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Antihyperlipidemic, Antiatherosclerotic and Cardio-protective Activity of Leaves and Aerial Roots of *Heritiera fomes* (Sundari) Extract on Alloxan-induced Diabetic Sprague Dawley Rats

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Abstract

Background: Hyperlipidemia is one of the leading causes of death in developed as well as in developing countries like Bangladesh. Hyperlipidemia is a major risk factor for early development of atherosclerosis and its cardiovascular complications. The objective of the study was to evaluate antihyperlipidemic, antiathersclerotic and cardio-protective activities of the leaves (LE) and aerial root (AR) extracts of *Heritiera fomes* (Family: Malvaceae, local name: Sundari) which is a common species in the world's largest mangrove forest, Sundarbans, major part of which is located in Bangladesh.

Methods and findings: Extracts of the powdered LE and AR of *Heritiera fomes* were obtained using methanol, and the extracts were applied at doses of 250 and 500 mg/kg of body weight, to treat the diabetic rats. Alloxan Monohydrate was used to induce diabetic condition in Sprague dawley rats. Metformin Hydrochloride was also used as a standard drug to treat the diabetic rats in order to compare the efficacy of the plant extracts with that of the standard drug. After 21 days of treatment, the animals were sacrificed and lipid profiles were estimated. The lipid levels were elevated in alloxan-induced diabetic rats as compared to the control rats. Total cholesterol, triglycerides, LDL and VLDL were increased in diabetic rats and the HDL level was significantly ($P<0.001$) decreased. After treatment with *Heritiera fomes* (LE and AR), the lipid levels of diabetic rats were returned to normal level and this reduction was dose-dependent, whereas HDL level was significantly ($P<0.001$) increased. LE and AR extracts also reduced atherosclerotic index and increased cardio-protective index as compared to the diabetic rats.

Conclusion: Conventional drugs available for the treatment of hyperlipidemia carry the risks that may lead to many adverse effects such as weight loss, hypoglycemia, muscle pain, muscle damage, liver damage etc. So, medicinal plants having antihyperlipidemic, antiatherosclerotic and cardio-protective properties should be given importance to avoid the adverse effects associated with modern allopathic lipid-lowering drugs. This study demonstrated that *Heritiera fomes* can be an excellent medicinal plant for the treatment of hyperlipidaemia, atherosclerosis and cardiovascular complications.

Keywords: *Heritiera fomes*; Hyperlipidaemia; Atherosclerotic; Cardio-protective; Alloxan

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Introduction

Hyperlipidemia, one of the common complications of diabetes mellitus, is associated with an increased level of total cholesterol (TC), triglycerides (TG) and low-density lipoprotein (LDL) as well as a lower level of high-density lipoprotein (HDL). These alterations in lipid profile significantly contribute to the initiation and development of atherosclerosis leading to coronary heart diseases [1,2]. Cardiovascular diseases are the principal causes of death in both developed and developing countries. Difficulties in lipid metabolism are the major risk factor for these diseases [3]. Diets enriched fat, inactive life style, lack of physical activities, and obesity are the risk factors for impaired lipid metabolism [4]. The anomalies in lipid metabolism lead to elevated levels of serum lipids and lipoproteins that play a crucial role in the development of early atherosclerosis affecting diabetic patients. Hypertriglyceridaemia and a low level of high density lipoprotein (HDL) cholesterol are the most common lipid associated problems in diabetic patients [5].

Many effective lipid-lowering synthetic drugs are available in the market, but none of them is effective for all lipoprotein disorders. Moreover, all such drugs are associated with some adverse effects such as heart disease, stroke, weight loss, hypoglycemia, muscle pain, muscle damage, liver damage, and many vascular diseases. Therefore, medicinal plants having antihyperlipidemic property should be prioritized in searching of new compounds, better efficacy and safety profile, to fulfill the need of avoiding the adverse effects of modern allopathic lipid-lowering drugs [6,7].

