

Available online on 15.04.2020 at <http://jddtonline.info>

Journal of Drug Delivery and Therapeutics

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Research Article

Evaluation of Antidiabetic and Antihyperlipidemic Effect of Ethanolic Leaves Extract of *Centratherum anthelminticum (L) Kuntze* against STZ Induced Diabetic Rats

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ABSTRACT

Objective: To evaluate the Antidiabetic & Antihyperlipidemic effect of *Centratherum Anthelminticum (L) Kuntze* extract against STZ induced diabetic rats.

Methods: The present study examined the influence of *Centratherum Anthelminticum* extract (CASE), on blood glucose level and antioxidant enzymes level in STZ induced diabetic rats. The possible effects of chronic administration of the extract on the fasting serum glucose, insulin, and total cholesterol and triglycerides levels in STZ-induced diabetic rats were also evaluated.

Results: The extracts showed a marked Antidiabetic & Antihyperlipidemic effect. *Centratherum anthelminticum ethanolic seeds extract* is capable of exhibiting glycemic control through its inhibitory effect on glycogenolytic enzyme glycogen phosphorylase, gluconeogenic enzymes such as glucose-6-phosphatase and fructose-1, 6-bis phosphatase and stimulatory effect on hexokinase, which is the key enzyme in the glycolytic pathway.

Antihyperlipidemic activity of *Centratherum anthelminticum ethanolic seeds extract* proved by restoration of altered lipid profile

Conclusions: The results of the study indicate that the extract of *Centratherum anthelminticum* possesses strong antidiabetic & antihyperlipidemic activity. This study also describes therapeutic effect of *Centratherum anthelminticum* in diabetes which will give a new direction for the future scientific research.

Keywords: Diabetes, *Centratherum anthelminticum*, Pharmacological, Antidiabetic, Antihyperlipidemic

Article Info: Received 19 Dec 2019; Review Completed 25 Feb 2020; Accepted 05 April 2020; Available online 15 April 2020



Cite this article as:

Goutam N, Kapoor R, Evaluation of Antidiabetic and Antihyperlipidemic Effect of Ethanolic Leaves Extract of *Centratherum anthelminticum (L) Kuntze* against STZ Induced Diabetic Rats, Journal of Drug Delivery and Therapeutics. 2020; 10(2-s):140-148 <http://dx.doi.org/10.22270/jddt.v10i2-s.4014>

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INTRODUCTION

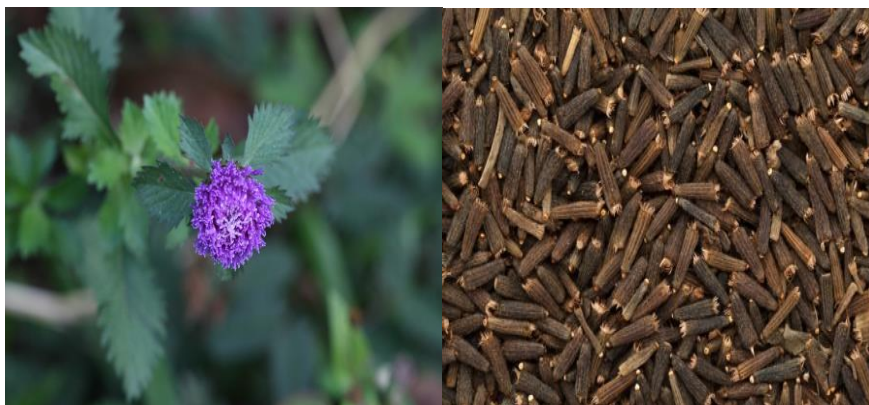
Diabetes mellitus is a metabolic disorder with high blood sugar levels over a prolonged period of time. Symptoms of high blood sugar may be polyuria, polydipsia and polyphagia. Diabetes mellitus is a chronic metabolic disorder characterized by a high blood glucose concentration - hyperglycemia (fasting plasma glucose >126 mg/dL, or plasma glucose >200 mg/dL 2 hrs after a meal) - caused by insulin deficiency, often combined with insulin resistance. In diabetic patients, oxidative stress also has been found to be mainly due to increased production of oxygen free radicals and sharp reduction of anti-oxidant defense¹. Hypoinsulinemia associated with diabetes increased the activity of enzyme, fatty acyl Coenzyme A oxidase which initiates the β -oxidation of the fatty acids, resulting in lipid peroxidation. Increased lipid peroxidation impairs membrane function by decreased membrane fluidity and

changing the activity of the membrane-bound enzyme and receptors². Its products (lipid radices and lipid peroxides) are harmful to the cell in the body and associated with atherosclerosis and brain damage. Diabetes Mellitus is associated with abnormalities in carbohydrates and lipid metabolism that results in excessive production of reactive oxygen species [ROS] and oxidative stress³.

Therefore the present study is undertaken to evaluate antidiabetic and anti-oxidant activity of *C. anthelminticum* in STZ induced diabetic rats.

The ethnobotanical information reports about 800 plants that may possess antidiabetic potential. Folk medicine for diabetes from Rayalaseema reports 26 plants with antidiabetic activity. One such plant is *C. anthelminticum* synonyms; *Vernonia anthelmintica*, *Conyza anthelmintica* belongs to the family Asteraceae. In Hindi it is known as Kalizeeri⁴.

