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Original Article

ANTIDIABETIC AND ANTIHYPERLIPIDEMIC POTENTIAL OF STANDARDIZED EXTRACT, FRACTION AND SUBFRACTION OF *CINNAMOMUM INERS* LEAVES

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ABSTRACT

Objective: *Cinnamomum iners* leaves are used in the Malaysian local traditional medicine for the treatment of diabetes and its complications. However, no scientific data is available to validate the folklore claim. Hence the present study was designed to evaluate the antidiabetic and antihyperlipidemic effect of *C. iners* leaves.

Methods: Firstly, the antidiabetic activity of petroleum ether, chloroform, methanol and water extract of *C. iners* leaves was evaluated. Next, the potential extract which was the methanol extract (ME) was fractionated to obtain ethyl acetate, n-butanol, chloroform and aqueous fraction. The active chloroform fraction (CF) was subjected to subfractionation process to obtain subfraction 1(SF-1) and subfraction 2 (SF-2). The antidiabetic effect of all the extract, fractions and subfractions were screened by using streptozotocin (STZ)-induced-diabetic rats for 12 days. Blood glucose levels and body weights were monitored at specific intervals and different biochemical parameters were determined at the end of treatment.

Results: The data showed the significant increase in the body weight and HDL level and decrease in the blood glucose, TC, TG, LDL and VLDL levels. The increment in body weight and HDL and reduction in various biochemical parameters were prominent after the purification of ME, CF and SF-1.

Conclusion: *C. iners* leaves showed antidiabetic properties and hypolipidemic effects. The increased antidiabetic potential of CF over ME and SF-1 over CF is presumably due to its partial purification achieved by fractionation which resulted in increase in quantity of cinnamic aldehyde.

Keywords: *Cinnamomum iners*; cinnamic aldehyde; antidiabetic; antihyperlipidemic; subfractionation.

INTRODUCTION

The number of diabetic patients is increasing worldwide and it is estimated that the number of diabetics will reach 2.48 millions by 2030 [1]. It is predicted that this disease will become one of the world's main disablers and killers within the next 25 years [2]. Diabetes mellitus is characterized by hyperglycemia sometime accompanied by hyperlipidemia, hypertension and an increased risk of complications from vascular disease [3, 4]. The plant kingdom is a potential hypoglycemic or hypolipidemic agent that would be useful to combat diabetes [5].

In Malaysia, varieties of plants have been historically employed for the treatment of diabetes due to the effectiveness, minimal side effect and relatively low cost. These include *C. iners* which grows wild in lowland of Malaysia, India, Myanmar, Indonesia, Thailand, Singapore, Brunei and Philippines. It is locally known as *kayu manis hutan*, *medang kemangi* and *teja*. The plant leaves have been heavily promoted for a wide range of pharmacological uses; from fever, headache, for digestive system problem, rheumatism, wound healing and diabetes [6, 7]. Although natural supplements derived from plants such as *C. iners* play important role in the treatment of diabetes, scientific research to validate its efficacy are still lacking [8]. Thus, the present study was aimed to investigate the antidiabetic and antihyperlipidemic action of various extract, fractions and subfractions of *C. iners* leaves on STZ-induced diabetic rats. In addition, the correlation between antidiabetic potential and predominant compound, cinnamic aldehyde presence in the active extract, fraction and subfraction was also investigated.

MATERIALS AND METHODS

Plant material

C. iners leaves were collected at USM (Universiti Sains Malaysia). The authentication work was carried out by a botanist from School of Biological Sciences, USM where the plant material was deposited. The voucher specimen number is 11014.

Extraction

The plant leaves were washed with water to remove dirt prior to the drying process. The leaves were then crushed into fine powder. Powdered dried leaves (500 g) of the plant were serially macerated in petroleum ether (2500 mL), chloroform (2500 mL) and methanol (2500 mL) for 3 days each. The residue after methanolic extraction was macerated in water (2500 mL) for 24 h to obtain water extract. All the leaves extracts (pet.ether, chloroform, methanol and water) were filtered and concentrated under reduced pressure at 55 °C in a rotary evaporator. The concentrated extracts obtained were placed in the oven at 50 °C for 3 days to remove the remaining solvent. Then it was placed in the freeze drier for 24 h which yielded a sticky material.