Medicinal Plants have healing properties because they contain biologically active chemical components such as essential oils, flavonoids, alkaloids, saponins, tannins and other chemical compounds. Many plants and their different parts have long been used as traditional medicines for the treatment of hyperlipidemia in many parts of the world [8]. Hypoglycemic agents from herbal plants could be less toxic and free from the side effects associated with the modern day's hypoglycemic drugs [9]. Herbs are considered to be a major source of the most effective lipid-lowering drugs, and more than two hundred types of herbal drugs have been identified that contain anti-lipid properties [10].

Heritiera fomes belongs (local name: Sundri) to Malvaceae family and grows abundantly in the Sundarbans. This tree can reach up to 25 m in height and its trunk can be about 50 cm in diameter. This mangrove forest is named after the tree Heritiera fomes, and it is grown plentifully in this mangrove forest. H. fomes plays an important role in traditional medicine. In the treatment of diabetes, diarrhea, dysentery, goiter and other diseases, this plant is traditionally used by the people living near the Sundarbans [11]. No report on the antihyperlipidemic, antiatherosclerotic and cardio-protective potential of this plant extract has been published yet. The objective of this study was to assess the antihyperlipidemic, antiatherosclerotic and cardio-protective effects of methanolic extracts of the leaves and aerial roots of Heritiera fomes in alloxan-induced diabetic rats.

Materials and Methods

Plant collection

First of all, the plant was identified and documented properly. Fresh leaves and aerial roots of *Heretiera fomes* (Sundari) were collected from this mangrove forest, Sundarbans (Karamjol area). The collection was performed under a specialist supervision. This plant Heretiera fomes (Bengali name -Sundari) was authenticated by a Botanist.

Preparation of the extract

The fresh leaves and aerial roots were washed carefully with distilled water to remove any extraneous materials. The fresh leaves and aerial roots were air-dried under a shade for 7 days followed by drying in an oven at a temperature of 65°C. The dried leaves and aerial roots were pulverized into coarse powder. About 1 kg of each of the two types of powders was extracted with 2.5 L of methanol by cold maceration for 48 hours using a Soxhlet apparatus [12]. After removal of the solvent from the samples by a rotary evaporator, the extract left behind was stored at 4°C in a refrigerator.

Experimental animals

Laboratory female Sprague Dawley rats (120-180 g) were obtained from the Department of Pharmacy, Jahangirnagar University, Savar, Dhaka. The animals were housed in plastic cages and maintained under the standard laboratory conditions of temperature 22 ± 3°C and 12-hour light/dark cycle. The rats were fed with a standard commercial pellet diet and water throughout the experimental period.

Drugs

Metformin hydrochloride as an active pharmaceutical ingredient (API) was purchased from the Square Pharmaceuticals Limited, Bangladesh.

Induction of diabetes mellitus

Alloxan monohydrate was used to induce diabetes in rats. Diabetes was induced by injecting intraperitoneally at a dose of 150 mg/kg of Alloxan monohydrate. The alloxanized rats were kept for 7 days with free access to food and water. The rats were fasted on the 8th day for 12 hours and their blood glucose levels were determined using On Call EZ II Glucometer (Acon Laboratories, inc. San Dego, USA). Rats with glucose levels above 7 mmol/L or 126 mg/dl were used for this study [13].

Design of experiment

The rats were divided into seven groups comprising of five rats in each group.

Group I: Negative control group rats that were treated with neither alloxan nor the extracts and served as the normal control, and were given only 0.5 ml water daily.

Group II: Positive control group rats that were treated with only alloxan to induce diabetes in the rats that served as Diabetic Control and were given 0.5 ml water only.

Group III: Alloxan-induced diabetic rats treated with 250 mg/kg of Heritiera fomes leaves extracts orally once daily.

Group IV: Alloxan-induced diabetic rats treated with 500 mg/kg of Heritiera fomes leaves extracts orally once daily.

Group V: Alloxan-induced diabetic rats treated with 250 mg/kg of Heritiera fomes aerial root extracts orally once daily.

Group VI: Alloxan-induced diabetic rats treated with 500 mg/kg of body weight of Heritiera fomes aerial root extract orally once daily.

