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EVALUATION OF ANTI-DIABETIC AND ANTI-HYPERLIPIDEMEDIC ACTIVITY OF *CRATAEVA NURVALA* (BUCH-HAM.) LEAVES AND ROOT BARK METHANOLIC AND AQUEOUS EXTRACT

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ABSTRACT

Diabetes and Hyperlipidemic disease effects many people around the world and their development is a result of the high glucose level and high cholesterol levelin many of the people effecting with cardiac arrest and myocardial infarction. The presence of various phytoconstituent such as glycosides, tannins, flavonoids, saponins, alkaloids, steroids, and triterpeniods were evident in CNLE and CNRBE. The presence study was undertaken to investigate the anti diabetic and anti hyperlipidemic effect of Crataeva nurvala leaves and Root bark of CNLE and CNRBE (250 and 500mg /kg, b.w , p.o) respectively following 7-days treatment in streptozotocin induced in albino wistar mice and 31 days treatment in Triton X-100 induced model in albino wistar rats. Metformin HCL 10mg/kg was taken as standard and Streptozotocin and Atrovastatin 10mg/kg was taken standard Triton X-100 was taken as inducers as drugs. The Diabetic effect of the extract is attributed to have lowered the glucose level and Hyperlipidemic effect of extract is attributed to have lower cholesterol level in histological studies. The anti diabetic and hyperlipidemic activity is recognized by a reduction in glucose and cholesterol level.

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INTRODUCTION

Herbal medicines serve as good cure for various ailments and are crucial in maintaining human health all over the world. According to WHO as much as 4 billion people (80%) in world depend on traditional medicine chiefly obtained from plants. This includes a wide spectrum of age old practices applied for health care like folk /tribal practices, as well as, Ayurveda, Siddha, Amchi and Unani. These curing procedures took their root from very ancient time and slowly expanded based on practical success, with least relation to today's scientific approach. Though traditional drugs are worthy in cure of different diseases, frequently these drugs are unscientifically exploited and /or improperly used. Therefore, those plants drugs deserved detailed studies in the light of modern sciences.

Diabetes mellitus is a group metabolic disorder characterized by chronic hyperglycemia. The metabolic disturbance involves the disturbance in the metabolism of fats, proteins and carbohydrates, reflecting a state of insulin deprivation and possibly abnormally high amounts of glucagon and other counter regulating hormones such a glucagon hormone, Sympathomimetic amines and corticosteroids. This occurs due to deficient insulin secretion and also to factors opposing the tissue effects of insulin or both. Diabetes mellitus is usually irreversible while it allows the patient to have reasonably normal lifestyle, its complications results in a considerably reduced life expectancy.

OBJECTIVE AND PLAN OF WORK

Since, there are very less reports available in the scientific literature regarding the Antidiabetic Activity and Anti-Hyperlipidemic activity of the plant *Crataeva nurvala* (Buch-Ham.) Leaves and Roots Bark, either in preclinical or clinical studies carried out so far, the present study is planned with the following objectives to evaluate the Antidiabetic and Anti -inflammatory effect of *Crataeva nurvala* (Buch-Ham.) Leaves and Root bark Methanolic Extract (CNLME, CNRBME) and Aqueous Extract (CNLAE, CNRBAE) in Swiss Albino Mice and Albino Wistar Rats.

- ❖Procurement, Collection and Authentication of the plant material *Crataeva nurvala* (Buch-Ham.) Leaves and Roots bark.
- ❖Drying and grinding of *Crataeva nurvala* Leaves and Root bark.
- ❖Extraction of *Crataeva nurvala* Leaves and Root Bark powder by Soxhlet extraction method using methanol as solvent to obtain CNLME, CNRBME and Aqueous Extract CNLAE, CNRBAE.
- ❖Preliminary Phytochemical screening of CNLME, CNRBME, CNLAE and CNRBAE.
- ❖To Evaluate the Anti-diabetic and Anti-Hyperlipidemic effect of CNLME, CNRBME, CNLAE and CNRBAE in Swiss Albino Mice and Albino Wistar Rats by using the experimental models like:

I) Anti-Diabetic Activity Models:

- Oral Glucose Tolerance Test (OGTT).
- Streptozotocin induced model (STZ).

II) Anti-Hyperlipidemic Activity Models:

- Triton X-100 Induced Hyperlipidemia.
- High Fat Diet Induced Hyperlipidemia.

MATERIALS AND METHODS

Collection and Authentication of the Plant Material:

Crataeva nurvala (Buch-Ham.) Plant and its Leaves and roots bark belonging to the family Capparaceae were purchased and collected from Dr.K Madhava Chetty, Assistant Professor, Department of Botany, Sri Venkateshwara University, Tirupati, A.P, (India) for carrying out the project research work. The Specimen (plant and its leaves and roots bark) was further confirmed by preparing a herbarium and was authenticated as *Crataeva nurvala* (Buch-Ham.) Ref No: BSI/DRC/2013-2014/Tech./867, by Mr. P.Venu, Additional Director, Botanical Survey of India (BSI), Deccan Regional Centre, Hyderabad - 500 048, Telangana. A specimen of the plant bearing the assigned reference no. was deposited at the institutional herbarium for future reference.

Preparation of Plant Material:

The collected Leaves and Roots bark of *Crataeva nurvala* (Buch-Ham.) were washed to make them free from soil, small insects and any other adhering foreign material. The roots were dried under shade for about 5-7 days, and were then chopped and grinded into a coarse powder using a mechanical grinder (Preeti mixer and grinder, PMG/AUCOP-1) and passed through sieve No. 40 to get the powder of desired coarseness. The powdered plant material weighing app. 985gm was then preserved in an air tight container for further processing and use.

***Crataeva nurvala* (Buch-Ham.) Leaves and Leaves Powder.**

***Crataeva nurvala* (Buch-Ham.) A root barks and Root barks powder.**

Extraction of the Powdered Plant material (Leaves and Roots bark):

- **Preparation of *Crataeva nurvala* (Buch-Ham.) Leaves and Root Bark Methanaolic Extract by Soxhlet Extraction Method:**

About 300 gm of *Crataeva nurvala* (Buch-Ham.) Leaves and Roots bark powder was extracted by Soxhlet extraction using low to high polarity solvent such as petroleum ether, chloroform and methanol as a solvent in the ratio of 1:4.The process was continued until the extraction was complete indicated by dark brown colour and then subject to continue with distilled water for Aqueous extraction. The obtained Methanaolic extract is again Soxhlet for recovery. The methanaolic extract was collected and the solvent was initially evaporated off on water bath at 60º C and the extract was then further concentrated on Rotary Vaccum Evaporator, EVATOR # EV11 under reduced pressure at 45-50ºC to obtain Methanaolic extract. The concentrated extract was then weighed and the percentage yield was calculated. The yield obtained for crude methanaolic extract was found to be 79.6%w/w w.r.t the initial dry leaves and roots bark powder. The yield obtained for crude aqueous extract was found to be 75.3% w/w w.r.t the initial dry leaves and root bark powder. *Crataeva nurvala* (Buch-Ham.) Leaves and root extract was kept in refrigerator at 2-8 ºC for until subjected to Preliminary Phytochemical Investigation and biological evaluation.

