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INVESTIGATING THE TREATABLE EFFECT OF *LAVANDULA OFFICINALIS* L. ETHANOLIC EXTRACT IN ALLOXAN-INDUCED MALE DIABETIC RATS

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Abstract

The aim of authors in current study is based on evaluation the antihyperglycaemic and antioxidant capacity of *Lavandula officinalis* medicinal plant in laboratory animals' model. *Lavandula officinalis* ethanolic extract (LOE) sample was obtained from the aerial parts of the plant and analyzed by GC–MS. Rats were divided into four groups: Healthy Control (HC); Diabetic Control (DC); First treatment (FT) and Second treatment (ST). Antidiabetic and antioxidant activities were evaluated after subacute intraperitoneal injection of *Lavandula officinalis* ethanolic extract (100 and 200 mg/kg. b.w., i.p.) to rats during 21 days consecutively. The principal compounds detected are: Polyphenyl ether (12.79%), Octane (14.27%), Epoxycaryophyllene (10.70%), β-caryophyllene (7.05%), NSC407740 (5.62%), α-piene (2.32%), β-piene (2.19%), Linalool (1.23%), Camphor (1.79%), Carvacrol (3.01%) and Thymol (2.22%). The ethanolic extract of *Lavandula officinalis* also contained smaller percentages of other chemical. Furthermore, the results showed that LOE significantly protected against the increase of blood glucose as well as its reduction effect in HDL, VLDL, and TG level. LOE treatment in first dose (100 mg/kg.bw.i.p.) could increase the level of LDL and cholesterol nevertheless in second dose treatment it could diminish the level of them. On the other hand, LOE treatment could decrease the level of alkaline phosphatase (ALP) enzyme in both treated groups (FT and ST). LOE treatment diminished the bilirubins level as well as healthy rats. LOE treatment induced a decrease of lip peroxidation as well as an increase of antioxidant enzyme activities. LOE treatment in second dose (200 mg/kg.bw.i.p.) had positive effect in Langerhans islets reformation. The antidiabetic and antioxidant effect of LOE are two main results of present study.

Key words: Alloxan monohydrate, Antioxidant, Blood glucose, Diabetes mellitus, Extract, Medicinal plant.

Introduction

Diabetes mellitus (DM) is a syndrome of impaired carbohydrate, fat, and protein metabolism caused by either lack of insulin secretion or decreased sensitivity of the tissues to insulin. In 2012, an estimated 1.5 million deaths were directly caused by diabetes (WHO). On the other hand, world health organization declares that diabetes will be the 7th leading cause of death in2030. DM is a chronic and very widespread disease which effecting the majority of citizens including developed and developing countries [1]. Despite acceptable progress in diabetes treatment by oral medications, search for better ones continues since the current drugs have several limitations due to their wide range side effects. Perhaps 90% of the world's population still relies completely on raw herbs and unrefined extracts as medicines [4]. A 1997 survey showed 25% of modern pharmaceutical drugs include plant ingredients [2]. There are a vast amount of medicinal plants in the all part of the world which some of them have been discovered but most of them are in their wild environment. The herbal drugs with antidiabetic capacity are yet to be commercially synthesized as modern medicines, even though they have been applauded for their therapeutic effects in the traditional system of medicine [3]. Plants have synthesized the basis for the system of traditional medicine, which have been used for thousands of years in countries such as China [4] and India [5]. The use of plants in traditional medicine systems of many cultures has been extensively documented. These herb-based systems continue to play an essential role in health care and the World Health Organization [WHO] estimates that 80% of the world's inhabitants continue to rely mainly on traditional medicines systems for their health care. It is clear that Nature has played and will continue to play, a critical role in the drug discovery process [6]. Despite of all progress in medicine and drug discovery in recent decades, medicinal plants and herbal based medications didn't lose their role in man healthy because of their high capacity in chemical materials production. *Lavandula officinalis* which is known as lavender, is a medicinal plant from Lamiaceae family. This plant is native of South Europe [7]. *Lavandula officinalis* have been used as herbal remedies for centuries. Today, the plant is most commonly used in the form of lavender essential oil to treat a wide variety of situation such as muscle aches, respiratory ailments, anxiety and insomnia. In present study, the aim was based on evaluation the antidiabetic and antioxidant activity of *Lavandula officinalis* ethanolic extract in male diabetic rats.

Methods

Collection and preparation of medicinal plant

Lavandula officinalis L. medicinal plant was collected from uncultivated lands from the suburbs of Shiraz city, Iran. The plants were identified by Dr. Zehtab (Professor of medicinal plant) from Department of plant Eco physiology, Faculty of Agriculture, University of Tabriz, Tabriz, Iran. All the plants were collected on July, 2015. The areal parts of plants were washed with continuous flow of distilled water for 15 minutes and allowed to dry at laboratory ambient temperature (20±5°C). The plant materials have been kept under shade for drying and crushed into a powder form by using a blender. The plant powder was packaged in dark polyethylene bags and kept in cold place (5 ± 2 °C) until extraction.