Group VII: Alloxan-induced diabetic rats treated with 100 mg/kg of body weight of Metformin Hydrochloride orally once daily [14].

Preparation of serum samples

After the experimental period of 21 days, the rats of different groups were sacrificed by decapitation under mild anesthesia. Blood was collected from the animals by cardiac puncture using a syringe and the serum was separated by centrifugation. The serum samples were collected in separate containers for biochemical analysis.

Estimation of triglycerides (TG)

Reagents and materials: Pipes buffer pH 6.8 (50 mM), 4-Chlorophenol (4.2 mM) 4-aminoantipyrine (0.35 mM), ATP (2 mM), Magnesium aspartate (40 mM0), Glycerol kinase (≥800 U/L), Glycerol-3-phosphate oxidase (≥2000 U/L), Peroxidase (≥500 U/L), Lipase (≥9000 U/L), Standard2.29 mmol/L (200 mg/dL), Micro-centrifuge tube, Micropipette and pipette, Disposable tips, QCA mini Discrete Random Access Analyzer, Spain.

Procedure: Serum and reagents were taken in specific cup or cell. They were arranged serially. Then ID number for each test was entered in the autoanalyzer. The instrument was calibrated before estimation. The autoanalyzer was programmed for estimation of cholesterol and allowed to run with the following procedure: 10 µL sample and 1000 µL reagent were mixed and incubated at 37°C for 5 minutes within the auto lab. The reaction occurred in the reaction cell or cup. Absorbances of the sample and the standard against the reagent blank were measured at 505 nm within 60 minutes [15].

Calculation: Concentration of triglycerides in the samples was calculated by using the software program (of the autoanalyzer) with the following formula.

Triglycerides concentration (mg/dL)=(A(Sample))/(A (Standard)) × Concentration of standard.

Estimation of total cholesterol (TC)

Reagents and materials: Mes buffer pH 6.5 (75 mM), Phenol (6 mM), 3, 5-dichlorophenol (0.2 mM), 4-aminoantipyrine (0.5 mM), Cholesterol esterase (≥500 kU/L), Cholesterol oxidase (≥300 kU/L), Peroxidase (≥1200 kU/L), Microcentrifuge tube, Micropipette and pipette, Disposable tips, QCA mini Discrete Random Access Analyzer, Spain.

Procedure: Serum and reagents were taken in specific cup or cell. They were arranged serially. Then ID number for each test was entered in the autoanalyzer. The instrument was calibrated before estimation. The autoanalyzer was programmed for the estimation of cholesterol and allowed to run with the following procedure: 10 µL sample and 1000 µL reagent were mixed and incubated at 37°C for 5 minutes within the auto lab. The reaction occurred in reaction cell or cup. Absorbances of the sample and the standard against the reagent blank were measured at 505 nm within 60 minutes [16].

Calculation: Concentration of cholesterol in the sample was calculated by using the software program (of the autoanalyzer) with the following formula.

Cholesterol concentration (mg/dL)=(A(Sample))/(A (Standard)) × Concentration of standard.

Estimation of high density lipoprotein cholesterol (HDL-c)

Reagents (A): 4-amiono antipyrine (0.95 mM), Plyanion/Polymer (0.8 mM).

Reagents (B): Cholesterol esterase (≥550 KU/L), Cholesterol oxidase (≥300 KU/L), POD (≥1500 KU/L), DSBmT(N,N-bis(sulphbutyl-m-toluidine) (1.2 mM).

Procedure: Serum and reagents were taken in specific cup or cell. They were arranged serially. Then ID number for each test was entered in the autoanalyzer. 3 µl sample, 300 µl reagent A were mixed and incubated at 37°C for 5 minutes within the auto lab. The reaction occurred in the reaction cell or cup. The absorbance (E1) of the sample of the sample and the standard against the reagent blank were measured at 600 nm. Then 100 µl reagent B were mixed and incubated at 37°C for 5 minutes within the auto lab. The absorbance (E2) of the sample of the sample and the standard against the reagent blank were measured at 600 nm [17].