BIOLOGICAL EVALUATION

Evaluation of Anti-Diabetic And Anti-Hyperlipidemic Activity of *Crataeva nurvala* (Buch-Ham.) Leaves and Root bark Methanolic and Aqueous Extract (CNLME, CNLAE, CNRBME, and CNRBAE.)

Experimental Study Protocol:

The present biological study was designed to evaluate the Anti-Diabetic and Anti-Hyperlipidemic activity by the test *Crataeva nurvala* (Buch-Ham.) Leaves and Roots Bark Methanaolic and Aqueous Extract (CNLME, CNLAE, CNRBME, and CNRBAE 250 & 500 mg/kg.b.w,p.o) and compare it's effect with the marketed drug formulation Metformin (10 mg/kg.b.w.p.o) for anti-diabetic activity & Atrovastatin (10 mg/kg.b.w.p.o) for Anti-Hyperlipidemic activity as standard drugs respectively. The Experimental study protocol was approved by the Institutional Animal Ethics Commitee (IAEC) (AUCOP/IAEC/2015/1/P.N-18).

Determination of CNE Acute Toxicity:

Acute toxicity study was.carried out according to the method described in the literature [P.K. Naik]. The limit dose test of up and down system according to OECD test guidelines on acute oral toxicity (No.423) was studied at a limit dose of 10mg/kg body weight, p.o for CNLME, CNLAE, CNRBME, and CNRBAE. Three animals were selected (1 Male and 2 Female) such that weight differences do not exceed ± 10% of the mean initial weight of the sample population. The rats were fasted for food overnight with free access to water prior to administration of CNLME, CNLAE, CNRBME, and CNRBAE (10mg/kg), suspended in 1.0% w/v CMC and access to food was reinstate after 3-4hr. Thereafter, individual rat was observed after dosing at least once during the first 30 mins, periodically during the first 24 hr. with special attention given during the first 4 hr and daily for a total of 14 days. The rats were observed for systemic and behavioural toxicity patterns as described in OECD test guidelines.

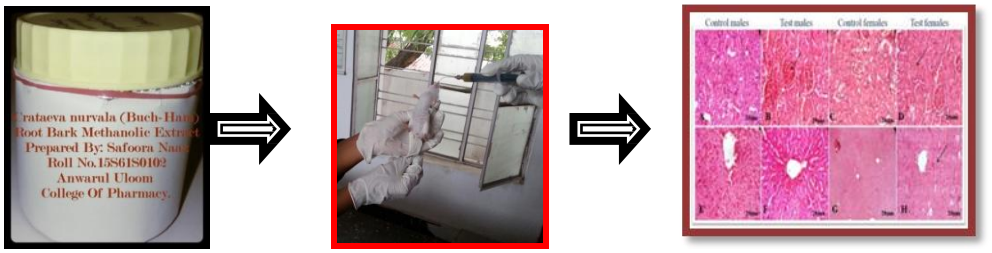
Procurement and Maintainance of Experimental Animals:

The experimental animals required for the study i.e., Swiss Albino Mice of either sex (2-3 months old, weight 20-30g) for Anti-diabetic activity and Albino Wistar Rats of either sex (2-3 months old, weight 180-200 g) for Anti-hyperlipidemic activity models were procured from the Sanzyme Bio-Analytical Laboratory, A Division of Sanzyme (P) LTD. Plot No. 8, SY. NO.542, Kolthur (V), Shameerpet (M), R.R Dist, biotech Park Phase-II, Alexandria Knowledge Park, Hyderabad-500078 Tel:040-48589999. Registered breeding and sale of animals bearing Regestration. No.1722/RO/ERe/S/13/CPCSEA. The procured animals were acclamatised to laboratory conditions for a period of 15days before performing the experiment. The animals were housed in polypropylene cages maintained at (Temperature 25±2˚C, 12-h light, 12-h dark cycle) under standard laboratory conditions in CPCSEA approved institutional animal house facility (Reg.No. 1534/PO/a/11/CPCSEA). The animals were fed with standard pellet diet (Nutrivet Life Sciences, Pune, M.S, India.) with free access to clean drinking water *ad-libitum*.

RESULTS

ANTI-DIABETIC ACTIVITY (Including Acute and Chronic Model)

CNLME **Route of Administration** **Histopathological Studies in mice**



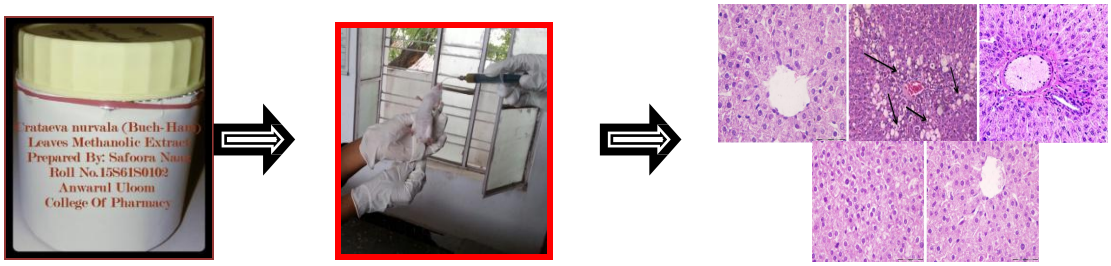
CNRBME **Route of Administration** **Histopathological Studies in mice**

CNLAE **Route of Administration** **Histopathological Studies in mice**

CNRBAE **Route of Administration** **Histopathological Studies in mice**

Flow chart Representation of Anti Diabetic activity (Including Acute and Chronic model).

ANTI-HYPERLIPIDEMIC ACTIVITY: (Including Actue And Chronic Model)



CNLME **Route of Administration** **Histopathological Studies in rats**

CNRBME **Route of Administration** **Histopathological Studies in rats.**

CNLAE **Route of Administration** **Histopathological Studies in rats.**

CNRBAE **Route of Administration** **Histopathological Studies in rats.**

Flow chart Representation of Anti Hyperlipidemic activity (Including Acute and Chronic model)

Percentage (%) Extractive Value of *Crataeva nurvala* Plant Extracts:

The percentage Extractive value of *Crataeva nurvala* (Buch-Ham.) Leaves and Root Bark Methanolic Extract by Soxhlet Extraction and Aqueous Extraction by Decocation method was calculated and the results obtained are as follows:

Percentage Extractive values result of *Crataeva nurvala* Plant Extracts.

