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Assessment of Antidiabetic efficacy of isolated compounds from roots of *Aerva lanata* and rhizomes of *Curcuma caesia* in animal model

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ABSTRACT:

AIM: In this study, streptozotocin (STZ)-induced diabetic rats were used to assess the antidiabetic efficacy of isolated compounds from the roots of Aerva lanata Linn. (IAL) and the rhizomes of Curcuma caesia Roxb. (ICC).

Materials and Methods: Standard procedures were used to estimate biochemical parameters such as body weight, OGTT, total cholesterol, triglycerides, HDL (VLDL and LDL), total protein, albumin, ACP, ALP, SGPT, and SGOT. The concentrations of IAL and ICC (5 mg/kg b.w., p.o.) and the combination of IAL + ICC (5 mg/kg b.w., p.o.) were administered orally.

Results and Discussion: The findings pertaining to antidiabetic actions reveal significant changes in physiological and biochemical indices. When compared to the standard drug glibenclamide at a dose of 500 µg/kg, the combination dose of 5 mg/kg of Aerva lanata Linn. (IAL) and Curcuma caesia Roxb. (ICC) showed a progressive decrease in body weight, blood glucose level, triglycerides, HDL, total protein, albumin, ACP, ALP, and SGPT and SGOT levels. The results of the study show that dosing the STZ-induced diabetic rats with IAL + ICC separately at a dose of 5 mg/kg body weight resulted in a significant increase in cholesterol levels and a significant drop in total cholesterol, triglyceride, and LDL cholesterol levels.

Conclusion: This study provides scientific support and a foundation for the development of an antidiabetic medication by demonstrating the antidiabetic potential of isolated compounds containing the combination of Aerva lanata (IAL) and Curcuma caesia (ICC).

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

1. INTRODUCTION

An important metabolic disease that can be treated with medicinal herbs is diabetes mellitus. Some traditional plants are said to offer potent anti-diabetic properties without any unfavourable side effects. They are rich in anti-diabetic compounds such flavonoids, alkaloids, phenolics, and tannins, which improve the function of pancreatic cells by increasing insulin secretion or reducing glucose absorption through the intestinal tract.[1] Only 109 of the more than 420 medicinal plants with anti-diabetic properties that have been experimentally confirmed have had their entire mechanism thoroughly examined, according to the literature.

Numerous medicinal plant extracts have been shown to modulate metabolic pathways such as glycolysis, gluconeogenesis, Krebs cycle, glycogen formation and degradation, insulin synthesis and release, cholesterol synthesis, carbohydrate metabolism, and absorption.[2]

Elevated blood glucose levels are a hallmark of diabetes mellitus (DM), a chronic endocrine disease that can alter how lipids, proteins, and carbs are metabolised.[3] Either insufficient insulin generation by the pancreatic Langerhans islet cells or poor insulin absorption in peripheral organs are the causes of it.[4] The pancreas releases the hormone insulin in response to an increase in blood glucose levels after a meal. Insulin causes the liver to metabolise glucose and the muscle and fat cells to remove glucose from the blood, causing the blood sugar level to return to normal. Because of the pancreas' inability or inefficiency in producing insulin, diabetes raises blood sugar levels.[5] India is known as the "capital of diabetes" due to the fact that over 61 million people there have the condition. Effective treatment of diabetes and its accompanying complications remains a major challenge for India due to a number of issues, such as an inadequate healthcare system, inadequate facilities, and etc.[6]

Herbal formulations are chosen over synthetic pharmaceuticals to reduce the negative effects of diabetes and its subsequent complications since they have fewer side effects and are less expensive.[7] The current study aims to evaluate the anti-diabetic efficaciousness of isolated compounds from two Indian medicinal plants in combination.

2. MATERIALS AND METHODS

The herbal medicinal plants and their parts such as roots and rhizomes of *Aerva lanata* and *Curcuma caesia* respectively were collected from Bhimbetka Bhojpur, Bhopal, and Madhya Pradesh. The selected plants parts were further authenticated by expert botanist Department

of Botany, Barkatullah University, Bhopal (MP). The plant specimens were compared with voucher specimen.

2.1 Extraction

The extract of crude drug was prepared using Soxhlet extraction method. A thimble containing approximately 100g of powdered plant material was evenly packed, and 500 ml of each of the several solvents was used for the extraction process. Depending on how polar each solvent was, a different solvent was utilised. The extraction process lasts for a whole day, or until the solvent in the extractor's syphon tube turns colourless. Subsequently, the extract was placed in a beaker and heated at between 30 & 40°C, until the solvent had evaporated. The dried extract was stored at 4ºC in the refrigerator in preparation for its usage in phytochemical analysis.[8]

2.2 Qualitative phytochemical analysis

The qualitative chemical tests were performed for the presence of active chemical constituents using standard testing methods.[9, 10, 11]

2.3 Isolation of Phytoconstituents from the selected fractions of both the plants

1. The basified Toluene sub-fraction was refined to extract alkaloids as the active ingredient from *Aerva lanata* roots. The preparative TLC method was used to isolate the chemicals.

