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Biochemical and histopathological studies of the anti-hyperglycemic and anti-hyperlipidemic effects of some extracts of aerial flowering parts of *Onopordum alexandrinum Boiss*. in alloxan-induced diabetic rats

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Abstract

Diabetes mellitus (DM) is a metabolic abnormality linked to a higher risk of heart disease and stroke. The objective of this research is to see how three extracts of *Onopordum alexandrinum Boiss* affect serum glucose and blood lipids in diabetic rats that have been given alloxan. For 21 days, 48 mature male Sprague-Dawely albino rats (125-135 g) were chosen at random and placed into six groups of eight animals each: (1) non-diabetic control group, (2) diabetic control group, (3) diabetic group + Glibenclamide (10 mg/kg body weight), (4) diabetic group + petroleum ether extract of *O. alexandrinum* (480 mg/kg body weight), (5) diabetic group + ethyl acetate extract of *O. alexandrinum* (420 mg/kg body weight), and (6) diabetic group + ethanol (70%) extract of *O. alexandrinum* (600 mg/kg body weight). Triglycerides(TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), very low-density lipoprotein (VLDL) and glucose levels were measured in the serum of all individuals at 0, 3, 7, 14, and 21 days. In comparison to the control group and diabetic control group, administration of 480, 420, and 600 mg/kg body weight of petroleum ether, ethyl acetate, and 70 % ethanol *O. alexandrinum* extracts resulted in significantly lower glucose, TC, TG, LDL-C, VLDL levels, and increased HDL levels ($p<0.05$). Histological evaluation of groups treated with different *O. alexandrinum* plant extracts revealed that the injured pancreas was reconstructed. These findings suggest that *O. alexandrinum* extracts had a substantial influence on blood lipids and glucose levels in diabetic rats at all doses, suggesting that they could be effective in preventing and treating diabetes.

Keywords: Anti-diabetes, alloxan, blood glucose, lipid profile, anti-hyperlipidemic, *Onopordum alexandrinum Boiss*, Asteraceae.

Introduction

The most prevalent carbohydrate metabolism problem is diabetes mellitus. It suffocated a big portion of the world's population. Currently, 2.8 % of the world's population has diabetes, which would be projected to rise to 4.4 % by 2030 **(Farzaei et al., 2017).** It is caused by problems with insulin secretion, insulin action, or both **(Ritter et al., 2014).** There are two main types of DM: Type1 which is characterized by an absolute deficiency of insulin as a result of autoimmune destruction of pancreatic B cells **(Dipiro et al., 2014).** Type 2 is associated with insulin resistance and insulin secretion. Type 2 DM patients are often obese **(Ritter et al., 2014).** Chronic increase in blood glucose levels leads to microvascular and macrovascular complications. It is the leading cause of morbidity and mortality associated with diabetes **(Johansen et al., 2005)**. One such complication is hyperlipidemia, which is characterized by elevated cholesterol and triglyceride levels and changes in lipoproteins **(Rajaei et al., 2015).**

According to the International Diabetes Federation in Africa, more than 14 million people in Africa suffer from diabetes, and this number is expected to double by 2040 **(International Diabetes Federation-Africa 2015).** It has been reported that

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traditional medicines are particularly useful in developing countries because most individuals have limited resources and access to treatment **(Ali et al., 2006).** In addition to traditional medicines have recently been gaining popularity in both developing and developed countries due to their fewer side effects and natural origin **(Modak et al., 2007).** Many plant-derived active substances such as flavonoids, Tannins, alkaloids, glycosides, and saponins are found possessing anti-hyperglycemic and anti-hyperlipidemic activity **(Grover et al., 2002; Ojiako et al., 2013; Mannan et al., 2014).**

Asteraceae plant family is also used to be known as the Compositae plant family, is known as one of the largest plant families with thousands of plant species. Its large production as angiosperm phylogeny is in Asterideae. The Asteraceae plant family consists of 24,000 accepted species. It also has about 1,600 to 1,700 of its genera is distributed around the world, excluding Antarctica. This family is also known as a cosmopolitan family, as it has a great concentration of species in different areas such as temperate, cold-temperate, and subtropical. Asteraceae consist of three subfamilies; Asteroideae, Barnadesioideae, and Cichorioideae **(Medeiros-Neves et al., 2018).** There are 97 genera in Egypt, including 230 species **(Boulos, 2002).** Onopordum is a genus of about 50 species. In Egypt, there are only two species of Onopordum: *O. alexandrinum* and *O. ambiguum* **(Boulos, 2002; Kawashty et al., 1996).**

Onopordum alexandrinum Boiss. (Family: Asteraceae) It is distributed naturally in the State of Israel, the Hashemite Kingdom of Jordan and Egypt. Its tuberous roots, when eaten by the inhabitants of the Egyptian Western Desert, cause hallucinations and even death in some cases in high doses **(Sugimoto et al., 2017; Abd El-Moaty et al., 2016).** *O. alexandrinum* is a short-lived perennial plant with jagged, spiny leaves and visible spiny winged stems. Many species of Onopordum have been studied chemically and biologically **(Cardona et al., 1992).** Sesquiterpenoids and lignans were isolated from *O. laconicum* and *O. acanthium*, respectively, **(Lazari et al., 1998; Lajter et al., 2015)** and cynarine, a quinic acid ester with antioxidant activity, was isolated from *O. Illyricum* **(Topal et al., 2016).** Flavonoids with hepatoprotective effects were isolated from the flowers of *O. alexandrinum* **(Salama et al., 2011).** However, a detailed anti-diabetic and anti-hyperlipidemic activity of the whole aerial parts of *O. alexandrinum* including leaves, stems, and flowers has yet to be conducted. Herein, the purpose of the present study was to carry out assessed the acute toxicity, anti-hyperlipidemic and antidiabetic activities of the whole aerial flowering parts of *O. alexandrinum* in alloxan-induced diabetic rats.

2. Materials and methods

2.1. Materials

2.1.1. Plant material

The aerial flowering parts of Carthamus glaucus M.Bieb were collected from Alex-Marsa Matrouh Road, 62Km west of El-Hammam town at the recorded site 30 45 47.88828ºN, 29 11 8.0826 ºE during the period of investigation in March 2020. The plant was identified by the Herbarium, at Desert Research Center. The plant parts were washed by distilled water then were shade dried at lab-temperature till constant weight. The dried parts were then grounded into fine powdery form, sieved and finally stored in dry glass jar at room temperature for further use.