Extraction of medicinal plant

500 g of plant material powder was suspended in 2500 mL (1:5) of ethanol solution (96%) for 10 days, and all the time the solution had replaced after saturation. The extraction affair done at room temperature. The solution was filtered via a fine muslin cloth followed by filter paper (Whatman No. 1) for removing the plant debris. The solvent was completely evaporated under vacuum at 50°C in a rotary evaporator. After evaporation of ethanol, the plants pure extract remained. The ethanolic extract was stored in aluminum foil wrapped and air-tight plastic bottles at ≤ 4 °C until use.

Phytochemical composition of medicinal plant extract

For identifying the chemical composition of LOE, 50 micro liter of extract was dedicated to analysis by GC-MS method. Forty six chemical composition identified by GC-MS method which will come in results part.

Experimental animals

Forty adult male albino (Wistar) rats, weighting 250± g used in the present study. Animals were obtained from the animal house of Tehran Pastor Institute, Tehran, Iran. Animals were housed at room temperatures of 24 ± 5°C, 30-55% of relative humidity on a 12-hours light/12-hours dark cycle. Water and standard commercial feed was available all the time. Animals were left to acclimatization for 10 days before research. The handling of the animals was in accordance with the standard principles of laboratory animal care of the United States National

Institutes of Health (NIH 1978).

Induction of diabetes/Experimental model

Hyperglycemia was induced in the experimental rats by single intraperitoneal (i.p.) injection of 90 mg/kg body weight of alloxan monohydrate 98% (Sigma-Aldrich, Acros. Organics, New Jersey, USA) in PBS solution (pH = 7.4) (8). The twenty one animals with fasting plasma glucose concentration > 110 mg/dL for 5 consecutive days were considered hyperglycemic and selected for the study. A total of 28 (twenty one diabetes and seven healthy) male Wistar rats were distributed into 4 groups of 7 rats each. The animals were deprived of food and water for an additional 16 hours prior to the commencement of treatment as described elsewhere [9].

Treatment

Twenty one diabetic and seven healthy rats divided in to four groups as follow:

Healthy control groups (HC), which included seven healthy rats without any diabetes induction and just received saline solution (0.9%) during the experiment. Diabetic control group (DC), which included seven diabetic rats without treatment by extract during the experiment. First treatment group (FT), which included seven diabetic rats with treatment by first dose of extract (100mg/kg.bw i.p.) [10]. Second treatment group (ST), which included seven diabetic rats with treatment by second dose of extract (200mg/kg.bw i.p.) [10].

The treatment duration was three weeks (21 days) consecutively.

Chemicals

Alloxan monohydrate (Sigma-Aldrich, Acros. Organics, New Jersey, USA) was applied from Kimia Eksir Co, Iran, containing 98% active ingredients. Thiobarbituric acid (TBA), trichloroacetic acid (TCA), H_2O_2 (30%), ethylenediaminetetraacetic acid (EDTA), Tris–HCl, 2,2´-dinitro-5,5´dithio-dibenzoic acid (DTNB), ethanol of technical grade and ethanol (96%) for extraction and the other chemicals used in this study were procured from Merck Co, Germany.

Blood glucose

The blood glucose of the all experimental rats measured by ACCU-CHECK-PERFOMA- glucometer after 21 days treatment to evaluate the effects of LOE on the blood glucose. This affair done under 16 hours fasting situation. The usage organ for measuring the blood glucose was the rat's tail.

Lipid peroxidation

Malondialdehyd (MDA), as a sign of lipid peroxidation and oxidative stress, was estimated by measuring the Thiobarbituric acid-reactive substances (TBARS) in pancreases biopsy. In this method, MDA was measured by its reactivity with TBA in acidic conditions to create a pink colored chromophore, which was read spectrophotometrically. In brief, the samples were mixed with 1 mL 10% trichloroacetic acid and 1 mL 0.67% Thiobarbituric acid. Then, the samples were heated in boiling water for 15 min, and N-butanol (2:1, v: v) was added to the solution. After centrifugation (900g, 5 min), TBARS were determined from the solution absorbance at 532 nm [11].

Lipid profiles

Obtained blood samples were centrifuged at 3000 rpm for 10 min at 4°C and plasma was collected. The levels of triglyceride (TG) and very low density lipoprotein (VLDL) in serum were determined by enzymatic kits (Ziest Chem Diagnostic kits, Iran) with utilizing glycerol as a standard. Furtherly, total cholesterol (TC), high density lipoprotein (HDL) and low density lipoprotein (LDL) levels were determined according to enzymatic methods by diagnostic kits, (Ziest Chem, Iran), with utilizing cholesterol as a standard [12].

