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In vivo Anti-hyperlipidemic Potential of Chloroform Extract of *Fagonia indica* in Streptozotocin-induced Rats

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AuR designed and supervised the study. He revised critically and finalized the manuscript for submission. SuH interpreted the study and conducted the experiments with AuR, IR statistically analys the data. BM and QK wrote the first version of the manuscript. Also helped in the GC-MS analysis of the sample. IuH conducted an animal study with AuR. All the authors read and approved the final manuscript.

Key words
Anti-hyperglycemic, Antihyperlipidemic, *F. indica*, Streptozotocin (STZ), Gas chromatography-mass spectroscopy (GC-MS), Alkaline phosphatase-ALP

ABSTRACT

The plant *Fagonia indica* is used in the treatment of various diseases. The purpose of this study was to elucidate the *in vivo* anti-hyperglycemic and antihyperlipidemic activity of chloroform extract of this plant in streptozotocin (STZ) induced diabetic rats. This extract was also investigated for acute toxicity and phytochemical analysis using gas chromatography-mass spectroscopy (GC-MS). Wistar albino rats were given an injection of STZ to induce diabetes. The diabetic rats received chloroform extract of *F. indica* (250 mg/kg b.w and 500 mg/kg b.w.) by oral route for three weeks after three days of inducing diabetes using *in vivo* STZ-induced diabetic rat model. Blood glucose levels (BGLs) were checked regularly. The investigation of chloroform extract on BGLs, biochemicals, and lipid profile was assessed along with its phytochemical evaluation by GC-MS. The diabetic rats given chloroform extract of *F. indica* (250mg/kg and 500 mg/kg) lowered fasting BGLs (223.4 ± 1.59 and 178.2 ± 2.96 mg/dL) significantly (P<0.05) after the 21st day of treatment. This extract also reduced lipid profile and biochemical parameters significantly (P<0.05) in diabetes-induced rats. GC-MS analysis identified 10 compounds in this extract belonging to fatty acids and fatty esters. *F. indica* is effective in managing diabetes and hyperlipidemia due to high BGLs. These activities are because of the presence of chemical compounds in chloroform extract.

INTRODUCTION

Diabetes is a broad category of endocrine and metabolic disorders marked by hyperglycemia brought on by a lack or a reduction in the efficacy of insulin, its secretion, or both. The occurrence of diabetes is much augmenting to date. According to WHO, diabetes mellitus will be one of the top ten major causes of all death all over the world by the year 2030 (Lee and Rosen, 2016). It is because it engages in the advancement of other complications of health like stroke, heart attack, and kidney failure (Davies *et al*., 2018).

Hyperlipidemia (dyslipidemia), a pathological condition of a major socioeconomic problem is considered by an increase in lipoproteins and cholesterol levels in the blood plasma. Globally it is generally characterized by increased levels of lipids (total cholesterol-TC, triglycerides-TG, low-density lipoprotein cholesterol-LDL-C, and a decreased level of high-density lipoprotein cholesterol-HDL-C. It is considered to be a significant preventable risk factor for cardiovascular disease (CVD) (Li *et al*., 2022). It leads to cardiovascular problems such as angina pectoris, myocardial infarction, hypertension, atherosclerosis, and congestive heart failure (Ghori *et al*., 2015). In hyperlipidemia, high intensities of LDL are deposited in the sub-endothelial region of arteries leading to plaque formation and inflammation. This finally results

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in a diminished functional capacity of metabolic organs (kidney and liver), hypertension, and also diabetes (Jain and Saraf, 2010).

Diabetes and hyperlipidemia are correlated. Persons suffering from diabetes type 2 are at much risk of developing CVD and atherosclerosis. The rate of mortality due to heart problems is two to four times more in patients suffering from diabetes in comparison to non-diabetic patients (Khan *et al*., 2018). A variety of antidiabetic and antihyperlipidemic drugs are available. These drugs are often encountered with different side effects including nausea, lactic acidosis, peripheral edema, weight gain, and hypoglycemia (Blanco-Montenegro *et al*., 2007). The researchers are trying to find the herbal products used to normalize the level of blood glucose and dyslipidemia in diabetes. Medicinal plants are thought to be effective against health issues with few side effects, affordable costs, and widespread availability. Plant products have acquired prominence as the source of bioactive substances including hypoglycemic, antioxidant, and hypolipidemic may be crucial in the search for novel therapeutic agents (Zhang and Reddy, 2018). Many phytochemicals derived from plants are now employed as essential medications and can prevent diseases all over the world (Magaji *et al*., 2020). Plant extracts are employed as a potential pathway for the creation of new bioactive chemicals. Phytomedicines are more accessible, less expensive, safer, and occasionally more effective compared to synthetic substances.