2.1.2. Laboratory animals

A total of 420 male albino mice weighing 25 - 30 g and 48 adult male Sprague-Dawely albino rats (125 -135 g) were used throughout this study and purchased from Egyptian Organization for Biological Products and Vaccines (VACSERA). Animals were housed in separate screen bottom cages (8/cage) under controlled environmental conditions (20-25 ºC, 55-60% relative humidity and 12 hours light dark cycle), where food and water were offered *ad-libitum.* Animals were maintained on a commercial pellet diet having the following composition: protein (21% w/w), barley (37% w/w) and corn oil (15% w/w) were the main constituent **(Lewi and Marsboom, 1981).** Animals were accommodated to the laboratory conditions for two weeks before starting the experiments as an acclimatization period.

2.1.3. Chemicals and kits

Alloxan monohydrate (2,4,5,6 tetraoxy-pyrimidine; 5-6-dioxyuracil) was obtained from LOBA CHEMIE PVT.LTD. Glibenclamide (Daonil tablets) was produced by Sanofi Egypt under license

of Sanofi-aventis GmbH/Austria one of Sanofi aventis/France affiliates. All other chemicals used in the present study were of high analytical grade purchased from El-Nasr Pharmaceutical Chemical Co. (ADWIC), Egypt and Aldrich-Sigma Co. (U.S.A). Kits were purchased from Biodiagnostic Co. (Egypt).

2.2. Methods

2.2.1. Preparation of extract

3000 gm dry powder of *O. alexandrinum* aerial flowering parts subjected to successive extraction using different organic solvents according to their polarity starting with petroleum ether followed by ethyl acetate then 70% ethanol giving 68.8 gm (2.3%), 62.8 gm (2.1%), 198 gm (6.6%), respectively. Then purified according to standard procedures reported by **(Mabry et al., 1970).** and **(Harborne, 1984).** Combined filtrates were evaporated under reduced pressure using rotavapour apparatus until a minimum amount of solvent remained. The residue (greenish sticky) was stored in a refrigerator at 5 ºC and kept for using in different analysis.

2.2.2. Acute Toxicity study

The acute toxicity of 480, 420 and 600 mg/kg body weight of petroleum ether, ethyl acetate and 70% ethanol *O. alexandrinum* extracts, respectively was estimated in 420 male mice orally by dividing animals into 13, 13 and 16 groups (10 mice each) for petroleum ether, ethyl acetate and 70% ethanol *O. alexandrinum* extracts, respectively. The animals were observed for 24 h for effects of toxicity and number of deaths. At the end of the study period, expired animals were counted for the calculation of LD_{50}. The arithmetic method of **Karber, (1931)** was used for the determination of LD_{50}.

2.2.3. Evaluation of antidiabetic activity

2.2.3.1. Induction of diabetes

Prior to initiation of this experiment, the animals were fasted for 8-12 hours, diabetes was induced by a single intraperitoneal injection of 120 mg/kg body weight of 'Alloxan monohydrate' in distilled water according to **(Pareek et al., 2009).** Induction of diabetes was confirmed by measuring fasting plasma glucose levels after 72 h of alloxan injection. Rats with plasma glucose levels > 250 mg dL-1 were considered as diabetic and used for the experiments.

2.2.3.2. Experimental design

Animals in all groups were treated for 21 days according to **Oloyede et al., (2015),** with some modifications. Animals were randomly divided into six equal groups (n = 8) as follows:

Group I: non-diabetic control rats

Rats served as normal-control and received the vehicle (0.5 ml Tween 80 (10%) /day/rat).

Group II: alloxan-induced diabetic control rats

Rats served as diabetic-control and received the vehicle (0.5 ml Tween 80 (10%) /day/rat).

Group III: Glibenclamide treated diabetic rats

Rats (diabetic) were administered *Glibenclamide* (10 mg/kg b.wt./day) in distilled water as a fine aqueous suspension orally.

Group IV: Fraction of petroleum ether of *O. alexandrinum*

Rats (diabetic) were administered *petroleum ether* (480 mg/kg b.wt./day) in Tween 80 (10%) as a fine aqueous suspension orally.

Group V: Fraction of ethyl acetate of *O. alexandrinum*

Rats (diabetic) were administered *ethyl acetate* (420 mg/kg b.wt./day) in Tween 80 (10%) as a fine aqueous suspension orally.

Group VI: Fraction of 70% ethanol of *O. alexandrinum*

Rats (diabetic) were administered *70% ethanol* (600 mg/kg b.wt./day) in Tween 80 (10%) as a fine aqueous suspension orally.

2.2.4. Specimens Collection & Storage

2.2.4.1. Blood sampling

The rats were fasted before the blood sample was taken, and blood samples were taken by tail sterilization 10% alcohol and then nipping the tail at the start of the experiment and repeated after 3, 7 and 14 days. After the operation, the tips of the tail were sterilized by swabbing with 70% ethanol. On day 21 blood samples were drawn by puncturing retro-orbital venous sinus of each rat and kept for 30 min at 37 °C. Sera were separated by centrifugation of blood

samples at 2500 rpm for 15 min. The serum was then separated and used fresh or kept frozen at -80 °C until used.

2.2.4.2. Histological examination

After a 3-week experimental period and the last blood sampling, the whole pancreas was removed after sacrificing the animal and was immersed in 10% formalin for histopathological examination. Sections were cut and stained with hematoxylin and eosin for histological examination **(Nagappa et al., 2003).**

2.2.5. Biochemical analyses

Serum glucose was determined by the glucose oxidase method using a commercial enzymatic kit **(Young et al., 1972).** Serum total cholesterol (TC), and high-density lipoprotein cholesterol (HDLc) were estimated using the procedure outlined in commercial kits **(Tietz, 1976).** Triglycerides (TAG) was measured according to **(Fossati and Prencipe, 1982).** Low-density lipoprotein cholesterol (LDLc) and very lowdensity lipoprotein cholesterol (VLDLc) were calculated using the following formula **(Friedewald et al., 1972):**

$$VLDLc = 0.2 \times TAG$$

$$LDLc = TC - (HDLc + VLDLc)$$

Statistical analysis

Statistical calculations were done using computer programs Microsoft excel version 365 and Minitab version 19 statistical programs. at 0.05 level of probability **(Snedecor and Cochran 1982).** Quantitative data with parametric distribution were done using analysis of variance the One-way ANOVA and Post hoc-Tukey's test **(Rosner, 2006).** The confidence interval was set to 95% and the margin of error accepted was set to 5%.

3. Results

3.1. Acute toxicity & Median lethal dose (LD50)

Acute toxicity test was carried out for all the extracts, using **(Karber, 1931).** LD_{50} calculated for petroleum ether, ethyl acetate and 70% ethanol extracts of *O. alexandrinum* was 4800, 4200 and 6000 mg/kg body weight, respectively. Hence, the doses selected for the evaluation of anti-diabetic activity of petroleum ether, ethyl acetate and 70% ethanol extracts of *O. alexandrinum* were 480, 420 and 600 mg/kg, body weight (1/10 of 4800, 4200 and 6000 mg/kg body weight), respectively.

