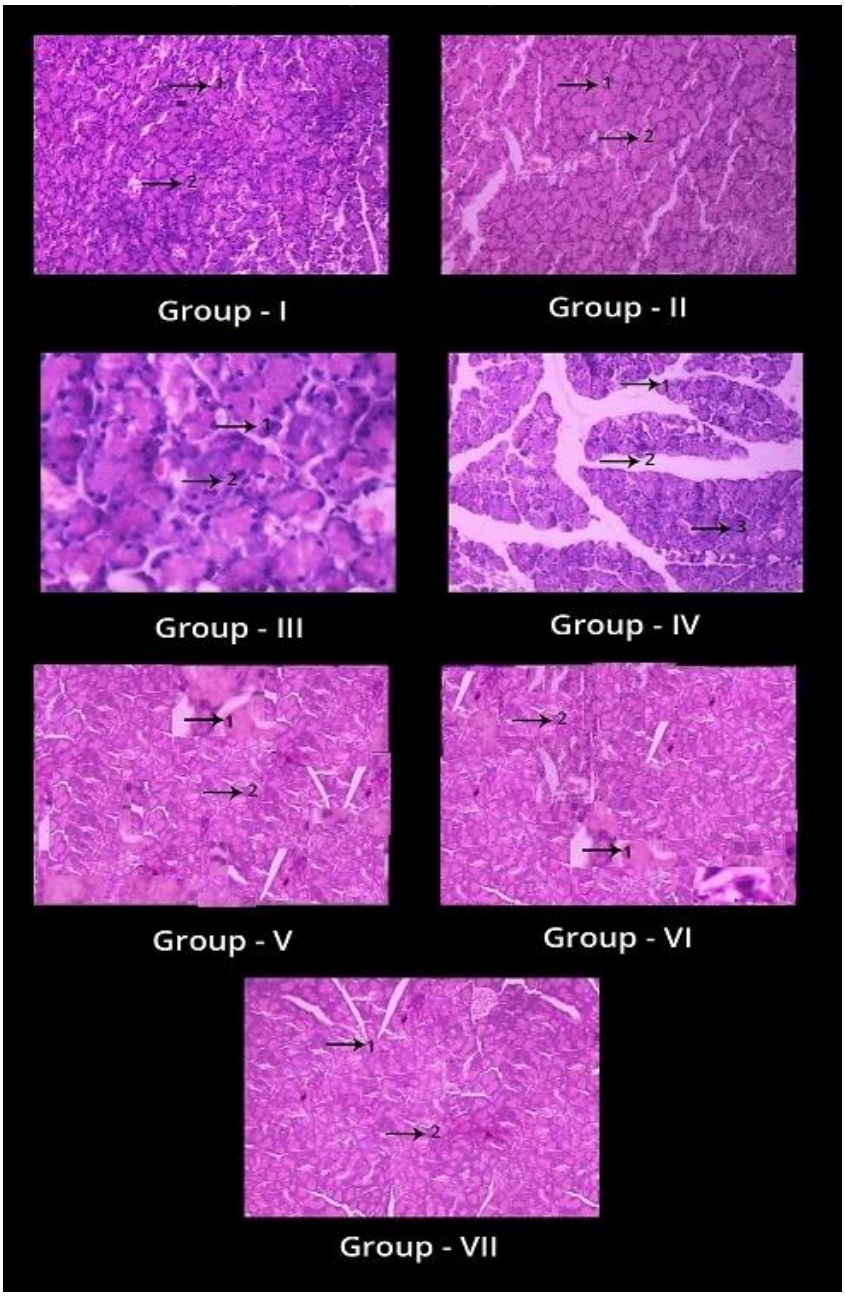
GROUP-I (Control Rats) 1. Normal Glomeruli 2. Tubular Epithelial cells 3. Bowmans capsule	GROUP-II (Normal Rat with Rhizome Aqueous Extract) 1. Normal Glomeruli Tubular Epithelial cells	GROUP-III (Normal Rat with Rhizome Methanol Extract) Normal Glomeruli Tubular Epithelial cells
GROUP-IV (Diabetic Control Rats) 1. Complete degeneration of Glomeruli 2. Enlarged tubules Intouchbular vacuolization	GROUP-V (Diabetic Rats treated with Rhizome Aqueous Extract) Atropic glomeruli Regeneration of tubular epithelial cells Picnotic nuclei	GROUP-VI (Diabetic Rats treated with Rhizome Methanol Extract) Atropic glomeruli Regeneration of tubular epithelial cells Picnotic nuclei
	GROUP-VII (Diabetic Rats treated with Glibenclamide) Regenerating architecture of glomeruli Picnotic nuclei	

HISTOPATHOLOGICAL STUDIES OF LIVER



GROUP-I (Control Rats) Normal Central Vein Normal Liver Cells	GROUP-II (Normal Rat with Rhizome Aqueous Extract) Normal Central Vein Normal liver cells Vacuolization	GROUP-III (Normal Rat with Rhizome Methanol Extract) Normal central vein Normal Liver cells Vacuolization
GROUP-IV (Diabetic Control Rats) Central vein with high hemorrhages Dense Kupper cells	GROUP-V (Diabetic Rats treated with Rhizome Aqueous Extract) Normal Central vein with mild degenerative changes Vacuolization	GROUP-VI (Diabetic Rats treated with Rhizome Methanol Extract) Normal Central vein with mild degenerative changes Vacuolization
	GROUP-VII (Diabetic Rats treated with Glibenclamide) Central vein with mild hemorrhages Normal Liver cells	