Extract	CNLAE	CNLME	CNRBME	CNRBAE
Colour	Greenish (Amber colour)	Dark Green (Amber colour)	Dark Brownish (Amber colour)	Brownish (Amber colour)
Consistency	Semisolid liquid	Semisolid Liquid	Syrupy liquid	Syrupy liquid
%Yield (w/w)	69.6 %w/w	79.6 %w/w	75.6 %w/w	59.6 %w/w

Phytochemical Screening:

The results of the Preliminary Phytochemical Screening of the *Crataeva nurvala* Methanolic and Aqueous Extracts are as follows:

Sl.No	Name of the test	CNLME	CNLAE	CNRBME	CNRBAE
	Test for Carbohydrates:				
1.	Molisch's Test	-	+	-	+
2.	Fehling's Test	+	+	-	+
3.	Benedict's Test	+	-	+	+
	2. Test for Amino Acids:				
4.	Ninhydrin Test	-	-	+	+
5.	Test for Tyrosine	+	+	-	-
6.	Test for Cysteine	-	+	+	+
	3. Test for Steroids and Triterpenoids:				
7.	Salkowski Reaction	+	-	-	-
8.	Liebermann Burchard Reaction	+	-	+	+
	4. Test for Cardiac Glycosides:				
9.	Baljet's Test	-	-	+	+
10.	Legal's Test	+	-	+	+
11.	Keller Killiani Test	+	+	+	+
	5. Test for Anthraquinone Glycosides:				
12.	Borntrager's Test	-	-	+	+
13.	Modified Borntrager's Test	+	-	-	-
	6. Test for Saponin Glycosides:				
14.	Foam Test	-	+	+	+
	7. Test for Flavonoids:				
15.	Shinoda Test	-	+	+	+
16.	Alkaline Reagent Test	+	+	+	+
17.	Zinc HCL Test	-	-	+	+
	8. Test for Alkaloids:				
18.	Dragendroff's Test	+	-	-	+
19.	Mayer's Test	+	+	+	+
20.	Hager's Test	+	-	+	+
21.	Wagner's Test	+	+	+	+
	9. Test for Tannins:				
22.	Ferric Chloride Test	-	-	+	+
23.	Vanillin HCL Test	-	-	+	+
24.	Bromine Water Test	-	+	+	+
25.	Acetic Acid Solution Test	+	-	+	+
26.	Lead Acetate Solution Test	+	+	+	+
27.	Gelatin Solution Test	+	+	+	+
28.	Potassium Dichromate Test	+	+	+	+
29.	Dilute Iodine Solution Test	-	-	+	+
30.	Dilute HNO_3	-	+	+	+

EFFECT OF THE EXTRACTS ON BLOOD GLUCOSE LEVELS IN SWISS ALBINO MICE:

Effect of methanaolic extracts of Leaves and Roots bark of Crataeva nurvala (CNLME, CNRBME):

The results are shown in Table 4.3 and Fig.4.1. All doses of CNLME, CNRBME. Significantly (P<0.05) reduced the blood glucose levels after 6 and except the lower dose. However CNLME at doses 250 and 500 mg\kg b.w. significantly (p<0.05) lowered the fasting blood glucose levels after 4h and the effects was highly significant (p<0.01; 21.1±5.2% and 23.6±3.1%) after 6h and the hypoghycemic effect of CNRBME was significant (p<0.05; 28.8 ± 4.3% and 30.92± 5.0%) up to 8h at the dose 250 and 500 mg\kg b. w. The effect of CNLME on impairment of blood glucose levels was marked and dose dependent at each time point. The hypoglycemic effect of Metformin HCL (10 mg\kg b.w.), the referenc drug was significant (p<0.05; 19.4+2.1%) after 2h and highly significant (p<0.01;33.01+3.8%) after 4h and was significant (p<0.05: 18 ± 5.4 %) up to 6 h only. Though there is a difference in the significant (p<0.05) on set of action between the reference drug (10 mg/kg b.w.) and CNRBME (250 and 5 00 m g/kg b.w.) CNRBME produced prolonged significant (p<0.05) hypoglycemia at 250 and 500 mg/kg b.w. doses.

Effect of Methanaolic Extracts on Blood glucose level In Streptozotocin Induced Type 2 Diabetic Rats

Effect of Methanolic extract of roots of *C. nurvala* (CNLME, CNRBME)

The results are shown in Table 4.5 and Fig. 4.3. CNLME, CNRBME exhibited dose dependent effect on reduction of blood glucose levels in streptozotocin induced hyperglycemia. CNLME at 250 mg/kg b.w. altered blood glucose levels non significantly, while 500 mg/kg b.w. dose exhibited the effect significantly (p< 0.05:19.4±3.2 %) after 6 h. However CNRBME at doses 250 and 500 mg/kg b.w. significantly (p<0.05) lowered blood glucose levels after 4h, 6h and 8h. The effect was highly significant (p< 0.01) after 6h only. The highly significant (p<0.01; 26.9±5.3 and 31.5±3.1%) effect of CNRBME at 250 and 500 mg/kg, b.w. doses after 6 h was comparable to that of Metformin HCL (10 mg/kg b.w.), the reference drug.

Effect of Aqueous extract of leaves of *C. nurvala* (CNLAE, CNRBAE)

The results are shown in Table 4.6 and Fig. 4.4. CNLAE, CNRBAE showed dose dependent reduction in blood glucose levels. CNLAE at 250 and 500 mg/ kg b.w. doses decreased the blood glucose levels significantly (p<0.05; 24.8 ±5.9 %) after4 h only while at 250 and 500 mg/kg b.w. doses, exhibited the effect significantly after 2h, 4h and 6h. Maximum reduction in blood glucose level was also seen at doses of 250 and 500 mg/kg b.w.(p<0.01: 30.5±5.5 and 32.7±4.6 %) after 4 h. The significant (p<0.05) and highly significant (p<0.01; 18 to 32 %) effect of CNRBAE at 2 5 0 and 500 mg/kg b.w. doses after 2 h and 4 h respectively were comparable to the of glibenclamide (10 mg/kg b.w.) the reference drug.

Effect of CNLME, CNLAE, CNRBME and CNRBAE on different parameters in sub acute study (28 Days) of Streptozotocine induced Type 2 Diabetes in rats

The observations made on the results of different parameters studied are as follows.

Body weight:

There was a diminution in body weight of animals in diabetic control group. The body weight of 'animals in extracts treated and reference drugs treated groups increased significantly (p<0.05) after 28 days. The gradual increase in weight of animals in EAFKR (100 mg/kg b.w.) group after 7 days to 28 days was comparable to that of glibenclamide (10 mg/kg b.w.) and metformin (250 mg/kg b.w.), the reference drugs. The significant effect of CNLME, CNLAE, CNRBME and CNRBAE on body weight of the animals was comparable to that of both the reference drugs.

Blood glucose level:

The extracts and reference drugs lowered the blood glucose level gradually after 7 days. The highly significant anti hyperglycemic effect was observed after 14 days in CNRBAE treated group and after 21 days in CNLME group, to till the end of 28 days. The maximum reduction in blood glucose level (44.0- 46.5 %) in CNRBME (100 mg/kg b.w.) and CNLAE (100 mg/kg b.w.) groups at the end of 28 days was comparable to that of the reference drugs,
Glibenclamide (10 mg/kg b.w.; 45.9 %) and Metformin (250 mg/kg b.w.; 44.36 %) groups. The antihypergiycemic effect of CNRBME (100 mg/kg b.w.) was comparable to glibenclamide (10 mg/kg b.w.) at any time point of the study.

Serum triglyceride level:

In extracts treated and reference drugs treated groups, a gradual decrease in triglyceride level was recorded. After 7 days, to till the end of 28 days. The two extracts, CNRBE and CNLEexhibited anti hyper triglyceridemic effect with '13' values less than 0.05 and 0.01 after 21 days and 28 days respectively, and was comparable to that of both the reference drugs, glibenclamide and Metformin.