3.2. Alloxan Induced Diabetes

The different solvent extracts (petroleum ether, ethyl acetate and 70% ethanol) of *O. alexandrinum* were subjected to *in vivo* antidiabetic effect. The results of blood glucose level in each experimental group of study are incorporated in **table (1)** and **fig.(1)**, Diabetic control rats had significantly elevated ($p<0.05$) serum levels of glucose compared to non-diabetic control rats. On the other hand, treatment with either glibenclamide (10 mg/kg/day), petroleum ether, ethyl acetate or 70% ethanol resulted in a significant reduction ($p<0.05$) in blood glucose level compared to diabetic control rats. It is worth to be mentioned; the 70% ethanol extract showed more effective lowering of blood glucose level as compared to other extracts of the plant.

Table 1: Level of blood glucose (mg/kg body weight) of alloxan-induced diabetic rats following 3-weeks oral administration of petroleum ether, ethyl acetate and 70% ethanol of aerial flowering extracts of *O. alexandrinum*

Days	Non-diabetic	Diabetic	Glibenclamide	Petroleum ether (mg/kg)	Ethyl acetate (mg/kg)	Ethanol (70%) (mg/kg)	*p*-value
0	88.31±1.10^{a}	88.20±1.11^{a}	70.73±0.51^{a}	80.70±1.01^{a}	89.33±1.3^{a}	85.71±1.21^{a}	0.278
3	83.66±1.06^{b}	490.01±1.13^{a}	442.33±1.59^{a}	391.66±2.04^{a}	385.11±1.97^{a}	387.66±2.01^{a}	0.001
7	78.33±0.76^{c}	487.67±1.87^{a}	299.67±2.39^{b}	259.00±1.78^{b}	218.33±1.68^{bc}	244.33±1.81^{b}	0.001
14	77.00±1.16^{d}	462.00±1.91^{a}	208.33±0.99^{b}	130.66±1.33^{c}	101.33±1.43^{cd}	112.33±1.01^{cd}	0.001
21	75.66±1.05^{c}	457.66±1.39^{a}	156.33±1.08^{b}	114.67±1.56^{c}	94.66±1.25^{c}	91.66±0.67^{c}	0.001

Data are mean of eight determinations ± SE. Values with superscripts different from the control are significantly different ($p< 0.05$).

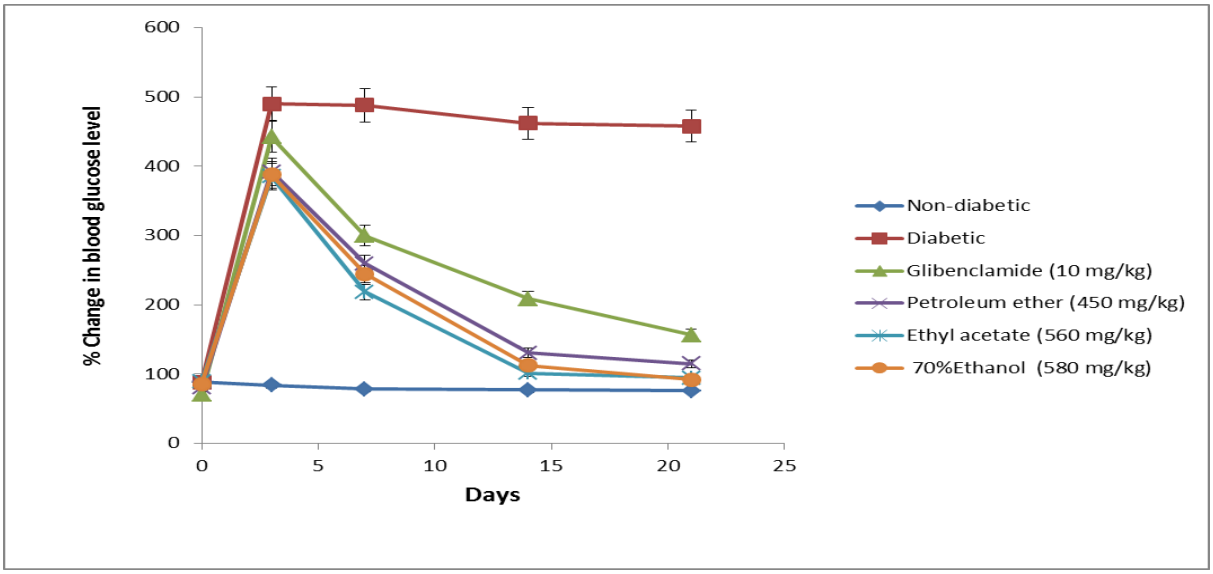


Fig. 1: Mean percentage change in blood glucose levels of Petroleum ether, ethyl acetate and 70% ethanol *O. alexandrinum* extracts orally administered in alloxan induced diabetic rats.

3.3. Lipid profile

The results of blood lipid profile in each experimental group of study are incorporated in **table 2** and **fig.(2,3,4,5,6).** Diabetic control rats had significantly elevated ($p<0.05$) TC, TG, LDL-C, and VLDL while had significant reduction ($p<0.05$) in HDL-C levels compared to non-diabetic control rats.

On the other hand, treatment with either glibenclamide (10 mg/kg/day), petroleum ether, ethyl acetate or 70% ethanol resulted in a significant reduction ($p<0.05$) in TC, TG, LDL-C, and VLDL levels and significant elevated ($p<0.05$) in HDL-C levels compared to diabetic control rats. It is worth to be mentioned; the 70% ethanol extract showed more effective lowering of serum TC, TG, LDL-C, VLDL levels and increasing in serum HDL–C level as compared to other extracts of the plant

