Evaluation of Antidiabetic activity of herbal extracts of roots of *Aerva lanata* and rhizomes of *Curcuma caesia* in animal model

Ritesh Agrawal, Dr. M A Naidu

Faculty of Pharmacy, Mandsaur University, Mandsaur, MP, India - 485001

ABSTRACT:

Objective: The objective of research paper was to evaluate the antidiabetic activity of methanolic extract of roots of *Aerva lanata* Linn. (MEAL) and rhizomes of *Curcuma caesia* Roxb. (MECC) in streptozotocin (STZ)-induced diabetic rats.

Materials and Methods: The concentration of MEAL and MECC (200 mg/kg b.w, p.o) and combination of MEAL + MECC (200 mg/kg b.w, p.o) was administered orally and evaluated for the estimation of biochemical parameters such as body weight, OGTT, Total Cholesterol, Triglycerides, HDL (VLDL and LDL), Total protein, Albumin, ACP, ALP and SGPT and SGOT by using standard procedures.

Results and Discussion: The antidiabetic activities data indicate substantially altered physiological and biochemical parameters. The dose of *Aerva lanata* Linn. (MEAL) and *Curcuma caesia* Roxb. (MECC) 200 mg/kg and combination dose shown the progressive decrease in body weight, blood glucose level, Triglycerides, HDL, Total protein, Albumin, ACP, ALP and SGPT and SGOT level as compared to standard drug glibenclamide at a dose of 500 µg/kg. Study result indicates that significant decrease in total cholesterol, triglyceride, and LDL cholesterol levels was observed after treating the STZ-induced diabetic rats with MEAL + MECC individually 200 mg/kg body weight leading to a significant increase in the cholesterol levels.

Conclusion: This study demonstrates the antidiabetic potential of combination of *Aerva lanata* and *Curcuma caesia* offers scientific validation and basis to develop antidiabetic drug.

Key words: *Aerva lanata* , *Curcuma caesia* , Antidiabetic activity, cholesterol, streptozotocin

1. INTRODUCTION

Diabetes mellitus is a major metabolic illness, and medicinal herbs are important in treating it. There are claims that traditional plants have powerful antidiabetic capabilities without any negative side effects. They are abundant in anti-diabetic substances such flavonoids, alkaloids, phenolics, and tannins, which increase insulin secretion or decrease glucose absorption through the intestinal tract, improving the function of pancreatic cells.[1]According to the literature, there are over 420 medicinal plants with anti-diabetic qualities that have been experimentally verified, but only 109 of them have had their whole mechanisms fully analysed.

The modulation of metabolic pathways like glycolysis, gluconeogenesis, Krebs cycle, glycogen production and their degradation, insulin synthesis and release, cholesterol synthesis, carbohydrate metabolism, and absorption has been demonstrated for a number of medicinal plant extracts.[2]

A chronic endocrine illness called diabetes mellitus (DM) is characterised by elevated blood glucose levels that can affect how carbohydrates, proteins, and fats are metabolised.[3] It results from either inadequate insulin synthesis by the pancreatic -Langerhans islet cells or from impaired insulin absorption in peripheral organs.[4] Following a meal, an increase in blood glucose causes the pancreas to release the hormone insulin. The blood sugar level drops to normal levels as a result of insulin stimulating the liver to metabolise glucose and the fat and muscle cells to eliminate glucose from the blood. Diabetes results in an elevated blood sugar level because the pancreas fails to produce any insulin or produces it ineffectively.[5] India is regarded as the "capital of diabetes" because there are more than 61 million individuals living with the disease there. Due to a variety of problems, including an inadequate healthcare system, a lack of adequate facilities, etc., effective treatment of diabetes and its related consequences continues to be a significant challenge for India.[6]

Due to their fewer side effects and lower cost, herbal formulations are preferred over synthetic medications to lessen the negative consequences of diabetes and its secondary problems.[7]The purpose of the current research is to assess the combined anti-diabetic effectiveness of two Indian medicinal herbs.

2. MATERIALS AND METHODS

Plant material were collection in the month of October 2020, *Curcuma caesia* rhizomes and *Aerva lanata* roots were identified and harvested from Bhimbetka Bhojpur, Bhopal (Madhya Pradesh). The selected plant material was authenticated from Department of Botany Barkatullaha University in Bhopal (M.P).