Antioxidant enzymes

Pancreas tissues were removed from the animals under ketamine anesthesia after 21 days of treatment and washed with cold saline buffer. Then washed tissues were immediately stored at -80°C. To obtain the enzyme extract, tissues were homogenized in ice-cold KCl 1.15% to yield 10% (W/V) homogenate. Then the homogenates were centrifuged at 1000 rpm for 10 min at 4°C. The supernatants were separated and used for enzyme activity of SOD and CAT which was expressed in international units per mg protein (IU/mg protein). Biomarkers for tissue damage were measured using the UV kinetics methodology and total protein was determined using bovine serum albumin (BSA) as standard and the values were expressed as mg/dl. Super oxide dismutase (SOD) was determined according to the method described by Ukeda (13). Catalase (CAT) was measured by monitoring the decomposition of hydrogen peroxide, as described by Aebi (14). Protein was measured by the method of Lowry (15) using bovine serum albumin as standard.

Bilirubins and alkaline phosphatase (ALP)

Bilirubin (direct bilirubin, indirect bilirubin, total bilirubin) determined in present study. The rats obtained blood, centrifuged in 3000 rpm for 10 min and supernatants liquid used to bilirubins determination by spectrophotometric method [16]. ALP activity measured with a modification of the method of Bessey, Lowry and Brock [17] using p-nitro phenyl phosphate as substrate, in the presence of magnesium, zinc and cobalt ions as activators [18] Readings were performed with a Beckman -Spinco microspect colorimeter. One unit of alkaline phosphatase is defined as the activity hydrolyzing 1 µ mole substrate/min at 37˚C.

Serum albumin

The rats obtained blood samples, centrifuged in 3000 rpm for 10 min to separate the blood serum which had been used to albumin determination by direct spectrophotometric method [45,46].

Pancreases histopathology

On the last day of the experiment, the rats were anesthetized by ketamine and the complete pancreas were removed and kept in 10% formaldehyde. Dehydration and clearing of the tissues were formed automatically. The paraffin blocks of pancreases were provided, and histological sections of 5 - 6 µm in thickness were prepared. The samples were stained with hematoxylin-eosin solution and langerhans islets changes have been studied under a light microscope.

Statistical analysis

Statistical analysis was performed by Tukey's post hoc test (level of significance $P < 0.05$) using SPSS version 18, statistical program. The results are expressed as MEAN ± SEM, and were obtained from 7 rats in each group. Statistical analysis was based on comparing the values between the DC group and control group (HC), while diabetic-treated groups (FT and ST) by LOE, were compared with DC group.

Results

Phytochemical components of LOE

The results obtained by GC–MS analyses of the LOE are presented in Table 1. Forty six compounds were identified. The principal compounds detected are: Polyphenyl ether (12.79%), Octane (14.27%), Epoxycaryophyllene (10.70%), β-caryophyllene (7.05%), NSC407740 (5.62%), α-piene (2.32%), β-piene (2.19%), Linalool (1.23%), Camphor (1.79%), Carvacrol (3.01%) and Thymol (2.22%) (Table1).

Blood glucose

Significant increase ($p<0.01$) in blood glucose level was observed in alloxan-induced diabetic rats (DC) compared to healthy nondiabetic rats (HC). LOE treatment in both diabetic-treatment groups (FT and ST) diminished the level of blood glucose significantly ($p<0.01$) in compared with DC group (Figure1).

Alkaline phosphatase

Significant increase ($p<0.01$) in serum ALP level was observed in alloxan-induced diabetic rats (DC) compared to healthy nondiabetic rats (HC). LOE treatment in both diabetic-treatment groups (FT and ST) diminished the level of serum ALP significantly ($p<0.01$) in compared with DC group (Figure2).

Serum albumin

Nonsignificant reduction in serum albumin protein level was observed in alloxan-induced diabetic rats (DC) compared to healthy nondiabetic rats (HC). LOE treatment in FT diabetic-treatment groups diminished the level of serum albumin protein significantly ($p<0.05$) in compared with DC group and LOE treatment in ST diabetic-treatment group diminished the level of serum albumin protein significantly ($p<0.01$) in compared with DC group.