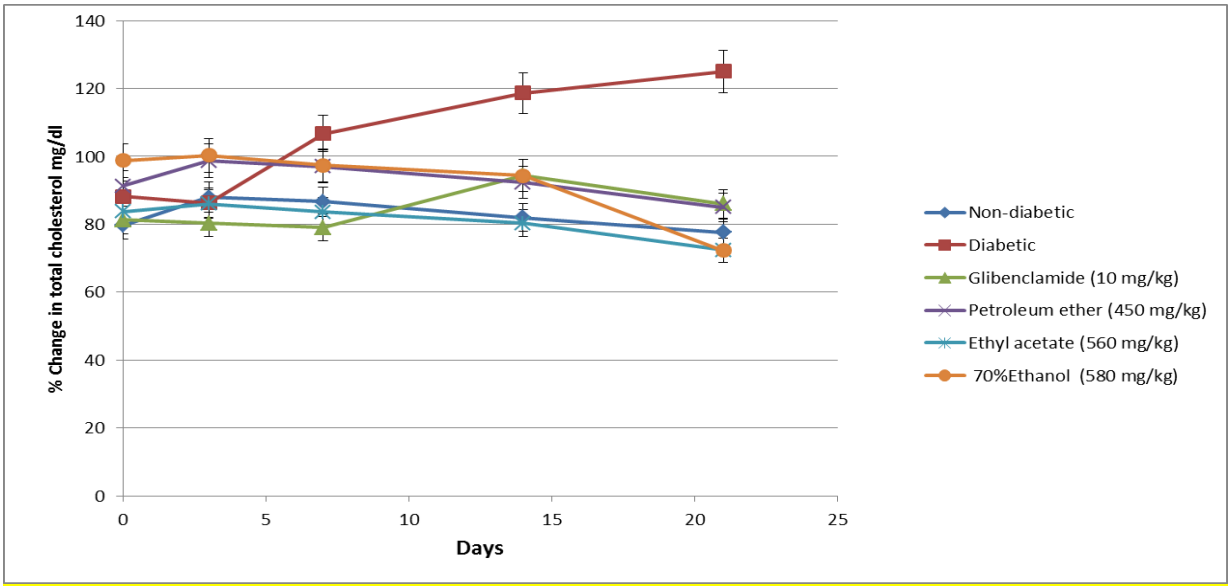


Fig. 2: : Mean percentage change in cholesterol levels of Petroleum ether, ethyl acetate and 70% ethanol *O. alexandrinum* extracts orally administered in alloxan induced diabetic rats

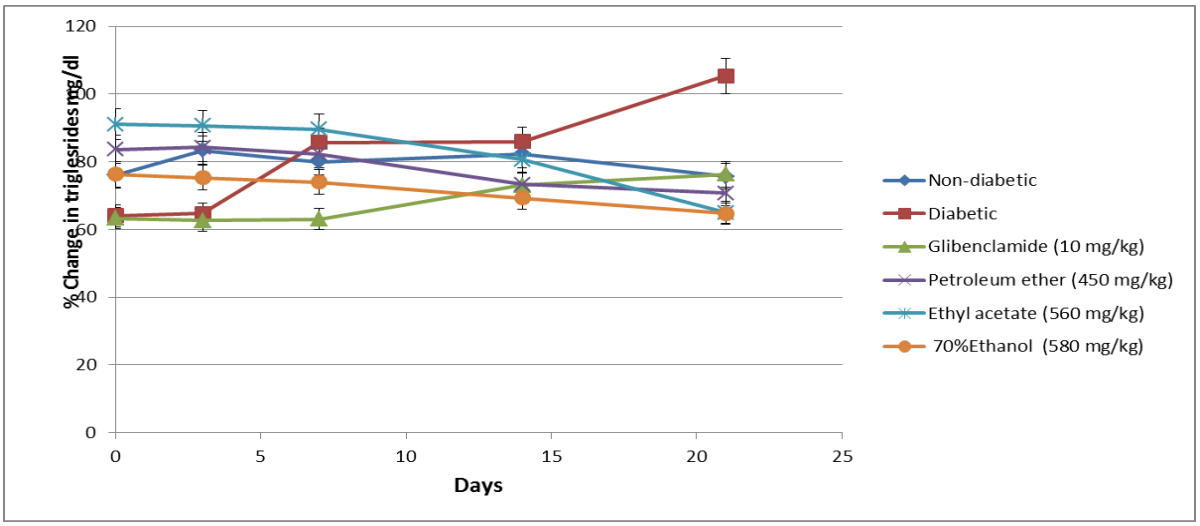


Fig. 3: Mean percentage change in triglycerides levels of petroleum ether, ethyl acetate and 70% ethanol *O. alexandrinum* extracts orally administered in alloxan induced diabetic rats.

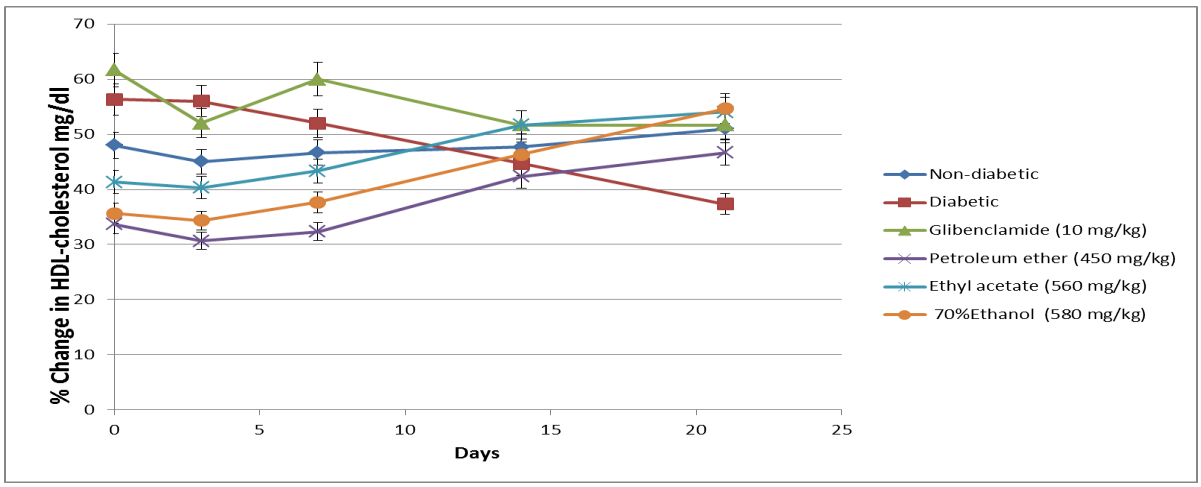


Fig. 4: Mean percentage change in HDL-C levels of petroleum ether, ethyl acetate and 70% ethanol *O. alexandrinum* extracts orally administered in alloxan induced diabetic rats.