2. For the purpose of isolating phenolic components from *Curcuma caesia* rhizomes, ethyl acetate extract was optimised using the preparative TLC method.

2.4 Animals

The antidiabetic trial employed 180–220 g Wistar albino rats of both sexes. The animals were acquired from the College of Veterinary Science and Animal Husbandry's animal house in Mhow, MP. Every rat was housed in a typical plastic rat cage with a coverlid made of stainless steel and bedding made of wheat straw. The animals were kept in conventional conditions, with a temperature of 25 ± 2°C and a photoperiod of 12:12 hours of light and dark. They received water on a constant basis along with commercial rat and mouse food. The Institutional Animal Ethical Committee approved both the study protocols and the usage of these animals.

2.5.1 Dose selection for Animal Study.[12]

The Organisation for Economic Cooperation and Development (OECD) and the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India, provided draft guidelines 423, which were followed in conducting the acute oral toxicity studies and dose selection. *Aerva lanata* Linn. and *Curcuma caesia* Roxb.'s LD50 were ascertained through an acute toxicity investigation using healthy Wistar rats of both sexes, weighing between 180 and 220 g. Prior to dosing, the animals were housed in their cages for seven days to provide acclimation to the laboratory environment. They were also randomly chosen and marked to enable individual identification. *Aerva lanata* Linn. and *Curcuma caesia* Roxb. did not show any harmful symptoms in the acute toxicity investigation up to a level of 3000 mg/kg body weight. Every animal exhibited typical behaviour. There was no discernible behavioural or neurological impact. Up to a 14-day trial period, no deaths were reported.

2.5.2 Antidiabetic Studies.[13]

Animal grouping for antidiabetic studies

The rats will be split up into groups of six, one for each group. Diabetes was produced in Groups II–VI with 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, following an overnight fast (devoid of food for 16 hours but with unrestricted access to water). The identical volume of sodium citrate buffer (0.1 M) was given to the control rats. Overnight, the animals were given 5% glucose solution to help them overcome the hypoglycemia caused by the medication. After 72 hours of STZ injection, blood glucose levels were estimated to establish the presence of diabetes. For the investigation, animals with fasting blood glucose levels greater than 250 mg/dl were chosen.

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 (IAL) 5 mg/kg/day p. o.	6
Group-V	Diabetic rats received (ICC) 5 mg/kg/day p. o.	6
Group -VI	Diabetic rats received combination of **IAL** + **ICC** (5 mg/kg/day p. o.)	6
	Total no. of animals used for the study	36

2.6 Biochemical analysis

The digital balance was used to determine the body weight of the experimental rats both before and after treatment, or on the first and last day of the treatment. On days 0, 8, and 21 after treatment, the blood glucose levels of fasted rats were measured before and after the intervention.

At the conclusion of the experiment, cervical decapitation was used to kill every experimental rat. After being drawn, blood samples were left to coagulate. A variety of biochemical characteristics were examined after the serum was separated by centrifuging it for 15 minutes at 2500 rpm. The Hitachi-902 automated biochemistry analyzer was used to examine biochemical parameters.

2.7 Oral glucose tolerance test estimation.[14]

The rats were fasted for eighteen hours before the oral glucose tolerance test was administered. The six groups of rats received the above-mentioned treatment. After the medications were given, 30 minutes later, glucose (2 g/kg, p.o.) was given. After administering glucose for 30, 60, and 120 minutes, blood was removed from the retro orbital sinus while under ether anaesthesia. Serum glucose levels were then measured using a commercial kit using the glucose oxidase (GOD) method.[15, 16]