Fat profiles

Significant reduction ($p<0.01$) in serum triglyceride level was observed in alloxan-induced diabetic rats (DC) compared to healthy nondiabetic rats (HC). LOE treatment in both diabetic-treatment groups (FT and ST) diminished the level of serum triglyceride significantly ($p<0.01$) in compared with DC group (Table2).Nonsignificant reduction in serum cholesterol level was observed in alloxan-induced diabetic rats (DC) compared to healthy nondiabetic rats (HC). LOE treatment in FT diabetic-treatment groups increased the level of serum cholesterol significantly ($p<0.01$) in compared with DC group, nevertheless LOE treatment in ST group diminished serum cholesterol level significantly ($p<0.01$) in compared with non-treatment diabetic rats group (DC) (Table2). Significant increase ($p<0.01$) in serum high density lipoprotein (HDL) level was observed in alloxan-induced diabetic rats (DC) compared to healthy nondiabetic rats (HC). LOE treatment in both diabetic-treatment groups (FT and ST) diminished the level of serum high density lipoprotein (HDL) significantly ($p<0.01$) in compared with DC group (Table2). Significant reduction ($p<0.01$) in serum low density lipoprotein (LDL) level was observed in alloxan-induced diabetic rats (DC) compared to healthy nondiabetic rats (HC). LOE treatment in FT

diabetic-treatment groups increased the level of serum low density lipoprotein (LDL) significantly ($p<0.01$) in compared with DC group, although LOE treatment in ST group had nonsignificant effect in compared with non-treatment diabetic rats group (DC) (Table2). Significant reduction ($p<0.01$) in serum very low density lipoprotein (VLDL) level was observed in alloxan-induced diabetic rats (DC) compared to healthy nondiabetic rats (HC). LOE treatment in both diabetic-treatment groups (FT and ST) diminished the level of serum very low density lipoprotein significantly ($p<0.01$).

Serum bilirubins

Significant increase ($p<0.01$) in serum total bilirubin level was observed in alloxan-induced diabetic rats (DC) compared to healthy nondiabetic rats (HC). LOE treatment in both diabetic-treatment groups (FT and ST) diminished the level of serum total bilirubin significantly ($p<0.01$) in compared with DC group (Table3). Significant increase ($p<0.01$) in serum indirect bilirubin level was observed in alloxan-induced diabetic rats (DC) compared to healthy nondiabetic rats (HC). LOE treatment in both diabetic-treatment groups (FT and ST) diminished the level of serum indirect bilirubin significantly ($p<0.01$) in compared with DC group (Table3). Significant increase ($p<0.01$) in serum direct bilirubin level was observed in alloxan-induced diabetic rats (DC) compared to healthy nondiabetic rats (HC). LOE treatment in both diabetic-treatment groups (FT and ST) diminished the level of serum direct bilirubin significantly ($p<0.01$) in compared with DC group (Table3).

Pancreases antioxidant enzymes

Significant reduction ($p<0.01$) in pancreases SOD enzyme level was observed in alloxan-induced diabetic rats (DC) compared to healthy nondiabetic rats (HC). LOE treatment in both diabetic-treatment groups (FT and ST) increased the level of pancreases SOD enzyme level significantly (0.01) in compared with DC group (Table4). Significant reduction ($p<0.01$) in pancreases CAT enzyme level was observed in alloxan-induced diabetic rats (DC) compared to healthy nondiabetic rats (HC). LOE treatment in both diabetic-treatment groups (FT and ST) increased the level of pancreases CAT enzyme level significantly ($p<0.01$) in compared with DC group (Table4).

Pancreases lipid peroxidation

Significant increase ($p<0.05$) in pancreases MDA level was observed in alloxan-induced diabetic rats (DC) compared to healthy nondiabetic rats (HC). LOE treatment in both diabetic-treatment groups (FT and ST) diminished the level of pancreases MDA level significantly ($p<0.05$) in compared with DC group (Table4).

Pancreases histopathology

In histopathology study of Langerhans islets in four experimental group (HC, DC, FT, ST) the positive effect of LOE in second dose (200mg/kg.bw) is probable in regeneration of Langerhans islets. The destructed islets in the pancreases of DC group rats is remarkable in compared with the pancreases of HC group rats. In the first dose of LOE there were no improvement condition in islets (Figure4).

Discussion

Plants effective materials are bioactive components which have been used in healing vast range of clinical disorders and diseases widely [19,20] whose pathogenesis are directly or indirectly related with oxidative stress [21,22,23]. Bioactive principles of various plant origins have been experimentally described to exhibit blood glucose control through different of mechanisms such as dampening the activity or gene expression of enzymes related to antioxidant glucose, and lipid homeostasis (Shaker et al. 2010) stimulating insulin secretion/ mimicry, [21,26,27,28], improvement of hepatic glutathione concentration, (21,29), inhibition of intestinal a-glucosidase, pancreatic lipase, and cholesterol esterase activities, facilitated muscle uptake of glucose, [28,31] regeneration / proliferation of b-cells, [32,33,34] and promoting the secretions of insulin and adrenaline and anti-oxidative capability [25,35,36]. It is write worthy that the components of *Lavandula officinalis* extract in Iran was in semi agreement with the prior report [37] but overall the principle of components were the same. . Alloxan monohydrate usage not only caused diabetes mellitus by destruction of pancreatic β-cells [38] but also changes in metabolic variables amount. The diabetes inducing feature of alloxan monohydrate are partly related to the specific cytotoxic action interceding by reactive oxygen species generation leading to the damage of large number of β-cells which follow by a decrease in endogenous insulin secretion. Present study declare that administration of alloxan increased blood glucose, serums ALP, HDL, total bilirubin, direct bilirubin, indirect bilirubin and MDA. On the other hand, alloxan monohydrate i.p. injection decreased serum triglyceride, LDL, VLDL, pancreases SOD and CAT enzyme and had not significant changes on serum cholesterol and

