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Original Article

ANTIDIABETIC AND ANTIDYSLIPIDEMIC ACTIVITY OF ETHYL ACETATE FRACTIONS OF *XYLOCARPUS GRANATUM* AND *XYLOCARPUS MOLLUCCENSIS* ON HIGH FRUCTOSE HIGH FAT AND HIGH SUCROSE HIGH FAT FED-LOW DOSED STREPTOZOTOCIN TREATED DIABETIC RATS

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ABSTRACT

Objectives: The present study was carried out to investigate the antidiabetic and antidyslipidemic effect of standardized fractions of *X. granatum* (CDR-134 F194) and *X. molluccensis* (CDR-267 F018) by measuring the status of blood glucose, serum insulin, lipid levels, hepatic and renal function markers of high fructose high fed streptozotocin treated rats and high sucrose high fat diet fed-low dosed Streptozotocin treated diabetic rats.

Methods: Male rats of Sprague Dawley strain of body weight around 150 g when kept on high fructose high fat diet and high sucrose high fat diet for two weeks, respectively, showed abnormal glucose tolerance, dyslipidemia and obesity and at this stage when streptozotocin was given intraperitoneally at 45.0 mg/kg body weight caused persistent hyperglycemia in them addition to dyslipidemia along with impairment in their hepatic and renal functions.

Results: The standardized fractions of *X. granatum* (CDR-134 F194) and *X. molluccensis* (CDR-267F018) when given to these high fructose high fat fed low dosed streptozotocin treated diabetic rats or high sucrose high fat diet fed-low dosed streptozotocin treated diabetic rats for 10 consecutive days showed significant improvement in their glucose intolerance, decline in their serum triglycerides and LDL-cholesterol levels. These CDR-134 F194 and CDR-267 F018 treated rats also showed elevation in their HDL-cholesterol levels and improvement in their hepatic and renal functions as evidenced by decline in SGOT, SGPT, urea, uric acid and creatinine levels.

Conclusion: The present study thus concludes that the antidiabetic efficacy of standardized fractions of *X. granatum* (CDR-134 F194) and *X. molluccensis* (CDR-267F018) have favorable effect in bringing down the severity of hyperglycemia, hyperlipidemia, decline the increased level of renal and hepatic function markers and also improving glucose tolerance activity.

Keywords: HFD fed streptozotocin treated diabetic rats, HSD fed streptozotocin treated diabetic rats, Type 2 diabetes mellitus, Antihyperglycaemic activity, Dyslipidaemic activity, Liver function test, Renal function test.

INTRODUCTION

Diabetes mellitus is a serious chronic metabolic disorder that has a significant impact on the health, quality of life, and life expectancy of patients, as well as on the health care system. The World Health Organization (WHO), has projected that the global prevalence of type 2 DM will more than double from 135 million in 1995 to 300 million by the year 2025 [1]. With the present population of 19.4 million diabetics and a projected increase of 300% and thereby leading to approximately 60 million by the year 2025, India would rank first in sharing global burden of diabetes [2].

Traditional medicines most often applies to plants are being employed as adjuvants in the management of diabetes mellitus in many of the Asian countries including India. India has a rich history of using various potent herbs and herbal components for treating diabetes. Medicinal plant or herb has a variety of metabolites, aliphatic and aromatic compounds have the basic skeleton of organic molecules which have various functional groups that makes their ability to alter the various metabolic pathways makes them medicinally important. Herbal drugs are prescribed widely because of their effectiveness, less side effects and relatively low cost [3]. Therefore, investigation on such agents from traditional medicinal plants may become extremely useful [4].

Two species of *Xylocarpus* i. e. Granatum and moluccensis (*X. granatum*, *X molluccensis*) are found in abundance in the coastal region of India, Bangladesh, Burma, Ceylon and Malaya. Each part of the plant is used as astringent, but the bark and root are more widely used. The bark is used in dysentery, diarrhoea, and other abdominal troubles and febrifuge. It has earlier been reported that *X. moluccensis* has been used in the treatment of cholera and fever whereas the fruits are aphrodisiac. The kernels are used in tonics and in relieving colic. The seeds or peels of the fruits are utilized to poultice swellings and ash of the seeds is applied to itch. The fruits are used as a cure for swellings of the breast and in elephantiasis. The bark pressings are used to treat fevers including those caused by malaria. A recent review reveals the various biological activity, chemical constituents and other properties in the *Xylocarpus* genus [5]. The aqueous extracts of different parts of this plant X*. granatum* have been reported to have significant antifilarial activity [6], whereas DPPH radical scavenging activity has been reported in the methanolic extract [7].