2.1 Extraction

Solvent extraction Crude plant extract was prepared by Soxhlet extraction method. About 100gm of powdered plant material was uniformly packed into a thimble and extracted with 500 ml of different solvents separately. Different Solvents were used according to their polarity. The process of extraction continues for 24 hours or till the solvent in siphon tube of an extractor become colourless. After that the extract was taken in a beaker and kept on hot plate and heated at 30-40ºC till all the solvent got evaporated. Dried extract was kept in refrigerator at 4ºC for their future use in phytochemical analysis.[8]

2.3 Qualitative phytochemical analysis

The extract was tested for the presence of bioactive compounds by using following standard methods .[9,10,11]

2.4 Animals

Wistar albino rats of both sexes (180–220 g) were used for antidiabetic study. The animals were obtained from the animal house of College of Veterinary Science and Animal Husbandry, Mhow, M.P. All the rats were kept in standard plastic rat cages with stainless steel coverlids and wheat straw was used as bedding material. The animals were facilitated with standard environmental condition of photo period (12:12 h dark: light cycle) and temperature (25 ± 2°C). They were provided with commercial rat and mice feed and water given *ad libitum*. The use of these animals and the study protocols was approved by Institutional Animal Ethical Committee.

2.4.1 Selection of the Doses for Animal Study.[12]

The acute oral toxicity studies and selection of doses were carried out as per guidelines of Organization for Economic Cooperation and Development (OECD), draft guidelines 423 received from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India. Healthy Wistar rats of either sex

weighing between 180 and 220 g were used for acute toxicity study to determine LD_{50} of *Aerva lanata* Linn. and *Curcuma caesia* Roxb. The animals were randomly selected, marked to permit individual identification, and kept in their cages for 7 days before dosing to allow for acclimatization to the laboratory condition. In acute toxicity study, no toxic symptoms were observed for *Aerva lanata* Linn. and *Curcuma caesia* Roxb. up to dose 3000 mg/kg body weight. All animals behaved normally. No neurological or behavioural effect could be noted. No mortality was found up to 14 days study.

2.4.2 Antidiabetic Studies.[13]

Animal grouping for ant diabetic studies

Rats will be divided into different groups, each group consisting of six animals. After overnight fasting (deprived of food for 16 h had been allowed free access to water), diabetes was induced in Groups II–VI by intraperitoneal injection of streptozotocin (STZ) dissolved in 0.1 M sodium citrate buffer at pH 4.5, at a dose of 55 mg/kg body weight. The control rats received the same amount of 0.1 M sodium citrate buffer. The animals were allowed to drink 5% glucose solution overnight to overcome the drug-induced hypoglycaemia. Diabetes status was confirmed by estimating blood glucose levels after 72 h of STZ injection. Animals showing fasting blood glucose levels above 250 mg/dl were selected for study.

GROUP	**TREATMENT**	**No. of ANIMALS (n)**
Group-I	Normal	6
Group-II	Diabetic control received only STZ (negative control)	6
Group-III	Diabetic rats received glibenclamide orally at dose of 500 µg/kg b.wt for 14 days	6
Group-IV	Diabetic rats received methanolic extract of *Aerva lanata* Linn. (200 mg/kg/day p.o.)	6
Group-V	Diabetic rats received methanolic extract of *Curcuma caesia* Roxb. (200 mg/kg/day p.o.)	6
Group -VI	Diabetic rats received combination of **MEAL + MECC** (200 mg/kg/day p.o.)	6
	Total no. of animals used for the study	36

2.5 Biochemical analysis

Body weight of the experimental rats was taken on pre- and post-treatment, that is, initial and final day of post-treatment by digital balance. The blood glucose level of fasted rats was taken pre- and post-treatment, that is, 0, 8th, and 21th day of post-treatment.

At the end of experimental time, all the experimental rats were sacrificed by cervical decapitation. Blood samples were collected and allowed to clot. Serum was separated by centrifuging at 2500 rpm for 15 min and analyzed for various biochemical parameters. Biochemical parameters were studied using automated biochemistry analyzer Hitachi-902.

2.6 Estimation of oral glucose tolerance test.[14]

The oral glucose tolerance test was performed in overnight fasted (18 h) rats. Rats were divided into six groups and treatment was given as mentioned above. Glucose (2 g/kg, p.o.) was fed 30 min after the administration of drugs. Blood was withdrawn from the retro orbital sinus under ether anaesthesia at 30, 60 and 120 min of glucose administration and serum glucose levels were determined using a commercial kit following glucose oxidase (GOD) method.[15, 16]

2.7 Estimation of total cholesterol (TC)

TC in serum was estimated using CHOD/PAP methods. Cholesterol is a main component of cell membranes and lipoprotein and it is the precursor for steroid hormones and bile acids synthesizing. [15]

The stimation of triglycerides (TGs) ,Triglyceride Assay Protocol, High-density lipoprotein (HDL) cholesterol in serum was estimated using PEG method, Estimation of total protein content, Estimation of albumin content, Estimation of acid phosphatase (ACP) activity, Estimation of alkaline phosphatase (ALP) activity, Estimation of serum glutamate pyruvate transaminase (SGPT), Estimation of serum glutamic oxaloacetic transaminase (SGOT) was conducted by using standard pathological procedures in Gupta Diagnosis laboratory ,Bhopal (M.P). [17]

3. RESULTS

Body weights of animals in all groups were performed at the onset of study and end of the study. Body weight of animals was significantly ($P < 0.05$) maintained in all treated groups (glibenclamide 500 µg/kg p.o., *Aerva lanata* Linn. 200mg/kg/p.o., *Curcuma caesia* Roxb. 200 mg/kg/p.o. and combination of both extract 200 mg/kg/p.o.) [Figure 1].