An important medicinal plant is known as *Fagonia indica* Burm. f. is found throughout Indo-Pakistani subcontinent (Farheen *et al*., 2015). This plant is associated with numerous ethnomedicinal practices and is used to cure a variety of ailments, including urinary problems, fever, asthma, skin conditions, thirst, typhoid, toothaches, and stomach problems. It has a long history of usage in managing diabetes mellitus (Miranda *et al*., 2022). The significance of this plant in traditional therapy has led to extensive pharmacological and chemical studies on it. This plant has been reported to have several biological activities. The cytotoxic potential of the plant chemical compounds indicacin and fagonicin against the human cancer cell line H-29 was evaluated. At a dosage of 6.25 mM/mL, indicacin exhibited 51.41% and 39.3% cytotoxicity, respectively (Farheen *et al*., 2015). The hepatoprotective effect of methanol extract of this plant in carbon tetrachloride was evaluated and it had a noticeable effect on the liver (Bagban *et al*., 2012). *F. indica* was tested for antidiabetic activity and it showed a hypoglycemic effect (Rehman *et al*., 2019). Using an electron spin resonance technique antioxidant activity of this plant was checked and it showed this activity effectively (Eman, 2011).

The techniques of gas chromatography-mass spectrometry (GC-MS) and nuclear magnetic resonance (NMR) spectroscopy are common analytical instrumental techniques used to identify the biologically active compounds (Satapute *et al*., 2019). These techniques offer the higher level of sensitivity, separation, and repeatability required for the analysis of a complex bioactive mixture (Murugesu *et al*., 2018). The chemical compounds in the chloroform extract of the plant responsible for hypoglycemic and hypolipidemic activity must be identified and characterized by the analytical technique. There are no reports of the presence of chemical compounds in the chloroform extract of this plant having hypoglycemic and hypolipidemic activities. The antihyperlipidemic potential of *F. indica* in streptozotocin-induced diabetes rats is not yet reported in the literature. No previous study is available on this important plant regarding hypoglycemic and hypolipidemic activity in diabetic-induced rats. Therefore, this study was aimed at the evaluation of the antihyperglycemic and antihyperlipidemic activity in chloroform extract of *F. indica* (var. *indica*) whole plant in STZ-induced diabetic rats. A phytochemical investigation of the same extract was done for the presence of chemical compounds by GC-MS analysis. There is a need for the development of new drug-lead compounds used in the treatment of diabetes and hyperlipidemia. As there is no information about the toxicity of this extract in the literature, it was also needed to check its acute toxicity in albino rats.

MATERIALS AND METHODS

Study area

The study area was carried out at the Pharmacology and Pharmaceutical Chemistry Laboratory, University College of Pharmacy, University of the Punjab, Allama Iqbal Campus, 54000, Lahore, Pakistan. The study was also carried out in the animal house of Faculty of Pharmacy, The University of Lahore, Lahore, Pakistan. The study was carried out in 2018-2019.

Plant material

The plant (1 kg) was collected from the district of Khanewal, Pakistan in the month of July. Identification and authentication of the plant were done by the Botanist of GC University, Lahore and the voucher specimen (GC-Herb-Bot. 2967) was kept at the Herbarium of the same University.

Drying, pulverization and extraction

After being cleaned, the plant was dried in the shade and powdered using a grinder. The powder material was placed in an airtight container for *in vivo* and GC-MS

analytical study (Namani *et al*., 2016).

The dried plant powder (1 kg) of *F. indica* was extracted by maceration process using n-hexane in a glass jar for seven days with continuous shaking at room temperature. Then the residue was re-extracted with chloroform using the same technique. The chloroform extract was then filtered using Whatman filter paper and was concentrated using a rotary vacuum evaporator (Heidolph Laboratory 4002, Germany) at 40°C temperature and low pressure. It was stored at 4°C in sample bottles for further studies.

Chemicals and solvents

Analytical-grade chemicals and reagents were used during this study. Streptozotocin (STZ) was taken from the Sigma-Aldrich Company (St. Luis, Mo, USA). Ethanol, paraffin, water, eosin, and hematoxylin stain were purchased from Bayer AG (Wuppertal, Germany). Triglyceride reagent, COBAS (C 111) cholesterol enzyme, alanine transaminase reagent, aspartate aminotransferase, and high-density lipoproteins (HDL) kits of Roche Diagnostics, USA were used for the analysis of biochemicals.