Description of Plant



Centratherum Anthelminticum (L) Kuntze plant and closed Seeds

Ethno medicinal & traditional uses of different parts of *Centratherum anthelminticum*

The Indications of Leaves of *C. anthelmintic* are rheumatism, chronic persistent fevers, phlegmatic cough, and dropsy. The indications of Seed of *C. anthelmintic* are kidney troubles, pustules, skin irritation, hiccup, eye itching, cold phlegmatic inflammation, chronic persistent fevers, dysuria, rheumatism, leucoderma, psoriasis, colic, vitiligo, sores, asthma, and pimples.

Roots of *C. anthelmintic* are used in rheumatic swellings. Seed (powder) of *C. anthelminticum* is used in pimples, thread worms, irritative skin & round worms ⁵.

Herb Paste of *C. anthelminticum* is used in skin eruptions, rheumatism. The Indications of infusion (seed) of *C. anthelmintic* are phlegmatic cough, dropsy, rheumatic affections, and chronic persistent fevers ⁶.

Significance of the Study

C. anthelminticum is an important drug of indigenous system of medicine and has been known for a number of medicinal properties in Ayurveda. *C. anthelminticum* is used as folk-medicine for diabetes in Rayalaseema of Andhra Pradesh of India but still its pharmacological potential for anti-diabetic activity has not been fully explored ⁷. This study will try to provide scientific basis for the further development of antidiabetic activity of herbal drugs.

MATERIAL AND METHOD

- Collection & identification of plant material *C. anthelminticum (L) kuntze*.
- Preparation of ethanolic extract of *C. anthelminticum*.

The powdered seeds (200 g) are extracted with 1 l of 95% ethanol in a soxhlet extractor for 24 h. The solvent is recovered by distillation in vacuo which yielded a dark brown oily semisolid residue. The oil is separated from the extract on standing and the resultant extract is used for further studies ⁸.

Chemicals and reagents

STZ, RIA kit serum insulin level, GOD-POD kit serum glucose level, Glibenclamide, Ammonium thiocyanate, 1,1-diphenyl-2-picryl-hydrazyl (DPPHd), 3-(2-Pyridyl)-5,6-bis(4-phenyl-sulfonic acid)-1,2,4-triazine (Ferrozine), Griess reagent, Colchicine, Ellman's Reagent (DTNB), acetylthiocholine iodide, bovine serum albumin, thiobarbituric acid and 1, 1, 3, 3-tetraethoxypropane (MDA) were purchased from Sigma chemical. Butylated hydroxytoluene (BHT), hydroxylamine,

ferrous chloride, Ascorbic acid (Vitamin C), nitro blue tetrazolium, EDTA, β -carotene, Tween-80, trichloroacetic acid (TCA), hematoxylin and eosin were purchased from Himedia Co. Chloral hydrate, isotonic saline, sodium carbonate, 4% paraformaldehyde, paraffin, were purchased from Central Drug House Laboratory (CDH). All others unlabelled chemicals and reagents were analytical grade ⁹.

Plant materials

Fresh leaves and flowers of *C. anthelminticum* seed were collected from the khari baoli; chandni chowk Delhi. The plant was authenticated by **Dr. Anjula Pandey, Principal Scientist** National Herbarium of Cultivated Plants (NHCP) Division of Plants Exploration and Germplasm Collection, National Bureau of Plant Genetic Resources, New Delhi. The voucher specimen (NHCP/NBPGR/2018/39) of the plant material has been deposited in the Department.

Preparation of ethanolic extract

C. anthelminticum seed were dried under shade coarsely powdered and passed through 40 mesh sieves. Successive solvent extraction scheme was followed for the preparation of different extracts ¹⁰. Coarsely powdered *C. anthelminticum* seed were defatted with petroleum ether using Soxhlet apparatus. The dried marc was further extracted with ethanol using Soxhlet apparatus. Solvent was removed under reduced pressure using rotary evaporator to yield dried ethanolic extract ¹¹.

Induction of diabetes & Experimental design ¹²

Assessment of antihyperglycemic effect of ethanolic extract of *C. anthelminticum* seeds in normal and STZ induced diabetic rats:

Body weights of the animals were recorded and they were divided into 7 groups of 6 animals each as follows

Group I: Normal rats were administered with CMC

Group II: Diabetic control - Rats were induced with Streptozotocin in normal saline at a dose of 60 mg/kg body weight IP.

Group III: Groups of diabetic rats treated with ethanolic extract of *C.A* (100mg/kgbw) orally for 60 days

Group IV: Groups of diabetic rats treated with ethanolic extract of *C.A* (200mg/kgbw) orally for 60 days

Group V : Groups of diabetic rats treated with ethanolic extract of *C.A* (300mg/kgbw) orally for 60 days

Group VI: Normal rats were administered orally the ethanolic extract of *Centratherrum anthelminticum* (300mg/kgbw) for 60 days.

Group VII: Groups of diabetic rats treated with Glibenclamide (5 mg/kgbw/oral) for 60 days

Animal care and selection

Adult male Swiss albino mice were obtained from the suitable source. They will be housed in polypropylene cages in groups of six mice per cage and kept in a room maintained at 25 ± 2 °C with a 12-h light/dark cycle. They will be allowed to acclimatize for 1 week before the experiments and will be given free access to standard laboratory feed and water ad libitum. Protocol is approved by Institutional animal ethics committee.

Acute toxicity study

The acute toxicity study will be performed according to OECD guideline 423. The adult Albino Swiss mice 20-25 g will be randomly divided into five groups containing three animals in each group. The animals will be fasted overnight, and the *C. anthelminticum* seeds extracts will be administered orally of various dose levels (mg/kg, BW) suspended in 0.5% Carboxymethyl cellulose. Fifth group will be maintained as control and administered the vehicle only. The animals will be observed continuously for 2 h, then occasionally for a further 24 h, and finally any mortality. The behaviour of the animals and any other toxic symptoms will be observed for 72 h, and they were kept under observation for one week¹³.