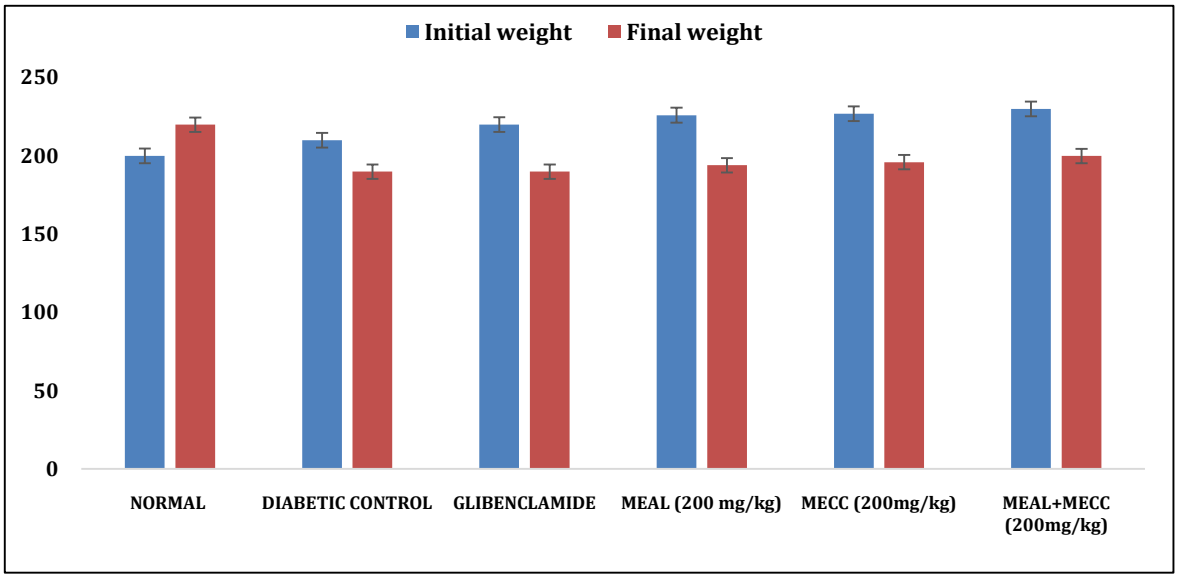

Figure 1: Mean body weight change

Blood glucose level of animals in all groups was recorded at 0, 8th, and 21st day. Progressive decrease in blood glucose level was found in all treatment groups during study. At the end of experiment glibenclamide 500 µg/kg p.o., *Aerva lanata* Linn. 200 mg/kg/p.o., *Curcuma caesia* Roxb. 200 mg/kg/p.o. and combination of both extract 200 mg/kg/p.o. (111.00 ± 6.50; 117.00 ± 6.00 and 119.00 ± 5.50) treated group blood glucose level was decrease significantly (P < 0.05) at 21st days, respectively [Figures 2-8].