Fractionations of the active extract (methanol extract)

ME (2 g) was suspended in distilled water (500 mL). Then, the suspension obtained was placed into a 1L separatory funnel. Firstly, the solution was extracted with chloroform (3×250 mL). Next, the aqueous layer was extracted with ethyl acetate and n-butanol (3×250 mL) to obtain three respective fractions. All fractions obtained were concentrated using the rotary evaporator. Concentrated fractions were kept in freeze dryer for 24 h to remove the remaining solvents.

Sub- fractionations of the active fraction (chloroform fraction)

CF (0.5 g) was further extracted in hexane-chloroform mixture (1:3; 100 mL). The supernatant formed was collected, filtered and concentrated using rotary evaporator and freeze dried to obtain SF-1. The residue was dried and then similarly washed with chloroform (100 mL) until no colour was formed. Again, this supernatant was filtered, concentrated using rotary evaporator and freeze dried to obtain SF-2.

Standardization of active extract, active fraction and active subfraction using cinnamic aldehyde

The chemical composition of the active ME, CF and SF-1 was analysed using Agilent Gas Chromatography (GC 6890N, China), and

Agilent Mass Spectrometer (MS 5973I, USA). HP-5MS column (30 m x 0.25 mm x 0.25 µm) was used. The inlet temperature was set at 220 ºC and MSD transferline heater at 225 ºC. GC was performed in splitless mode. The flow rate of carrier gas (helium) was maintained at 1 mL/min. The initial temperature of oven was 80 °C and then increased to 170 °C by 10 °C/min and maintained for 2 min. The standard cinnamic aldehyde was prepared at a concentration of (0.25-2.00 mg/mL) in methanol by serial dilution of stock solution. Samples were prepared at 1 mg/mL in methanol.

Animals

Healthy male Sprague Dawley (SD) rats between 2 to 3 months of age, and weighing 200-250 g were obtained from Animal Research and Service Centre (ARASC), USM, Penang. The animal were kept in clean and dry cages and maintained in a well-ventilated animal transit room with 12 h-light-12 h dark cycle. Rats were fed with rat pellet and water *ad libitium*. The study was approved by the Animal Ethic Committee of USM (Reference number: USM /Animal Ethics Approval / 2012 / (78) (393). For experimental purpose, animals were fasted (12 h for diabetic rats and 6 h for normal rats) but had free access to water.

Induction of diabetes

Diabetes was induced by intraperitoneal injection (single dose) of STZ (55 mg/ kg body weight) in 0.9% NaCl to the rats. Blood glucose level was measured after 72 h of STZ injection. Rats with fasting blood glucose concentrations within 12-22 mmol/L was considered as diabetic and used for the study [9, 10].

Antihyperglycemic test of extract, fractions and sub-fractions

All the animals were randomly divided into groups with six animals in each group. The first group was the normal control (non-diabetic rats) group which was given cosolvent (10 ml/kg b.w., 20% Tween-20 in distilled water). The rest of the groups were STZ-induced diabetic rats. Group 2 served as negative control (diabetic rats) group received cosolvent (10 ml/kg b.w.). Group 3 served as positive control which received metformin (500 mg/kg b.w.). The rest of the group were treated orally with either extracts (petroleum ether, chloroform, methanol and water extract; 1 g/kg b.w.), fractions (ethyl acetate, n-butanol, chloroform and aqueous; 500 mg/kg b.w.) or active extract (1 g/kg b.w.), active fraction (500 mg/kg b.w.), subfractions (250 mg/kg b.w.) and cinnamic aldehyde (20 mg/kg b.w.), which served as the standard compound for *C. iners*. The dosages of the plant extracts were determined from preliminary dose–response study in our laboratory (data not shown). All rats were administered with either cosolvent, tested samples or standard drugs twice daily for 12 days. Blood sample (approx. 10µl) was collected from tail vein of each rat on day -3, 0, 3, 6, 9 and 12. Blood glucose level was measured using Accu-check Advantage II Clinical Glucose meter [11].

Antihyperlipidemic potential of active extracts, active fractions and sub-fractions

On day 12, all rats were euthanized using carbon dioxide not higher than 8 psi and blood was collected by cardiac puncture. The blood was allowed to clot and spinned to obtain the serum for the determination of TG, LDL, HDL and TC. Serum estimations for TC, TG, LDL and HDL were done spectrophotometrically using commercial kits available (Erba Diagnostics).