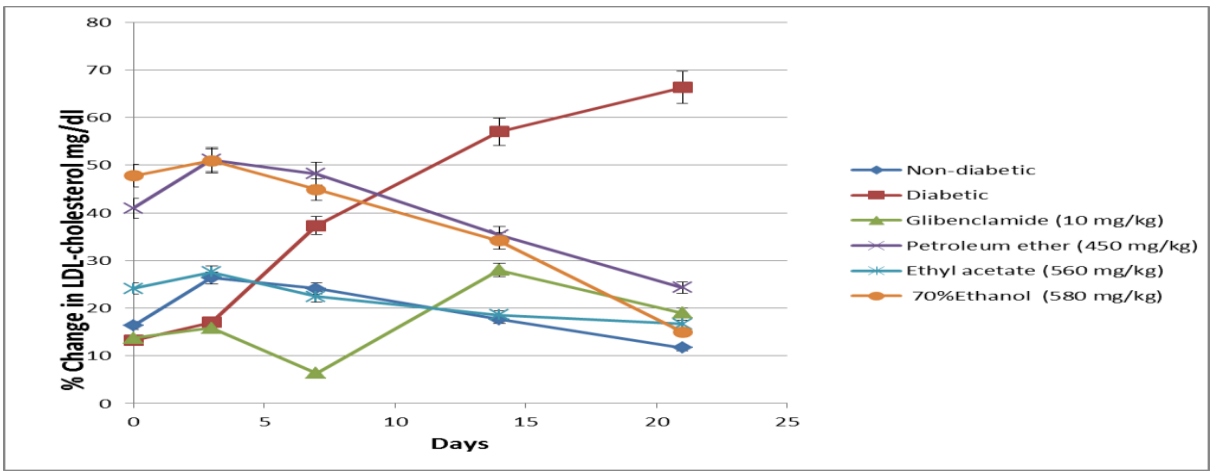


Fig. 5: Mean percentage change in LDL-C levels of petroleum ether, ethyl acetate and 70% ethanol *O. alexandrinum* extracts orally administered in alloxan induced diabetic rats.

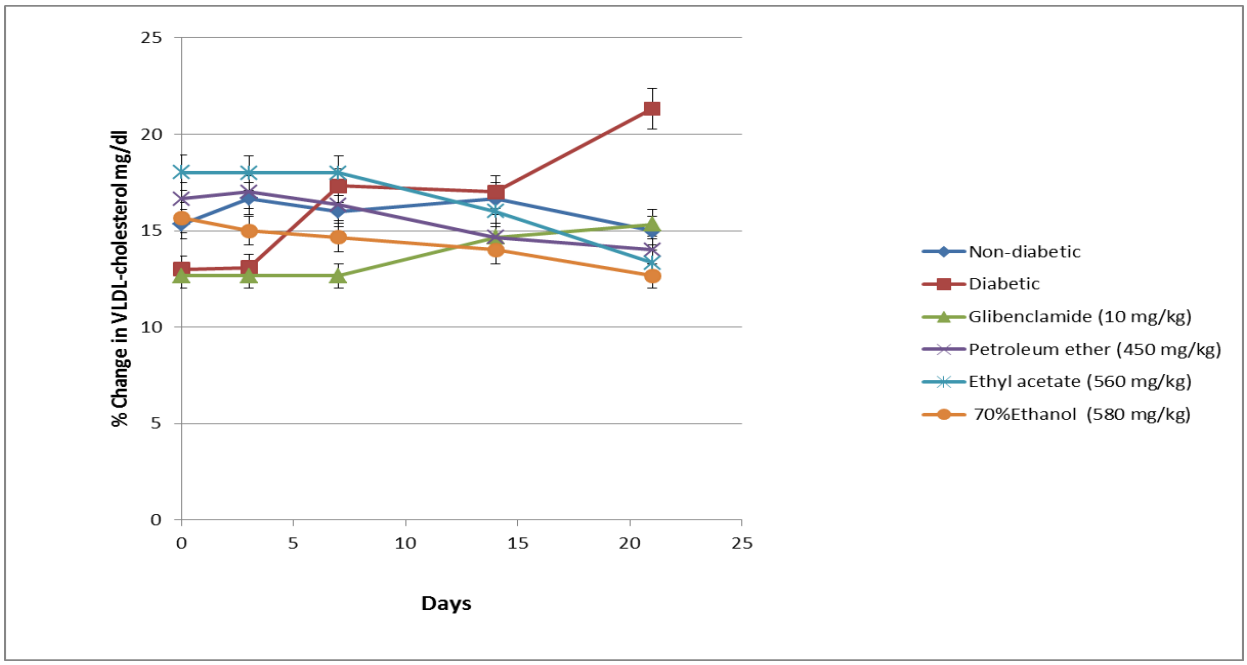


Fig. 6: Mean percentage change in VLDL-C levels of petroleum ether, ethyl acetate and 70% ethanol *O. alexandrinum* extracts orally administered in alloxan induced diabetic rats.

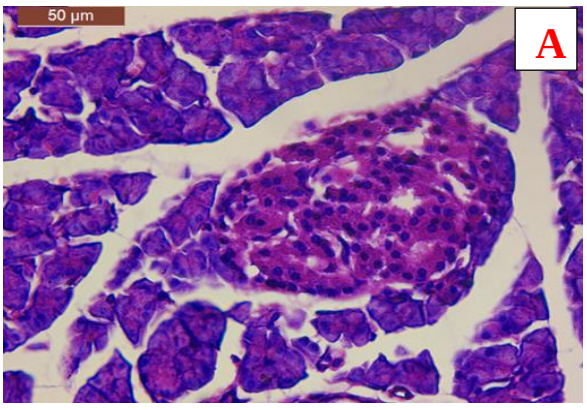
3.4. Histopathology

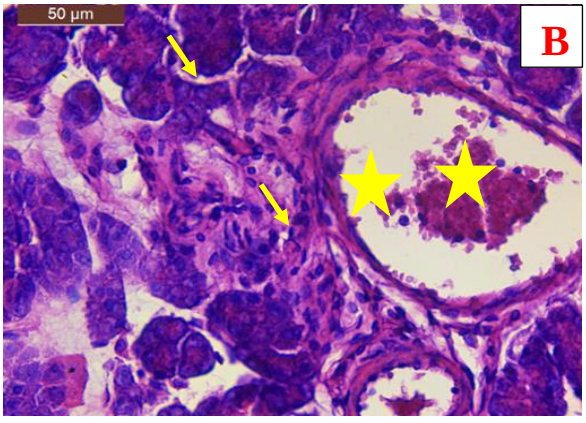
A photomicrograph from a section of pancreas of control rat group showing normal structure of endocrine and exocrine portions, the endocrine part show pale stained islets of Langerhans (asterisk) scattered between the exocrine acini (arrow), most of its cells were centrally located with rounded nuclei **fig.7(A).** A photomicrograph of rat pancreas of from a section of pancreas of diabetic group showing congested dilated blood vessels (asterisk) and the exocrine portion revealed focal acinar damage represented by cytoplasmic vacuolation (arrow) **fig.7 (B).** Also atrophy of the endocrine portion of the pancreas associated with a vacuolation (arrow) and pyknotic nuclei of in the islet's cells (arrowhead) **fig.7 (C)**. In glibenclamide group showing normal shape and islets were centrally located (β cells) and exocrine acini **fig.7 (D).** Diabetic rats that had been treated with the 480 mg/kg body weight extract showing appeared similar to the control and most of the Islets of Langerhans cells were intact were centrally located (β cells) and exocrine acini) **fig.7 (E).** While diabetic rats that had been treated with the 420 mg/kg body weight extract of ethyl acetate group showing normal architecture. Few islet cells show deeply stained pyknotic nuclei (arrow). Pancreatic exocrine acini appear nearly (EX) **fig.7 (F).** On the other hand diabetic rats that had been treated with the 600 mg/kg body weight extract of 70% ethanol group showing normal structure of islet cells and pancreatic exocrine acini **fig.7 (G).**