Xylocarpus species contain a special class of bitter substances termed as 'limonoids'. These limonoids are mostly hydroxylated and occur either as esters or as free hydroxyl derivatives. The limonoids are usually defined as a triterpene derivative in which the side chain has become a furan ring by the loss of four carbons, hence alternatively called as tetranortriterpenoids. The most characteristic of the genus Xylocarpus are xyloccensins, a class of limonoids. Perhaps even most limonoids are active as insect antifeedants, but most of these are not directly insecticidal. Some limonoids have also been found to be active against some types of cancer. A number of limonoids mainly of phragmalin-type and a few sterols have been reported from *X. granatum* [8-17], lignins, tannins alkaloids [18], and sterols [19, 20] were reported from *Xylocarpus granatum*. Isolation and identification of two limonoids, gedunin (1) and 1α-hydroxy-1,2-dihydrogedunin (2) has been recently reported. Gedunin, a Limonoid from *Xylocarpus granatum*, inhibits the growth of CaCo-2 colon cancer cell line *in vitro* [21].

The present paper reports anti hyperglycaemic as well as antidyslipidemic activity in ethyl acetate fraction of the epicarp portion of the fruits of *X. granatum* (CDR-134 F194) and *X. molleccensis* (CDR-267 F018) on high fructose high fat fed and high sucrose high fat fed low dosed streptozotocin treated diabetic rats.

MATERIALS AND METHODS

Animals

Eight to ten weeks old male albino rats of Sprague Dawley strain and Syrian golden hamsters were procured from the animal colony of Central Drug Research Institute, Lucknow, India. Research on these animals was conducted in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) formed by the Government of India in 1964. Rats were always placed in groups of three to five in polypropylene cages. The following norms were always followed for animal room environment: temperature 23 ± 2°C; humidity 50-60%; light 300 Lux at floor level with regular 12 h light cycle; noise level 50 decibel; ventilation 10-15 air changes per hour. The animals were fed *ad libitum* standard pellet diet and had free access to water.

Chemicals

Streptozotocin, metformin, and Fenofibrate were obtained from Sigma Chemical Company, St. Louis, USA. Glucose, fructose, sucrose and cholesterol were obtained from Sisco Research Laboratory (India). Biochemical kits were obtained from the Roche diagnostic kits. The fraction CDR-134 F194, CDR-267 F018 used in the present study was an ethyl acetate fraction of 95% and 50% ethanolic extract respectively, obtained from the plant source and was processed in the Chemical Technology Division of this Institute. All other chemicals were of the highest purity grade.

Collection of Plant material and preparation of ethyl acetate fractions

The whole fruits of *X. granatum* and X. *mollucensis* were collected during January to March from coastal region of India, and authenticated in the Botany Division of the Institute. The collection details and representative voucher specimen of the plants have been documented in the herbarium of the Botany Division, Central Drug Research Institute, Lucknow for future reference. 1.0 kg of the fruits of each i. e. *X. granatum* and X. *mollucensis* were shade dried, powered in disintegratar.

The powder of the *X. granatum* was submerged in 50% ethyl alcohol whereas in case of *X. mollucensis* was submerged in 95% ethyl alcohol and this process was repeated several times. The combined extracts of respective plants were filtered and concentrated in a rotavapour under reduced pressure below 50^{0}C. Finally the extracts were dried under high vacuum to remove the last traces of solvents present in them. The ethanol extracts of each plant were macerated with hexane separately at room temperature and hexane insoluble fraction was further macerated with ethyl acetate. The ethyl acetate soluble fractions of each plant were concentrated in a rotavapour as above. The ethyl acetate fractions thus obtained were coded as CDR-134F194 for *X. granatum* and CDR-267-F018 for *X. molluccensis*, respectively. The HPLC profiles of CDR-134 F194 and CDR-267 F018 are shown in fig. 1 and fig. 2.

Experimental regimen

Effect of CDR-134F194 and CDR-267F018 on Normal, High fructose high fat diet fed/high sucrose high fat diet fed streptozotocin treated diabetic rats

The rats were divided into three major groups. Rats of one group were kept on normal diet (ND), one group was kept on HFHFD and one group was kept on HSHFD for two weeks. Composition of the Normal, HFHF and HSHF diets are shown in table 5. The animals showing high serum triglycerides and cholesterol levels in the later two groups and all the animals in normal group were administered streptozotocin intraperitoneally at a dose of 45 mg/kg.

The animals of the later two groups had free access to HFHFD and HSHFD and water for a further period of two weeks whereas the first group was always on normal diet. The animals of the HSHF and HFHF diet groups showing impaired oral glucose tolerance test in each diet group were separated and sub grouped into five. Each sub group consists of six animals. Rats of experimental groups were treated with CDR-134 F194 and CDR-267 F018 at 100 mg/kg respectively for 10 days. The standard drug treated groups were given metformin and Fenofibrate, respectively at 100 mg/kg doses. The rats of control group received 1.0 % gum acacia. The oral glucose tolerance of each group was followed for two hours post glucose load on day 0 and day 10th respectively. At the end of experiment the animals were bled; serum was separated and analyzed for insulin, triglycerides, cholesterol, HDL and LDL-cholesterol, GOT, GPT, urea, uric acid, creatinine levels by diagnostic kits from Roche on fully automated clinical analyzer (Cobas Integra 400) according to instructions provided by the manufacturer.