Experimental animals

Twenty adult Wistar albino rats weighing 180-220 ± 10 g were bought from Bahauddin Zakariya University, Multan. The animals were placed in a ventilated animal transit room under controlled environmental conditions (23 ± 2°C) with a relative humidity of 50 ± 5% on 12 h light and 12 h dark for seven days. The rats were given water and a standard pellet diet *ad libitum* and given an acclimatization period for laboratory environmental conditions before being allowed to participate in the experiment.

Acute oral toxicity study

Mature healthy female rats (non-pregnant) weighing between 180 and 220 g were used to analyze the chloroform extract of the plant by the most recent Organization for Economic Co-operation and Development (OECD) guidelines No. 425 (Saleem *et al*., 2017). Two groups of rats were randomly divided. Each group contained 5 animals. All the rats were placed for seven days to acclimatize to the standard lab conditions for 12 h light/12 h dark in stainless steel cages. The rats were given a breeder feed received from Hi-tech Feeds (Pvt.) Ltd., Lahore with No. Feed APL-413, dated 13/8/18, and also given running tap water. One rat from each group was selected and chloroform extract ranging from 50, 100, 250, and 1000 mg/kg/ b.w was given by oral route. In the first four hours, the rats were watched for half an hour at random, and then every four hours on the first day. Next, the identical dose was administered to the final four animals in each group. For two weeks, the rats were watched daily, and any indicators of toxicity were looked for and recorded. Body weight, mortality, behavioral changes, neurological changes, and symptoms of toxicity were continuously observed for 24h (Parasuraman *et al*., 2015). Changes in the nasal mucous membrane, skin, respiratory system, eyes, fur, and central nervous system were among the other daily findings. The body weight (b.w.) was measured at the start and after each dose administration for seven days. A record of any fatality was visible. Every performance of the animal was observed.

In vivo antihyperglycemic and Antihyperlipidemic potentials of *Fagonia indica* in streptozocin-induced diabetic rats

Chloroform extract was investigated for the potential of these effects in STZ induced diabetic rats using *in vivo* rat model (Gobinath *et al*., 2022).

Induction of diabetes

Intraperitoneal (i.p) injections of STZ (55 mg/kg/ b.w) combined with citrate buffer (0.05 M) were given after a one-week acclimatization period to induce diabetic mellitus (Lin *et al*., 2019). To prevent hypoglycemia mortality, the rats were administered oral glucose solution 5% w/v (2 mL/kg/b.w.) after twenty-four hours to induce diabetes mellitus. Three days following the injection of STZ, diabetes was checked by measuring BGLs using ACCU-CHEK® (Active glucometer, Roche Diagnostics, Germany). The rats with BGLs greater than 250 mg/dL were considered to have diabetes.

Experimental design

Twenty adult Wistar albino rats (20) were distributed into four groups.

Group I: Normal control (untreated) rats received starch only.

Group II: Diabetic control (with STZ to induced diabetes)

Group III: Chloroform extract (250 mg/kg b.w) given by oral route to diabetic-induced rats for three weeks

Group IV: Chloroform extract (500 mg/kg b.w) given by oral route to diabetic-induced rats for three weeks

Group I and Group II consisted of normal and diabetic control rats who were given 10% Tween 20.

After the 21st day of treatment and 12h of fasting 3mL blood samples were collected from the experimental rats by puncturing them into activator tubes (gel clot). Blood glucose levels (BGLs) were checked using a glucometer. Separation of serum was done by centrifuging (3000 rpm) for five minutes and stored at -40 ºC for biochemical analysis following the method (Ahmad *et al*., 2017) after minor modification.

Analysis of biochemicals

Serum samples were analyzed for estimating biochemical such as TC, TG, HDLP, ALP, AST, ALT, and TP using respective assay kits of Chemistry Analyser Cobas C111 (Roche Diagnostic). The levels of LDL-C and VLDL-C were determined using the formula (Friedewald *et al*., 1972).

LDL- C = TC - (HDLC - VLDL - C)

VLDL-C = Triglycerides/5

Atherogenic index (AI) = TC-HDL-C/HDL-C (Munshi *et al*., 2014).

Histopathological analysis

Finally, after one month, the rats were sacrificed and the liver was taken out for histopathological study. The liver was washed with normal saline, fixed in formalin 10%, prepared for embedding in paraffin. 5µm thick sections were cut and then stained with hematoxylin and eosin. The sections were examined under a light microscope (Olympus Corp., Tokyo, Japan).