Serum cholesterol:

CNLME, CNLAE, CNRBME and CNRBAE lowered cholesterol level significantly (p<0.05) after 28 days. The maximum reduction in serum cholesterol (37.1±1.8 %) was observed in CNLE group, when compared to the other groups of the study. The significant (p<0.05) anti hypercholesterolemic effect of the extracts was comparable to that of the reference drugs.

Serum insulin

In CNLME, CNLAE, CNRBME and CNRBAE groups, very significant ($p<0.001$) increase in serum insulin level after 28 days was observed, comparable to glibenclamide. The percent increase in serum insulin level was ranging from 18 to 124. There was no significant change observed in serum insulin level in both CNLE and metformin treated groups.

Serum Glutamate Oxaloacetate Transaminase (SGOT) and Serum Glutamate Pyruvate Transaminase (GPT) level:

After 28 days of CNLME, CNLAE, CNRBME and CNRBAE supplementation, there was a significant diminution in serum GOT and PT level. The significant effect ($p<0.05$) of the extracts was comparable to that reference drugs.

Serum total protein:

The effect of CNLME, CNLAE, CNRBME and CNRBAE on serum total protein level was significant ($p<0.05$) after 28 days of the study, comparable to the of glibenclamide and metformin, the reference drugs. The increase in serum total protein level was ranging from 40-70% and was maximum in CNLE group.

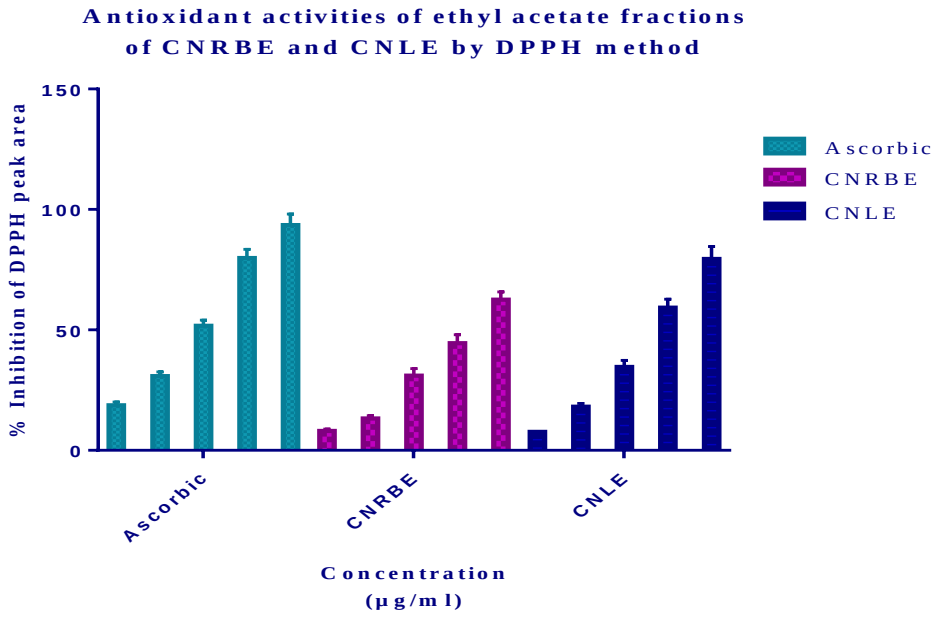
Antioxidant studies on CNRBE and CNLE

***In vitro* study by DPPH induced diabetic rats**

The percentage inhibition of DPPH peak area by ascorbic acid, CNLME, CNLAE, CNRBME and CNRBAE are summarized in Table 4.13. and Fig.4.17. Antioxidant activity was measured in terms of percentage inhibition of DPPH peak area. The test extract, ascorbic acid, the reference compound increased percentage inhibition of DPPH peak area ina concentration dependent manner. CNLME, CNLAE, CNRBME and CNRBAE at 100 µg/ml concentration showed 62.56% and 79.42% inhibition respectively, where as the reference compound, ascorbic acid at the same concentration exhibited 93.5% inhibition. Among the two extract, CNRBE was found to be the most effective antioxidant (IC_{50} -67.5 µg/ml) followed by CNLE (IC_{50} -59.74 µg/ml) However, the IC_{50} value of ascorbic acid (IC_{50} - 21.07 µg/ ml) was found to be lower then those of the test extracts.

Antioxidant activities of ethyl acetate fractions of CNRBE and CNLE by DPPH method.

Concentration (µg/ml)	% Inhibition of DPPH peak area		
	Ascorbic acid	CNRBE	CNLE
5	18.69+1.4	8.06±0.8	7.62±0.2
10	30.81±1.8	13.25±1.2	18.07±1.4
25	51.75±2.3	31.04±2.9	34.63±2.7
50	79.8±3.6	44.53±3.5	59.18±3.6
100	93.5± 4.6	62.56±3.2	79.42+5.2



Antioxidant activities of ethyl acetate fractions of CNRBE and CNLE by DPPH method.

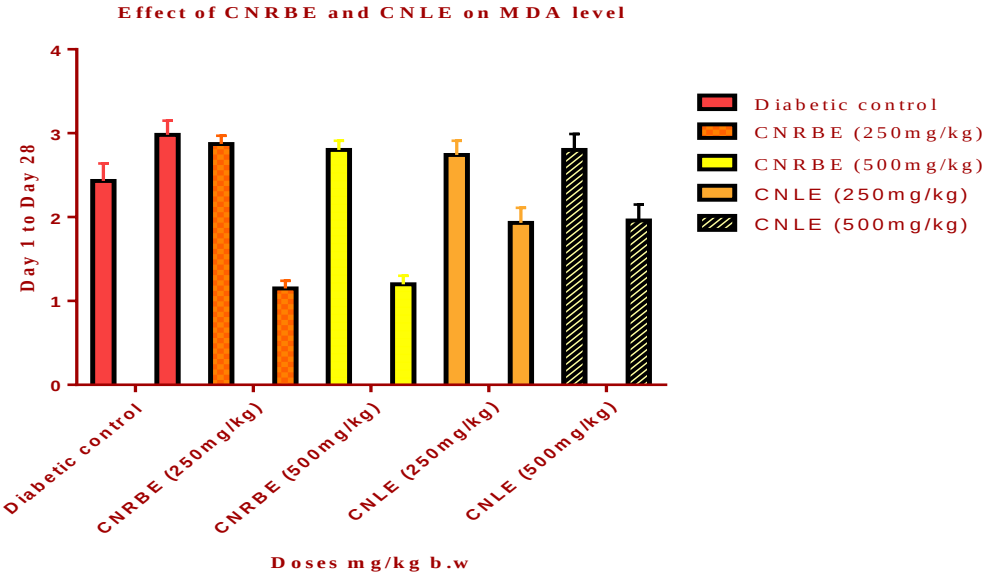
***In vivo* study in streptozotocin induced diabetic rats (sub acute model) by estimation of lipid-peroxidation product (malondialdehyde)**

The level of malondialdehyde was significantly increased in diabetic control rats. Administration of CNRBE and CNLEto diabetic rats daily at 100 mg/kg b.w. dose for 28 days significantly ($p<0.001$) decreased the level of lipid peroxidation marker, malondialdehyde after 28 days. CNRBE and CNLEexhibited the same degree of antioxidant activity.