Serum insulin measurement

Serum insulin was estimated using the radioimmunoassay kit for rat serum insulin provided by Linco Research Inc, USA. The assay method is based upon the competition of unlabelled insulin in the standard or samples and radio-iodinated (I-[12]^5) insulin for the limited binding sites on a specific antibody.

Statistical analysis

Quantitative glucose tolerance of each animal was calculated by area under curve (AUC) method using Prism Software. The area under curve of the control group and experimental group was compared to determine the percentage antihyperglycaemic activity. Each serum parameters was expressed as mean ± SEM**.** Statistical comparison between groups was made by student's t test followed by Dunnett's test.

Table 1: Blood glucose lowering and serum lipid profile of high fructose high fat fed streptozotocin induced diabetic rats

Groups	Fructosamine (µmol/l)	Biochemical profiles (blood+/serum++)					
		OGTT (AUC)	Insulin (µU/ml)	TG (mg/dl)	Cholesterol (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)
Sham treated Control	326.4 ±19.2	56430±3093	24.5±2.6	391.0±52.5	275.2±28.7	67.2±9.50	89.2±12.4
CDR-134F194 (100 mg/kg)	256.3±12.8 (-21.5)*	38440±1825 (-31.9)**	23.1±1.3 (-5.70)	254.4±24.6 (-35.0)**	212.2±16.4 (-22.9)	84.5±7.60 (+25.7)*	69.2±7.3 (-22.4)*
CDR-267F018 (100 mg/kg)	241.1±11.4 (-26.1)*	41220±1527 (-26.9)*	23.5±1.5 (-4.10)	241.2±23.4 (-38.4)**	226.4±17.2 (-17.8)	87.6±7.08 (+30.4)*	64.0±5.40 (-28.3)*
Metformin (100 mg/kg)	275.2.0±18.4 (-15.9)	39750±1630 (-29.6)**	22.2±1.4 (-9.40)	359.1±37.4 (-8.20)	260.4± 22.6 (-5.45)	75.8±12.1 (+12.8)	82.2±11.3 (-7.95)
Fenofibrate (100mg/kg)	289.5±22.5 (-11.3)	51990±1980 (-7.86)	25.2±3.2 (+2.85)	261.2±22.6 (33.3)**	238.6±20.2 (-13.5)	89.5±8.20 (+33.2)*	62.1±5.50 (-30.4)**

Values are mean± S. E of six rats.

Statistical significance *p<.05, **p<.01, ***p<.001

Table 2: Liver and renal function markers of high fructose high fat diet fed streptozotocin treated diabetic rats

Groups	Body weight (g)	Liver function marker (Serum)				Renal function marker (Serum)		
		T-Bil (mg/dl)	ALT (U/L)	AST (U/L)	ALP (U/L)	Uric acid (mg/dl)	Creatinine (mg/dl)	Urea (mg/dl)
Control	193±8.80	1.72±0.18	252.5±25.6	241.3±14.6	277.5±37.4	3.65±0.64	3.36±0.18	46.2±4.05
CDR-134F194 (100 mg/kg)	173±7.5 (-10.4)	1.35±0.10 (-21.5)*	245.0±28.5 (-2.97)	225.1±15.5 (-6.75)	318.9±35.8 (+14.9)	3.10±0.22 (-15.1)*	2.60±0.14 (-22.6)*	32.2±2.20 (-30.3)*
CDR-267F018 (100 mg/kg)	171±7.2 (-11.4)	1.21±0.08 (-29.6)**	188.6±10.9 (-25.5)*	201.0±11.3 (-16.7)*	251.4±26.8 (-9.40)	2.86±0.18 (-21.6)*	2.41±0.12 (-28.3)*	28.7±1.85 (-37.9)*
Metformin (100 mg/kg)	185±9.2 (-4.14)	1.45±0.10 (-15.7)*	206.0±14.5 (-18.4)*	204.3±12.8 (-15.5)	18.2±20.2 (-21.4)*	2.99±0.28 (-10.9)	2.78±0.20 (-17.3)	34.5±2.60 (-25.3)*
Fenofibrate (100mg/kg)	165.5±6.6 (-14.5)	1.50±0.10 (-12.7)	209.8±12.3 (-16.9)*	210.0±10.2 (-12.9)	232.0±22.2 (-16.4)	3.03±0.26 (-16.9)	2.89±0.30 (-13.9)	38.7±5.70 (-16.2)