Calculation: Concentration of the HDL-c in the sample was calculated by using software program (of the autoanalyzer) with the following formula.

HDL Cholesterol concentration (mg/dl)=(E2-E1 Sample)/(E2-E1 Calibrator) × Concentration of standard.

Estimation of low density lipoprotein cholesterol (LDL-c)

The LDL-c level in serum was calculated by using Friedewald formula [18].

LDL Cholesterol={Total Cholesterol-(HDL Cholesterol+1/5× Trigllyceride)}.

Estimation of atherosclerotic and cardio-protective index

Atherosclerotic index (AI) is a marker of atherosclerosis that has a direct correlation with the risk of cardiovascular diseases. It is calculated by the following formula [19].

AI=(TC-HDL)/HDL

Cardio-protective Index (CI) is a superior measure of the risk of cardiovascular diseases. It is calculated as the ratio of HDL-cholesterol to total cholesterol [20].

Statistical analysis

Data were expressed as mean ± standard error of means (SEM). Statistical comparison was performed by one-way ANOVA (SPSS Version 14) followed by LSD post hoc test and the values were considered as statistically significant when p values were <0.05.

Results

The effects of *Heritiera fomes* leaves extracts (both 250 mg and 500 mg) on lipid profile

The results from **Table 1** indicated that there were significant (P<0.001) rises in total cholesterol, triglycerides, LDL and VLDL levels, and a significant (P<0.001) decrease in HDL level in the diabetic control group as compared to the normal control group.

In addition, oral administration of *Heritiera fomes* leaves extracts (both 250 mg and 500 mg) reduced cholesterol, triglycerides, LDL and VLDL levels and increased HDL level as compared to the diabetic control group on the 21st day **(Figure 1)**. And, treatment with 100 mg/kg of Metformin Hydrochloride also led to a significant (p<0.001) improvement in the lipid profile, but this improvement was not better than that shown by the leaves extracts **(Figure 1)**.

The effects of *Heritiera fomes* aerial root extracts (both 250 mg and 500 mg) on lipid profile

Similar with the response shown by the leaves extracts, oral administration of *Heritiera fomes* aerial root extracts (both 250 mg and 500 mg) produced a reduction in total cholesterol, triglycerides, LDL and VLDL levels along with an elevation in HDL level as compared to the diabetic control group on the 21st day **(Table 1 and Figure 2)**. Similarly, treatment with 100 mg/kg of Metformin Hydrochloride also led to a significant (p<0.001) improvement in the lipid profile, but this improvement was not better than that shown by the aerial root extracts **(Figure 2)**.

Table 1: Effects of *Heritiera fomes* Leaves and Aerial Root Extracts (both 250 mg and 500 mg) on Total Cholesterol, Triglycerides, HDL, LDL and VLDL Levels.

Group	Total Cholesterol (mg/dL)	Triglycerides (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)
1.D+LE 250 mg	43.5 ± 1.61*	43 ± 5.94*	29.65 ± 2.62*	5.25 ± 2.58*	8.6 ± 0.53*
2.D+LE 500 mg	42.83 ± 2.03*	42.5 ± 6.65*	30.75 ± 2.27*	3.58 ± 3.19*	8.5 ± 0.59*
3.D+AR 250 mg	40.17 ± 2.11*	38 ± 3.11*	29.85 ± 2.66*	2.71 ± 1.48*	7.6 ± 0.28*
4.D+AR 500 mg	39.67 ± 2.69*	37.83 ± 3.29*	30 ± 1.29*	2.1 ± 1.16*	7.57 ± 0.29*
5.D+MET100	45.17 ± 1.34	70.5 ± 1.71	22.67 ± 1.25	8.4 ± 2.25	14.1 ± 0.15
6. DC	52.67 ± 2.14	90.83 ± 2.11	19.17 ± 3.66	15.33 ± 4.57	18.17 ± 0.19
7. NC	49 ± 4.62	75.5 ± 11.74	27.17 ± 4.99	6.77 ± 2.67	15.1 ± 1.05

Values represented as mean ± SEM, (n=6); *P<0.001, significant compared to the diabetic control group. NC: Normal Control, DC: Diabetic Control, D: Diabetic, LE: Leaves Extract, AR: Aerial Root Extract, MET100: Metformin Hydrochloride 100 mg.