Effect of CNRBE and CNLE on MDA level.

Group	Dose (mg/kg b.w.)	First Day (nM/m1)	Day 28 (nM/m1)
Diabetic control	-	2.43±0.21	2.98±0.17
CNRBE	250	2.87±0.10	1.15±0.09***
CNRBE	500	2.80±0.11	1.20±0.10***
CNLE	250	2.74±0.17	1.93±0.18***
CNLE	500	2.80±0.19	1.96±0.19***

***P< 0.001 in comparison with Diabetic control.



Effect of CNRBE and CNLE on MDA level

Evaluation of CNRBE and CNLE for glucose uptake stimulatory activity in 3T3-L1 adipocytes and insulin secretogogue property in RIN cells.

Induction of adipogenesis

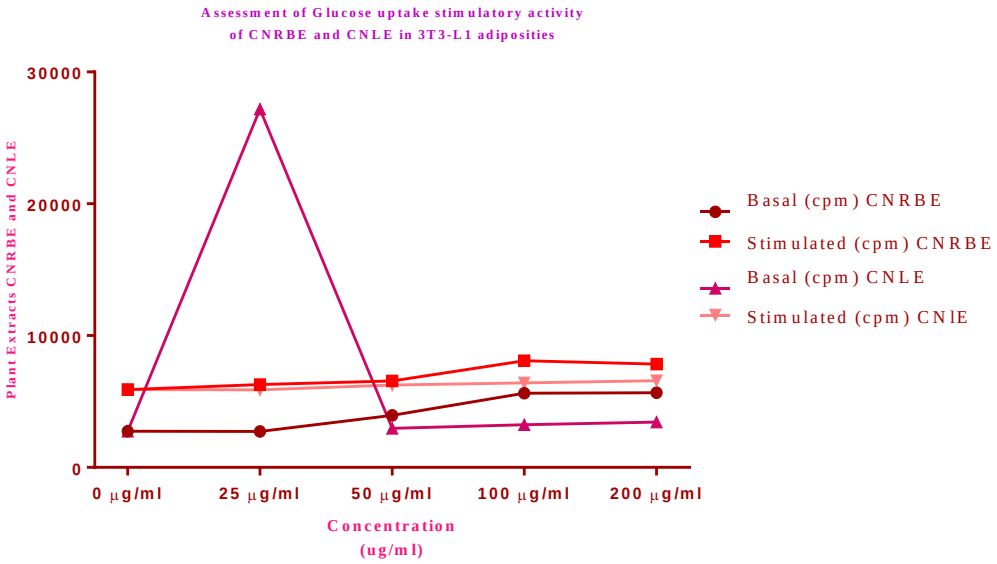
Differentiation of adipogenesis was started on day 4 and continued till day-12 of post induction and on day-10 cells were reached almost 90% of adipogenesis. Plates where cells were >90% differentiated were used for experiments between days 9-12 post-induction. Pioglitazone is the standard drug.

Effect of CNRBE and CNLE on cellular uptake of 2-deoxy-D-[3H]-glucose

Initially, insulin concentration that promotes higher glucose uptake was optimized by subjecting various concentrations of insulin to adipocytes and found that at 1 tM glucose uptake was maximized. Cellular uptake of glucose was significantly ($p<0.01$) elevated by the test extracts. The test extracts exhibited dose dependent glucose uptake, both basal and insulin stimulated. The order of degree of glucose uptake stimulation by the extracts was CNRBE =CNLE. Glucose uptake stimulating activity of the extracts is presented in table.4.15 and Fig.4.19.

Assessment of Glucose uptake stimulatory activity of CNRBE and CNLE in 3T3-L1 adiposities.

Concentration (ug/ml)	CNRBE		CNLE	
	Basal (cpm)	Stimulated (cpm)	Basal (cpm)	Stimulated (cpm)
0	2739 ± 135	5911±299	2739 ±135	5911±299
25	2736 ± 153	6283±65	27182	5883±122
50	3944 ± 73*	6563±366*	2966±82	6241±116
100	5626 ± 159***	8094±173***	3232±97	6407±142
200	5673±160***	7843±156***	3447±104	6580±186*



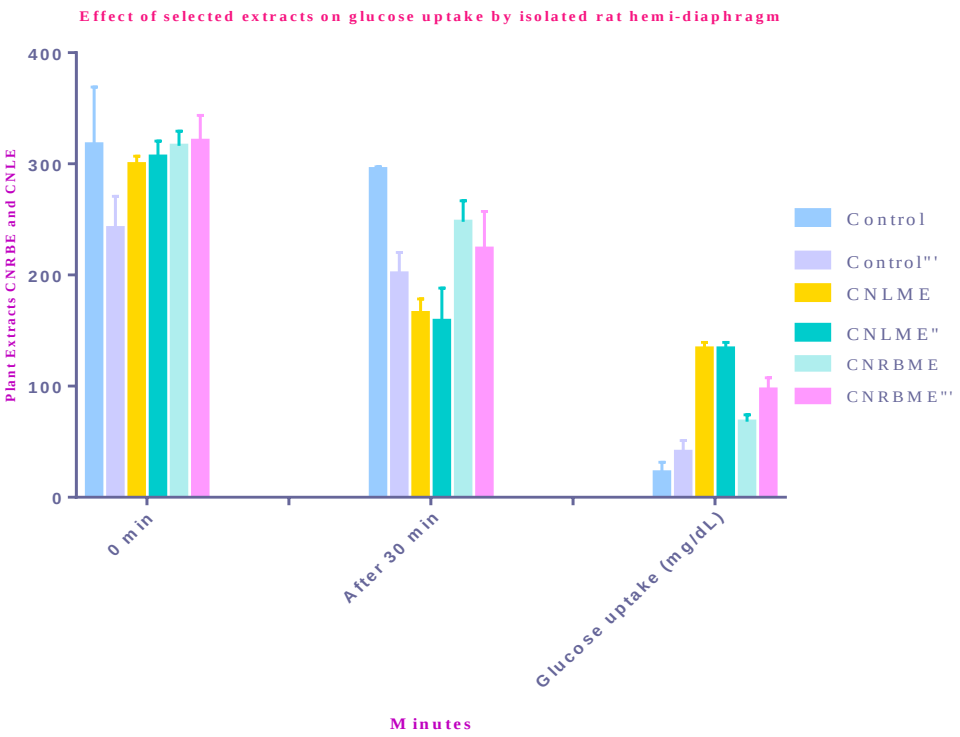
Assessment of Glucose uptake stimulatory activity of CNRBE and CNLE in 3T3-L1 adiposities.

Glucose uptake by isolated rat hemi-diaphragm:

The glucose uptake by rat hemi-diaphragm was significantly more in all the groups tested when compared to the control group. CNLE and CNRBE is more effect plant against diabetes. The effect was more in extracts and extract insulin treated groups than in the animals treated with insulin alone.