Values are mean± S. E. of six rats

Statistical significance **P*<.05, ***P*<.01, ****P*<.001

Table 3: Blood glucose and serum lipid profile of high sucrose high fat fed streptozotocin treated diabetic rats

Groups	Fructosamine (µmol/l)	Biochemical profile (Blood/Serum)					
		OGTT (AUC)	Insulin (µU/ml)	TG (mg/dl)	Cholesterol (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)
Control	342.5 ±24.4	51090±3308	21.9±2.3	488.4±82.6	275.4±29.4	106.4±9.60	123.5±12.5
CDR-134F194 (100 mg/kg)	268.2±14.6 (-21.7)*	39340±2238 (-22.9)*	17.6±1.3 (-19.6)*	232.1±44.5 (-52.5)**	232.4±20.2 (-15.6)	134.2±8.60 (+26.1)*	90.6±7.40 (-26.6)*
CDR-267F018 (100 mg/kg)	259.2±12.4 (-24.3)*	36210±1420 (-29.1)**	18.6±1.5 (-15.1)*	212.8±39.4 (-56.4)**	210.6±15.2 (-23.5)*	141.2±8.8 (+32.7)*	93.3±8.20 (-24.5)*
Metformin (100 mg/kg)	301.0±23.4 (-12.1)	37220±1329 (-27.2)**	20.1±1.4 (-8.20)	399.5±37.4 (18.2)	270.2±29.7 (-1.89)	109.8±10.5 (+3.20)	115.7±13.5 (-6.31)
Fenofibrate (100mg/kg)	188.5±6.6 (-15.1)	47830±1841 (-6.38)	21.4±3.2 (-2.30)	269.7±30.2 (44.8)**	253.4±22.4 (-7.99)	125.4±7.40 (+17.8)*	98.4±6.60 (-20.3)*

Values are mean± S. E. of six rats.

Statistical significance **p*<.05, ***p*<.01, ****p*<.001

Table 4: Liver function and renal function markers of high sucrose high fat diet fed streptozotocin treated diabetic rats

Groups	Body weight (g)	Liver function marker (Serum)				Renal function marker (Serum)		
		T-Bil (mg/dl)	ALT (U/L)	AST (U/L)	ALP (U/L)	Uric acid (mg/dl)	Creatinine (mg/dl)	Urea (mg/dl)
Control	222.0±10.4	1.68±0.10	212.2±21.6	236.0±16.5	287.0±32.4	2.99±0.56	4.77±0.36	33.4±3.70
CDR-134F194 (100 mg/kg)	184.0±6.80 (-17.1)*	1.40±0.10 (-16.7)*	168.1±10.8 (-20.8)	216.5±12.5 (-8.30)	312.2±35.4 (+8.78)	2.30±0.22 (-23.1)*	3.60±0.18 (-24.5)*	24.8±2.20 (-25.7)*
CDR-267F018 (100 mg/kg)	177±7.60 (-20.3)*	1.35±0.07 (-19.6)*	142.5±10.2 (-32.8)*	189.8±10.3 (-19.6)*	240.4±24.8 (-16.2)*	2.38±0.24 (-20.4)*	3.40±0.16 (-28.7)*	22.1±1.72 (-33.8)**
Metformin (100 mg/kg)	192.4±9.2 (-13.3)	1.47±0.12 (-12.5)*	180.0±18.4 (-15.2)*	194.2±9.80 (-17.7)*	232.3±21.2 (-19.1)*	2.89±0.28 (-3.34)	3.80±0.26 (-20.3)	25.0±2.40 (-25.1)*
Fenofibrate (100mg/kg)	188.5±6.6 (-15.1)	1.58±0.15 (-5.95)	190.5±20.3 (-10.2)*	207.5±13.2 (-12.1)	254.1±22.2 (-11.4)	2.73±0.26 (-8.69)	4.20±0.38 (-11.9)	28.4±4.15 (-14.9)

Values are mean± S. E. of six rats.

Statistical significance **p*<.05, ***p*<.01, ****p*<.001

Table 5: Composition of High Fructose/High Sucrose High Fat diet (HFHFD and HSHFD)

Constituents	Amount in g/kg diet
Fructose/Sucrose*	500
Casein*	190
Dalda† Vanaspati Ghee	110
Gram flour	150
Cholesterol*	5
Methionine#	3
Vitamin mix$	2
Mineral mixture@	40

*Purchased from SRL (Mumbai, India).

Purchased from Hi Media (Mumbai, India).

† Commercial preparation composed of different vegetable oils

$Vitamin mix (mg/kg of dry diet): retinol, 1.8; cholecalciferol, 0.019; thiamine, 6; riboflavin, 4.5; pantothenic acid, 21; pyridoxine, 3; inositol, 45; cyanocobalamin, 0.015; ascorbic acid, 240; DL-tocopherol, 51; menadione, 12; nicotinic acid, 30; paraminobenzoic acid, 15; folic acid, 1.5; biotin, 0.09.