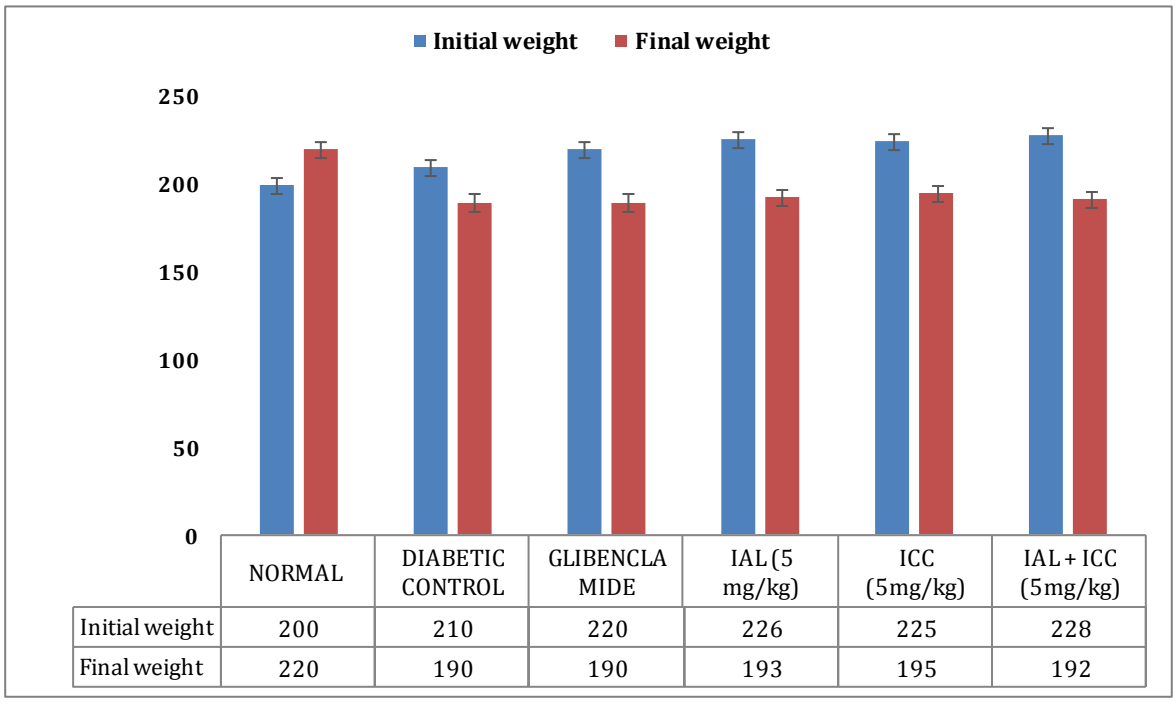
2.8 Estimation of total cholesterol (TC)

TC in serum was calculated with the CHOD/PAP techniques. A major component of lipoprotein and cell membranes, cholesterol serves as a precursor to the synthesis of bile acids and steroid hormones. [15]

The PEG method was used to measure serum levels of high-density lipoprotein (HDL) cholesterol, triglycerides (TGs), and TG Assay Protocol. Calculating the overall protein content, Calculating the albumin content Calculating the activity of alkaline phosphatase (ALP) and acid phosphatase (ACP), In Gupta Diagnosis laboratory, Bhopal (M.P.), serum glutamate pyruvate transaminase (SGPT) and serum glutamic oxaloacetic transaminase (SGOT) were measured using standard pathological techniques. [16, 17]

3. RESULTS

The animals body weight in all groups were performed at the onset and end of the study. Body weight of all animals were significantly ($P < 0.05$) maintained in all treated groups (glibenclamide 500 µg/kg p.o., isolated compounds of *Aerva lanata* Linn. (IAL) 5 mg/kg/p. o., isolated compounds of *Curcuma caesia* Roxb. (ICC) 5 mg/kg/p. o. and combination of both compounds 5 mg/kg/p. o.) [Figure 1].



	NORMAL	DIABETIC CONTROL	GLIBENCLAMIDE	IAL (5 mg/kg)	ICC (5mg/kg)	IAL + ICC (5mg/kg)
Initial weight	200	210	220	226	225	228
Final weight	220	190	190	193	195	192

Figure 1: Mean body weight change

All groups' animals' blood glucose levels were measured on days 0, 8, and 21. Throughout the research, all therapy groups experienced a progressive decline in blood glucose levels. At the end of experiment glibenclamide 500 µg/kg p. o., isolated compounds of *Aerva lanata* Linn. 5 mg/kg/p. o., isolated compounds of *Curcuma caesia* Roxb. 5 mg/kg/p. o. and combination of both isolated compounds of 5 mg/kg/p. o. shows (112. 00 ± 6. 50; 118. 00 ± 6. 00, 120.00 ± 5. 50 and 115. 00 ± 5. 50) treated group blood glucose level was decrease significantly (P <0.05) at 21st days, respectively [Figures 2-8].