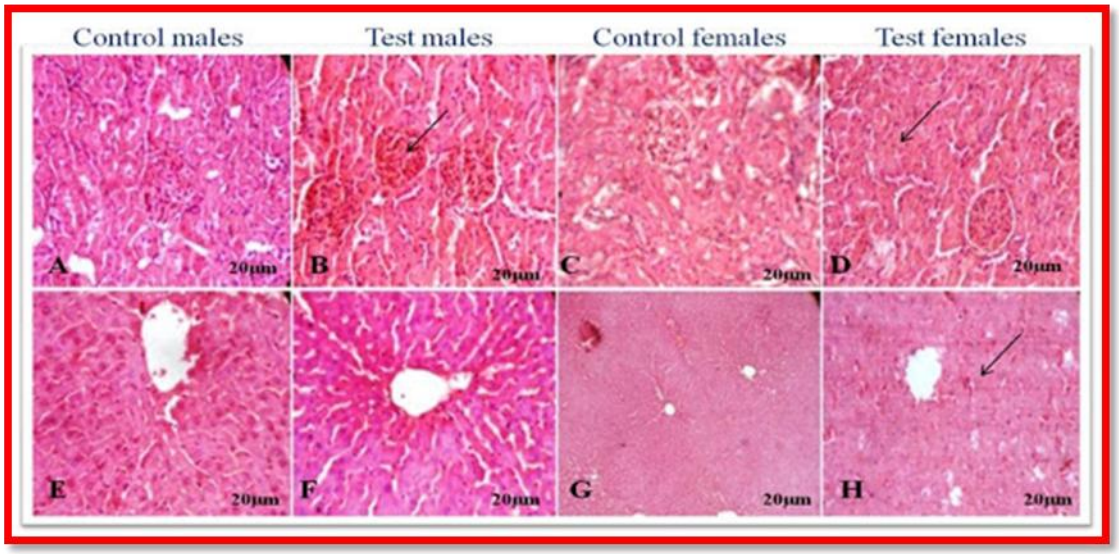
Effect of selected extracts on glucose uptake by isolated rat hemi-diaphragm.

	Control	Control"'	CNLME	CNLME"	CNRBME	CNRBME '"
0 minute	317.6 ±51.6	242.1±28.8	299.7±7.2	306.5 ±13.9	316.3 ±13.1	320.7±23.0
After 30 min	295.3±42.01	201.3±18.9	165.8±12.7	158.6±29.7	247.8±19.03	223.7±33.6
Glucose uptake	22.3±9.2	40.8±10.2	133.9±5.5***	147.9±16.2***	68.1±6.1*	97±10.6*



Effect of selected extracts on glucose uptake by isolated rat hemi-diaphragm.

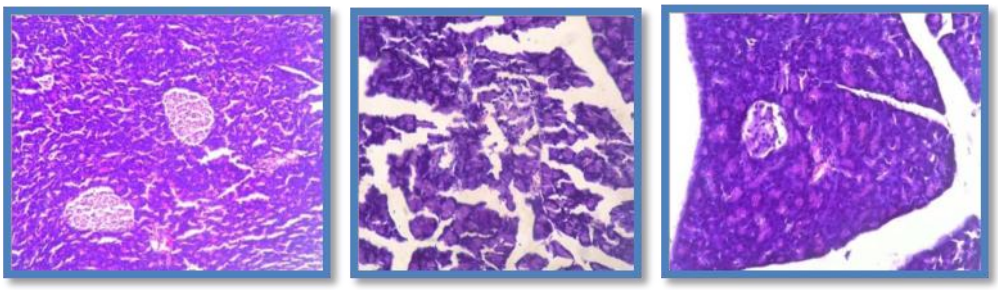
Histopathological studies of control and treated against C. *nurvala* of Methanaolic extract of Albino Wistar Mice.



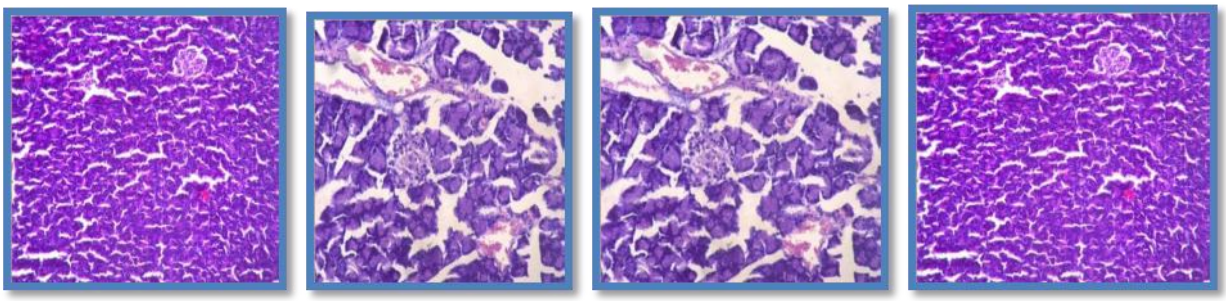
Histopathological studies of Anti Diabetic Activity In Albino Wistar Mice of Methanaolic extract.

A Control
B Standard Metformin 10mg/kg
C Test-I CNLME (250mg/kg)
D Test-II CNLME (500mg/kg)
E Test-III CNRBME (250mg/kg)
F Test-IV CNRBME (500mg/kg)
G Test-V CNLAE (250mg/kg)
H Test-VI CNRBAE (500mg/kg)

Histopathological studies of control and treated against C. *nurvala* of Aqueous extract of Albino Wistar Mice.