Table 2: Lipid profile of alloxan-induced diabetic rats following 3-weeks oral administration of petroleum ether, ethyl acetate and 70% ethanol of aerial flowering extracts of *O. alexandrinum*

Biochemical factor	Days	Non-diabetic	Diabetic	Glibenclamide	Petroleum ether	Ethyl acetate	Ethanol (70%)	*p*-value
TC (mg/dl)	0	79.71±0.64^{a}	82.33±0.67^{a}	81.33±0.41^{a}	91.30±0.59^{a}	83.71±1.39^{a}	98.73±1.38^{a}	0.278
	3	88.01±1.03^{a}	86.33±0.84^{a}	80.33±0.41^{a}	98.66±1.81^{a}	86.01±0.43^{a}	100.33±1.44^{a}	0.042
	7	86.67±1.29^{a}	106.67±1.31^{a}	79.00±0.89^{a}	97.00±1.77^{a}	83.67±0.36^{a}	97.33±1.42^{a}	0.229
	14	82.00±0.85^{b}	118.65±1.12^{a}	94.33±0.97^{ab}	92.33±1.63^{ab}	80.31±0.59^{b}	94.30±1.41^{ab}	0.046
	21	77.66±0.85^{b}	125.00±0.99^{a}	86.00±0.63^{b}	85.00±1.59^{b}	72.40±0.54^{b}	72.33±0.67^{b}	0.001
TG (mg/dl)	0	76.11±0.88^{a}	64.01±1.21^{a}	63.33±0.58^{a}	83.66±0.58^{a}	91.01±1.02^{a}	76.33±1.33^{a}	0.278
	3	83.41±0.71^{ab}	64.67±1.27^{ab}	62.67±0.85^{ab}	84.33±0.59^{ab}	90.67±1.13^{a}	75.33±1.37^{ab}	0.019
	7	80.00±0.83^{ab}	85.67±1.18^{ab}	63.00±0.92^{ab}	82.33±0.59^{ab}	89.67±1.03^{a}	74.00±1.32^{ab}	0.018
	14	82.31±0.95^{a}	86.00±0.97^{a}	73.00±0.93^{a}	73.31±0.67^{a}	80.66±0.78^{a}	69.33±1.14^{a}	0.336
	21	75.66±0.41^{ab}	105.33±0.88^{a}	76.33±0.88^{ab}	70.66±0.95^{b}	65.00±1.36^{b}	64.66±0.78^{b}	0.001
HDL-C (mg/dl)	0	48.01±0.91^{ab}	.56.33±0.52^{a}	61.66±0.76^{a}	33.66±0.68^{bc}	41.33±0.72^{abc}	35.66±0.96^{bc}	0.001
	3	45.01±0.25^{abc}	56.02±0.54^{a}	52.02±1.14^{ab}	30.67±0.78^{c}	40.33±0.72^{abc}	34.33±0.95^{bc}	0.001
	7	46.67±0.82^{abc}	52.00±0.63^{ab}	60.00±0.89^{a}	32.32±0.59^{c}	43.33±0.95^{bc}	37.67±0.92^{bc}	0.001
	14	47.67±0.79^{a}	44.67±0.59^{a}	51.67±0.58^{a}	42.31±0.82^{a}	51.67±0.92^{a}	46.33±0.96^{a}	0.336
	21	51.00±0.88^{a}	37.33±0.71^{b}	51.66±0.53^{a}	46.67±0.78^{a}	54.00±0.76^{a}	54.67±0.41^{a}	0.002
LDL-C (mg/dl)	0	16.33±0.67^{cd}	13.21±0.62^{d}	13.73±0.64^{cd}	40.93±0.66^{abc}	24.13±1.59^{bcd}	47.73±0.85^{ab}	0.001
	3	26.33±1.18^{bc}	17.04±0.89^{c}	15.81±1.06^{c}	51.13±1.86^{abc}	27.53±0.78^{bc}	50.93±0.94^{abc}	0.001
	7	24.11±1.38^{ab}	37.33±1.30^{ab}	6.32±0.59^{b}	48.20±1.79^{a}	22.40±0.91^{ab}	44.87±0.92^{ab}	0.019
	14	17.66±0.48^{b}	57.00±1.28^{a}	28.00±0.99^{ab}	35.33±1.62^{ab}	18.53±0.77^{b}	34.13±0.99^{ab}	0.009
	21	11.67±0.88^{b}	66.33±0.76^{a}	19.00±0.43^{b}	24.31±1.44^{b}	16.67±0.59^{b}	15.00±0.54^{b}	0.001
VLDL (mg/dl)	0	15.33±0.35^{a}	13.01±0.54^{a}	12.66±0.25^{a}	16.66±0.25^{a}	18.01±0.47^{a}	15.66±0.59^{a}	0.029
	3	16.67±0.25^{ab}	13.10±0.54^{ab}	12.67±0.41^{ab}	17.00±0.33^{ab}	18.00±0.47^{a}	15.00±0.62^{ab}	0.015
	7	16.00±0.33^{ab}	17.33±0.53^{a}	12.66±0.41^{ab}	16.33±0.25^{ab}	18.00±0.47^{a}	14.66±0.62^{ab}	0.014
	14	16.66±0.41^{a}	17.00±0.47^{a}	14.66±0.36^{a}	14.66±0.25^{a}	16.00±0.33^{a}	14.00±0.54a	0.454
	21	15.00±0.01^{ab}	21.33±0.41^{a}	15.33±0.43^{ab}	14.00±0.44^{b}	13.33±0.59^{b}	12.66±0.34^{b}	0.001

Data are mean of eight determinations ±SE. Cholesterol, triglycerides, high-density lipoprotein cholesterol; low-density lipoprotein cholesterol and very low-density lipoprotein cholesterol are expressed as mg/kg body weight. Values with superscripts ^{abc} for each parameter are significantly different ($p< 0.05$).