Evaluation of Biochemical Parameters

Estimation of serum glucose level (GOD-POD method), serum insulin level (RIA method), serum cholesterol (enzymatic method), HDL cholesterol (enzymatic method), serum triglyceride (enzymatic method), serum urea (Berthelot method) and creatinine (alkaline picrate method) has to done. VLDL-cholesterol and LDL- cholesterol are calculated as per Friedewald' equation¹⁴.

VLDL-cholesterol = total serum triglycerides/5

LDL-cholesterol= Total serum cholesterol- total serum triglycerides/5-HDL-C

Estimation of total cholesterol & triglyceride: 1.0 ml of isopropanol was mixed with 0.1 ml of sample and 0.1, 0.2, 0.3, 0.4 and 0.5 ml of standard solution. To this 0.4g of alumina was added and mixed well for 15 min. The content was centrifuged at 3000 rpm for 10 minutes and 0.5 ml of the supernatant was transferred to reaction tubes and heated at 65 °C for 15 min. Then

0.6 ml of the saponification reagent was added and cooled. Then 1.0 ml of sodium metaperiodate and 0.5 ml of acetyl acetone reagent were added and heated at 65 °C for 30 minutes. The contents were cooled and the colour developed was read at 430nm.

From the standard optical density values, the amount of triglyceride in the sample was calculated and expressed as mg/dl¹⁵.

Estimation of phospholipids

0.1ml of sample (tissue lipid) was digested with 0.2ml of perchloric acid over a sand bath. Digestion was continued till it was colorless. The liberated phosphorus was estimated. 4.3ml of deionised water was added to the digested sample followed by 0.5ml of ammonium molybdate. After 10min

0.2ml of ANSA was added. Tubes were well shaken and kept aside for 20mins. Blue colour read at 620nm. The total phospholipids were estimated by multiplying the value of Pi by 25 and expressed as mg/g wet tissue¹⁶.

Estimation of HDL cholesterol

To 1.0 ml of lipid extract, 0.18 ml of heparin manganese chloride reagent was added and mixed. This was allowed to stand in an ice bath for 30 minutes and then centrifuged in a refrigerated centrifuge 2500 g for 30 minutes. The supernatant contained HDL fraction. Aliquots of the HDL supernatant were estimated for cholesterol, phospholipids¹⁷.

Estimation of VLDL

To 1 ml of lipid extract was added to 0.15 ml of SDS solution. The contents were mixed well and incubated for 2 hours. The contents were centrifuged in a refrigerated centrifuge at 10,000 g for 30 minutes. VLDL aggregated as a pellicle at the top. The supernatant was a mixture containing HDL and LDL fractions. The fractions of lipoproteins were assayed after heparin manganese chloride and SDS precipitation. The values are expressed in mg/dl plasma. After precipitation the cholesterol levels in supernatant was measured to get HDL cholesterol. SDS precipitated VLDL and the cholesterol content in the supernatant was measured for HDL cholesterol, LDL cholesterol and VLDL cholesterol¹⁸.

Evaluation of antioxidant Parameters

Animal are scarified at the end of 28 days treatment. The liver of animal is dissected out, rinsed with ice cold distilled water followed by sucrose solution (0.25 M). One gm of liver tissue is homogenized in 10 ml ice cold Tris hydrochloride buffer. The prepared homogenates are centrifuged and used for the determination of antioxidant parameters like Malondialdehyde (MDA), Superoxide dismutase (SOD), catalase, Reduced Glutathione (GSH) levels and total protein estimation. Liver and kidney are isolated from one animal of each group and used for histopathology¹⁹.

Measurement of MDA i.e. lipid peroxidation, was measured spectrophotometrically by the method of using 1, 1, 3, 3-tetraethoxypropane as standard. MDA is expressed as nanomoles per mg protein. GSH was determined by its reaction with 5, 5'-dithiobis (2-nitrobenzoic acid) (Ellman1959) to yield a yellow chromophore which was measured spectrophotometrically. GSH is expressed as µg/mg protein²⁰. Nitrite is a stable end product of nitric oxide metabolism (as RNI) was measured in brain homogenate by Griess method. Briefly, the following procedure was adapted. The sample and reagents were well mixed and incubated for 10 min at room temperature in dark. Then absorbance was taken at 540 nm. The concentration of nitrite in supernatant was determined from sodium nitrite standard curve²¹.

Histopathological studies

For histopathological study, the tissues were fixed in Bouin's fluid. The classical paraffin sectioning, and haematoxylin eosin staining techniques were used for histopathological studies. The various steps involved in the preparation of tissues for histopathological studies were: carried out²².

Statistical analysis

The results are expressed as mean±S.E.M. Statistical analysis of passive avoidance, Morris water maze and biochemical values were performed by comparison Duncan's multiple range test (DMRT).