GC-MS analysis of chloroform extract of F. indica

GC-MS analysis of chloroform extract was performed with the help of GC-MS (5975C Agilent system). A mass spectrometer of the MS model was connected to the GC (7890A). A capillary column (30 m length, 50 mm internal diameter, and 0.25 mm film thickness) was used in this experiment. Helium was used as a carrier gas with an injection volume (1uL) and a flow rate (0.7 mL/min) of 10°C/minute was used to maintain the column temperature, which ranged from 60 to 310°C. The injector and detector were both set to a temperature of 250°C with an ionization energy of 70 ev and a scan range of 50–650 m/z. In split less mode, a 4 L sample volume was injected and the mass spectra were collected. A mass hunter GC-MS program with the NIST library Agilent Technologies (5301 Stevens Creek Blvd Santa Clara, CA 95051 United States) was used.

The chemical compounds with their molecular weights and chemical structures were identified and compared with the GC-MS spectrum with the more than 62000 patterns in the NIST (National Institute of Standard Technology) database.

Statistical analysis

All the data were expressed as mean±SD and five animals were used in each group (n=5). Statistical difference was determined among the groups using one-way and two-way analysis of variance (ANOVA) which was followed by Tukey's Multiple Comparison as a *post hoc* test. The statistical analysis was performed using GraphPad Prism version 5. The values at the statistical difference of $P < 0.05$ were considered to be significant.

RESULTS

Acute toxicity studies

Chloroform extract prepared from the plant exhibited no signs and symptoms of toxicity or mortality up to1000 mg/kg dose level given via oral route to the rats. The result exhibited that the lethal dose is more than this dose. Hence, the chloroform extract of the plant was considered non-toxic and safe for further studies. Hence the doses of 250 and 500 mg/kg b.w of chloroform extract were administered by the oral route to the rats for *in vivo* study.

Antihyperglycemic activity of chloroform extract

Various doses of chloroform extract of *F. indica* showing the effect on fasting BGL in diabetic rats are presented in Table I. The fasting BGLs of diabetic control rats were significantly higher than the normal control group on days 0, 7, 14, and 21. The diabetic rats that were administered with the doses of 250 mg/kg and 500 mg/kg of chloroform extract significantly (P<0.05) lowered

Table I. Effect of chloroform extract of *F. indica* on fasting blood glucose level (BGL) in STZ-induced diabetic rats.

Groups	BGL (mg/dl)			
	0 day	**7th day**	**14th day**	**21st day**
GI (NC) (untreated, uncompromised)	99.5 ± 2.71	98.4 ± 3.15	97.2 ± 1.45	99.5 ± 2.32
GII Diabetic control (received STZ)	221.3 ± 3.45	248.1 ± 4.58	275.4 ± 3.95	298.3 ± 4.11
GIII (Diabetic + plant extract at 250mg/kg)	112.3 ± 2.14 [b]	128.4 ± 1.23 [c]	174.1 ± 2.35 [c]	223.4 ± 1.59 [c]
GIV (Diabetic + plant extract at 500mg/kg)	91.5 ± 1.97 [b]	103.9 ± 4.21 [c]	142.2 ± 3.25 [c]	178.2 ± 2.96 [c]

Data are presented as mean ± SD (n=5) with one-way and two-way analysis of variance (ANOVA) followed by *post hoc* test (Tukey's Multiple Comparison). Statistical significant results at b: ** $P < 0.01$, c: *** $P < 0.001$ were obtained compared to diabetic control.

Table II. Effect of oral administration of chloroform extract doses of *F. indica* on lipid profile in STZ-induced diabetic rats.

Groups	TC mg/dL	TG mg/dL	HDL-C mg/dL	AI	LDL/ HDL	LDL-C mg/dL	VLDL-C mg/dL
GI (NC) (untreated, uncompromised)	66.23 ± 2.08	73.49 ± 3.08	48.02 ± 1.24	0.37	0.68	32.90	14.69
GII(HC) Diabetic control (received STZ)	108.42 ± 4.17	115.98 ± 2.34	35.31 ± 2.21	2.07	2.72	96.30	23.19
GIII (Diabetic + plant extract at 250mg/kg)	68.12 ± 1.58^{c}	88.13 ± 4.56^{c}	42.63 ± 2.17^{b}	0.67^{c}	1.08	46.33^{c}	17.62^{c}
GIV (Diabetic + plant extract at 500mg/kg)	52.43 ± 2.71^{c}	68.34 ± 1.39^{ns}	50.83 ± 3.37^{c}	0.03^{c}	0.30	15.26^{c}	13.66^{c}

TC-Total cholesterol, TG-triglyceride, HDL-C-high-density lipoprotein cholesterol, LDL-C-low-density lipoprotein cholesterol, AI-atherogenic index, VLDL-C-very low-density lipoprotein cholesterol, mg/dL-milligram per deciliter, ns=non-significant.
For statistical details, see Table I.