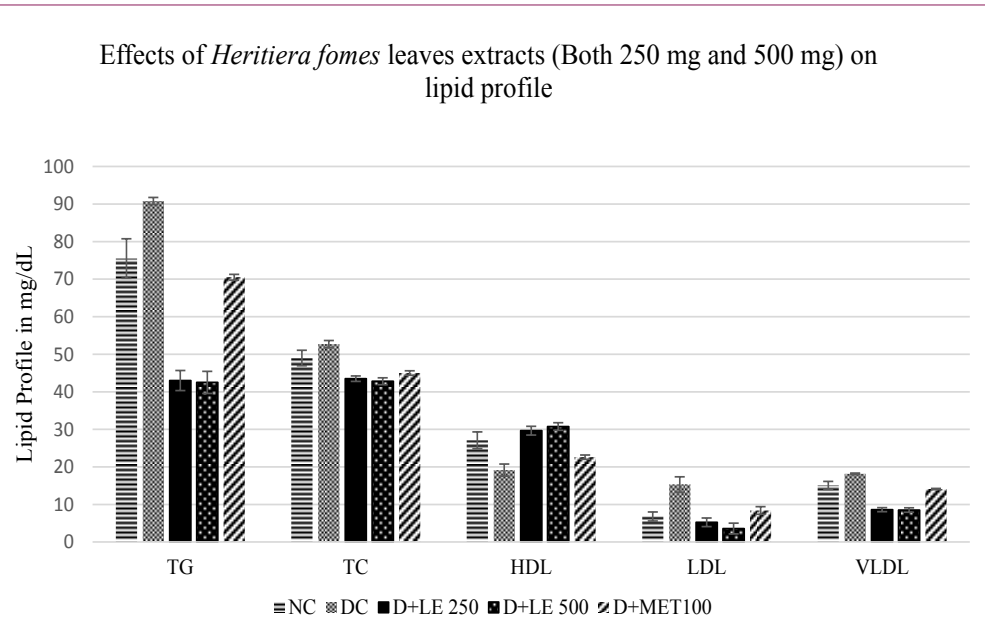

Figure 1 Effects of *Heritiera fomes* Leaves Extracts (Both 250 mg and 500 mg) on lipid profile (values represented as mean ± SEM, (n=6); *P<0.001, significant compared to the diabetic control group. TG: Triglycerides, TC: Total Cholesterol, NC: Normal Control, DC: Diabetic Control, D: Diabetic, LE: Leaves Extract, MET100: Metformin Hydrochloride 100 mg).

Effects of *Heritiera fomes* leaves and aerial root extracts on atherosclerotic and cardio-protective index

There were significant (p<0.001) rises in atherosclerotic and decrease cardio-protective index on diabetic group as compared to normal control **(Table 2)**. However, administration of *Heritiera fomes* leaves and aerial root extracts at the doses of 250 mg/kg, 500 mg/kg reduced atherosclerotic index and increased cardio-protective index as compared to diabetic Rats **(Figure 3)**. And treatment with metformin hydrochloride also led to a significant (p<0.001) reduction of atherosclerotic index and rise cardio protective index but not better than LE and AR extracts after 21st days **(Figure 3)**.