HISTOPATHOLOGICAL STUDIES OF PANCREAS



GROUP-I (Control Rats) Normal Islets of Langerhans β-cells	GROUP-II (Normal Rat with Rhizome Aqueous Extract) Normal Islets of Langerhans β-cells	GROUP-III (Normal Rat with Rhizome Methanol Extract) Normal Islets of Langerhans β-cells
GROUP-IV (Diabetic Control Rats) Destruction of islets of Langerhans Destruction of β-cells Small and shrunken islets	GROUP-V (Diabetic Rats treated with Rhizome Aqueous Extract) Normal Islets of Langerhans β-cells	GROUP-VI (Diabetic Rats treated with Rhizome Methanol Extract) 1. Normal Islets of Langerhans Mild recovery of β-cells
	GROUP-VII (Diabetic Rats treated with Glibenclamide) Almost normal Islets of Langerhans Mild recovery and abundant patches in the β-cells	

DISCUSSION

Ethanolic extract of *Alpinia galanga* showed potent scavenging activity on DPPH with IC_{50} value of 69.5 µg/ml; by lipid peroxidation IC_{50} value 77.1 µg/ml; hydrogen peroxide radical scavenging activity with IC_{50} value 55.1 µg/ml, ABTS radical scavenging IC_{50} value 0.086 µg/ml. Glucose up take of rat was significant at 400 mg/kg b.wt with marked increase in body weight; decreased serum glucose level, triglycerides and total cholesterol; increased total protein levels than diabetic control rats.[41]

Hypoglycemic and anti-hyperglycemic activity of the crude aqueous and methanolic rhizome extracts 1:4 ratio of *Curcuma amada* on Alloxan-induced diabetic mice showed decreased blood glucose levels in a dose and time dependent manner at 250 mg/kg b.wt after 8 hrs of treatment. No signs of toxicity observed upto 650 mg/kg b.wt.[42]

Aqueous rhizome extracts of C. longa and Abroma angusta daily oral administration of 300 mg/kg on STZ induced diabetic rats after 8 weeks of study showed a significant effect with decreased blood glucose, lipid peroxidation and on the antioxidant defense system with decreased radical formation in the liver, lung, kidney and brain tissues and also increased total haemoglobin was observed. Decrease Thiobarbitonic and reactive substances (TBARS) and increase in reduced Glutathione (GSH), SOD and CAT supports the antioxidant activity by which supports the antidiabetic activity in combination with both plant extracts than the individual plant extract. Fasting plasma glucose (FPG) values remained more or less same in normal rats as 93.4 mg/dL before and 89.4 mg/dL after 8 weeks of treatment. In diabetic rats after first week FPG values were higher 176 and increased upto 290.5 mg/dL by 8 weeks. After treatment with 300 ml of aqueous extracts of both plant extracts showed the high initial values 162.4 and further came back to normal level 89.4 mg/dL. The same was observed in the Fasting Blood Glucose (FBG) values in glucose tolerance test rats subjected after 8 weeks of study showed in normal 82.3; diabetic 295.5; and in diabetic treated 72.2 mg; in normal animals peak values 290.5 obtained within 30 minutes and returned back to initial values after 2 hours. Hypolipidemic effect on fasting blood glucose of normal 92.3 and untreated diabetic rats as 92.5 in treated diabetic rats 72.2 mg/dL with increased glucose tolerance levels after 8 weeks. Body weights were decreased in diabetic rats and in treated rats showed gain in weights than control animals. Observed effect on lipid peroxidation and decreased serum cholesterol levels in hyperlipidaemic rats also inhibits cyclooxygenase activity by lowering food intake and reduction in body weight and kidney mass. Increase in SOD in liver

at lower concentrations of glucose as the protective response by the liver cells to counter act the peroxidative stress in the tissue.[43]

Potent *in-vitro* antidiabetic activity by inhibiting α-amylase and α-glucosidase may be due to its action on carbohydrate binding regions of α-amylase and α-glucosidase that catalase hydrolysis of internal α-1, 4 glycosidic linkages in starch, resulting in suppression of postprandial hyperglycemia. The presence of such inhibitors in food stuffs may be responsible for impaired starch digestion.[44] The STZ induced diabetic rats showed significant increase in fasting blood glucose and decrease in body weight. The weight loss is due to increased muscle wasting and polyuria.[45-46] The decrease in phosphoglucoisomerase activity may inhibit the proportion of glucose 6 phosphate getting metabolized via glycolysis.[47] The activities of glycolitic enzymes, hexokinase and phosphoglucoisomerase were significantly decreased in diabetic rats. The first step in glycolysis is severely impaired in diabetic rats due to decreased activity of hexokinase because of insulin deficiency.[48]