albumin level. Alloxan-administered rats became hyperglycemic with hepatic glucose overproduction as a following phenomenon [39]. The most essential thing about present study is LOE effects in blood glucose reduction in diabetic rats. LOE may exert its antihyperglycaemic effect by stoutening plasma insulin activity, stimulating secretion or its release from bound form [40] Consumption of proteins as a source of energy is the last priority for the cells as mentioned before. Although the amount of albumin in diabetic group did not show a significant changes in compare with non-diabetic group, but the reduction of it in treatment groups (FT and ST) shows the positive role of LOE in cells stimulation to glucose use instead of sub priorities. SOD and CAT are two antioxidant enzymes which are against reactive oxygen species (ROS). These two enzymes are known to reduction in diabetic model as a result of oxidation [41]. The reduction of these two enzyme in diabetic rats' pancreases is a sign of tissue injury. The increased level of SOD and CAT in treatment group by LOE shows its antioxidant capacity which protect the tissues from destruction. Alloxan monohydrate induced oxidative stress causes lipid peroxidation of cell membrane polyunsaturated fatty acids [42]. Lipid peroxidation illustrates one of the most common reactions resulting of free radicals attacks to biological structures in addition to accumulation of oxidized lipids in the cell membrane [43]. The achieved results showed the reduction effect of LOE on ROS production. In this study, induced diabetic model by alloxan monohydrate significantly increased the pancreases MDA content which making the lipid peroxidation understandable. The significant decrease in the pancreases malondialdehyd content, as a marker of lipid peroxidation, confirmed the high capacity of LOE in protecting against alloxan-induced pancreases lipid peroxidation. Totally our results demonstrated that the levels of antioxidant enzymes (SOD, CAT), decreased in the diabetic (DC) group, were recovered by LOE treatment. The protective effects of LOE in holding the above enzymes close to the control group level increased the capacity of endogenous antioxidant defense as well as their firm state. These effects can also elevate the enzymes synthesis rates, conferring enhanced protection against oxidative stress. The increased alkaline phosphatase (ALP) level in diabetic rats' serum (DC group) is the hepatodestruction alarm, nevertheless the diminished level of it in treated groups (FT and ST) by LOE show its protective effect.

In other word, alkaline phosphatase (ALP) is an enzyme which was measured to evaluation the liver disorders. The increased level of ALP in non-treatment diabetic group (DC) can show easily hepatic problems which caused by alloxan monohydrate in diabetic rats. So LOE hepato-protective effect in the experimental animals is so clear which is understandable by reduction of ALP in treatment groups (FT and ST). Bilirubins are remarkable due to their powerful antioxidant capacity [44] in present study. The increased amount of three kind of bilirubins in DC group compensated in FT and ST groups by LOE treatment which can be another sign to antioxidant capacity of studied medicinal plant. In our study LOE decreased the serum triglyceride, VLDL and HDL levels in treatment groups (FT and ST) in both doses of LOE. The LDL and cholesterol level increased in FT treatment group by the first dose (100 mg/kg.bw i.p.) of LOE. But the second dose (200 mg/kg.bw i.p.) of LOE in ST group decreased the serum LDL and cholesterol level. At the end it is noteworthy that mechanism effect of LOE in diabetes model is by effecting on body metabolism probably due to fat profiles imbalance.

Conclusion

In this study two main results are understandable as the research conclusion:

1. The antidiabetic effects of LOE.
2. The antioxidant effects of LOE.

Abbreviation

ALP: Alkaline phosphatase **BSA**: Bovine serum albumin **CAT**: Catalase **DC**: Diabetic control group **DM**: Diabetes mellitus **DTNB**: 2,2-dinitro-5,5dithio-dibenzoic acid **EDTA**: Ethylenediaminetetraacetic acid **FT**: First treatment group **GC-MS**: Gas chromatography mass spectrophotometer **HC**: Healthy control group **HDL**: High density lipoprotein **i.p**: intraperitoneal **LDL**: Low density lipoprotein **LOE**: *Lavandula officinalis* extract **MDA**: Malondialdehyd **SOD**: Super oxide dismutase **ST**: Second treatment group **TBA**: Thiobarbituric acid **TCA**: Trichloroacetic acid **TBARS**: Thiobarbituric acid-reactive substances **TC**: Total cholesterol **TG**: Triglyceride **VLDL**: Very low density lipoprotein **WHO**: World health organization..