@Mineral mixture (g/kg diet) CaHPO4: 430 g; KCL: 100 g; NaCl: 100 g; MgO: 10.5 g; MgSO4: 50 g;

Fe2O3: 3 g; FeSO4. 7 H2O: 5 g; trace elements (Mn, Cu, Co, Zn, I): 10 g; quantity sufficient to 1000 g.

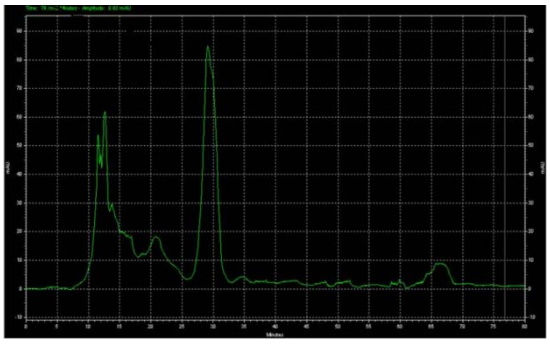

Fig. 1: HPLC profile of CDR-134 F194 showing gedunin peak

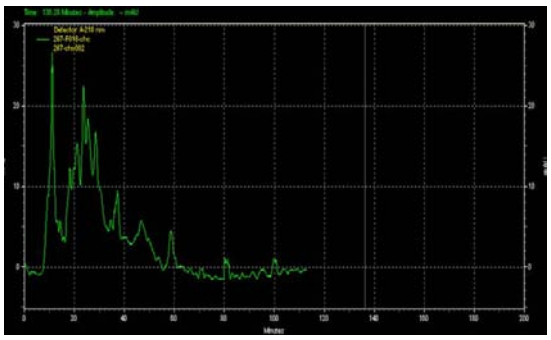

Fig. 2: HPLC profile of CDR-267 F018

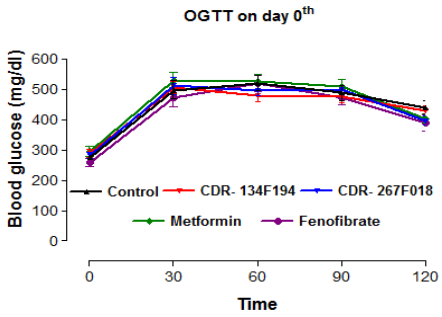

Fig. 3a: Oral glucose tolerance of HFD fed streptozotocin treated diabetic rats on day 0 and 10 post treatment

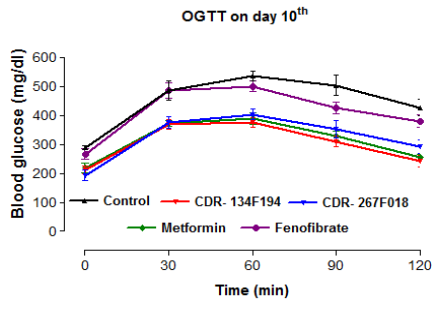

Fig. 3b: Oral glucose tolerance of HFD fed streptozotocin-induced diabetic rats on day 0 and 10 post treatment

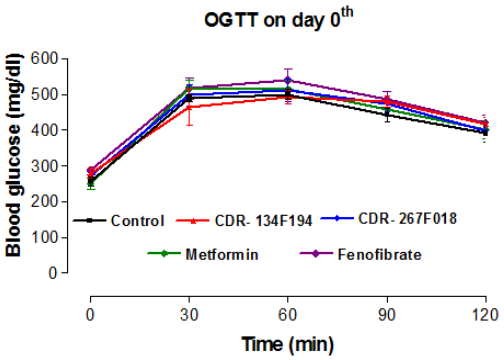

Fig. 4a: Oral glucose tolerance of HSD fed streptozotocin-induced diabetic rats on day 0 and 10 post treatment

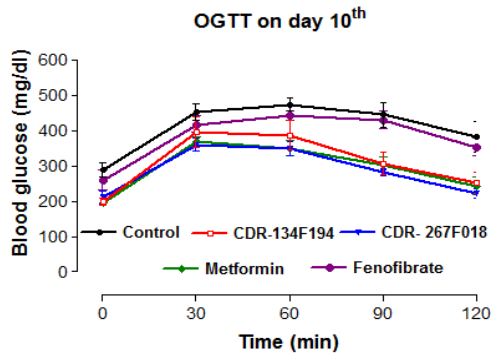

Fig. 4b: Oral glucose tolerance of HSD fed streptozotocin-induced diabetic rats on day 0 and 10 post treatment

RESULTS

Antihyperglycaemic activity of CDR-134F194 and CDR-267F018 on high fructose high fat diet fed streptozotocin treated diabetic rats

Effect on fasting blood glucose and oral glucose tolerance (OGTT)