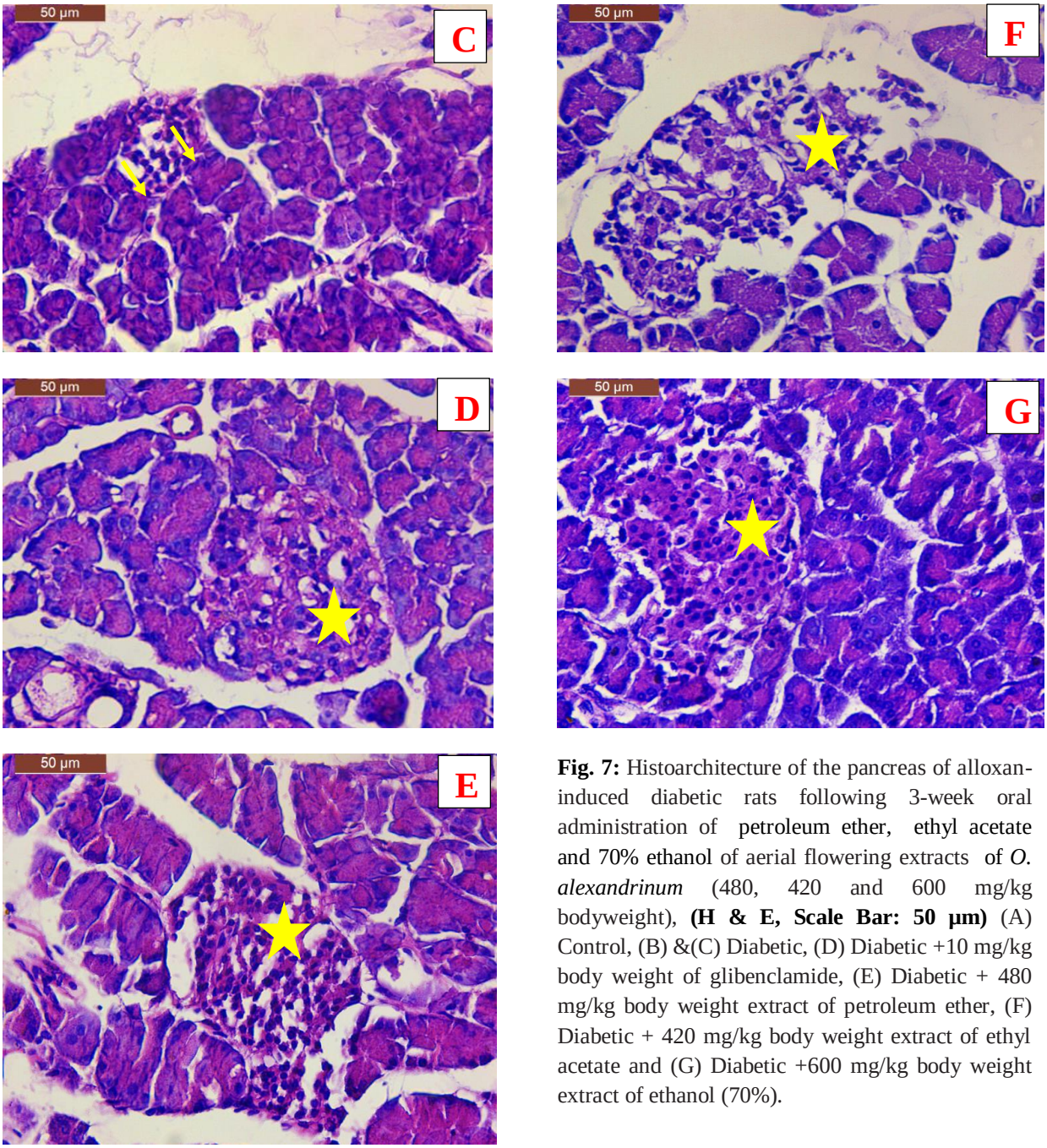


Fig. 7: Histoarchitecture of the pancreas of alloxan-induced diabetic rats following 3-week oral administration of petroleum ether, ethyl acetate and 70% ethanol of aerial flowering extracts of *O. alexandrinum* (480, 420 and 600 mg/kg bodyweight), **(H & E, Scale Bar: 50 µm)** (A) Control, (B) &(C) Diabetic, (D) Diabetic +10 mg/kg body weight of glibenclamide, (E) Diabetic + 480 mg/kg body weight extract of petroleum ether, (F) Diabetic + 420 mg/kg body weight extract of ethyl acetate and (G) Diabetic +600 mg/kg body weight extract of ethanol (70%).

4. Discussion

4.1. Blood glucose

This study investigated the effects of fractions of *O. alexandrinum* as a possible cure for diabetes disease, so we used diabetic rats induced by alloxan monohydrate injection. Alloxan, a well-known diabetogenic drug, is commonly used in animals to cause type 2 diabetes **(Viana et al., 2004).** With the generation of superoxide radicals, the medication and its reduction product dialuric acid form a redox cycle. Dismutation of these radicals to hydrogen peroxide occurs. The fenton reaction then produces highly reactive hydroxyl radicals. The rapid death of β cells is caused by reactive oxygen species along with a significant increase in cytosolic calcium concentration. **(Szkudelski, 2001).** Alloxan induced diabetes mellitus serve as a pathological biomodel for testing a substance with supposed antioxidant activities in vivo **(Bartošíková et al., 2003).** One of the targets

of the reactive oxygen species is DNA of pancreatic islets. Its fragmentation takes place in b cells exposed to alloxan **(Takasu et al., 1991).** The increase in oxygen free radicals in diabetic conditions is mainly because of the effect of the diabetogenic agent alloxan. Alloxan causes diabetes through its ability to destroy the insulin-producing beta cells of the pancreas **(Lenzen and Panten, 1988, Oberley, 1988).** *In vitro* studies have shown that alloxan is selectively toxic to pancreatic beta cells, leading to the induction of cell necrosis **(Jorns et al., 1997, LeDoux et al., 1986).** The cytotoxic action of alloxan is mediated by reactive oxygen species, with a simultaneous massive increase in cytosolic calcium concentration, leading to a rapid destruction of beta cells **(Szkudelski, 2001).** According to earlier studies, plant extracts causes antihyperglycemic effect by promoting regeneration of β-cells or by protecting these cells from destruction, by restricting glucose load as well as by promoting unrestricted endogenous insulin action. Antihyperglycemic effect may also be caused by the effect of plant extract on β-cells to release insulin or activate the insulin receptors to absorb the blood sugar and stimulate the peripheral glucose consumption **(Jadhav et al., 2009).** Our present experimental study reveal that the petroleum ether, ethyl acetate and 70% ethanol of *O. alexandrinum* (480, 420 and 600 mg/kg body weight), respectively administered orally for 21 days produced a significant decrease in the blood glucose level in the model of alloxan-induced diabetes. The comparable effect of the plant extracts with glibenclamide may suggest similar mode of action.

As compared to petroleum ether and ethyl acetate extracts, 70% ethanol extract exhibits strong anti-diabetic activity. The present investigation hence proves the traditional claim regarding *O. alexandrinum* for its anti-diabetic activity.