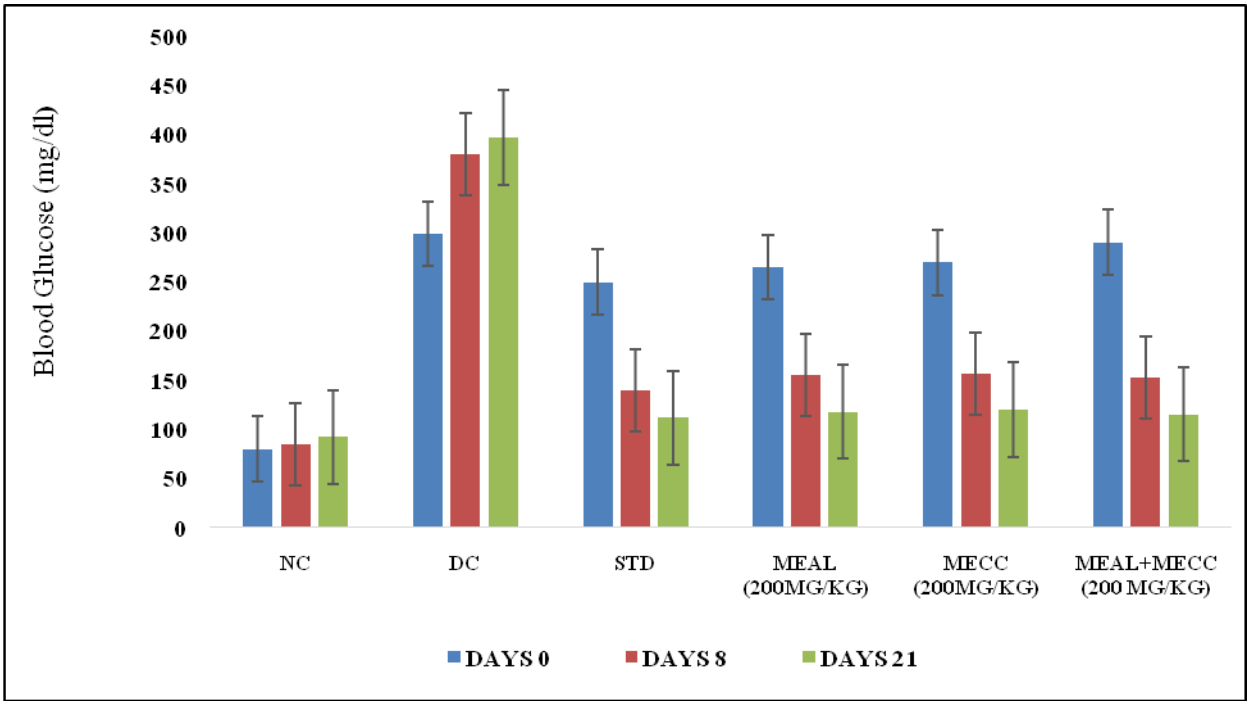

Figure 2: Antidiabetic activity of *Aerva lanata* Linn and *Curcuma caesia* Roxb. on blood glucose level in streptozotocin-induced diabetic rats

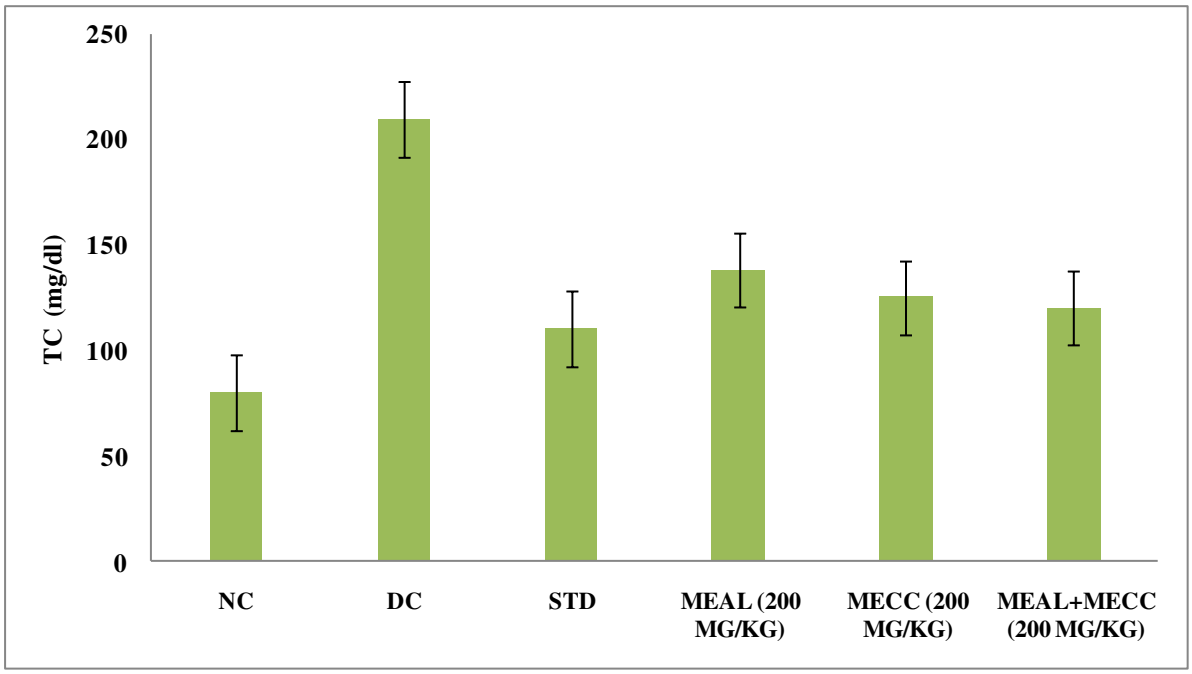

Figure 3: Effect of *Aerva lanata* Linn and *Curcuma caesia* Roxb. on total cholesterol level in streptozotocin-induced diabetic rats