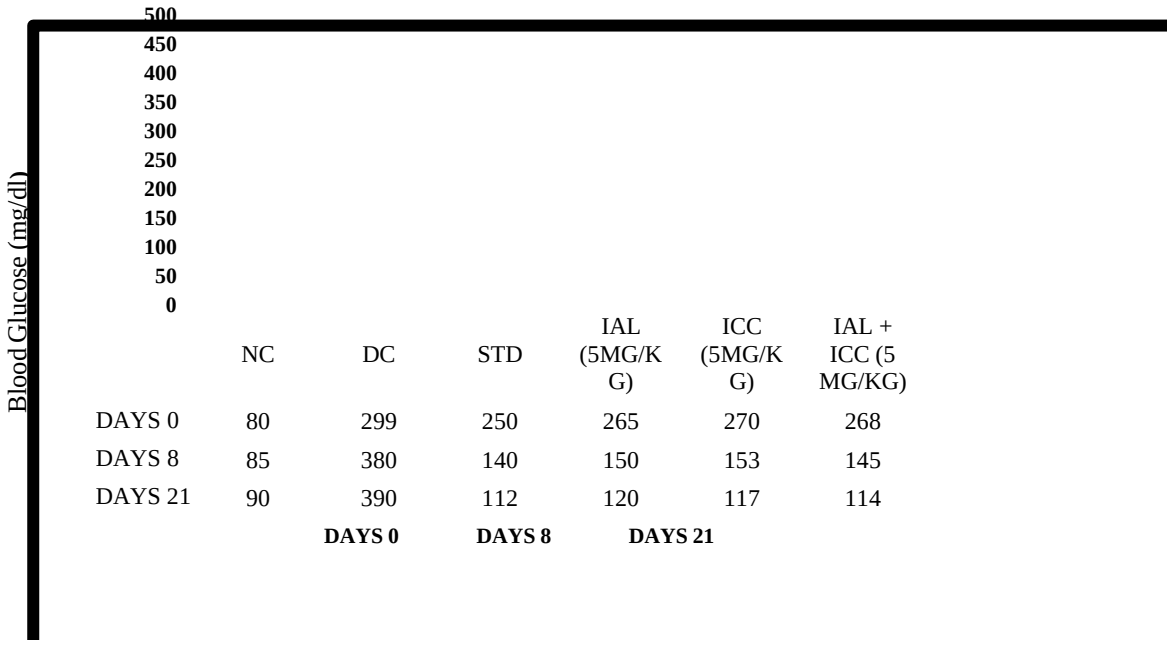


Figure 2: Antidiabetic activity of *Aerva lanata* Linn (IAL) and *Curcuma caesia* Roxb. (ICC) on blood glucose level in streptozotocin-induced diabetic rats

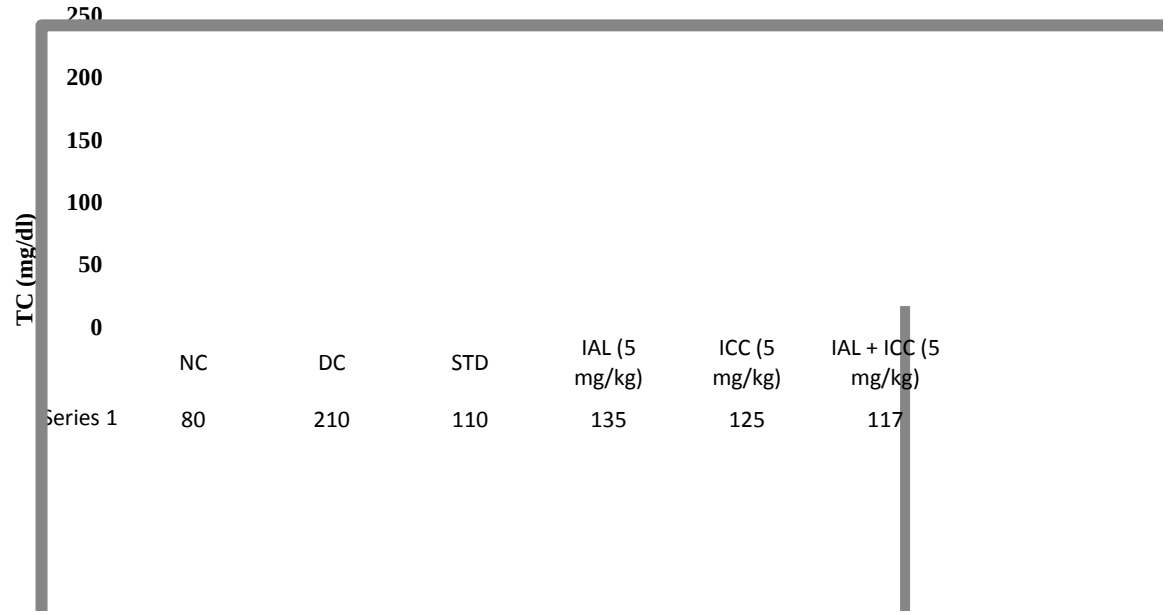


Figure 3: Effect of *Aerva lanata* Linn (IAL) and *Curcuma caesia* Roxb. (ICC) on total cholesterol level in streptozotocin-induced diabetic rats