Discussion

The use of medicinal plants to find out healing power is an ancient idea. Medicinal plants and harbs play an important role in the health care system [21]. Treatments using plants are familiar as they have minimal or no side effects [22]. H. fomes has significant antioxidant, antinociceptive, antihyperglycemic, antimicrobial, and anticancer activities. It is also beneficial in cardiovascular diseases [23]. The present study is a preliminary assessment of the antihyperlipidemic, antiatherosclerotic and cardio-protective activity of methanolic extract of of leaves (LE) and aerial roots (AR) of *H. fomes*. The administration of alloxan in animals causes diabetes and also induces hyperlipidemia [24]. Dyslipidemia which is common in type-2 diabetes mellitus is represented by hypercholesterolemia, hypertriglyceridemia, and low HDL cholesterol [25]. The rise of serum lipids characterize a risk factor for coronary heart disease [26]. In this study, there were significant (P<0.001) increase in the TC, TG and the LDL levels on diabetic rats. Increased synthesis of triglyceride rich lipoprotein particles (VLDL) in liver and reduced catabolism are responsible for the raised TG level in diabetic rat. Lack of insulin is related with excess lipolysis and augmented influx of free fatty acids to the liver [27]. Due to the free fatty acid influx, there is also excess production of LDL and VLDL by the stimulation of hepatic TG synthesis [28]. In this study, the methanolic extracts of leaves (LE) and aerial roots (AR) of *H. fomes* showed a significant dose-dependent reduction in the total cholesterol, triglycerides, LDL and VLDL levels besides increasing the HDL level **(Table 1)**. Moreover, this reduction of the total cholesterol, triglycerides, LDL, VLDL and increase of HDL levels by the LE and AR were higher than that obtained with the standard drug metformin hydrochloride at the dose of 100 mg/kg. The results indicated that the methanolic extracts of the LE and AR were more potent than metformin hydrochloride in providing a beneficial effect on lipid profile of the diabetic rats **(Table 1)**. The elevation of cholesterol level in human serum is one of the major factors in the progression of atherosclerosis [24]. There were an elevation of atherosclerotic index and reduction of cardio-protective index in diabetic rats. LE and AR extracts were also found to reduce

Table 2: Effects of *Heritiera fomes* Leaves and Aerial Root Extracts (Both 250 mg and 500 mg) on Atherosclerotic and Cardio-protective Index.

Group		Atherosclerotic Index (AI)	Cardio-Protective Index (CI)
1.	NC	0.85 ± 0.11	0.55 ± 0.04
2.	DC	1.85 ± 0.24	0.37 ± 0.03
3.	D+MET 100	1.00 ± 0.07	0.50 ± 0.02
4.	D+LE 250 mg	0.48 ± 0.06*	0.68 ± 0.03*
5.	D+LE 500 mg	0.40 ± 0.06*	0.72 ± .03*
6.	D+AR 250 mg	0.35 ± 0.03*	0.74 ± 0.02*
7.	D+AR 500 mg	0.32 ± 0.02*	0.76 ± 0.01*

Values represented as mean ± SEM, (n=6); *P<0.001, significant compared to the diabetic control group. NC: Normal Control, DC: Diabetic Control, D= Diabetic, LE: Leaves Extract, AR: Aerial Root Extract, MET100: Metformin Hydrochloride 100 mg.