Statistical analysis

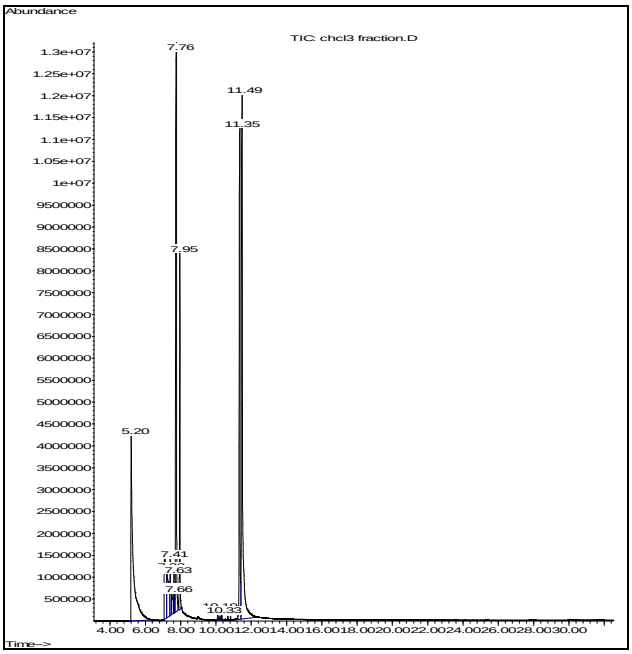
Data were expressed as mean ± standard error of mean (SEM) for six animals per group. Statistical analysis was made using one-way analysis of variance (ANOVA) followed by Dunnett's t-test for post-hoc analysis. *p*-values < 0.05 were considered to be statistically significance.

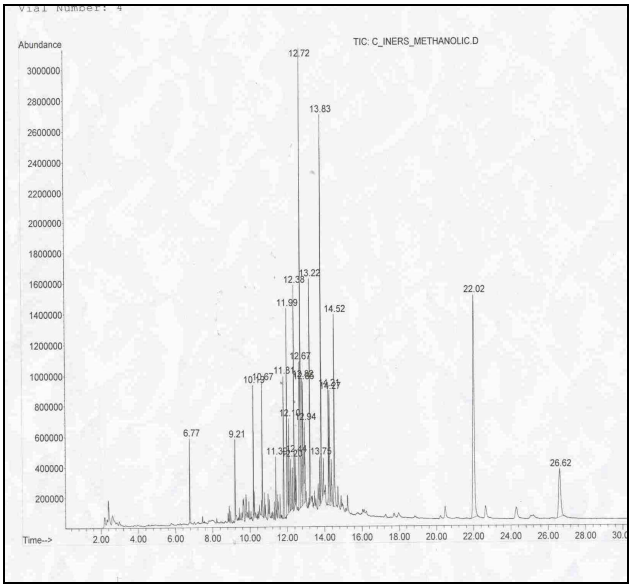
RESULTS

Main active constituent in *C. iners* leaves

GCMS profiles of methanol extract, chloroform fraction and SF-1 of *C. iners* leaves were compared with standard cinnamic aldehyde (Figure 1). The analyses show the presence of cinnamic aldehyde in all samples, but in varying proportions. Mass spectroscopy of cinnamic aldehyde identified in the samples showed major characteristic fragmentations pattern (m/z=45; m/z=59; m/z=69; m/z=77; m/z=89; m/z=103; m/z=111; m/z=119; m/z=131; m/z=152; m/z=161; m/z=179 M^+=204) exactly identical to the standard cinnamic aldehyde mass fragmentations pattern.

The retention time of pure cinnamic aldehyde was 10.12 min whereas the retention time of the samples were 10.2 ± 0.2 min. Calculation based on simple linear regression curve revealed that ME, CF and SF-1 of *C. iners* leaves contain 8.32%, 14.8 % and 33.2 % of cinnamic aldehyde, respectively.