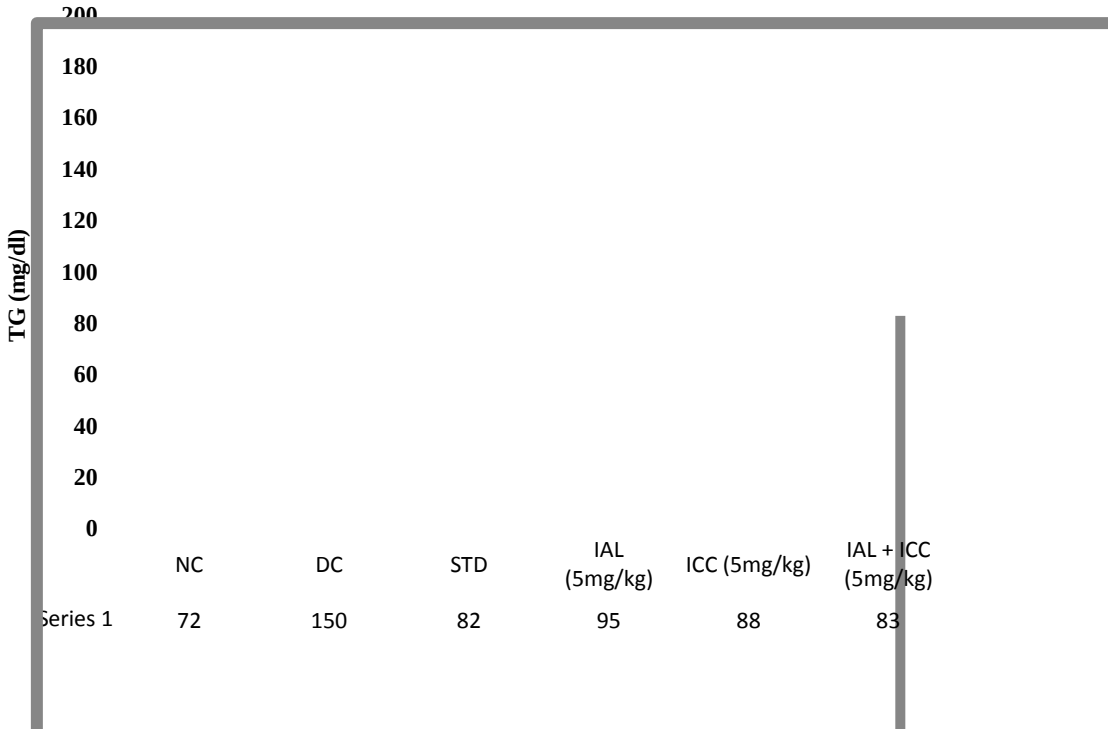


Figure 4: Effect of *Aerva lanata* Linn. (IAL) and *Curcuma caesia* Roxb. (ICC) on triglyceride level in streptozotocin-induced diabetic rats