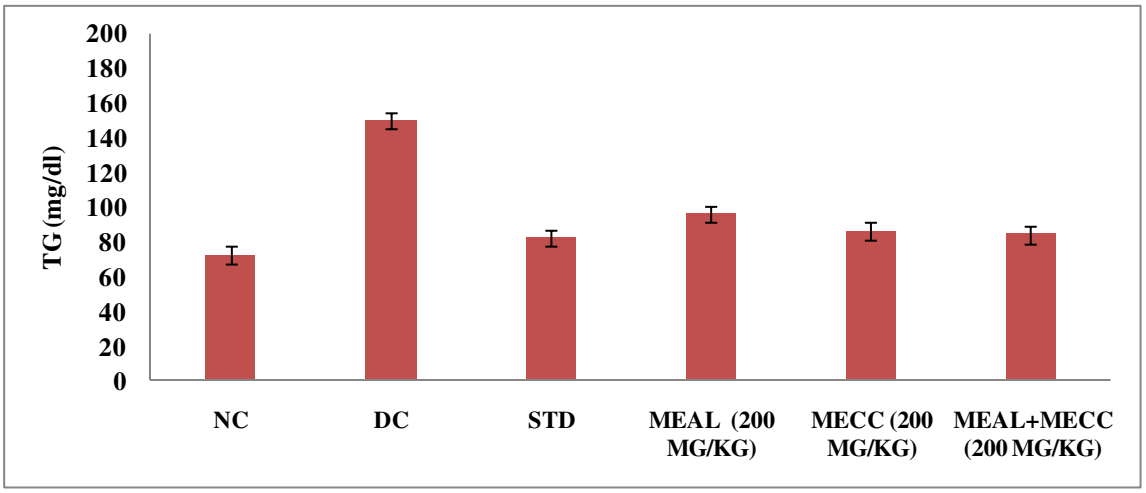

Figure 4: Effect of *Aerva lanata* Linn and *Curcuma caesia* Roxb. on triglyceride level in streptozotocin-induced diabetic rats

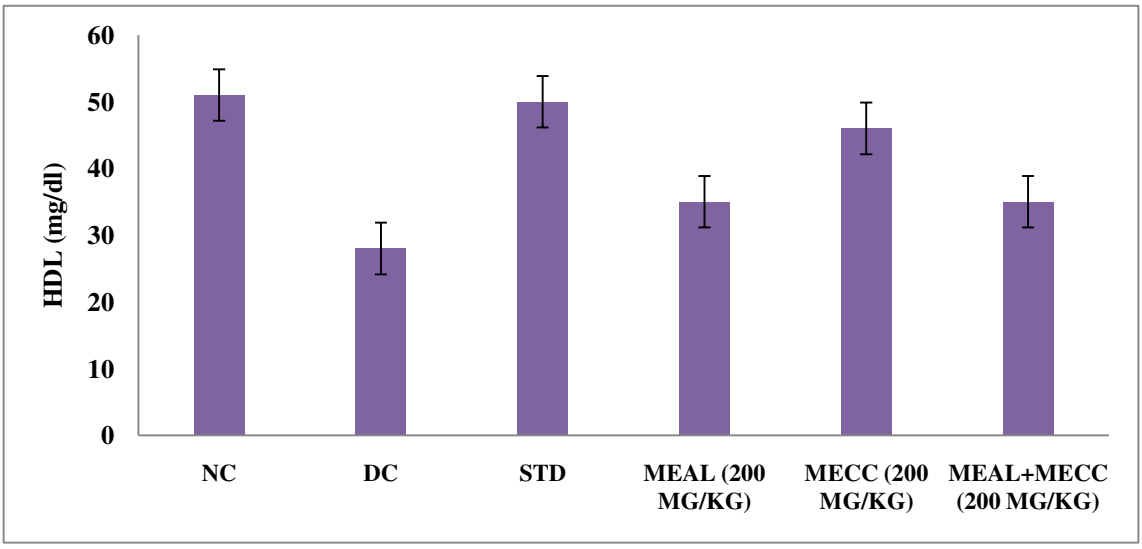

Figure 5: Effect of *Aerva lanata* Linn and *Curcuma caesia* Roxb. on high-density lipoproteins in streptozotocin-induced diabetic rats