RESULT AND DISCUSSION:**Anti-diabetic activity of ethanolic leaves extract of *Centratherrum anthelminticum*****Effect of ethanolic leaves extract on Body weight, of experimental animals**

Body weights of the animals were recorded and they were divided into 7 groups of 6 animals each as follows

Body weight

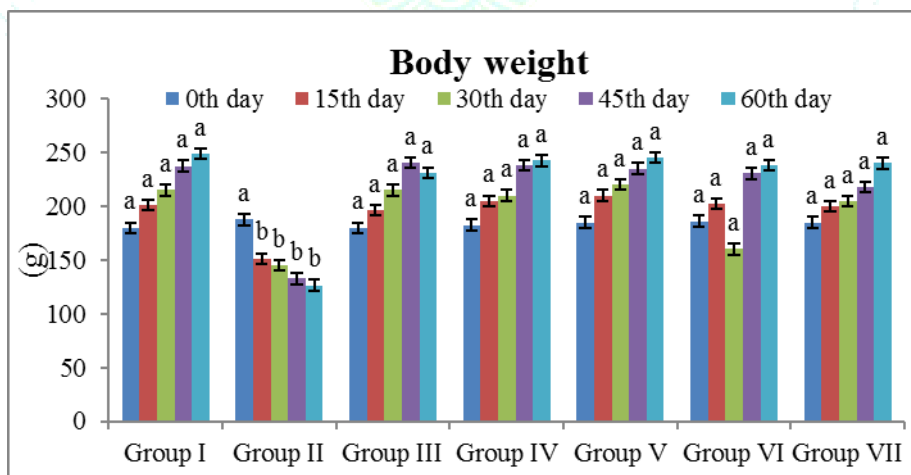
The effect of administration of different doses (100, 200 and 300mg/kg) of ethanolic leaves extract of *Centratherrum anthelminticum* and Glibenclamide on body weight of rats

during the experimental period is summarized on Table 4.15 & Fig 4.10. Diabetes- induced animals showed significant decrease ($p < 0.05$) in body weight day by day when compared with control animals. The ethanolic extract of *Centratherrum anthelminticum* treated animals were found to be have significant increase ($p < 0.05$) in body weight when compared with diabetes-induced animals. The results showed significant dose dependent increase in the body weight of the diabetic rats treated with the leaves extracts and the standard drug. The increase in ethanol extract treated group was similar to the normal group. There is no significant changes were observed in *Centratherrum anthelminticum* alone (without induction of diabetes) treated rats²³.

Table 1: Effect of ethanolic leaves extract on Body Weight of experimental animals

Groups	Body Weight(g)				
	0 th day	15 th day	30 th day	45 th day	60 th day
I	180.00±3.33 ^a	201.25±3.49 ^a	215.00±1.67 ^a	237.50±4.86 ^a	248.75±3.49 ^a
II	187.50±3.91 ^a	151.25±2.57 ^b	145.00±1.94 ^b	132.50±2.64 ^b	126.50±2.46 ^b
III	180.00±15.09 ^a	196.25±4.53 ^a	215.00±12.69 ^a	240.50±17.12 ^a	231.25±12.73 ^a
IV	182.50±13.49 ^a	205.00±15.46 ^a	210.00±26.77 ^a	238.00±30.55 ^a	242.50±25.47 ^a
V	185.00±12.69 ^a	210.00±10.74 ^a	220.50±20.10 ^a	235.00±20.28 ^a	245.50±28.94 ^a
VI	186.00±11.06 ^a	202.00±11.73 ^a	160.00±18.56 ^a	230.50±16.46 ^a	238.00±21.21 ^a
VII	185.00±2.36 ^a	200.00±13.49 ^a	204.50±20.38 ^a	217.50±21.95 ^a	240.00±21.86 ^a

Values are expressed as Mean ± SD for six rats. Mean values within a graph followed by different letters are significantly different from each other at $P < 0.05$ level comparison by Duncan's multiple range test (DMRT)

**Fig 1: Effect of ethanolic seeds extract on Body Weight, of experimental animals****Table 2: Effect of ethanolic seeds extract on Glucose (mg/dl) of experimental animals**

Groups	Glucose (mg/dl)				
	4 th day	15 th day	30 th day	45 th day	60 th day
I	106.40±1.62 ^a	103.60±0.68 ^a	103.75±0.66 ^a	103.75±0.66 ^a	104.00±0.66 ^a
II	178.00±4.43 ^b	222.60±0.47 ^b	225.60±0.47 ^b	228.60±0.47 ^b	231.60±0.47 ^b
III	151.00±5.85 ^c	212.45±0.34 ^c	207.45±0.34 ^c	202.45±0.34 ^c	197.45±0.34 ^c
IV	142.00±2.33 ^c	207.95±0.34 ^c	199.95±0.34 ^c	191.95±0.34 ^c	183.95±0.34 ^c
V	114.00±2.71 ^a	103.95±0.34 ^a	102.70±0.34 ^a	117.70±0.34 ^a	118.70±0.34 ^a
VI	107.60±1.57 ^a	103.45±0.68 ^a	103.60±0.66 ^a	104.23±0.66 ^a	104.35±0.66 ^a
VII	106.40±1.62 ^a	108.35±0.34 ^a	106.45±0.34 ^a	114.45±0.34 ^a	122.45±0.34 ^a

Values are expressed as Mean ± SD for six rats. Mean values within a graph followed by different letters are significantly different from each other at $P < 0.05$ level comparison by Duncan's multiple range test (DMRT)

Effect of *Centratherum anthelminticum* ethanolic seeds extract on HDL, LDL, VLDL, FFA, PL and TC of experimental animals.