This may be attributed to the phytochemical constituents of *O. alexandrinum*, eleven flavonoid compounds have been isolated from the leaves and stems of *O. alexandrinum* and identified as apigenin, luteolin, chrysoeriol, and their 7-galactosides and 7-glucosides together with the 7-diglucosides of apigenin and chrysoeriol **(Kawashty, et al. 1996).** Taraxasterol, lupeol, *β*-sitosterol, stigmasterol, scutellarein 4'-methyl ether, and takakin were isolated from the aerial parts of *O. alexandrinum* **(Salama et al., 2011).** Three lactones were isolated from the aerial parts of *O. alexandrinum*, and one of them was identified as onopordopicrin, in addition to two flavone rhamnosides **(Khafagy *et al.*, 1977).** The study of the nitrogenous bases of *O. alexandrinum* resulted in the isolation of stachydrine and choline **(Wassel, 1975).**

The observed hypoglycemic activity of *O. alexandrinum* is in agreement with a previously reported data on another example from the same family, namely *Vernonia amygdalina,* one study looked at the antidiabetic activity of different formulations of metformin (50 mg/kg) and aqueous extracts of *Vernonia amygdalina* leaves (100 mg/kg) at normal blood glucose and the results of alloxan-induced diabetic rats showed that the combinations of the extract and Metformin caused a greater drop in blood sugar than either of the agents acting alone in either from two classes of animals **(Michael et al., 2010).**

The second example is *Chiliadenus iphionoides* from the Asteraceae plant family, the results showed that The ethanolic extracts of *Chiliadenus iphionoides* aerial parts increased insulin secretion from β cells and glucose uptake by adipocytes and skeletal myotubes, in vitro **(Gorelick et al., 2011).**

4.2. Hyperlipidemia

Recently, cardiovascular disease (CVD), a complex and multifactorial disease, remains one of the serious diseases that threaten human health worldwide with increasing incidence and mortality year by year **(Nichols et al., 2014).** Unfortunately, 17 million people die to CVD each year and it is estimated to reach 24.8 million in 2030 in the world **(Estruch et al., 2013).** The most important risk factors of CVD are hypertension, high cholesterol, alcohol intake, and tobacco usage, etc. according to the World Health Organization statistics **(Wood, 2001).** Dyslipidemia including high total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and triglyceride (TG), as well as decreased high-density lipoprotein cholesterol (HDL-C) are important causes of CVD **(Miller, 2003).** It is well known that an increase in LDL-C concentration is a risk factor for CVD and is therefore considered to

be the primary goal of CVD prevention and treatment **(Jung et al., 2014).** However, nowadays, most of hypolipidimic drugs have relatively great side effects during the treatment of CVD. Thus, a certain component from food with slight side-effect or free of side effects has been paid more and more attentions on the treatment of hyperlipidemia.

Our present experimental study reveal that the petroleum ether, ethyl acetate and 70% ethanol of *O. alexandrinum* (480, 420 and 600 mg/kg body weight), respectively administered orally for 21 days produced a significant decrease in TC, TG, LDL-C, and VLDL levels and increase in HDL-C level in the model of alloxan-induced diabetes. The comparable effect of the plant extracts with glibenclamide may suggest similar mode of action.

As compared to petroleum ether and ethyl acetate extracts, 70% ethanol extract exhibits strong anti-hyperlipidemic activity. The present investigation hence proves the traditional claim regarding *O. alexandrinum* for its hyperlipidemic activity. The observed hypolipidimic activity of *O. alexandrinum* is in agreement with a previously reported data on another example from the same family, namely *Achillea arabica Kotschy* ethanolic extract was used to test its hypolipidimic effect in animals **(Mais et al., 2016).** The extract was from the aerial parts of the plant taken during its flowering phase. The high-fat diet was fed to adult male Golden-Syrian hamsters for ten days to cause hyperlipidemia. The dose of 400 mg/kg of the *A. arabica* ethanolic extract had reduced VLDL, cholesterol, LDL, and triglycerides level in the hamsters' serum. It had no significant effect on HDL. Total cholesterol and triglycerides in the hepatic were also reduced. The plant extract contains flavonoids, sesquiterpene lactones, and polyphenols.

Whereas, its essential oil contains a high amount of eucalyptol, camphor and piperitone. These may act as an inducer for the hypolipidimic effect of *A. arabica* upon the experimental hyperlipidemic hamster **(Mais et al., 2016).**

The second example is *Ageratum conyzoide L.* The hypolipidemic efficacy of methanolic extracts of *Ageratum conyzoide L.* root, leaf, and stem was investigated in rats in vivo. **(Mehrabi et al., 2017).** The extracts contain flavonoids, alkaloids, cardiac glycosides, triterpenes, saponins, carbohydrates, and tannins. Meanwhile, the leaf also consists of steroids. Fiber, saponins, and flavonoids have an underlying antihyperlipidemic effect. The methanolic extract in a concentration of 100 mg/kg was treated on rats. The extract reduced serum lipids, which is one of the insulin-releasing factors. Insulin inhibits lipolysis, thus causing a rise in uptake of fatty acids into adipose tissue and triglyceride synthesis. The diabetic rat had shown a significant reduction in total cholesterol, triglycerides, low-density lipoprotein cholesterol (LDL-C) levels, and increased high-density lipoprotein cholesterol (HDL-C) (cardioprotective lipid) levels **(Mehrabi et al., 2017).**

4.3. Histopathology

A significant improvement was observed in the histological findings of pancreas tissue following supplementation with *Onopordum alexandrinum*. Plant antioxidants such as alkaloid, terpenoids and sterol have been shown in previous studies to restore and regenerate pancreatic beta cells and reduce tissue inflammation in experimental models of diabetes **(Vesal et al., 2003, Zhang and Hamauzu, 2004).** Alloxan-induced diabetic rats produced sinusoidal dilation and necrosis with cell infiltration, which were annulled by the extract treatment. This could have resulted from the capability of the extract to enhance antioxidant activities. The extensive damage to the islets of Langerhans and reduced dimension of islets in the diabetic rats were restored back to normal cellular population size of islets with hyperplasia by Glibenclamide 10 mg/kg. Similarly regeneration of islet cells and mild expansions were observed in rats treated with *Onopordum alexandrinum* extracts at various doses investigated.

5. Conclusion

According to biochemical and histological results, we can conclude that *Onopordum alexandrinum Boiss*. aerial flowering parts has beneficial effects on blood glucose level as well as improving hyperlipidemia due to diabetes. The improvement in biochemical and histological activities of *Onopordum alexandrinum Boiss*. extracts need a large scale study aiming to use the pure substance of these extracts as a candidate for disease treatment.

Declaration of conflicting interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article**.**

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