Fig. 3 a and b depict the effect of CDR-134 F194 and CDR-267 F018 on OGTT post glucose load on day 0 and 10 post treatment in HFD fed streptozotocin treated diabetic rats. It was evident from results that both CDR-134 F194 and CDR-267 F018 lowered the fasting blood glucose level and improved oral glucose tolerance after 10 days of treatment. The respective lowering on blood glucose profile by CDR-134 F194 and CDR-267 F018 was calculated to be around 26.5 and 32.8 %, whereas the respective improvement on OGTT was calculated to be around 31.9 % ($p<.01$) and 26.9 % ($p<.05$) respectively by CDR-134 F194 and CDR-267 F018 at 100 mg/kg dose. The metformin and fenofibrate treated groups showed around 24.4 ($p<.05$) and 7.35 % lowering on fasting blood glucose profile and around 29.6 ($p<0.01$) and 7.86 % improvement on OGTT respectively (Table 1).

Effect on serum insulin level, fructosamine and lipid profile

Table 1 also showed the serum insulin, fructosamine and lipid profile of sham treated control, CDR-134 F194, CDR-267 F018, metformin and fenofibrate treated groups. The CDR-134F194 and CDR-267-F018 were found to mild decrease the serum insulin level in streptozotocin induced rats and was calculated around 5.70 % and 4.10 % respectively. Metformin also decrease the serum insulin level and was found nearly 9.40 %. The standard lipid lowering drug fenofibrate treatment resulted in the mild increase in the serum insulin level in HFD fed streptozotocin treated diabetic rats. It was also evident from table 1, the fractions CDR-134F194 and CDR-267F018 resulted around 21.5 % ($p<.05$) and 26.1 % ($p<.05$) reduction in the serum fructosamine value.

Table 1 also represents the effect of CDR-134F194 and CDR-267F018 on the serum lipid level. 35.0 % (p<.01) and 38.4 % (p<.01) reduction in serum triglycerides concentration, 22.9 %, 17.8% reduction in serum cholesterol level and 22.4 % (p<.05) and 28.3 % (p<.05) reduction in serum LDL-C concentration was seen respectively after 10 day post treatment. Treatment of fractions also resulted in the increase in serum HDL-C content and was calculated around 25.7 % (p<.05) and 30.4 % (p<.05) respectively. Fenofibrate treatment in these rats resulted 33.3 % (p<.01) reduction in serum triglyceride, 30.4 % (p<.01) reduction in serum LDL-C, 13.5 % reduction in serum cholesterol level and 33.2 % (p<.05) increase in HDL- C level. Metformin treatment results in the 8.20 % lowering in triglyceride, 7.95 % lowering in LDL- C, 5.45 % lowering in cholesterol and 12.8 % increase in HDL- C level.

Effect on liver and kidney function tests

Table 2 showed the effect of CDR-134 F194 and CDR-267 F018 on the liver and kidney function tests after 10 days post treatment. The fractions CDR-134F194 and CDR-267F018 resulted around 21.5 (p<.05) and 29.6 % (p<.01) lowering in the serum bilirubin as compare to sham treated control. 2.97 and 25.5 % (p<.05) lowering in the serum ALT and 6.75, 16.7 % lowering in the serum AST. 14.9 % increase in the serum ALP level was seen in CDR-134F194 treatment group and 9.40 % (p<.05) reduction in the serum ALP level was observed in the CDR-267F018 treatment group. Metformin and fenofibrate resulted in the 15.7 % (p<.05), 12.7 % reduction in bilirubin, 18.4 % (p<.05), 16.9 % (p<.05) lowering in ALT activity, 15.5 %, 12.9 % lowering in AST activity and 21.4 % (p<.05), 16.4 % reduction in the serum ALP activity respectively.

The fractions CDR-134F194 and CDR-267F018 also resulted around 15.1 % (p<.05), 21.6 % (p<.05) reduction in serum uric acid level, 22.6 % (p<.05), 28.3 % (p<.05) lowering in the serum creatinine level and 30.3 % (p<.05), 37.9 % (p<.05) lowering in serum urea was observed respectively. Metformin and fenofibrate were found to lower the 10.9 %, 16.9 % serum uric acid, 17.3 %, 13.9 % creatinine and 25.3 % (p<.05) and 16.2% lowering in the serum urease activity.

Effect on body weight

It was evident from table 2 that 10.4 % and 11.4 % decrease in body weight was obseved in the CDR-134F194 and CDR-267F018 treated group respectively. 4.14 % and 14.5 % decrease in body weight was shown in metformin and fenofibrate treated group respectively.