Total cholesterol (TC), Triglycerides (G), Phospholipids (PL), free fatty acid (FFA), Low Density Lipoprotein (LDL), High Density Lipoprotein (HDL) and Very Low Density Lipoprotein (VLDL) content was shown in Table 4.18 and Fig 4.14. Streptozotocin induced diabetic rats showed increased LDL (245.57mg/dl), TG (353.60mg/dl), VLDL (78.93mg/dl), FFA (2.77mg/dl), PL (1.96mg/dl), TC (392.00mg/dl) while decreased HDL (26.60mg/DL) content was observed²⁴. Administration of different doses (100, 200 and 300mg/kg) of ethanolic leaf extract of *Centratherum anthelminticum*

decreased content in LDL, TC, VLDL, FFA, PL, TC and increased HDL content was observed dose dependent manner. Among the various doses, the highest dose (300mg/kg) of leaf extract of *Centratherum anthelminticum* shows significantly ($p > 0.05$) restored the LDL (89.90mg/dl), TG (80.21mg/dl), VLDL (23.93mg/dl), FFA (1.43mg/dl), PL (1.41mg/dl), TC (140.54mg/dl) and increased HDL (70.80mg/DL) content to normal rats²⁵. The standard treated group also decreased LDL, VLDL, FFA, TC while increased HDL content. *Centratherum anthelminticum* alone (300mg/kg) treated rats did not show any significant variation in lipid profile throughout the experimental period²⁶.

Table 3: Effect of *Centratherum anthelminticum* ethanolic seeds extract on HDL, TG, LDL, VLDL, FFA, PL and TC of experimental animals

Groups	Total Cholesterol (mg/g)	Triglycerides (mg/g)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
I	136.40±1.65 ^a	62.00±0.65 ^a	77.20±0.93 ^a	39.00±7.87 ^a	10.33±2.07 ^a
II	392.00±1.12 ^b	353.60±1.31 ^b	26.60±0.98 ^b	245.57±49.12 ^b	78.93±11.79 ^b
III	327.60±1.02 ^c	312.60±0.80 ^c	46.31±0.74 ^c	213.90±42.81 ^c	62.10±10.42 ^c
IV	230.00±1.55 ^d	262.60±0.94 ^d	54.80±0.79 ^d	143.90±28.83 ^d	43.77±8.75 ^d
V	140.54±0.98 ^a	73.60±1.10 ^a	70.80±0.67 ^a	89.90±18.02 ^a	23.93±4.79 ^a
VI	138.60±1.77 ^a	80.21±0.94 ^a	77.80±0.79 ^a	68.73±13.90 ^a	20.27±4.06 ^a
VII	144.00±1.98 ^a	72.80±5.10 ^a	81.80±0.34 ^a	44.70±9.15 ^a	7.13±1.69 ^a

Values are expressed as Mean ± SD for six rats. Mean values within a graph followed by different letters are significantly different from each other at $P < 0.05$ level comparison by Duncan's multiple range test (DMRT)

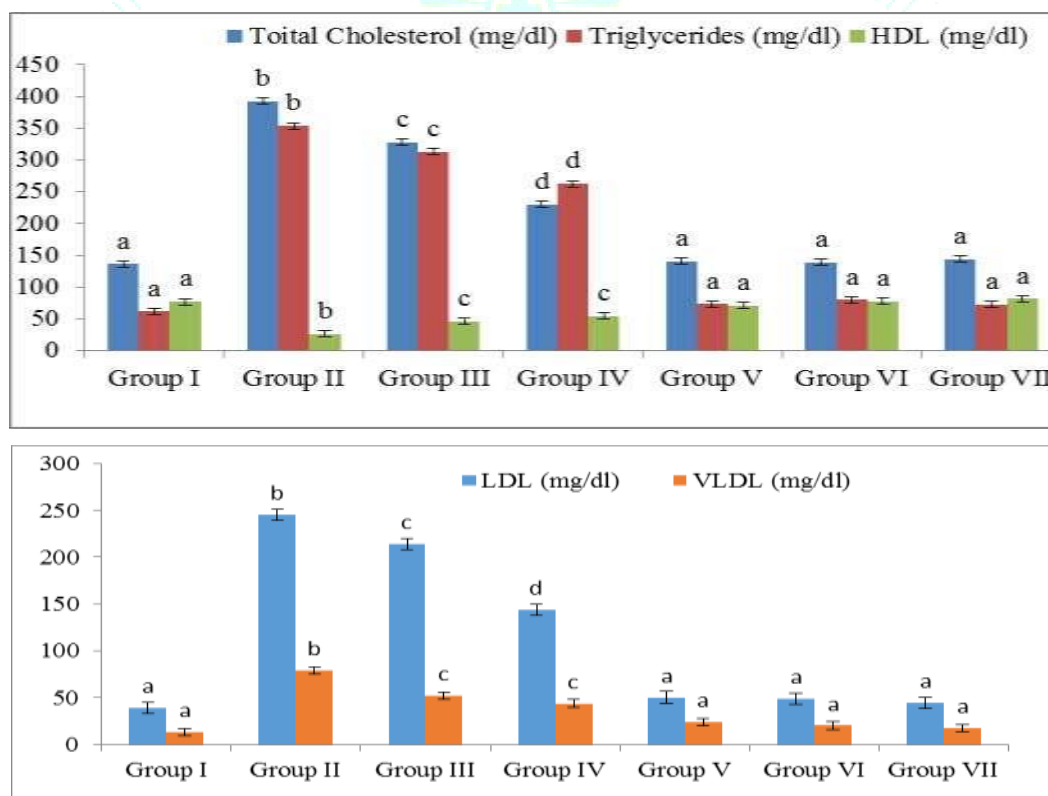


Fig 2: Effect of *Centratherum anthelminticum* ethanolic seeds extract on HDL, TG, LDL, VLDL, TC of experimental animals