Table III. Effect of chloroform extract of *F. indica* on liver function enzymes and protien in STZ induced albino rats of various groups (n=5).

Groups	ALT(IU/dL)	AST(IU/dL)	ALP(IU/dL)	TP(g/dL)
GI (NC) (untreated, uncompromised)	38.41 ± 3.02	145.05 ± 3.45	180.59 ± 8.06	5.4 ± 0.05
GII Diabetic control (received STZ)	74.32 ± 4.32	210.45 ± 12.28	210.91 ± 6.42	4.9 ± 0.12
GIII (Diabetic+ plant extract at Fr 250mg/kg)	50.49 ± 2.75^{b}	153.84 ± 4.52^{c}	181.48 ± 4.23^{c}	6.2 ± 0.32^{c}
GIV (Diabetic + plant extract at 500mg/kg)	25.41 ± 3.41^{b}	126.34 ± 8.46^{c}	165.35 ± 3.96^{c}	7.3 ± 0.2^{c}

AST, aspartate aminotransferase; ALT, alkaline aminotransferase; ALP, alkaline phosphatase; TP, total protein; IU/dL, international unit/deciliter; g/dL, gram per deciliter.
For statistical details, see Table I.

fasting BGLs (223.4 ± 1.59 and 178.2 ± 2.96 mg/dL) from 21st, 14th and 7th day of treatment in comparison to the diabetic control rats, respectively.

Lipid profile

Lipid profiles of diabetes-induced rats checked after 21 days of chloroform extract administration are shown in Table II. The diabetic rats showed an increase in TC, TG, LDL-C, and VLDL-C levels with decreased HDL-C levels compared to normal control rats. However, giving the chloroform extract (250 and 500 mg/kg b.w.) doses of the plant extract exhibited a significant ($P < 0.05$) reduction in TG, TC, LDL-C, VLDL-C levels and an increase in HDL-C levels compared to diabetes-induced rats.

Liver function parameters

Diabetes-induced rats increased the levels of AST, ALP, and ALT levels in comparison to the normal control group. However, giving chloroform extract for 21 days recovered ALT, ALP, AST, and TP in group III and group IV compared with diabetic-induced rats (Table III).

GC-MS analysis of the chloroform extract

The analysis of chloroform extract by GC-MS exhibited that there were ten compounds in the extract. The structures of these compounds are shown in Figure 1. The molecular weights and molecular formulae of these compounds are given in Table IV.

Histopathological analysis of the liver

Figure 2 shows the pathological effect of chloroform extract of *F. indica*. Group I shows a normal configuration of the liver with normal architecture. Group II (untreated STZ-induced hyperlipidemic rats) show severe congestion of hepatic blood vessels (arrows). There was fatty infiltration and granular degeneration. Group III (diabetic + chloroform extract 250 mg/kg) show mild congestion of hepatic blood vessels. Group IV (diabetic + chloroform extract 500 mg/kg) has mild to moderate cytoplasmic fatty infiltration and granular degeneration.