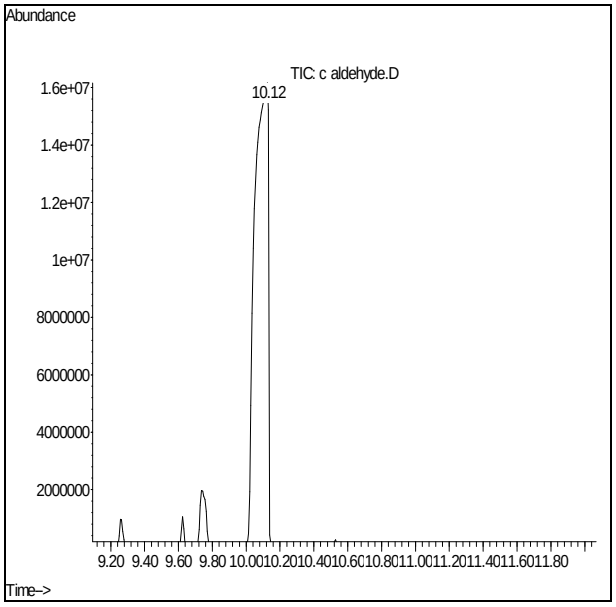


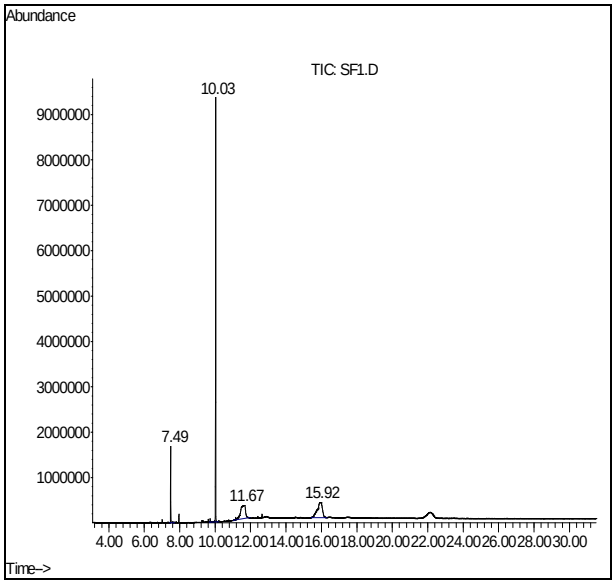


Antihyperglycemic effect of extract, fractions and sub-fractions

ME and chloroform extract treated diabetic rats showed significant decrease of blood glucose level as compared to diabetic control group on day 9 and day 12 as depicted in Figure 2. However, ME showed prominent reduction in blood glucose level. Hence, ME was considered as the most active and further fractionated to obtain various fractions. Antihyperglycemic test of fractions indicated that

CF showed significant reduction in blood glucose level on day 6 compared to diabetic control (Figure 3). The decrease observed began on day 6 of the study reach maximum reduction at the end of the experiment (day 12). CF was subjected to subfractionation to obtain SF-1 and SF-2. The antihyperglycemic activity of SF-1 and SF-2 were examined and compared with ME, CF and pure standard, cinnamic aldehyde (Figure 4). The ME, CF, and SF-1 showed





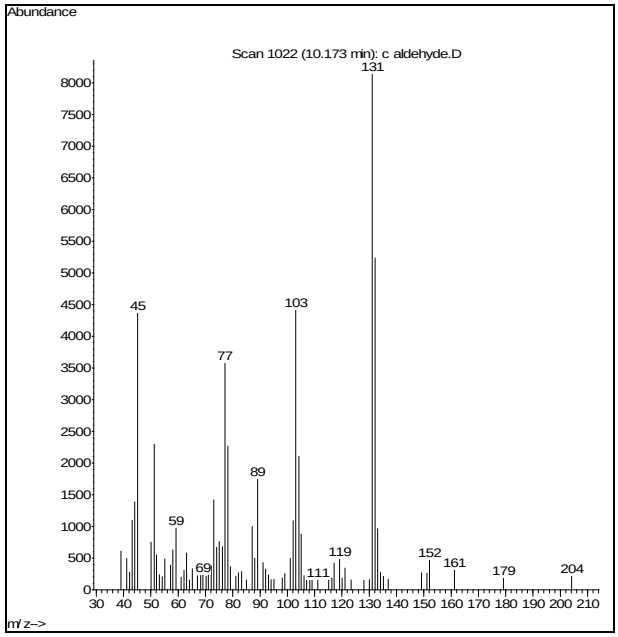


Fig. 1: GC chromatogram of (A) ME, (B) CF, and (C) SF-1 of *C. iners*. (D) GC chromatogram of standard cinnamic aldehyde and (E) is the mass spectrum of pure cinnamic aldehyde and samples.