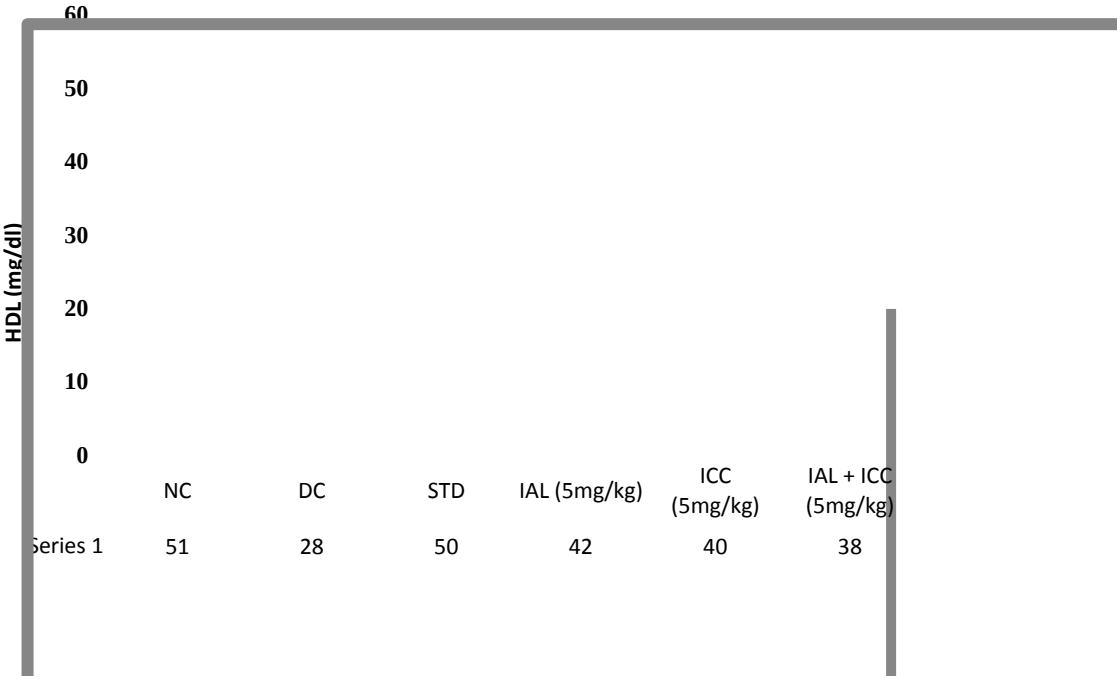


Figure 5: Effect of *Aerva lanata* Linn. (IAL) and *Curcuma caesia* Roxb. (ICC) on high-density lipoproteins in streptozotocin-induced diabetic rats

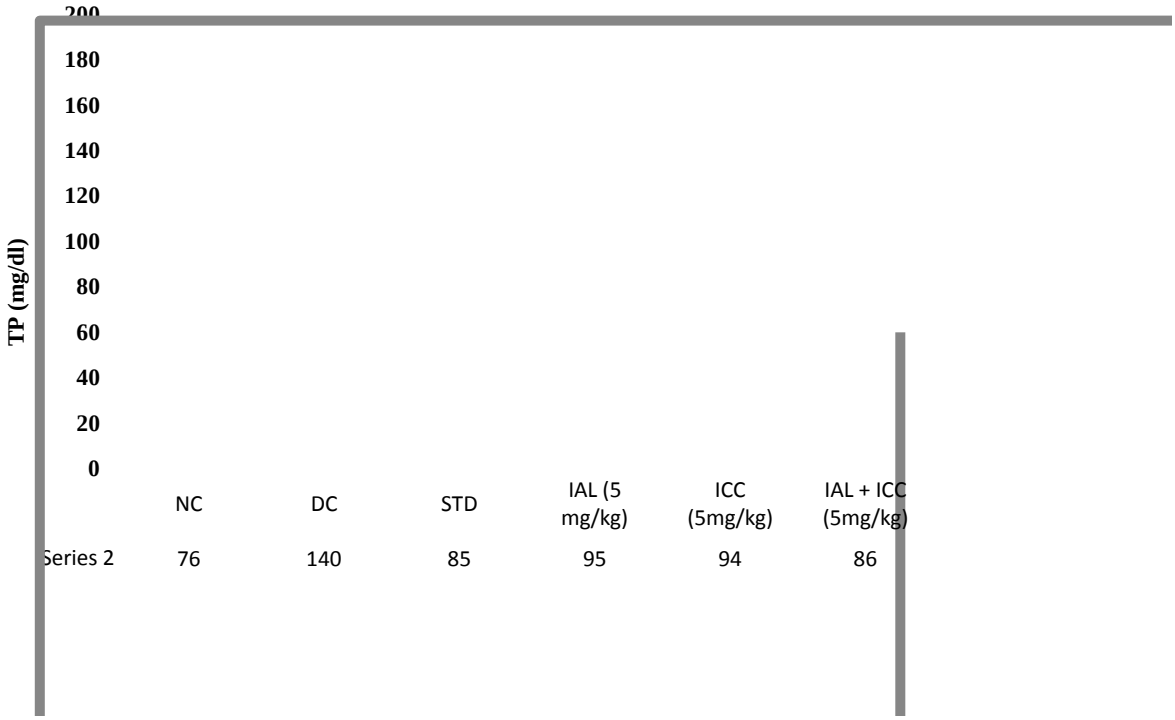


Figure 6: Antidiabetic effect of *Aerva lanata* Linn. (IAL) and *Curcuma caesia* Roxb. (ICC) on serum lipid profile, that is, total protein level in streptozotocin-induced diabetic rats