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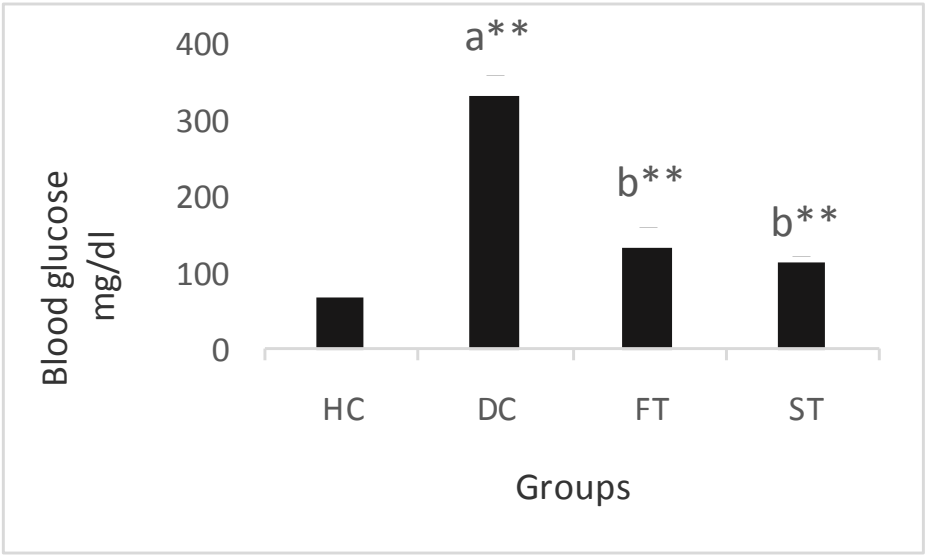

Figure 1. Effects of LOE treatment on blood glucose.
a p<0.01 compared to HC. b p<0.01 compared to DC.

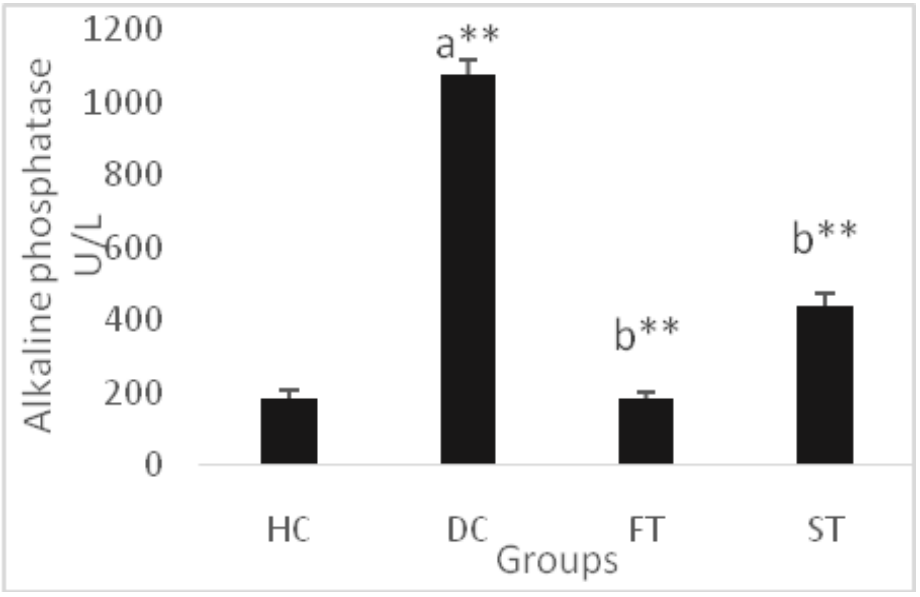

Figure 2. Effects of LOE on serum ALP enzyme.
a p<0.01 compared to HC. b p<0.01 compared to DC.

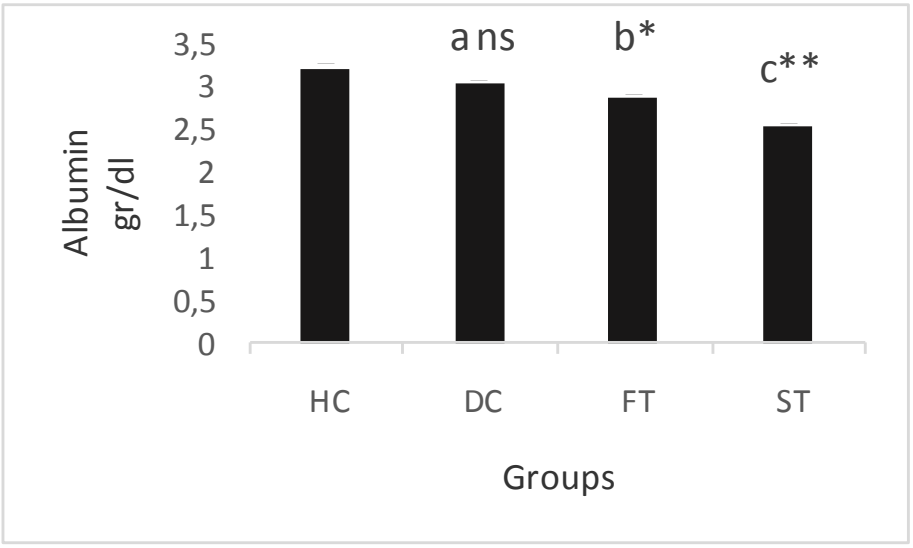

Figure 3. Effects of LOE treatment on serum albumin.
a ns compared to HC. b p<0.05 and c p<0.01 compared to DC.