significant decrease in blood glucose level as compared to diabetic control at different time intervals. ME, CF and SF-1 showed the antidiabetic effect beginning on day 9, 6 and 3 respectively. Our finding indicates that the onset of antidiabetic effect is increasing starting from ME, CF and SF-1. Cinnamic aldehyde showed almost similar antihyperglycemic effect to standard drug, metformin which lowered the blood glucose on day three and lasted to the end of day twelve study period.

Changes in body weight

STZ treatment produced significant loss in body weight as compared to non diabetic animals during the study (Table 1). Diabetic experimental group continued to lose weight till the end of the study while SF-1 and cinnamic aldehyde treated diabetic rats exhibited significant increase ($P < 0.05$) in body weight compared to diabetic control. Treatment with metformin also suppressed the decrease in the body weight as compared to diabetic control.

Antihyperlipidemic effect of methanol extract, chloroform fraction, sub-fraction 1 and cinnamic aldehyde.

When compared to the diabetic control rats, significant ($P < 0.05$) reductions of TC and LDL were observed by CF, SF-1 and cinnamic aldehyde (Figure 5). Similar result was observed in metformin treated group. All tested samples showed reductions in TG level as compared to diabetic control. Also, there was a significant ($P < 0.05$) increase of HDL cholesterol in ME, CF, SF-1 and cinnamic aldehyde treated diabetic rats.

Table 1: The effect of oral administration of active extract (ME; 1 g/kg b.w.), active fraction (CF; 500 mg/kg b.w.), SF-1 (250 mg/kg b.w.) of *C.iners* leaves and cinnamic aldehyde on body weight of STZ-induced diabetic rats for 12 days.

	Body weight (g)			
Groups	**Day -3**	**Day 0**	**Day 7**	**Day 12**
Normal control	224.6 ± 5.4	226.1 ± 4.6***	240.1 ± 3.8***	256.1 ± 4.6***
Diabetic control	231.5 ± 8.7	216.5 ± 16.5	196.5 ± 10.5	180.3 ± 14.6
ME	232.4 ± 8.8	219.4 ± 14.8	198.8 ± 11.4	195.2 ± 15.4
CF	225.6 ± 9.1	210.9 ± 15.1	200.5 ± 12.6	187.0 ± 16.2
SF-1	229.5 ± 8.8	212.7 ± 16.0	202.1 ± 13.2	199.8 ± 13.8*
Cinnamic aldehyde	232.6 ± 9.0	214.8 ± 13.2	207.6 ± 14.1	205.6 ± 12.5*
Metformin	235.9 ± 6.4	215.4 ± 11.8	210.2 ± 15.0	208.1 ± 11.7*

Values are the means ± S.E.M of six animals. * $p<0.05$ compared with diabetic control. ***$p<0.001$ compared with diabetic control.