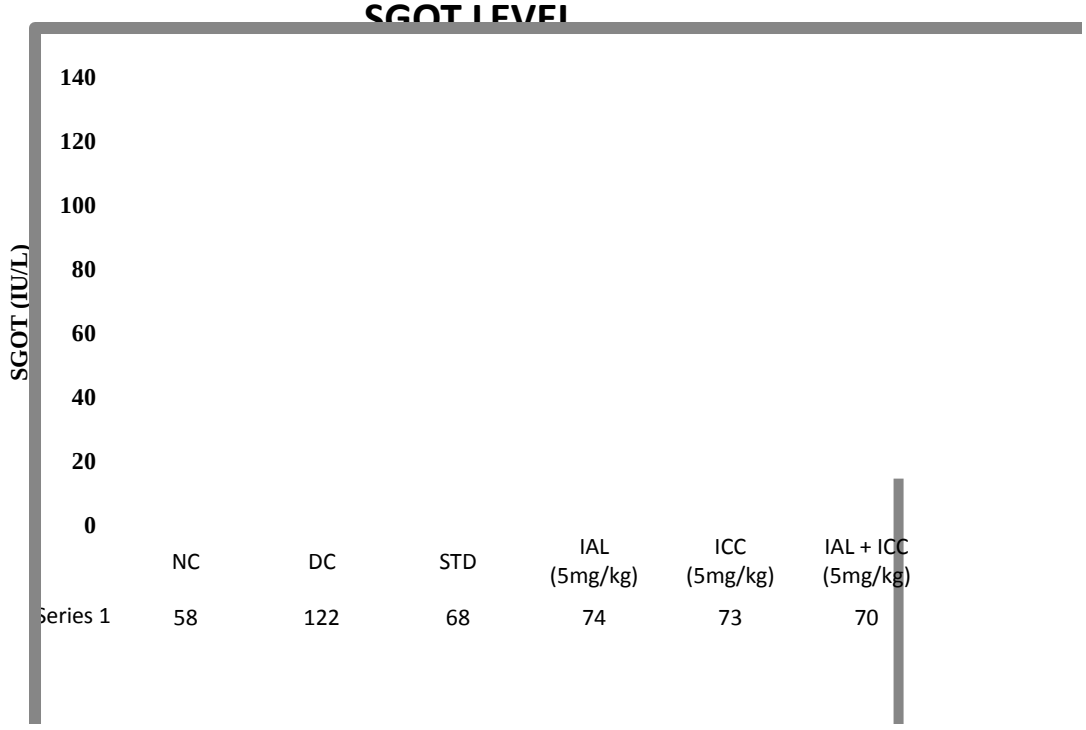


Figure 7: Effect of *Aerva lanata* Linn. (IAL) and *Curcuma caesia* Roxb. (ICC) on serum glutamic oxaloacetic transaminase in streptozotocin-induced diabetic rats

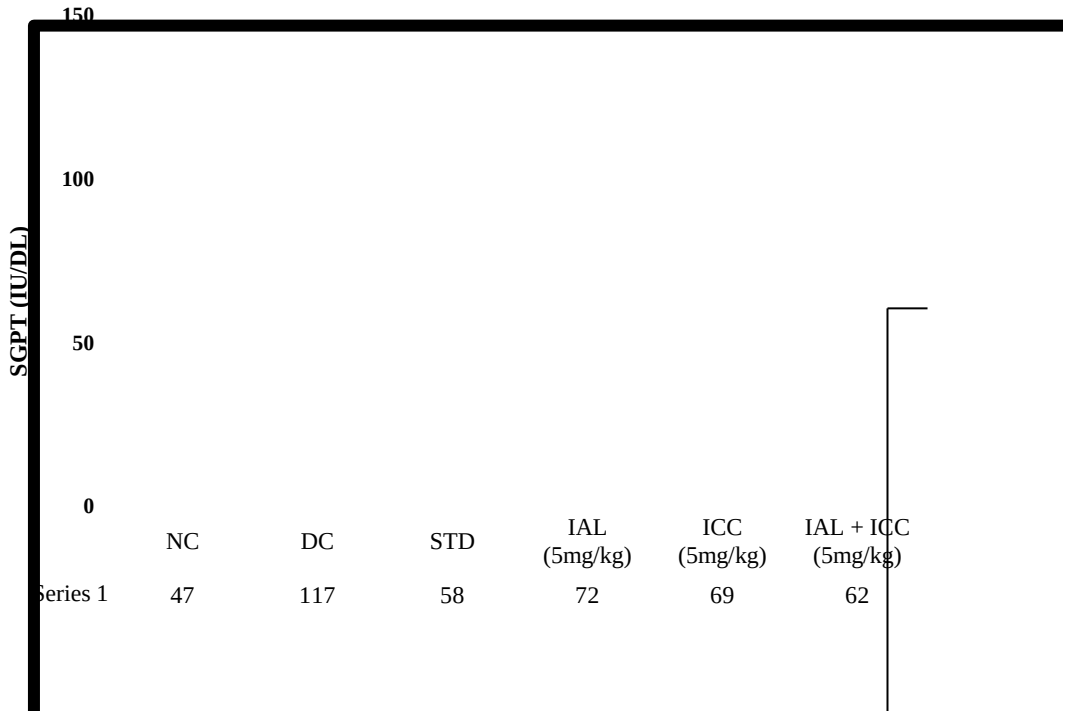


Figure 8: Effect of *Aerva lanata* Linn. (IAL) and *Curcuma caesia* Roxb. (ICC) on serum glutamate pyruvate transaminase in streptozotocin-induced diabetic rats