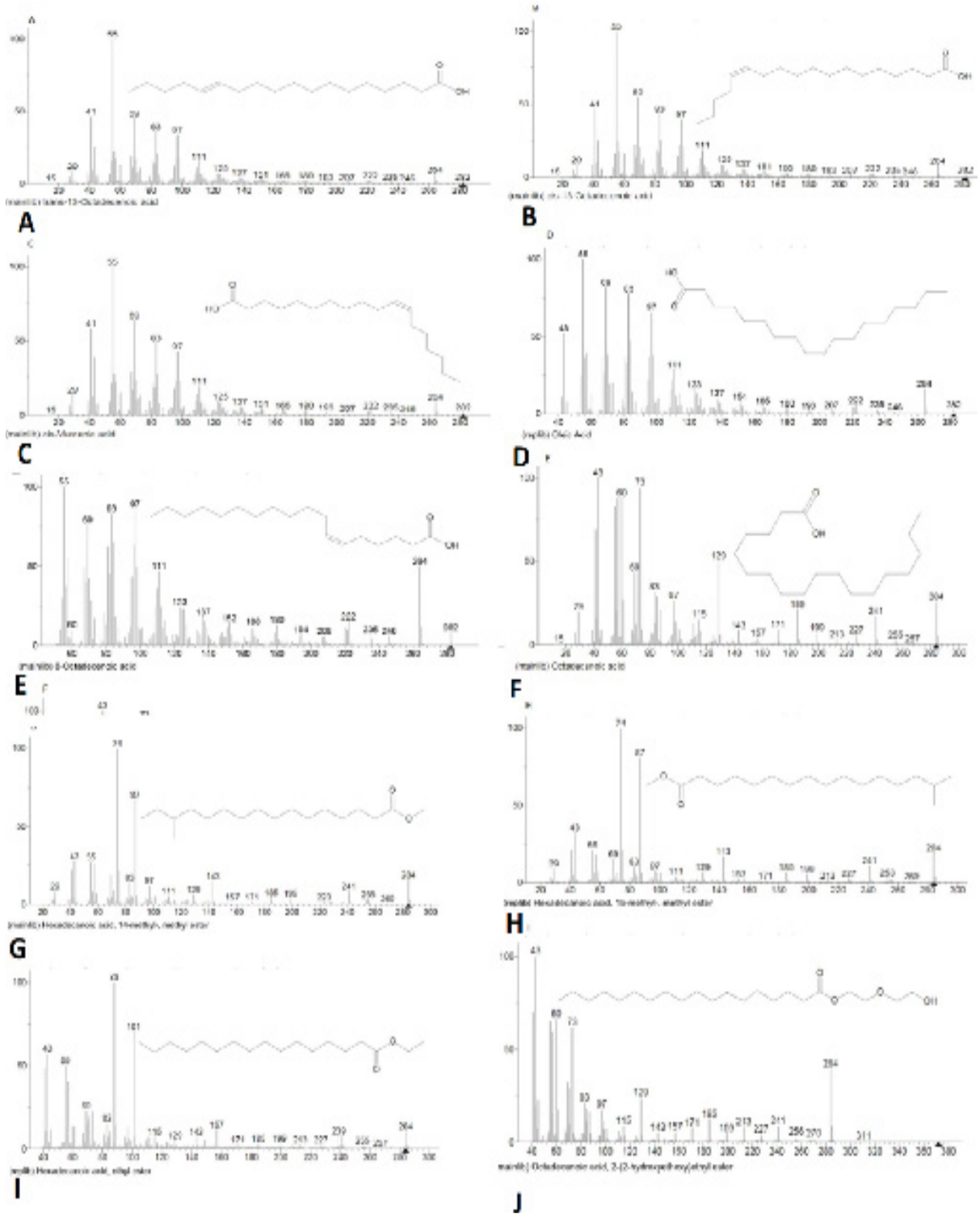

Fig. 1. Chemical structure of compounds identified in chloroform extract through GC-MS analysis A, Trans-13-octadecanoic acid ; B, Cis-13-octadecanoic acid; C, Cis-vaccenic acid; D, Oleic acid; E, 6-octadecenoic acid; F, Octadecanoic acid C18H36O2 358 G, Hexadecanoic acid, 14-methyl-, methyl ester; H, Hexadecanoic acid, 15-methyl-, methyl ester; I, Hexadecanoic acid, ethyl ester; J, Octadecanoic acid, 2-(2-hydroxyethoxy) ethyl ester.

Table IV. Chemical Compounds identified in chloroform extract of *F. indica* through GC-MS analysis.

S. No.	Retention time (RT) minute	Name of the chemical compound	Molecular formula (MF)	Molecular weight (MW)
1.	14.666	A) Trans-13-octadecanoic acid	$C_{18}H_{34}O_2$	282
		B) Cis-13-octadecanoic acid	$C_{18}H_{34}O_2$	282
		C) Cis-vaccenic acid	$C_{18}H_{34}O_2$	282
		D) Oleic acid	$C_{18}H_{34}O_2$	282
		E) 6-octadecenoic acid	$C_{18}H_{34}O_2$	282
2.	14.817	F) Octadecanoic acid	$C_{18}H_{36}O_2$	358
		G) Hexadecanoic acid, 14-methyl-, methyl ester	$C_{18}H_{36}O_2$	284
		H) Hexadecanoic acid, 15-methyl-, methyl ester	$C_{18}H_{36}O_2$	284
		I) Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	284
		J) Octadecanoic acid, 2-(2-hydroxyethoxy) ethyl ester	$C_{22}H_{44}O_4$	372