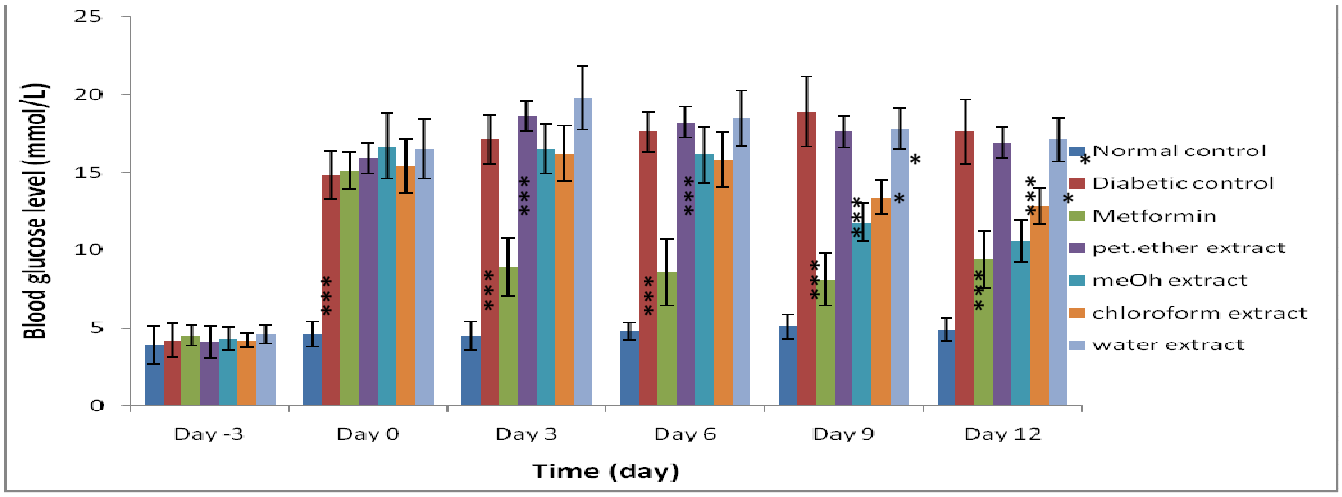


Fig. 2: The effect of oral administration of petroleum ether, methanol, chloroform and water extracts of *C. iners* leaves (1 g/kg b.w.) respectively on STZ-induced diabetic rats for 12 days. Values are the means ± S.E.M of six animals. * p<0.05 compared with diabetic control. ***p<0.001 compared with diabetic control.

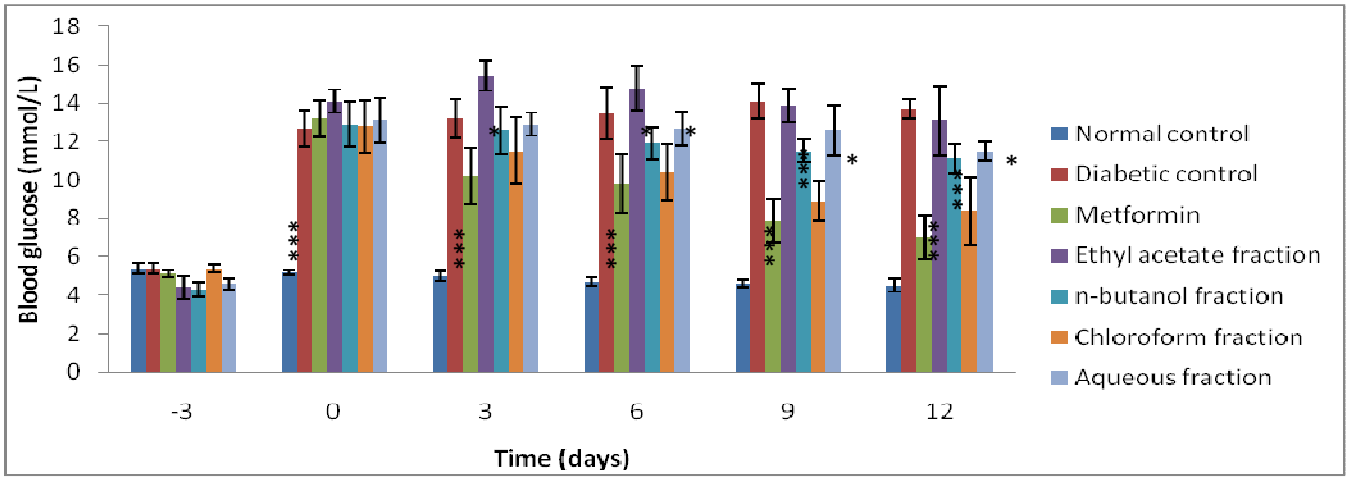


Fig. 3: The effect of oral administration of ethyl acetate, n-butanol, chloroform and aqueous fraction of *C. iners* leaves (500 mg/kg b.w.) respectively on STZ-induced diabetic rats for 12 days. Values are the means ± S.E.M of six animals. * p<0.05 compared with diabetic control. *** p<0.001 compared to diabetic control.