Treatment with standard Glibenclamide and Ethanolic extracts of C. raktakanta (ECR) restored the weight loss also observed with the administration of *C.longa* extracts.[49] C. raktakantha also revealed the presence of ethyl p- methoxycinnamate, α- pinene, β- pinene, camphor, terpinyl acetate tumerone and some oleoresins.[50] Gluconeogenic enzymes namely glucose 6 phosphate and fructose 1, 6 diphosphatase were found to show significantly elevated activities in liver of STZ induced rats showed insufficiency. Treatment with ECR for 30 days improvised the impaired glycolitic enzymes activity and limited the activity of gluconeogenic enzymes.[51] STZ induction is associated with formation and accumulation of free radicals which leads to a number of deleterious effects.[52] Continuous exposure of the system to free radicals results in decreased activities of SOD, CAT, Glutathione peroxidase (GPx) and GST. Depression of these antioxidant marker enzymes under diabetic condition may be due to radical induced inactivation of glycosylation. Decreased levels of vitamin C, a key non enzymatic antioxidant was observed in STZ induced diabetic rats which may be due to increased utilization in trapping the oxyradicals. Depletion in the antioxidant defense mechanism is also reflected by an increase in lipid peroxidation. However administration of ECR for 30 days resulted in a significant restoration of the antioxidant system which is quite evident from the lowered levels of Thiobarbituric acid reactive substances (TBARS), a lipid peroxidation marker.[53] The toxic effect of STZ is not restricted to abolishment of pancreatic β-cells but also cause renal injury and oxidative stress.[54-55] The antidiabetic and anti

hyperlipidemic activity with ECR might be due to the presence of numerous polyphenols which have been shown to possess antioxidant activities. The pharmacological properties of Curcuma species is highly attributed to the main active component, curcuminoid. So that the ECR has the potential to emerge as a novel remedy for the treatment of Non-Insulin Dependent Diabetes Mellitus (NIDDM).[56]

ECR on STZ induced (50 mg/kg b.wt) diabetic rats showed on effect on α-amylase 52% and on α-glucosidase 5% inhibition after 30 days at 224.22µg/ml and 961.54µg/ml by both aqueous and methanol extracts respectively. And also observed effective activity in controlling fasting blood glucose levels, and decreased activities of hexokinase and phosphoglucoisomerase, increased tissue vitamin-C levels, decreased levels of TBARS; lowered the levels of Total Cholesterol (TC), Triglyceride (TG) and Low Density Lipoprotein (LDL) with a simultaneous increase in HDL.[57]

Ethanolic extracts of C. xanthorrhiza and Guazuma ulmifolia combined extracts on alloxan induced diabetic mice observed after 28 days of administration the plasma glucose levels were significantly lowered at 12.5 and 25 mg/kg b.wt as 59.54 mg/dL to that of Glibenclamide 49.20 mg/dL b.wt may be due to increase in insulin production and also showed less damage of pancreatic cells.[58]

C. neilgherrensis rhizome extracts are already reported as efficient antimicrobial agents as that of Ampicillin and Nystatin, anthelmintic agent to that of Albendazole and also possess major secondary metabolites like phenolics, flavonoids, anthocyanidins, alkaloids, tannins, terpenoids and saponins in high quantities. Presence of protocatecheuic, caffeic, melilotic, salicylic acids; myricetin, apigenin, kaempferol, quercetin, peonidin and cyanidin compounds supported and proved as potent inhibitor of ulcer index to 5.0 with 63.5% ulcer protection at 500 mg/kg aqueous rhizome extracts on pyloric ligated gastric ulcers induced Albino rats equally to that of the standard drug Omeprazole.[59-61]

Antidiarrhoeal activity of C.neilgherrensis methanol and aqueous rhizome extracts and also its acute oral toxicity studies proved as harmless drug upto 5000 mg/kg b.wt without any behavioural changes on Albino wistar rats. Rhizome methanol extracts at 1000 mg/kg b.wt proved more effective to that of Atropine the standard drug with 70 % antidiarrhoeal activity and 60 % of fluid inhibition than the aqueous extracts may be due to the presence of tannins, terpinoids, glycosides, flavonoids and saponins.[62]

CONCLUSIONS

C. neilgherrensis antidiabetic activity has been proved as effective drug at the minimum dose levels of 250 mg/kg b.wt of rhizome extracts as safe drug and without toxic and there is no negative effects on body weights and behavioural aspects; without alteration of haematological and biochemical parameters equal to that of normal rats and Glibenclamide the standard drug treated rats. It is also more effective than the A.galanga antidiabetic activity at 400 mg/kg b.wt; *C.longa* + Abromine angusta at 300 mg/kg b.wt; *C.raktakanta* at 224.22µg/ml and 961.54 µg/ml; C.xanthorrhiza+Gauzuma ulmifolia at 12.5 and 25 mg/kg b.wt respectively. Presence of high quantities of phenols and flavonoid compounds like quercitin in C.neilgherrensis rhizome may acts as potent drug in controlling the lipid peroxidation which regulates diabetic effect. Hence it is recommended to design the drug against diabetic with wild turmeric aqueous and methanolic extracts.

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