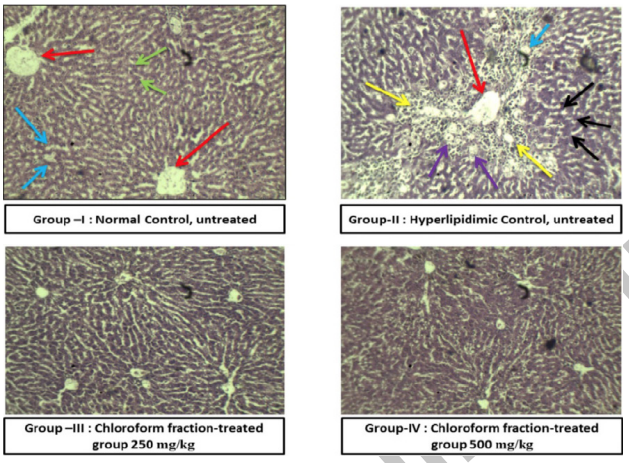

Fig. 2. Histopathological changes in the liver of animals induced by streptozocin. Group I showed a normal configuration of the liver with normal architecture (shown by green, blue and red arrows). Group II (untreated streptozocin-induced hyperlipidemic rats) had severe congestion of hepatic blood vessels (red, yellow, black and blue arrows). There was fatty infiltration and granular degeneration. Group III (diabetic + chloroform extract 250mg/kg) had mild congestion of hepatic blood vessels. Group IV (diabetic + chloroform extract 500mg/kg) had mild to moderate cytoplasmic fatty infiltration and granular degeneration.

DISCUSSION

Glucose concentrations were significantly different between normal and diabetic control rats. The fact is that STZ raises the glucose level in diabetes-induced rats. According to Montilla *et al.* (2005) diabetes causes reduced plasma insulin with increased BGLs and decreased urine glucose excretion. An increase in BGLs was concurrent with alterations in serum lipid levels in diabetes-induced rats. Induction of diabetes affected the lipid profile and the lipoprotein in these animals as supported by Shirali *et al.* (2013).

Secondary problems such as hypertriglyceridemia and hypercholesterolemia were brought on by an inactivated enzyme (lipoprotein lipase) caused by insulin deficit during diabetes condition. A rate-limiting enzyme (HMG-CoA reductase) breaks down LDL with elevated cholesterol. However, insulin inhibits HMG-CoA reductase, and insulin shortage results in dyslipidemia (Grice and Elmendorf, 2017).

STZ is a derivative of N-nitro glucosamine. It is a naturally occurring cytotoxic chemical abroad-spectrum antibiotic that is specifically toxic to the pancreas that produces insulin in β-cells of mammals (Alsayadi *et al.*, 2014). According to Gu *et al.* (1997) STZ decreases the ability of pancreatic β-cells to secrete insulin by causing damage. ROS are important in the onset and course of diabetes and pancreatic islets of diabetic rats have higher ROS levels (Sadri *et al.*, 2017). The oxidants (oxygen species) are known to increase glucose and lipids. It was believed that oxidative stress brought on by hyperglycemia plays an important role in the pathophysiology of injuries, which cause physiological alterations in particular tissues. STZ also causes diabetes by harming β-cells of pancreatic islets of Langerhans (Nasirian *et al.*, 2019). STZ administration was found to be sufficient to cause non-insulin-dependent DM type 2 in rats by causing resistance to insulin or decreasing insulin production from the pancreatic cells (Qinna and Badwan, 2015). Administration of chloroform extract of *F. indica* stimulated the regeneration of β-cells which secrete insulin eventually decreasing BGL in rats after the eradication of β-cells by STZ (Florence *et al.*, 2014).

In the following study, the effect of chloroform extract on BGLs, TG, TC, LDL-C, and HDL-C levels demonstrates its strong antihyperglycemic, antihyperlipidemic, and anti-atherogenic potential. By preventing the action of hormone-sensitive lipases by the extracts, insulin limits the release of free fatty acids and therefore slows lipolysis. Since enzyme activity is elevated in diabetes, more fatty acids are released into the bloodstream by lipolysis. A major factor in the disruption of the lipid profile in diabetes is hypoinsulinemia. Besides managing BGLs, chloroform extract showed potential antihyperlipidemic activity. This study showed that the lipid profiles of diabetic rats were improved by *F. indica* treatment which demonstrates its protective effects in complications related to diabetes like cardiovascular disease (Rani *et al*., 2019). Similar to *F. indica* the plant *Spirulina* restricted cholesterol absorption from the intestines or inhibited LDL-C oxidation and uptake, which would explain its hypocholesterolemic effects (Nasirian *et al*., 2019).