Effect of *Centratherum anthelminticum* ethanolic seeds extract on liver LPO, SOD, CAT, GPx, GST, GSH of experimental animals²⁷

The lipid peroxidation (LPO) content, Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx), Glutathione S transferase (GST) activity, Reduced glutathione

(GSH), Vitamin C and E content in liver was given in Table 4.19 and Fig 4.15, 4.16 and 4.71. Streptozotocin induced diabetic rats showed increased LPO content while decreased in the activities of SOD, CAT, GPx, GST and content of GSH, Vitamin C and E was observed. Administration of different doses (100, 200 and 300mg/kg) of ethanolic leaf extract of

Centratherrum anthelminticum decreased LPO content while increased in the activities of SOD, CAT, GPx, GST and content of GSH, Vitamin C and E was observed in dose dependent manner²⁴. Among the various doses, the highest dose (300mg/kg) of leaf extract of *Centratherrum anthelminticum* shows significantly ($p>0.05$) restored the LPO content, the

activities of SOD, CAT, GPx, GST and content of GSH, Vitamin C and E content to normal rats²⁸. The standard treated group showed no significant changes were observed in antioxidant enzymes. *Centratherrum anthelminticum* alone (300mg/kg) treated rats did not show any significant variation in antioxidant status throughout the experimental period²⁹.

Table 4: Effect of *Centratherrum anthelminticum* ethanolic seeds extract on liver LPO, SOD, CAT, GPx & GSH of experimental animals.

Groups	LPO (nmol MDA/mg protein)	SOD (Liver) (U/mg protein)	CAT (Liver) (U/mg protein)	GPx (U/mg protein)	GSH ($\mu\text{g/g}$ tissue)
Group I	2.77 \pm 0.09 ^a	1600.53 \pm 4.51 ^a	24.0 \pm 1.5 ^a	997.0 \pm 151.7 ^a	48636.4 \pm 5.0 ^a
Group II	13.91 \pm 0.08 ^b	825.67 \pm 2.69 ^b	8.8 \pm 2.3 ^b	434.5 \pm 251.6 ^b	25776.0 \pm 1.7 ^b
Group III	10.05 \pm 0.06 ^c	1186.00 \pm 10.97 ^d	15.6 \pm 1.5 ^c	584.5 \pm 234.6 ^d	32124.8 \pm 2.4 ^c
Group IV	7.47 \pm 0.21 ^d	1362.53 \pm 13.00 ^c	18.0 \pm 0.6 ^d	831.5 \pm 184.2 ^c	39684.0 \pm 2.0 ^d
Group V	3.02 \pm 0.04 ^a	1516.80 \pm 8.12 ^a	22.0 \pm 1.0 ^a	970.0 \pm 156.4 ^a	46418.7 \pm 1.0 ^a
Group VI	2.83 \pm 0.03 ^a	1599.33 \pm 6.59 ^a	23.0 \pm 0.7 ^a	995.5 \pm 151.6 ^a	47653.2 \pm 4.3 ^a
Group VII	3.23 \pm 0.07 ^a	1590.53 \pm 4.51 ^a	24.0 \pm 1.5 ^a	997.0 \pm 151.7 ^a	48614.3 \pm 2.0 ^a

Values are expressed as Mean \pm SD for six rats Mean values within a graph followed by different letters are significantly different from each other at $P < 0.05$ level comparison by Duncan's multiple range test (DMRT)

Effect of *Centratherrum anthelminticum* on AST, ALT, ALP activities and Direct Bilirubin, Total Bilirubin, protein content of experimental animals²⁴

The AST, ALT, ALP activities and direct bilirubin, total bilirubin, protein content was represented in normal and experimental rats represented in Table 4.20 and Fig 4.18. Streptozotocin induced diabetic rats showed significantly increased in the activities of AST, ALT, ALP and content of direct bilirubin, total bilirubin while decreased protein content was observed³⁰. Administration of different doses (100, 200 and 300mg/kg) of ethanolic leaf extract of *Centratherrum anthelminticum* significantly restored in the

activities of AST, ALT, ALP and content of direct bilirubin, total bilirubin while increased protein content was observed in dose dependent manner³¹. Among the various doses, the highest dose (300mg/kg) of leaf extract of *Centratherrum anthelminticum* shows significantly ($p>0.05$) restored the activities of AST, ALT, ALP and content of direct bilirubin, total bilirubin and protein content to normal rats³². The standard treated group showed no significant changes were observed in liver markers. *Centratherrum anthelminticum* alone (300mg/kg) treated rats did not show any significant variation in liver marker enzymes throughout the experimental period³³.