Table1. Phytochemical components of LOE.

NO	Components	IR *	Area (%)	NO	Components	IR *	Area (%)
1	Polyphenyl ether	1411	12.79	**24**	4-Carvamenthenol	1600	0.76
2	Z-Ethyl hexane	764	4.57	**25**	α-Terpineol	1185	0.59
3	Octane	800	14.27	**26**	Dodecane	200.12	1.31
4	Ethylbenzol	1125	1.41	**27**	Bornylacetate	1595	2.90
5	M-xylol	1132	0.48	**28**	Thymol	1287	2.22
6	P-xylol	1130	0.38	**29**	Carvacrol	2219	3.01
7	Origanene	1023	0.35	**30**	α-TerpinylAcetate	1679	0.22
8	α-piene	6.720	2.32	**31**	β-bourbonene	1380	0.37
9	Camphene	7.320	0.93	**32**	Tetradecane	236.21	0.36
10	3-methylnonane	983	0.28	**33**	Jasmone	1960	0.23
11	Sabinene	1133	0.55	**34**	β-caryophyllene	1591	7.05
12	β-piene	8.626	2.19	**35**	Trans-β-Farnesene	1663	0.53
13	Myrcene	1159	0.51	**36**	Humulene	1665	0.44
14	Decane	159.66	3.82	**37**	Ent-spathulenol	1575	4.31
15	Isopropyltoluene	1276	1.06	**38**	Epoxycaryophyllene	1418	10.70
16	Dipenten	1031	1.54	**39**	Decalin	1170	0.47
17	NSC407740	1198	5.62	**40**	(1Z, 4Z)-germacrene	1480	0.32
18	Ocimene	1247	0.92	**41**	Spathulenol	1575	0.38
19	Gamma-Terpinene	1243	0.53	**42**	β-Eudesmol	1630	1.20
20	Isodurene	1430	0.16	**43**	(+)-Volenanone	2514	1.80
21	Linalool	16.785	1.23	**44**	Methypalmitate	2202	0.47
22	Camphor	1142	1.79	**45**	Methyl Z-ethyl hexyl Phatalate	2053	0.31
23	Borneol	1166	1.01	**46**	Methyl oleate	2426	0.40

Forty six compounds were identified in LOE as a result of GC–MS analyze.
* Retention index.

Table 2. Effect of LOE on serum fat profiles.

	Triglycerides (mg/dl)	**cholesterol** (mg/dl)	**HDL** (mg/dl)	**L DL** (mg/dl)	**VLDL** (mg/dl)
HC	100.857 ± 4.056	75.857 ± 2.219	17.857 ± 0.7377	4 ± 0.2182	20.642 ±0.9005
DC	68.714 ± 0.6061 [a]	70.142 ±0.4041[c]	25 ±0.3086[a]	2.285 ± 0.1844[a]	13.385 ±0.1184[a]
FT	45.428 ± 2.458[b]	79.142 ±0.5084[b]	19.857 ±0.3401[b]	6 ± 0.2182[b]	9.071 ±0.4724[b]
ST	30 ± 0.8729[b]	57.428 ± 0.4809[b]	15.714 ± 0.5216[b]	3 ±0.2182[d]	6 ±0.2138[b]

[a]p<0.01 compared to HC group and [b]p<0.01 compared to DC group and [c] ns compared to HC group and [d] ns compared to DC group.

Table 3. Effect of LOE on serum bilirubins.

	Bilirubin total (mg/dl)	**Bilirubin direct** (mg/dl)	**Bilirubin indirect** (mg/dl)
HC	0.248±0.003401	0.037±0.001857	0.208±0.005948
DC	0.575±0.008959[a]	0.217±0.003595[a]	0.314±0.002974[a]
FT	0.288±0.002608[b]	0.087±0.003595[b]	0.201±0.002608[b]
ST	0.321±0.008845[b]	0.037±0.002857[b]	0.26±0.004364[b]

[a]p<0.01 compared to HC group and [b]p<0.01 compared to DC group.

Table 4. Effect of LOE on pancreases anti-oxidant enzymes (SOD, ACT) and malondialdehyd (MAD).