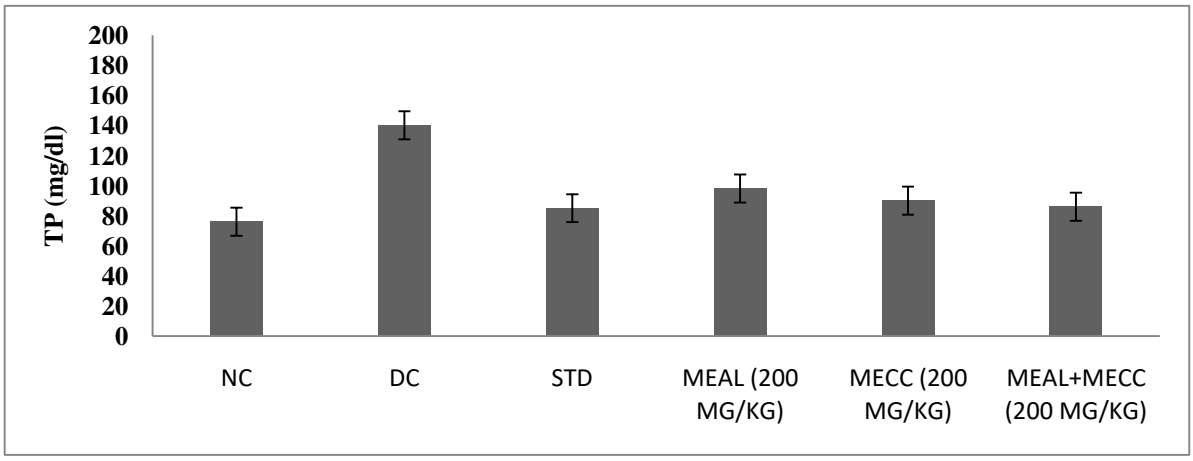

Figure 6: Antidiabetic effect of *Aerva lanata* Linn and *Curcuma caesia* Roxb. on serum lipid profile, that is, total protein level in streptozotocin-induced diabetic rats

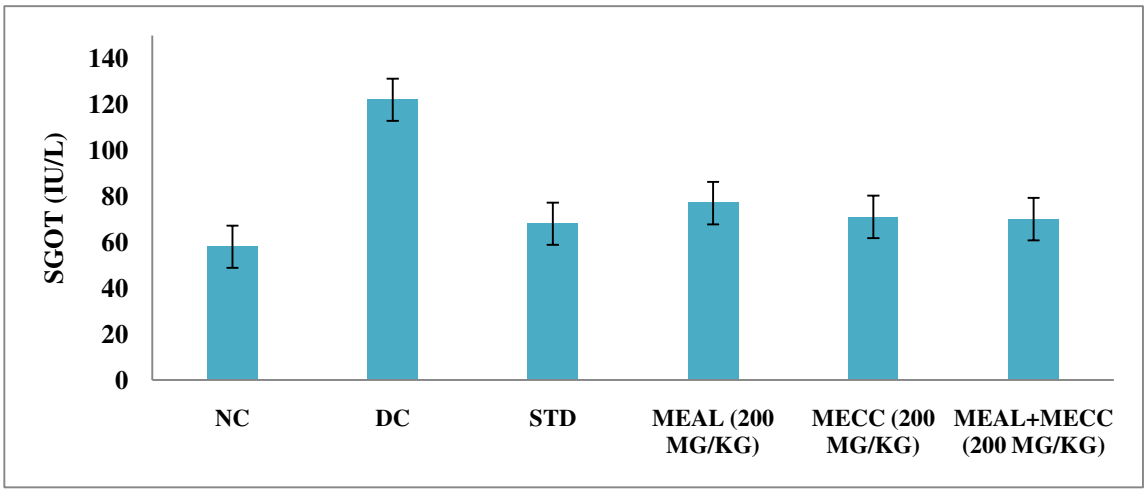

Figure 7: Effect of *Aerva lanata* Linn and *Curcuma caesia* Roxb. on serum glutamic oxaloacetic transaminase in streptozotocin-induced diabetic rats