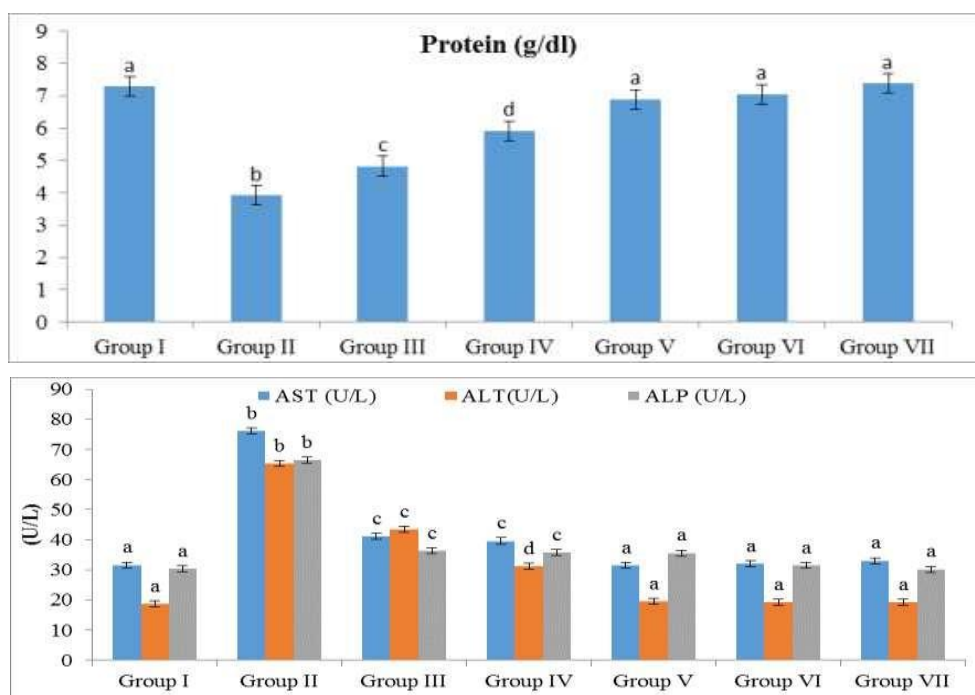


Fig 3: Effect of *Centratherrum anthelminticum* on AST, ALT, ALP activities and Direct Bilirubin, Total Bilirubin, protein content of experimental animals

Histology of Liver ³⁴

Photomicrographs of normal liver showed well visualized architecture with predominant nucleus. Liver sections of streptozotocin induced diabetic rats showed marked structural alterations in the liver ³⁵. The major alteration was periportal fatty infiltration, necrosis of hepatocytes ³⁶. This damage was reversed and regeneration of parenchyma cells by the leaf extract of *Centratherrum anthelminticum* (300mg/BW) and Glibenclamide treatments (plate.1)

Histology of pancreas ³⁷

Photomicrographs of pancreas showed normal acini and normal cellular population in the islets of langerhans in pancreas of control rats ³⁸. Pancreas sections of streptozotocin induced diabetic rats showed extensive damage to the islets of langerhans and reduced dimensions of islets ³⁹. Photomicrograph of Pancreatic Islets of Streptozotocin induced rat treated with *Centratherrum anthelminticum* (300mg/kg) and Glibenclamide (1mg/kgbw) showing regeneration of Islets of Langerhans, blood vessels and acinus (plate.2).

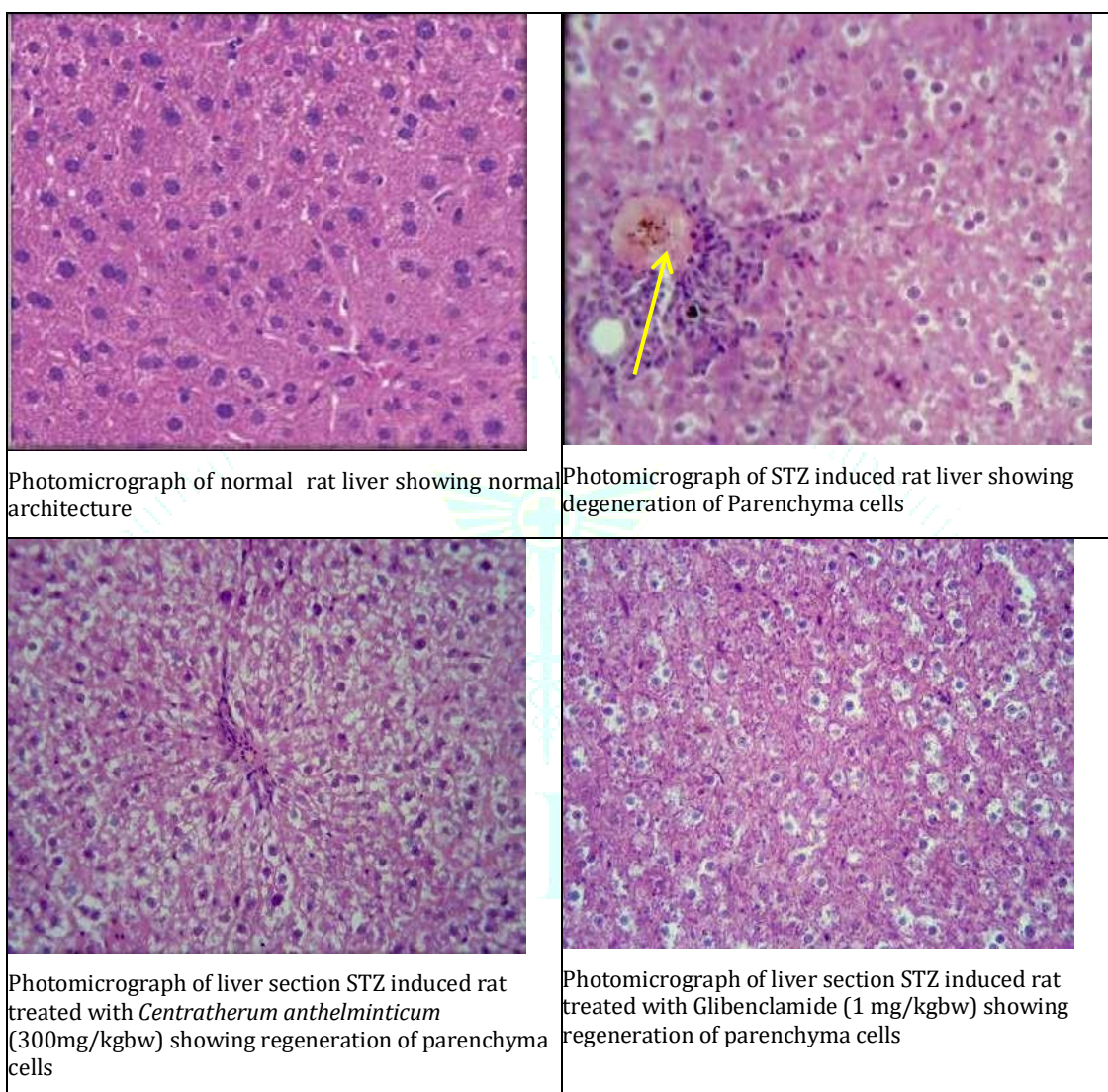


Plate.1: Histology of Liver in normal and experimental rats (40x Magnification)