Antihyperglycaemic activity of CDR-134F194 and CDR-267F018 in High sucrose high fat diet fed streptozotocin treated diabetic rats

Effect on fasting blood glucose and oral glucose tolerance (OGTT)

Fig. 4 a and b depict the effect of CDR-134 F194 and CDR-267 F018 on OGTT post glucose load on day 0 and 10 post treatment in HSD fed streptozotocin treated diabetic rats. Both the fractions CDR-134 F194 and CDR-267 F018 lowered the fasting blood glucose level and improved oral glucose tolerance after 10 days of treatment. The respective lowering on blood glucose profile by CDR-134 F194 and CDR-267 F018 was calculated to be around 31.3 and 26.4%, whereas the respective improvement on OGTT was calculated to be around 22.9 % (p<.05) and 29.1 % (p<.01), respectively, by CDR-134 F194 and CDR-267 F018 at 100 mg/kg dose. The metformin and fenofibrate treated groups showed 32.1 (p<.05) and 9.45 % lowering on fasting blood glucose profile and 27.2 (p<0.01) and 6.38 % improvement on OGTT, respectively (Table 3).

Effect on serum insulin level, fructosamine and lipid profile

Table 3 showed the serum insulin, fructosamine and lipid profile of sham treated control, CDR-134 F194, CDR-267 F018 metformin and fenofibrate treated groups. The CDR-134F194 and CDR-267-F018 treatment significantly decreased the serum insulin level in streptozotocin induced rats and was calculated around 19.6 % (p<.05) and 15.1 % (p<.05) respectively. Metformin and fenofibrate also resulted in decrease serum insulin level and was found nearly 8.20 and 2.30 % respectively. It was also evident from table 3, the fractions CDR-134 F194 and CDR-267 F018 resulted around 21.7 % (p<.05) and 24.3 % (p<.05) decline in the serum fructosamine value.

Table 3 also represents the effect of CDR-134F194 and CDR-267F018 on the serum lipid level. 52.5 % (p<.01) and 56.4 % (p<.01) reduction in serum triglycerides concentration, 15.6 %, 23.5 % (p<.05) reduction in serum cholesterol level and 26.6 % (p<.05) and 24.5 % (p<.05) reduction in serum LDL-C concentration was seen respectively. The treatment of these fractions also resulted in increase the serum HDL-C content and was calculated around 26.1 % (p<.05) and 32.7 % (p<.05) respectively. Fenofibrate treatment in these rats resulted 44.8 % (p<.01) reduction in serum triglyceride, 20.3 % (p<.05) reduction in serum LDL-C, 7.99 % reduction in serum cholesterol level and 17.8 % (p<.05) increase in HDL-C level. Metformin treatment results in the 18.2 % lowering in triglyceride, 6.31 % lowering in LDL- C, 1.89 % lowering in cholesterol and 3.20 % increase in HDL- C level.

Effect on liver and kidney function tests

Table 4 showed the effect of CDR-134 F194 and CDR-267 F018 on the liver and kidney function tests after 10 days post treatment in HSD fed streptozotocin treated diabetic rats. It was evident from table 4, the fractions CDR-134 F194 and CDR-267 F018 resulted around 16.7 % (p<.05) and 19.6 % (p<.05) lowering in the serum bilirubin as compare to sham treated control. 20.8 %, 32.8 % (p<.05) lowering in the serum ALT and 8.30 %, 19.6 % (p<.05) lowering in the serum AST. 8.78 % increase in the serum ALP level was seen in CDR-134 F194 treatment group and 16.2 % (p<.05) reduction in the serum ALP level was observed in the CDR-267 F018 treatment group. Metformin and fenofibrate resulted in the 12.5 % (p<.05), 5.95 % reduction in bilirubin, 15.2 % (p<.05), 10.2 % (p<.05) lowering in ALT activity, 17.7 % (p<.05), 12.1 % lowering in AST activity and 19.1 % (p<.05), 11.4 % reduction in the serum ALP activity respectively.

The fractions CDR-134F194 and CDR-267F018 also resulted around 23.1 % (p<.05), 20.4 % (p<.05) reduction in serum uric acid level, 24.5 % (p<.05), 28.7 % (p<.05) lowering in the serum creatinine level and 25.7 % (p<.05), 33.8 % (p<.05) lowering in serum urea was observed respectively. Metformin and fenofibrate were found to lower the 3.34 %, 8.69 % serum uric acid, 20.3 %, 11.9 % creatinine and 25.1 % (p<.05) and 14.9 % lowering in the serum urease activity.

Effect on body weight

It is evident from table 4 that around 17.1 % (p<.05) and 20.3 % (p<.05) decrease in body weight were observed in the CDR-134 F194 and CDR-267F018 treated groups, respectively, compared to 13.3 % and 15.1 % decrease in body weight in the case of metformin and fenofibrate treated groups, respectively.