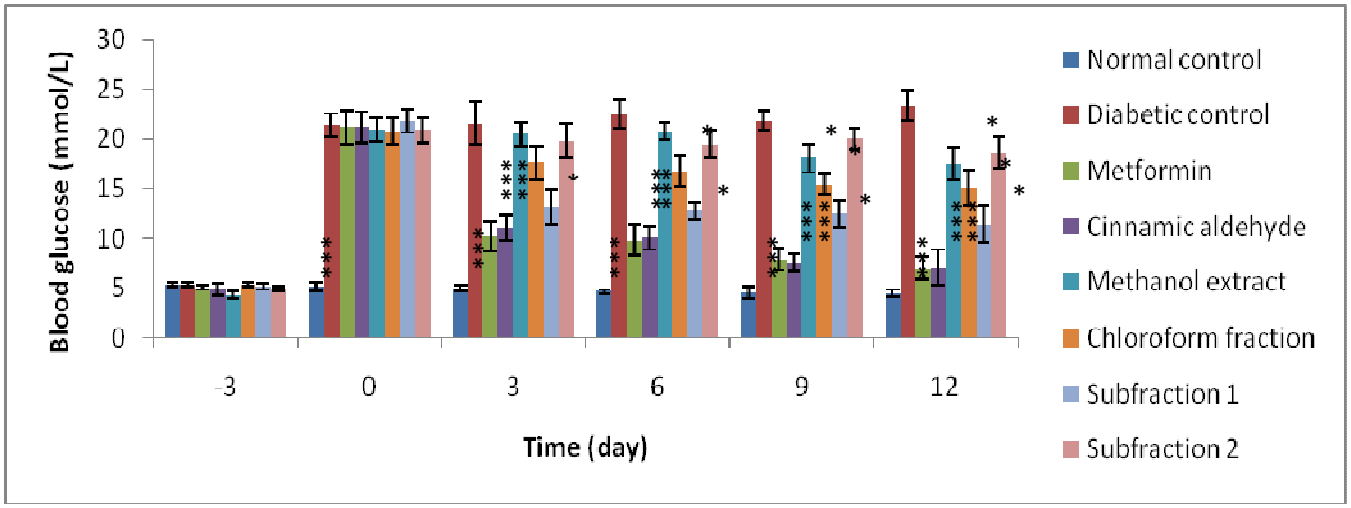


Fig. 4: The effect of oral administration of active extract (ME; 1 g/kg b.w.), active fraction (CF; 500 mg/kg b.w.), subfractions (250 mg/kg b.w.) of *C.iners* leaves and cinnamic aldehyde on STZ-induced diabetic rats for 12 days. Values are the means ± S.E.M of six animals. * p<0.05 compared with diabetic control. ***p<0.001 compared with diabetic control.