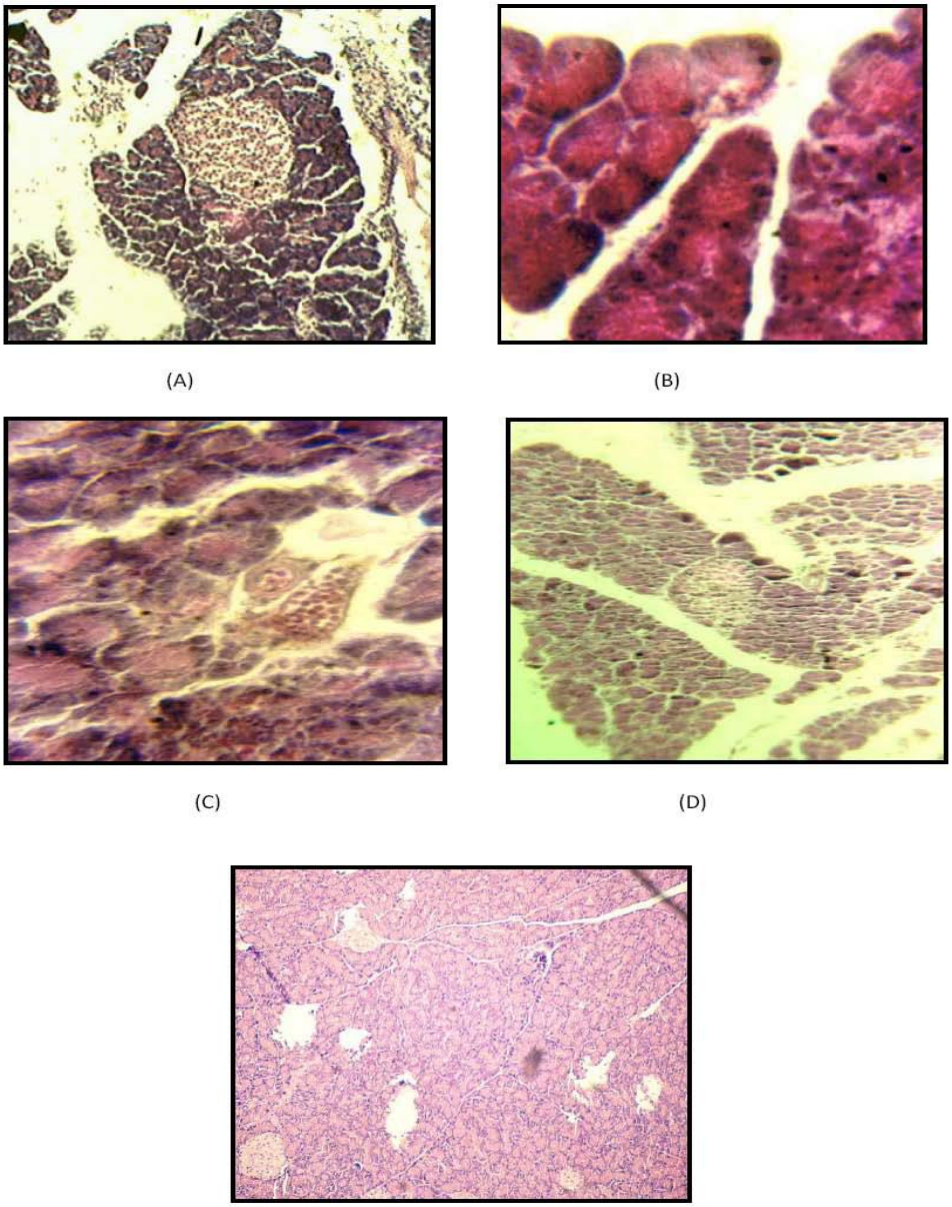


Normal Control Non-Diabetic **Positive Control Diabetic** **Metformin HCL (10mg/kg)**



Test-I 250mg/kg **Test-II 500mg/kg** **Test-III 250mg/kg** **Test-IV 500mg/kg**

Histopathological studies of Anti Diabetic Activity In Albino Wistar Mice of Aqueous extract.
Test-I CNLAE (250mg/kg)
Test-II CNLAE (500mg/kg)
Test-III CNRBAE (250mg/kg)
Test-IV CNRBAE (500mg/ kg)



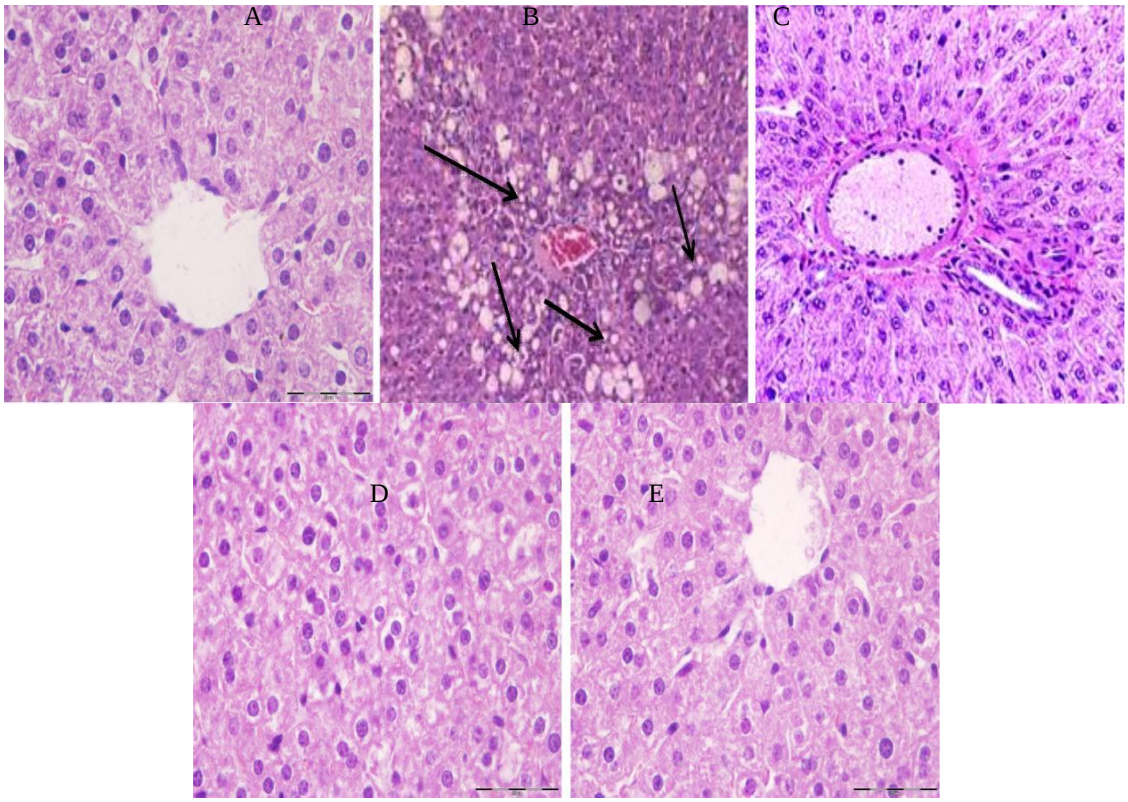
Effect of methanaolic extract of *C. nurvala* at 250 mg/kg and reference compound on pancreases.

(A) Normal islets with secretary granules (B) Clear and normal parenchyma (C) Degenerative and necrotic changes with shrunken islets of langerhans (D) Regeneration of islets of langerhans (E) Restoration of normal cellular size of the islet with hyperplasia.