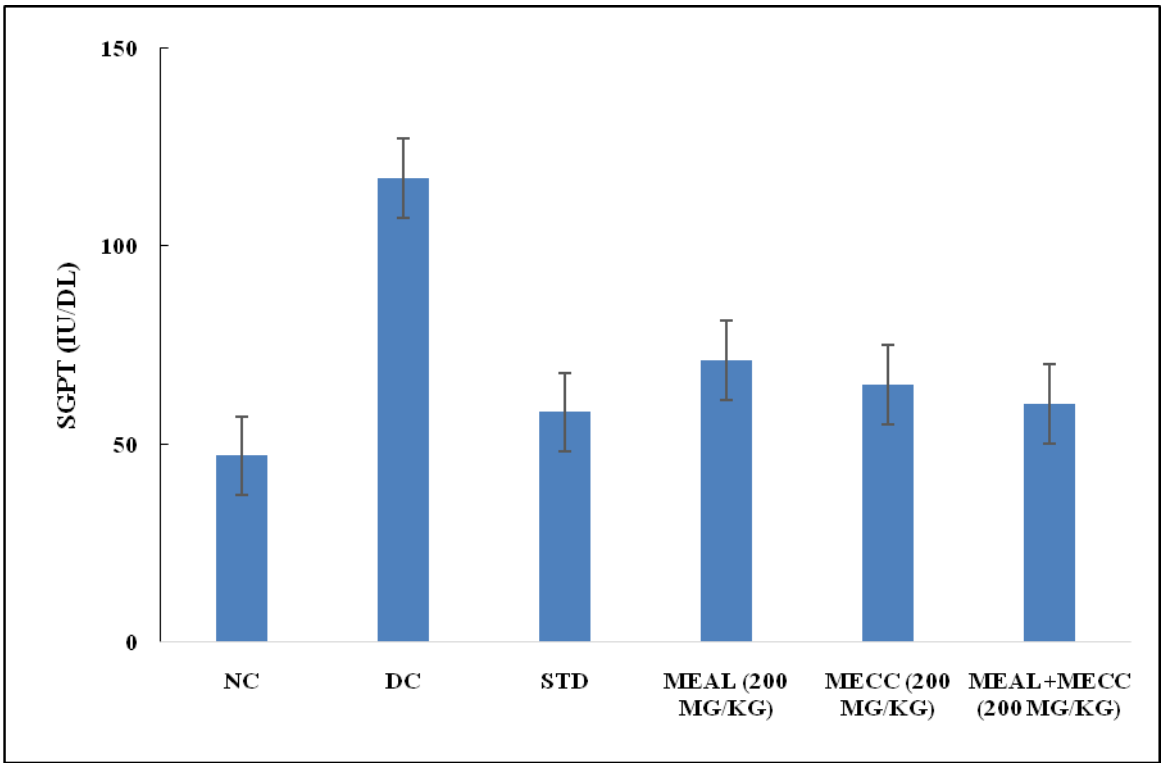

Figure 8: Effect of *Aerva lanata* Linn and *Curcuma caesia* Roxb. on serum glutamate pyruvate transaminase in streptozotocin-induced diabetic rats

DISCUSSION

This antihyperglycemic effect of *Aerva lanata* Linn. (roots) and *Curcuma caesia* Roxb. (rhizomes) on the STZ-induced diabetic rats suggests that its main mechanism may not be due to stimulating insulin release from pancreatic cells, but may exert a direct action by promoting glucose utilization by peripheral tissues. We would need much more research work to study the mechanism. Diabetes-induced hyperlipidemia is attributable to the excess mobilization of fat from adipose tissue. In diabetic condition, the activity of enzyme lipoprotein lipase is decreased resulting in increased levels of lipoproteins in the blood. In the present study, a significant decrease in TC, triglyceride, and LDL cholesterol levels was observed after treating the STZ-induced diabetic rats with *Aerva lanata* Linn. (roots) and *Curcuma caesia* Roxb. (rhizomes) at 200 mg/kg body weight leading to a significant increase in the HDL cholesterol levels.

The levels of SGOT and SGPT were significantly altered with the decrease in glucose levels than negative control in diabetic + *Aerva lanata* Linn. (roots) and *Curcuma caesia* Roxb. (rhizomes) group and overall condition of animals were indicative of classical anti-diabetic activity of *Aerva lanata*

Linn. (roots) and *Curcuma caesia* Roxb. (rhizomes). Diabetic + *Aerva lanata* Linn. (roots) and *Curcuma caesia* Roxb. (rhizomes) group showed significant glucose levels compare to negative control groups, also *Aerva lanata* Linn. (roots) and *Curcuma caesia* Roxb. (rhizomes) outcomes hypoglycemia. SGOT and SGPT levels were increased in the negative control group which indicates might be hepatotoxicity and it was in conjunction with the histopathological observations of minimal to mild hepatocyte degeneration and necrosis with hypertrophy and bile duct proliferation with cytoplasmic clarification (vacuolation) in this study. Diabetic + *Aerva lanata* Linn. (roots) and *Curcuma caesia* Roxb. (rhizomes) groups also showed significant ($P < 0.05$) as well as histologically minimal hepatocellular hypertrophy and degeneration as compared to negative control in SGOT. The SGOT level was found significant ($P < 0.001$) elevated in diabetic control group.

Moreover, *Aerva lanata* Linn. (roots) and *Curcuma caesia* Roxb. (rhizomes) 200 mg/kg (77.50 ± 5.50) treated group SGOT significantly decreased, and combination of *Aerva lanata* Linn. (roots) and *Curcuma caesia* Roxb. (rhizomes) 200 mg/kg (71.00 ± 5.00) treated group SGOT also decreased significantly ($P < 0.01$). In 500 mg/kg p.o. metformin (67.00 ± 4.00) treated group, SGOT was significantly decreased ($P < 0.001$), respectively, as compared with control group (122.0 ± 7.00). Although, at the end days of experiment, the serum transaminase such as SGPT level was significantly ($P < 0.001$) elevated in diabetic control group.