DISCUSSION

The present study clearly indicates antidiabetic, antidyslipidemic and insulin resistance reversal activity in the fruits of *Xylocarpus granatum* (CDR-134F194) and *Xylocarpus molluccensis* (CDR-267F018) on high carbohydrate high fat diet fed streptozotocin treated (HCHF-STZ) diabetic rats. It is well reported that high carbohydrate high fat diet (HCHF) is primarily responsible for development of dyslipidemia, obesity, insulin resistance and usually hypertension finally in the development of cardiovascular diseases in rodent animal models [22, 23, 24]. In the present study post feeding of HCHF diet for two weeks and mild dose of streptozotocin in these rats results in development of persistent hyperglycemia and other secondary diabetic complication i. e. elevated level of liver and kidney function parameters which is in accordance with the published reports [25, 26, 27, 28, 29, 30, 31]. Therefore, this model is a validated model for screening of wide variety of plant fractions and synthetic compounds for their antidiabetic, antidyslipidemic, insulin resistance reversal activity and also for the secondary complications like liver and kidney damage in type 2 diabetes mellitus.

Both the plant fractions (CDR-134F194, CDR-267F018) significantly lowers the area under curve value of glucose after 10 days

continuous feeding of fractions in the normal diet fed streptozotocin treated diabetic rats (ND-STZ), high fructose high fat fed streptozotocin diabetic rats (HFD-STZ), (fructose 60% fat 13%) and high sucrose high fat fed streptozotocin treated diabetic rats (HSD-STZ) ((sucrose 60%, fat 13%). These fractions also lowers the fasting blood glucose and significantly improve the glucose tolerance which means the fractions are antihyperglycaemic in ND-STZ diabetic rats, HFD-STZ diabetic rats and in HSD-STZ diabetic rats. The degree of antihyperglycaemic activity of CDR-134F194 was more than that of CDR-267F018 in these models i. e. ND-STZ rat model and in HFD-STZ rat model. The probable mechanism of antihyperglycaemic activity may be either insulin mimetic, insulin secretagogue or insulin resistance reversal activity. Since the ND-STZ rats were insulin deficient (streptozotocin causes damage of pancreatic β-cell resulting insulin deficiency), the treatment of both the fractions resulted in significant increase in insulin level indicate their insulin secretagogue activity. The standard drug metformin is an insulin sensitizer, also showed an increase in the insulin level because it normalizes the low level of insulin to the normal level. Therefore the fractions are thought as insulin mimetetic, insulin secretaceous and insulin sensitizers.

Both CDR-134 F194 and CDR-267 F018 fractions showed promising triglyceride and LDL-C lowering activity in ND-STZ, HFD-STZ and HSD-STZ rats. CDR-267F018 was found have more triglyceride lowering activity as compared to CDR-134F194 in HFD-STZ and HSD-STZ rat model. CDR-134F194 and CDR-267F018 also lowers the elevated level of cholesterol in HCHF-STZ and both fractions were found to have more triglyceride and cholesterol lowering activity as compare to fenofibrate treatment at the same oral dose. It was found that treatment with these fractions in ND-STZ diabetic rats resulted in the increase in the serum cholesterol level which is probably due to activity of normalizing the cholesterol level as the streptozotocin induced rats are very week and having low level of cholesterol and triglyceride as compared to lipid level of normal rats. The interesting feature is that continuous feeding of CDR-134 F194 and CDR-267 F018 results in the significant elevation of the cardio-protective HDL-C level in ND-STZ, HFD- STZ and HSD-STZ rats which is a favorable effect. The lipid lowering activity of these fractions was of as like the fenofibrate showed. Both the fractions were observed to have potent antidyslipidemic activity than that of the commercially available lipid lowering drug fenofibrate.

The high carbohydrate high fat diet fed streptozotocin rats showed liver and renal damage. The treatment of both the fractions showed improvement in the liver and renal functions. CDR-267F018 showed enormous ability to improve the liver and renal functions as evidenced by decline in the levels of SGOT, SGPT, urea, uric acid and creatinine levels in the serum thus improving the liver and kidney functions. Metformin also showed ability to improve liver functions in the present animal model. Metformin is well known in the treatment of non alcoholic steatohepatitis [32, 33] and renal function parameters. Fenofibrate treated group showed mild improvement in the liver and renal functions indicating that it is a weak agent for improving liver and kidney functions. CDR-134 F194, CDR-267 F018 and metformin also decrease the levels of serum fructosamine i. e. improving the diabetic injury and the degree of improvement in diabetic injury by fractions is greater than that to metformin.

It may be considered from the results of the present study that CDR-134F194 and CDR-267F018 have strong potential to lower down the fasting blood glucose level, improve glucose tolerance, have antidyslipidemic, hepatic and nephro protective activities on high carbohydrate high fat diet fed streptozotocin treated diabetic rats, it offer a exciting possibility to identify the active ingredients in the CDR-134 F194 and CDR-267 F018 fractions which can be utilized in the design and synthesis of plant based antidiabetic drugs in the management of type 2 diabetes mellitus.

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CONFLICT OF INTERESTS

Declared None

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