RESULTS OF ANTI-HYPERLIPIDEMIC ACTIVITY

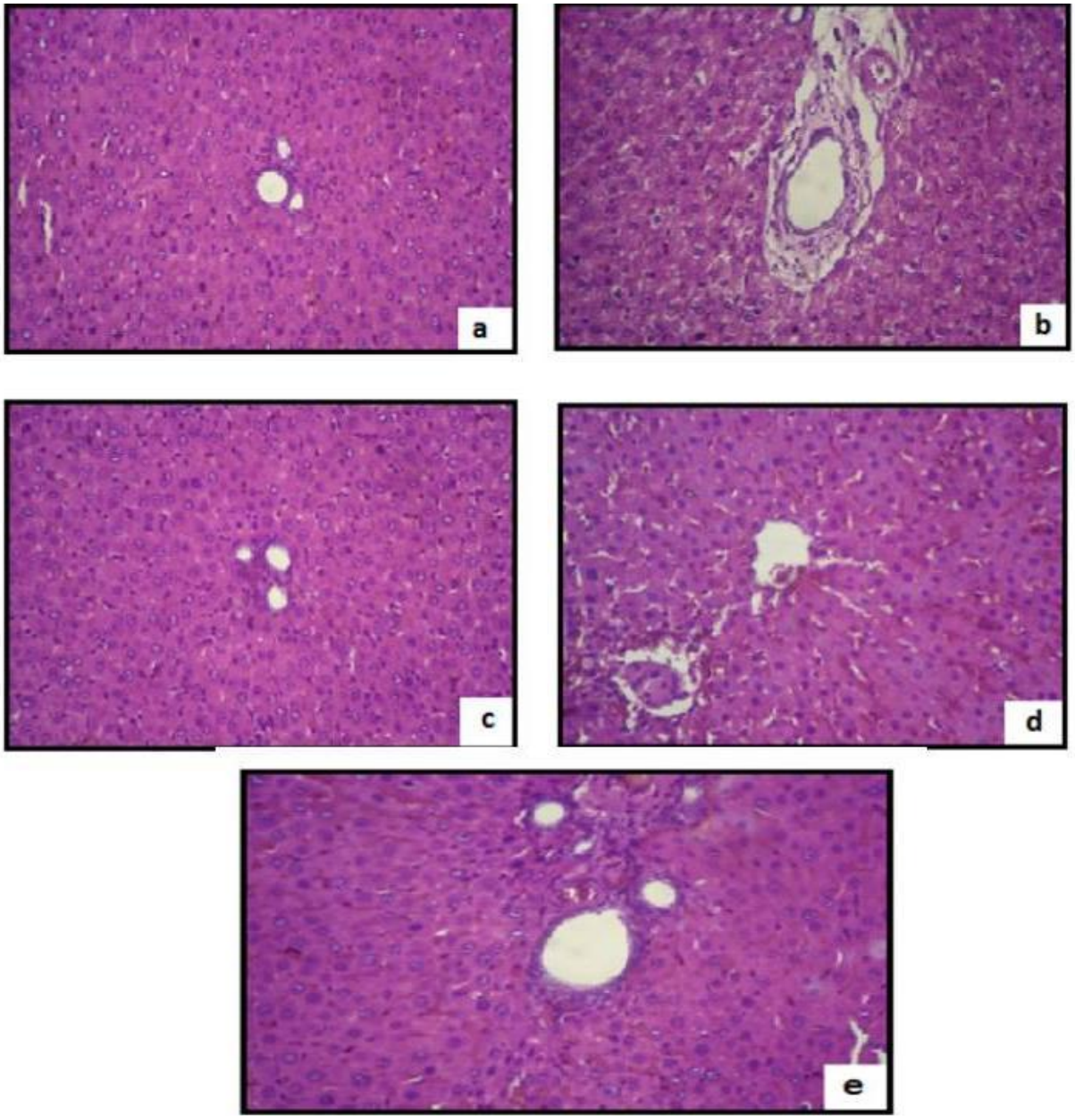
The results of *in-vivo* anti hyperlipidemic studies of the formulation (at 250 mg/kg and 500 mg/kg b.w.) like the changes in body weight, serum lipid parameters and action on hepatic enzymes. A significant decrease ($p<0.01$) in the TC, TG and LDL-C levels were observed in the treatment groups with an increase in HDL-C. The serum AST, ALT and ALP levels were also decreased in groups treated with the formulations in a dose dependent manner.

Histopathology of Liver of control and treated animals



Histopathological sections of the liver (40X) of normal diet rat showing normal hepatocytes of Methanaolic Extract.

(A), AD fed rat showing fatty liver with peripheral located nucleus (B), Atrovastatin treated rat showing normal hepatocytes (C), CNLME – 250 mg/kg treated rat hepatocytes (E). CNRBME – 500mg/kg treated rat hepatocytes.



Histopathological sections of the liver (40X) of normal diet rat showing normal hepatocytes of Aqueous Extract.

(A), AD fed rat showing fatty liver with peripheral located nucleus (B), Atrovastatin treated rat showing normal hepatocytes (C), CNLAE – 250 mg/kg treated rat hepatocytes (E). CNRBAE – 500mg/kg treated rat hepatocytes.

CONCLUSION

- Methanolic extract of roots of *C. nurvala* leaves and roots bark.
- Methanolic and aqueous were found to be non-toxic in albino mice up to 2 g/kg b.w. p.o.
- CNLME and CNRBME at 250 and 500 mg/kg b.w dose exhibited significant hypoglycemic activity in normal healthy rats.
- CNLAE and CNRBAE at a dose of 250 and 500mg/kg b.w. showed significant oral glucose tolerance in normal rats and antihyperglycemic activity in Triton Induced Hyperlipidemia in rats.
- Hypoglycemic and antidiabetic effect of the extracts (250 mg/kg b.w. p.o) are comparable to that of standard drug, metaformin HCL (10 mg/kg b.w. p.o).
- Methanol fraction of CNL (CNLME) and CNRB (CNRBME) at 200 mg/kg b.w. significantly decreased the blood glucose level in streptozotocin induced diabetic mice.
- CNLAE and CNRBAE at dose, 250 mg/kg b.w. showed significant oral glucose tolerance in streptozotocin induced diabetic mice.
- In sub acute study of CNLME and CNRBME at 250 mg/kg b.w. dose significantly decreased the elevated levels of blood glucose, cholesterol and trigiyeerides, SGOT, SGPT and increased the diminished body weight, total protein, insulin (except in case of CNL, CNRB) in streptozotocin induced diabetic rats, which are desirable in diabetes therapy.
- In invitro study CNLME and CNRBME exhibited a concentration dependent free radical scavenging activity. In sub acute study in streptozotocin induced diabetic rats, CNLAE and CNRBAE showed antioxidant activity by significantly decreasing the level of lipidperoxidative - marker, malondialdehyde (MDA), a protective action against cell damage required in diabetes therapy.
- Preliminary phytochemical investigations showed the presence of bioactive compounds like glycosides, sterols, terpenoids and phenolic compounds in selected plant namely, *Crataeva nurvala*, using two parts leaves and roots bark.
- The phenolic content of the selected plants were determined and were found to be in the order CNLME (Methanolic extract of *C. nurvala* leaves)> CNRBME (Methanolic extract of *C. nurvala* roots bark) >CNLAE (Aqueous extract of *C. nurvala* leaves) > CNRBAE (Aqueous extract of *C. nurvala* roots bark).
- The plant extracts showed no toxicity at a maximum dose of 2000 mg/kg.
- All the extracts tested showed significant antioxidant activities in DPPH, Nitric oxide and lipid peroxidation methods in a dose dependant manner.
- Among the four extracts tested for anti diabetic and anti hyperlipidemic activity *in-vitro*, four extracts showed significant activity in the order CNLME> CNRBME> CNLAE> and CNRBAE.
- All the four extracts tested *in-vivo* showed decrease in lipid profiles and atherogenic index in a dose dependent manner. The efficacy was in the order, CNRBME> CNRBAE> CNLAE. However CNLME did not show good activity. The decrease in the levels of hepatic enzymes ALT, AST, ALP and lipid peroxidation indicate the hepatoprotective nature of the extracts.
- The alcoholic extracts of selected herbal drugs could be formulated into effective hypolipidemic dosage form. The formulation possessed significant activity than the individual extracts. This may be due to the synergistic effect of the combined extracts.
- Thus the results of the present investigation clearly indicated that the selected medicinal plants possess good antihyerlipidemic activity in atherogenic diet induced hyperlipidemic rats and led to the development of new Herbal formulation possessing antihyperlipidemic and antidiabetic activities. This is the first study which investigates the hypolipidemic activity of *Crataeva nurvala*. The results found are encouraging for further studies on the selected plants and to identify the bioactive compounds.

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