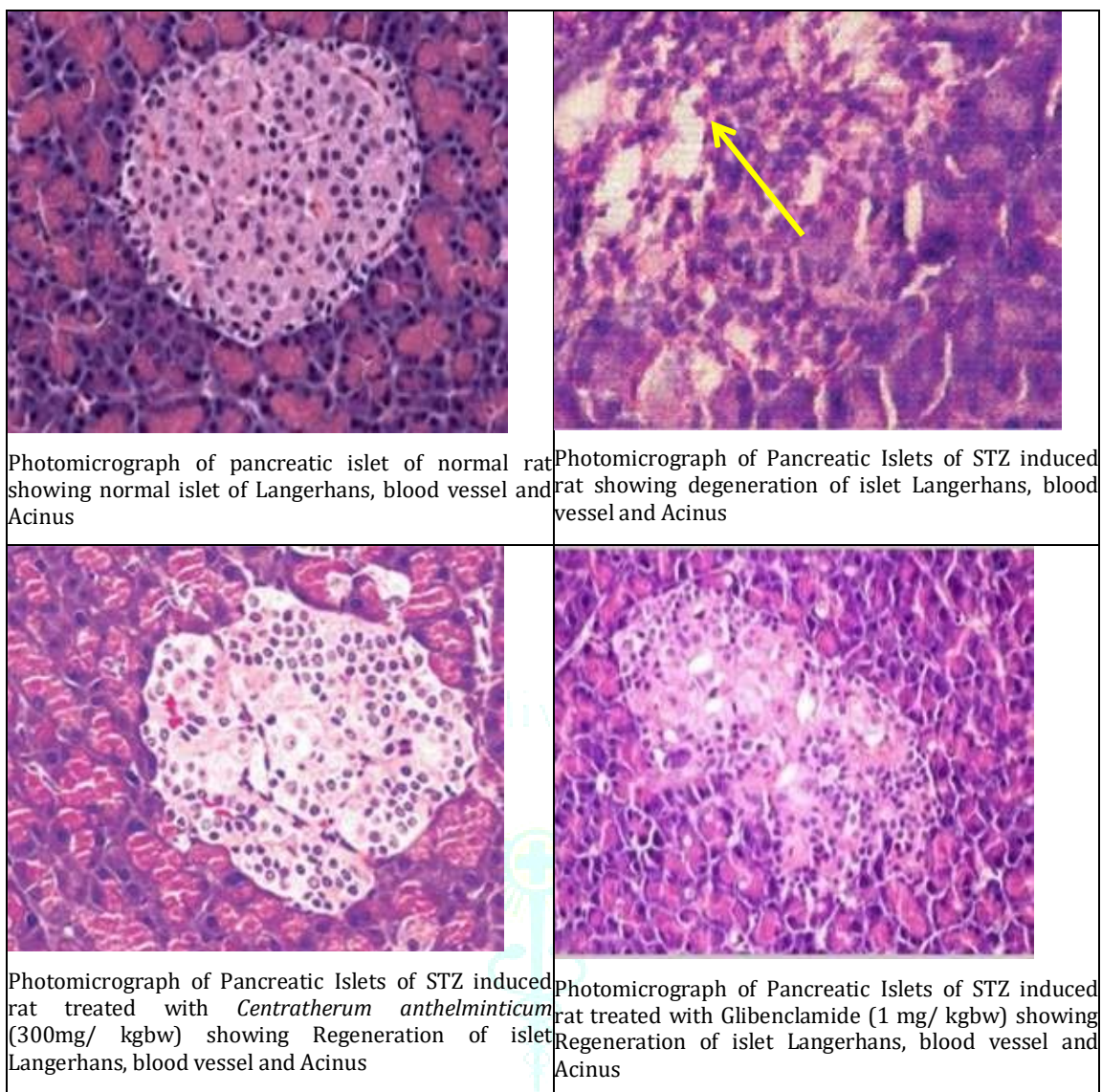


Plate.2: Histology of pancreas in normal and experimental rats (40x Magnification)

SUMMARY

Overall, the experimental studies suggest that *Centratherum anthelminticum* ethanolic seeds extract has potential antioxidant and antidiabetic activity⁴⁰. This study is the first scientific report that provides convincing Phytochemical and ethno pharmacological evidence for the relevance of *Centratherum anthelminticum*, thus providing scientific validity to its traditional consumption by the local populace of south India. The antioxidant and antidiabetic activity of *Centratherum anthelminticum* ethanolic seeds might be present in bioactive compounds⁴¹.

Acknowledgement: The authors are thankful to L.T.R. Institute of Technology, Baghat Road, Meerut, Uttar Pradesh 250002 for assistance towards this research work.

Conflict of Interest:

The authors declare no conflict of interest.

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