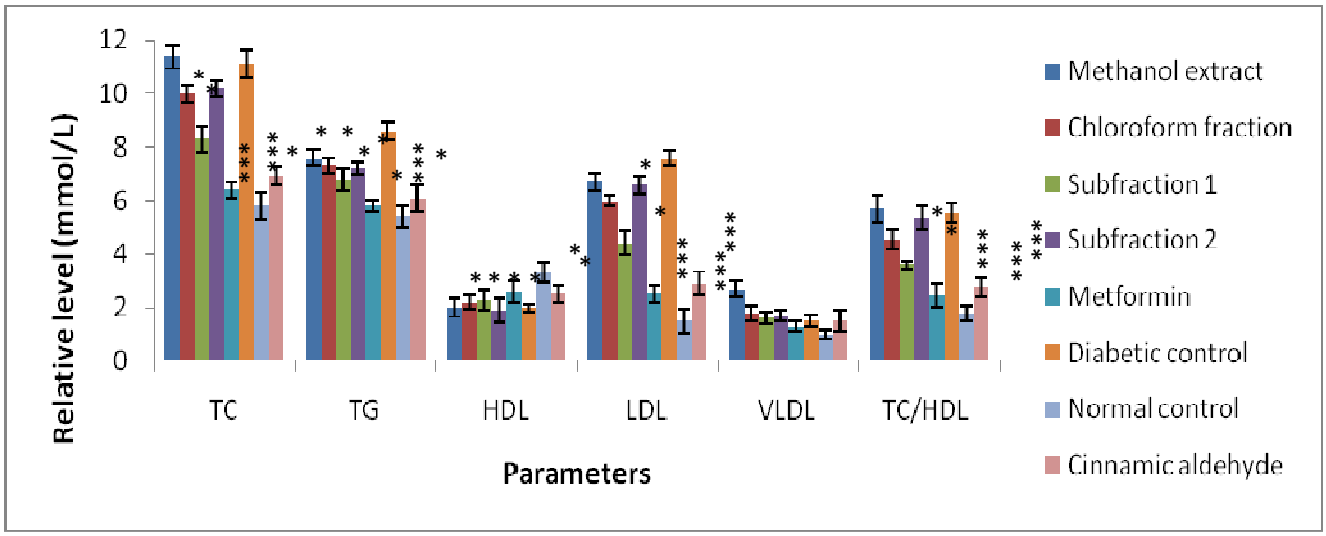


Fig. 5: Effect of ME (1 g/ kg b.w.), CF (500 mg/kg b.w.), subfractions (250 mg/kg b.w.) of *C. iners* leaves extract on serum lipid profile. Values are the means ± S.E.M of six animals.*p<0.05, ***p<0.001 significant to diabetic control.

In the case of untreated diabetic rats, there was a reduction in HDL level compared to normal control rats. None of the samples nor metformin could lower the VLDL level. TC/HDL ratio indicated that CF, SF-1 and cinnamic aldehyde significantly reduce the ratio as compared to diabetic control. Similarly, standard drug, metformin decreased the TC/HDL ratio.

DISCUSSION

Fasting blood glucose in diabetic rats represents an important sign of diabetic status. The present manuscript clearly indicates the antidiabetic effect of various extracts, fractions and subfractions of *C. iners* leaves as it reduces the fasting blood sugar level. ME, CF and SF-1 lowered the blood glucose level in diabetic rats on different days of treatment. The effect of the ME, CF and SF-1 were retained in the body till the last day of the post treatment (day 12), indicating its longer tolerance to bio recycling in the body. The increased antidiabetic potential of CF over ME and SF-1 over CF is due to its partial purification achieved by fractionation. Our GCMS evidence highlighted that this is due to the increase in the quantity of cinnamic aldehyde. Cinnamic aldehyde is a well established compound of Cinnamomum species for antihyperglycemic properties and it has been proven to promote hypolipidemic effect [12-16]. However to the best of our knowledge, this is the first report on the antidiabetic activity on *C. iners* and the identification of its respective antidiabetic compound, cinnamic aldehyde. The antihyperglycemic action of *C. iners* might be similar with cinnamaldehyde which results from the secretion of insulin from existing b-cells of the pancreas. Untreated STZ induced diabetic rats showed decrease in body mass because STZ leads to loss of body weight due to the increased muscle wasting and loss of tissue proteins [17, 18]. Conversely, animals treated with SF-1, cinnamic aldehyde and metformin exhibited significant increase in body mass. Hypercholesterolemia and hypertriglyceridemia are the most important disorder involved in the development of coronary heart disease and atherosclerosis which serve as the secondary complications of diabetes [19]. Under normal condition, triglycerides are being hydrolyzed by lipoprotein lipase which was activated by insulin [20]. Moreover, insulin reduces elevated cholesterol by different mechanisms such as increasing uptake of fatty acids into peripheral tissue and inhibiting lipolysis. In case of insulin resistance circumstances, lipolysis is not inhibited and leads to hyperlipidemia [20]. CF and SF-1 significantly reduced serum TG and TC in STZ-induced diabetic rats. A marked decrease in LDL was also observed in diabetic rats treated with CF and SF-1, while increase in HDL cholesterol has been recorded in SF-1 treated diabetic rats. HDL is beneficial as it prevent hardening of the arteries which causes atherosclerosis by transporting cholesterol from peripheral tissues into the liver. Our finding showed that CF and SF-1 could lower the TC/HDL ratio which is directly proportional in lowering the risk of heart attack. Thus, it is reasonable to conclude that *C. iners* leaves fraction and subfraction could modulate blood lipid abnormalities and could offer protection against hyperlipidemia.

CONCLUSION

Data of this study clearly highlighted that cinnamic aldehyde is the active component of ME, CF and SF-1 that possessed antidiabetic effect. *C. iners* leaves help in preventing diabetic complications and serves as a natural oral agent, with potential antidiabetic and hypolipidemic effects. This study support the traditional usage of this plant leaves for diabetes in folk medicines.

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