Aerva lanata Linn. (roots) and *Curcuma caesia* Roxb. (rhizomes) 200 mg/kg (71.00 ± 5.00) treated group SGPT significantly decreased, and *Aerva lanata* Linn. (roots) and *Curcuma caesia* Roxb. (rhizomes) 200 mg/kg (60.00 ± 5.00) treated group SGPT also decreased significantly ($P < 0.01$). In 500 mg/kg p.o. metformin (58.00 ± 5.00) treated group, SGPT was significantly decreased ($P < 0.001$), respectively, as compared with control group (117.0 ± 6.00).

The present study showed that biochemical parameters did not show any of the adverse effect of *Aerva lanata* Linn. (roots) and *Curcuma caesia* Roxb. (rhizomes) in rats. Liver enzymes such as SGOT and SGPT are considered to be biochemical markers for assessing liver function. Combination of *Aerva lanata* Linn. (roots) and *Curcuma caesia* Roxb. (rhizomes) significantly reduced the liver enzymes levels in experimental animals show that combined therapy has a hepatoprotective effect. During the experimentation, Wistar rats did not show any mortality or any other adverse effects when the rats fed orally with *Aerva lanata* Linn. (roots) and *Curcuma caesia* Roxb. (rhizomes) at the doses of 200 and 300 mg/kg. Hence, *Aerva lanata* Linn. (roots) and *Curcuma caesia* Roxb. (rhizomes) has a good safety.

CONCLUSION

It is evident that methanolic extract of combination of *Aerva lanata* Linn. (roots) and *Curcuma caesia* Roxb. (rhizomes) in streptozotocin (STZ)-induced diabetic rats capable of reducing the blood glucose level.

REFERENCES

1. Kooti W, Farokhipour M, Asadzadeh Z, Ashtary-Larky D, Asadi-Samani M. The role of medicinal plants in the treatment of diabetes: a systematic review. Electronic physician. 2016 Jan;8(1):1832.
2. Prabhakar PK, Doble M. A target based therapeutic approach towards diabetes mellitus using medicinal plants. Current Diabetes Reviews. 2008 Nov 1;4(4):291-308.
3. Bastaki S (2005) Diabetes mellitus and its treatment. Int J Diabetes Metab 13:111–134
4. Al-Goblan AS, Al-Alfi MA, Khan MZ. Mechanism linking diabetes mellitus and obesity. Diabetes, metabolic syndrome and obesity: targets and therapy. 2014 Dec 4:587-91.
5. Dean L, McEntyre J (2004) The genetic landscape of diabetes [Internet]. National Center for Biotechnology Information (US), Bethesda
6. Viswanathan V, Rao VN (2013) Problems associated with diabetes care in India. Diabetes Manag 3(1):31–40
7. Modak M, Dixit P, Londhe J, Ghaskadbi S, Paul A, Devasagayam T (2007) Indian herbs and herbal drugs used for the treatment of diabetes. J Clin Biochem Nutr 40(3):163–173.
8. Yadav RN, Agarwala M. Phytochemical analysis of some medicinal plants. Journal of phytology. 2011 Dec 14;3(12).
9. Sofowra, A. 1993. Medicinal Plants And traditional Medicine In Africa. Spectrum Books Ltd., Ibadan, Nigeria, pp. 191-289.
10. Trease, G.E., Evans, W.C. 1989. Pharmacognosy, 11th edn., Bailliere Tindall, London, pp. 45-50.
11. Harborne, J.B. 1973. Phytochemicals Methods. Chapman and Hall Ltd., London, pp. 49-188.
12. P'ng XW, Akowuah GA, Chin JH. Acute oral toxicity study of Clinacanthus nutans in mice. International Journal of Pharmaceutical Sciences and Research. 2012 Nov 1;3(11):4202.
13. Mir MA, Sawhney SS, Jassal MM. In-vitro antidiabetic studies of various extracts of Taraxacum officinale. The Pharma Innovation. 2015 Mar 1;4(1, Part B):61.

14. Emeka EJ, Oluwatoyin AE, Adekunle OI, Ignis IO. Preliminary phytochemical screening and evaluation of hypoglycemic properties of the root extract of Uvaria chamae. ||| Bangladesh Journal of Pharmacology|||. 2015 Apr 11;10(2):326-1.
15. Mandukhail SU, Aziz N, Gilani AH. Studies on antidyslipidemic effects of Morinda citrifolia (Noni) fruit, leaves and root extracts. Lipids in Health and Disease. 2010 Dec;9:1-6..
16. Corcoran RM, Durnan SM. Albumin determination by a modified bromcresol green method. Clinical chemistry. 1977;23(4):765-6.
17. Tyagi CK. Antidiabetic evaluation of methanolic extract of Lagerstroemia parviflora Roxb. in streptozotocin-induced diabetic Wistar albino rat model. International Journal of Green Pharmacy (IJGP). 2021 Apr 9;15(1).