ALP, AST, ALT, and TP levels in the serum were shown to be elevated in STZ-induced hepatotoxicity. The findings of other studies that STZ raises ALP, AST, and ALT levels in serum and kidney supported this study (Nasirian *et al*., 2019). Also, the ratio LDL/HDL is helpful in the prediction of cardiovascular (CV) risk. A higher ratio showed an increased tendency towards CV disease. Diabetes-induced rats had shown a maximum ratio for LDL/HDL but the rats who received chloroform extract (500 mg/kg) have lower LDL/HDL ratio. The value of AI is the prediction of heart coronary artery diseases and atherosclerosis in the patient. However, the rats treated with chloroform extract reduced AI index and exhibited a dose-dependent cardioprotection with 67.63% from 250 mg/kg and 98.55% from 500 mg/kg doses, respectively. Thus, chloroform extract decreased AI value and the risk of atherogenesis. The results were further supported by a histopathological study. The rats induced by STZ showed changes in the histology of the liver as supported by Petchi *et al*. (2014). The animals treated with chloroform extract showed decreased pathological variations induced by STZ.

Most of the compounds identified through GC-MS are known to have antidiabetic and antihyperlipidemic activity. Trans-13-octadecanoic acid, Cis-13-octadecanoic acid, oleic acid, 6-octadecenoic acid, octadecanoic acid have antidiabetic, antihyperlipidemic activity and lowers cholesterol and triglyceride levels. The compounds 6-octadecenoic acid, Octadecanoic acid, hexadecanoic acid, 14-methyl- methyl ester, and hexadecanoic acid have hypocholesterolemic effects. Hexadecanoic acid, 14-methyl- methyl ester, hexadecanoic acid, 15-methyl-, methyl ester and hexadecanoic acid, ethyl ester and octadecanoic acid, 2-(2-hydroxyethoxy) ethyl ester have antidiabetic and hypocholesterolemic activity (Merugesu *et al*., 2018; Marella *et al*., 2013; Tyagi and Agarwal, 2017; Agada *et al*., 2021; Mehdi *et al*., 2021). Oleic acid has a beneficial effect on type 2 diabetes (Vassiliou *et al*., 2009). It has a positive effect in decreasing the risk of CVD by increasing HDL-C and decreasing TG levels in the blood of animals (Nogoy *et al*., 2020). Cis-vaccenic acid lowers cholesterol and triglyceride levels and has hypolipidemic activity. It lowers cholesterol and triglyceride levels (Fagbemi *et al*., 2022). These compounds are fatty acids and fatty esters which are not reported in this extract. The observed hypolipidemic activity in the present study might be due to the presence of mentioned compounds or due to the synergistic activity of these compounds (Uthirapathy *et al*., 2021). Further studies are recommended for assay-guided isolation, identification, and structure elucidation of active compound(s) responsible for the antidiabetic and antihyperlipidemic activities of this plant. There is a need to check the mechanism(s) of the antihyperlipidemic activity of chloroform extract of the plant for an effective therapeutic entity. There is a need to perform clinical trials for the assessment of the evidence of other effects of chloroform extract on health.

CONCLUSIONS

F. indica as an ethnomedicinal plant possesses anti-diabetic and anti-hyperlipidemic properties in an animal model and is useful for treating diabetes and hyperlipidemia. It can potentially be useful for patients with diabetes who are required to control their BGLs and diminish the risks of CVD. More research will be required on the relationship between *F. indica* and dyslipidemia. The compounds in the plant are responsible for hypoglycemic and hypolipidemic effects. Further studies are needed for assay-guided fractionation, isolation, identification, and structure elucidation of active compound(s) responsible for the antidiabetic and antihyperlipidemic effects of this plant. This study is beneficial in the transfer of natural remedies to an evidence-based product.

DECLARATIONS

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Ethical statement and IRB approval

The experimental protocol was approved by the

institution Animal Ethical Committee, University College of Pharmacy (UCP), Allama Iqbal Campus, University of Punjab (UOP), Lahore Pakistan (Approval no. AEC/PUCP/1076, dated 03/05/2018). The study was done as per the Animal Research Review Guidelines.

Statement of conflict of interest

The authors have declared no conflict of interest.

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