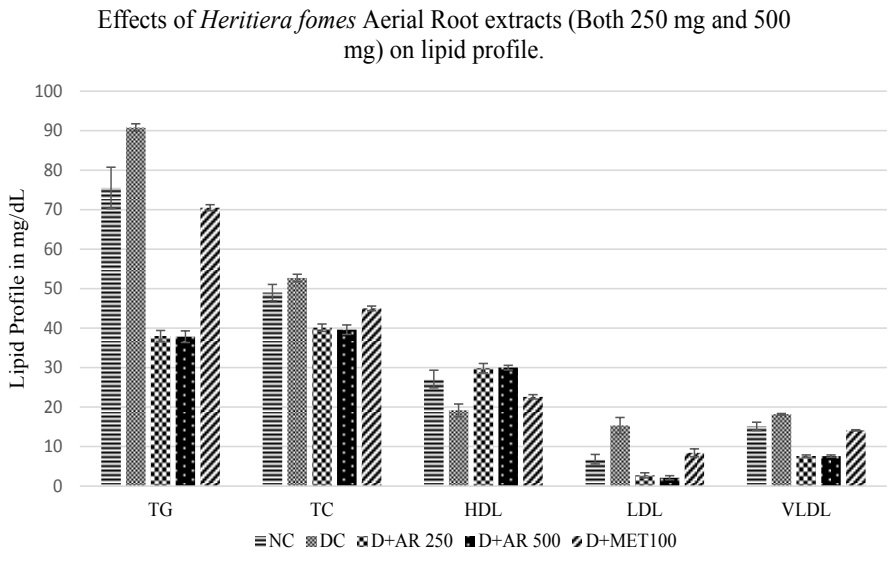

Figure 2 Effects of *Heritiera fomes* Aerial Root Extracts (both 250 mg and 500 mg) on lipid profile (values represented as mean ± SEM, (n=6); *P<0.001, significant compared to the diabetic control group. TG: Triglycerides, TC: Total Cholesterol, NC: Normal Control, DC: Diabetic Control, D: Diabetic, AR: Aerial Root Extract, MET100: Metformin Hydrochloride 100 mg).

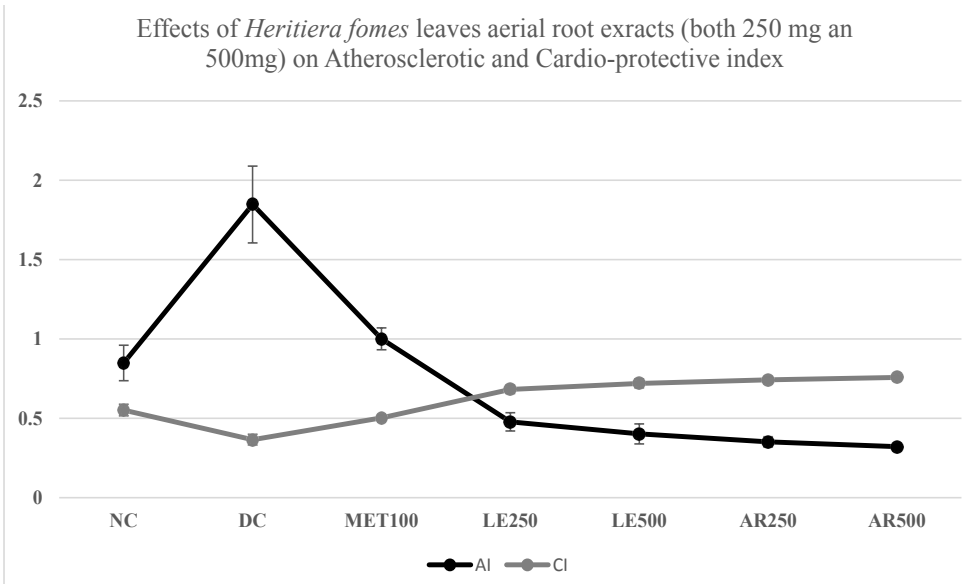

Figure 3 Effects of *Heritiera fomes* Leaves and Aerial Root Extracts (both 250 mg and 500 mg) on Atherosclerotic Index and Cardio-protective Index (values represented as mean ± SEM, (n=6); *P<0.001, significant compared to the diabetic control group. AI: Atherosclerotic Index, CI: Cardio-protective Index, NC: Normal Control, DC: Diabetic Control, LE: Leaves Extract, AR: Aerial Root Extract, MET100: Metformin Hydrochloride 100 mg).

atherosclerotic index and increase cardio-protective index, as compared to the diabetic rats **(Table 2)**. The AR also showed a better impact on lipid profile, atherosclerotic and cardio-protective activity than that provided by the LE **(Tables 1 and 2)**.

Conclusion

It can be concluded from the data that leaves (LE) and aerial roots (AR) extracts of *H. fomes* possesses potent antihyperlipidemic, antiatherosclerotic and cardio-protective activity. Bangladesh has already a large number of hyperlipidemic patients, but our healthcare system lacks an effective model for managing the disease including identification and treatment. Most of the people are not aware of this disease. Moreover, a number of effective allopathic medications that are available in Bangladesh for the treatment of hyperlipidemia, can cause serious side effects after chronic use. Thus, *Heritiera fomes* plant can be an excellent alternative for the treatment hyperlipidemia, atherosclerosis and can be used as a cardio-protective means and needs further investigations.

Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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