DISCUSSION

Aerva lanata Linn. (roots) and *Curcuma caesia* Roxb. (rhizomes) have an antihyperglycemic effect on STZ-induced diabetic rats. This suggests that the primary mechanism of action may not be to stimulate insulin release from pancreatic cells, but rather to directly promote glucose utilisation by peripheral tissues. Excess fat mobilisation from adipose tissue is the cause of diabetes-induced hyperlipidemia. The lipoprotein lipase enzyme is less active in diabetics, which raises the blood levels of lipoproteins. In the current study, treating the STZ-induced diabetic rats with isolated compounds of *Aerva lanata* Linn. (roots-IAL) and *Curcuma caesia* Roxb. (rhizomes-ICC) at 5 mg/kg body weight resulted in a significant increase in HDL cholesterol levels and a significant decrease in TC, triglyceride, and LDL cholesterol levels.

The diabetic + *Aerva lanata* Linn. (roots) and *Curcuma caesia* Roxb. (rhizomes) group showed significantly lower glucose levels than the negative control, and the animals' general health was consistent with the traditional anti-diabetic effects of these plants (rhizomes and roots). In addition to *Aerva lanata* Linn. (roots) and *Curcuma caesia* Roxb. (rhizomes) causing hypoglycemia, the diabetic + *Aerva lanata* Linn. (roots) group also demonstrated substantial glucose levels when compared to negative control groups. The negative control group had higher SGOT and SGPT values, which may indicate hepatotoxicity and it was

combined with the histological findings in this study of bile duct proliferation with cytoplasmic clarity (vacuolation) and modest to mild hepatocyte degeneration and necrosis with hypertrophy. In comparison to the negative control in SGOT, the diabetic + Aerva lanata Linn. (roots-IAL) and Curcuma caesia Roxb. (rhizomes-ICC) groups similarly demonstrated significant ($P < 0.05$) and histologically limited hepatocellular enlargement and degeneration. In the diabetic control group, the SGOT level was found to be significantly ($P < 0.001$) higher.

Furthermore, SGOT significantly decreased in the groups treated with *Aerva lanata* Linn. (roots-IAL) and *Curcuma caesia* Roxb. (rhizomes-ICC) at a dose of 5 mg/kg (77.50 ± 5.50); SGOT also significantly decreased in the group treated with a combination of *Aerva lanata* Linn. (roots-IAL) and *Curcuma caesia* Roxb. (rhizomes-ICC) at a dose of 5 mg/kg (71.00 ± 5.00) ($P < 0.01$). SGOT was considerably lower ($P < 0.001$) in the 500 µg/kg p.o. glibenclamide (67.00 ± 4.00) treatment group than in the control group (122.0 ± 7.00). Nevertheless, the diabetes control group's blood transaminase level, such as SGPT level, was considerably ($P < 0.001$) higher at the end of the experiment.

Significant reductions in SGPT were observed in the *Aerva lanata* Linn. (roots-IAL) and *Curcuma caesia* Roxb. (rhizomes-ICC) 5 mg/kg (71.00 ± 5.00) treated group, and in the *Aerva lanata* Linn. (roots-IAL) and *Curcuma caesia* Roxb. (rhizomes-ICC) 5 mg/kg (60.00 ± 5.00) treated group ($P < 0.01$). When compared to the control group (117.0 ± 6.00), the SGPT was considerably lowered ($P < 0.001$) in the 500 µg/kg p.o. glibenclamide (58.00 ± 5.00) treatment group.

The results of this study demonstrated that *Aerva lanata* Linn. (roots-IAL) and *Curcuma caesia* Roxb. (rhizomes-ICC) had no negative effects on rats' biochemical markers. Liver enzyme tests, like SGOT and SGPT, are thought to be useful biochemical indicators for evaluating liver health. The hepatoprotective effect of combined therapy was demonstrated by the considerable reduction of liver enzyme levels in experimental mice when *Aerva lanata* Linn. (roots-IAL) and *Curcuma caesia* Roxb. (rhizomes-ICC) were given. Hence, *Aerva lanata* Linn. (roots) and *Curcuma caesia* Roxb. (rhizomes) has a shown the expected results.

CONCLUSION

It is evident that combination (IAL + ICC) of isolated and individual compounds from *Aerva lanata* Linn. (roots-IAL) and *Curcuma caesia* Roxb. (rhizomes-ICC) in streptozotocin (STZ)-induced diabetic rats capable of reducing the blood glucose level.

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