SOD (U/mg protein)	**CAT** (n mol H2O2/min/mg protein)	**MDA** (n mol/mg protein)
HC	13.07±0.2906	45.375±1.097	0.67±4.8092
DC	5.39±0.2443[a]	20.355±0.3811[a]	0.92±0.0001587[c]
FT	19.33±0.5058[b]	33.581±0.7283[b]	0.66±0.0007431[d]
ST	31.37±0.7685[b]	50.082±0.8836[b]	0.67±0.0004358[d]

[a]p<0.01 compared to HC group and [b]p<0.01 compared to DC group and
[c]p<0.05 compare d to HC group and [d] p<0.05 compared to DC group.

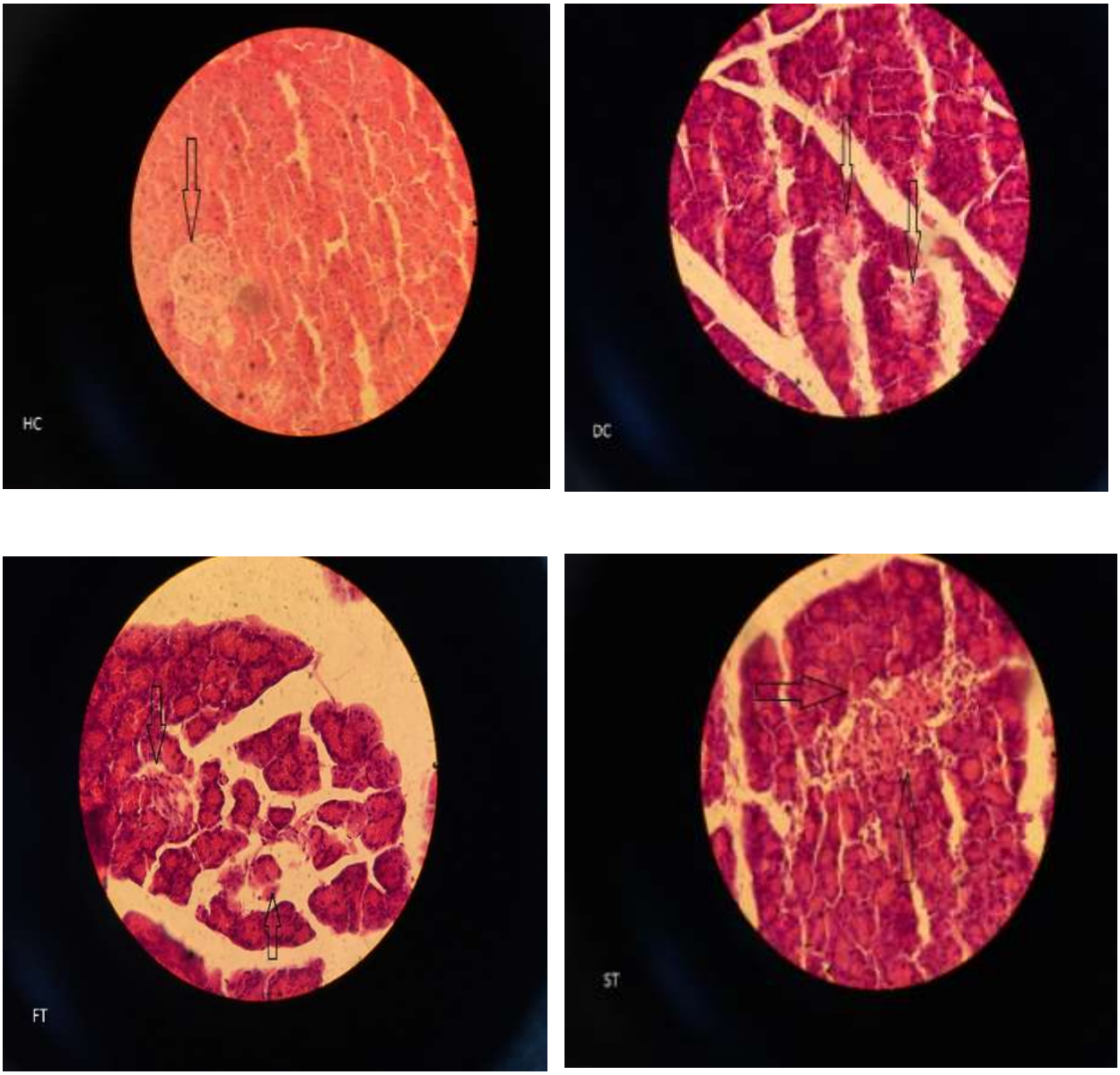

Figure 4. Langerhans islets in cross section of pancerases (←). HC: healthy rats, DC: diabetic rats without treatment, FT: treated diabetic rats by 100 mg/kg.bw of LOE, ST: treated diabetic rats by 200 mg/kg.bw of LOE. (Hematoxylin and eosin stain,40x).