JOURNAL OF COMPLEMENTARY MEDICINE RESEARCH, 2018
VOL 7, NO. 2, PAGE 115–130
10.5455/jcmr.20180121122758

eJManager

ORIGINAL RESEARCH

Open Access

Therapeutic potential of *Achillea fragrantissima* extracts in amelioration of high-fat diet and low dose streptozotocin diabetic rats

Abeer I. Abd El-Fattah[1], Sanaa A. Ali[2], Hanan F. Aly[2], Howaida I. Abd-Alla[3], Nagwa M. Shalaby[3], Marwa H. Saleh[2]

[1]Biochemistry Department, Faculty of Pharmacy (Girls), Al-Azhar University, Cairo, Egypt

[2]Department of Therapeutic Chemistry, Division of Pharmaceutical Industries Research National Research Centre (NRC), Dokki, Giza, Egypt

[3]Department of Chemistry of Natural Compounds, Division of Pharmaceutical Industries Research National Research Centre (NRC), Dokki, Giza, Egypt

ABSTRACT

Aim: *Achillea fragrantissima* is a perennial herb used by Arabic Bedouin for the treatment of several pathologies. Type 2 diabetes mellitus (T2DM) is a metabolic disorder that is usually associated with hyperlipidemia into what is nowadays known as the metabolic syndrome epidemic. This study aim is to evaluate the effects of alcoholic and ethyl acetate extracts of *A. fragrantissima* in metabolically manipulated T2DM rats.

Materials and Methods: Induction of T2DM in rats was through high-fat diet for 12 consecutive weeks followed by a single injection of streptozotocin. The activities of both *A. fragrantissima* extracts were assessed in comparison with glibenclamide and fenofibrate as reference drugs.

Results: Elevated blood glucose levels, serum lipid profile, liver functions, and kidney functions witnessed a significant reduction by treatment with the extracts. Meanwhile, Oxidative stress markers (malondialdehyde, glutathione peroxidas, and superoxide dismutase), pro-inflammatory biomarkers (tumor necrosis factor-alpha and interleukin-6), and adhesion molecules (intercellular adhesion molecule-1 and vascular cell adhesion molecule-1) assessments recorded significant improvement after treatment with *A. fragrantissima* extracts. The results were also associated with the improvement in histopathological examination of pancreas, liver, and kidney tissues.

Conclusion: The current study suggests that *A. fragrantissima* extracts might be considered as a promising natural supplements for management of T2DM manifestations.

ARTICLE HISTORY

Received January 21, 2018
Accepted February 22, 2018
Published March 03, 2018

KEYWORDS

Achillea fragrantissima; adhesion molecules; antioxidant, pro-inflammatory cytokines; T2DM; chemical composition

Introduction

Type 2 diabetes mellitus (T2DM) is usually associated with obesity leading to what is recently has been known as metabolic syndrome which is the clustering of interconnected biochemical, physiological, clinical, and metabolic factors manifested through hyperglycemia, central obesity, hypercholesterolemia, and elevated triglycerides (TG) due to processed food consumption [1]. It is estimated that 415 million (8.8%) of adults aged 20–79 years old are type 2 diabetic patients, while it is predicted that by 2040 about 642 million or 10% of adults aged 20–79 years old will have diabetes [2].

Achillea fragrantissima (Forssk.) Sch. Bip. is a member of the Asteraceae or Compositae family, Spermatophyta superdivision, Angiospermae division, Dicotyledoneae class. *Achillea fragrantissima* has many synonyms and common names in different languages such as lavender cotton in English and Qaysūm in Arabic, and it is a perennial herb with yellow petals flower. *Achillea* species has been known and used by the Arabic Bedouinas folk for

Contact Marwa H. Saleh ✉ mh.saleh_nrc@yahoo.com Department of Therapeutic Chemistry, Division of Pharmaceutical Industries Research National Research Centre (NRC), Dokki, Giza, Egypt.

treatment of respiratory diseases, skin diseases, gastro-intestinal disturbances, high blood pressure, stomach aches, hepatobiliary complaints, and as analgesic [3–6]. Moreover, *A. fragrantissima* extract demonstrated *in-vitro* α-glucosidase inhibitory effect [7].

Induction of T2DM through introduction of high fat and low dose of streptozotocin (STZ) is based on simulating the natural pathology stages of diabetes by directing the model through two main key stages which include induction of insulin resistance state and insulin insufficiency state. Insulin resistance state is induced by subjecting the rats to high-fat (high energy) diet based on the theory of characterization of obesity as a state of chronic low-grade inflammation [8] that can lead to propagation of insulin resistance state through body cells, which in turn leads to T2DM [9]. The second stage which is insulin insufficiency, that is developed by incompetency of β-cell in performing their function by manipulating with low dose of STZ after being exhausted by the high-fat diet (HFD) period [10].

Association of T2DM and hyperlipidemia is usually treated by prescribing both anti-diabetic drug and hypolipidemic medications, concurrently. Glibenclamide is a potent hypoglycemic sulfonylurea derivative for management of T2DM, while fenofibrate is a fibric acid derivative which is commonly prescribed as a lipid-lowering agent [11].

The main target of conducting this study was to assess the activity of alcoholic and ethyl acetate extracts of *A. fragrantissima* in the treatment of T2DM, hyperlipidemia, and other metabolic repercussions, which could shed light on developing a potential natural alternative to minimize the side effects and drug-drug interactions that evolve when multiple medications has been consumed simultaneously.

Materials and Methods

Chemicals

Analytical grade solvents were used for extraction and chromatographic separation purchased from Sigma-Aldrich (St Louis, MO), Merck (Darmstadt, Germany), BDH (Poole, UK), Riedel de Haën (Seelze, Germany), and Fluka (Buchs, Switzerland). STZ (PubChem CID: 29327) and cholesterol (PubChem CID: 5997) powders were purchased from Sigma-Aldrich Co. (St. Louis, MO). Glibenclamide was purchased from Sanofi-Aventis, Egypt. Fenofibrate was purchased from Minapharm under license of Fournier, Egypt, where crushed tablets were suspended in distilled water.

Plant material

The aerial parts of *Achillea fragrantissima* Sch. Bip. (family: Asteraceae) were collected from Sinai desert, Wadi Al Gady, Egypt during the flowering stage. Authentication of the plant was performed, where the voucher specimen was deposited in the herbarium of National Research Centre (NRC), Cairo (Egypt).

Preparation of extracts

Aerial parts of *A. fragrantissima* approximately (3.2 kg) were air-dried for a week until constant weight, and subsequently, powdered and exhaustively extracted using ethanol (80%) by using continuous extraction apparatus. The total ethanol extract (Alc. Ext) was evaporated under vacuum at 40°C to yield brown oily viscous residue, which was divided into two portions: the first portion was diluted with distilled water to a concentration of 100 mg/ml, while the second portion of the residue was partitioned with ethyl acetate to yield the ethyl acetate extract (Eth. Ac. Ext.), which was evaporated under reduced pressure to yield a residue and was diluted with distilled water into a concentration of 100 mg/ml extract.

Phytochemical investigation

Quantitative estimation of total polyphenolic and flavonoid contents

Total polyphenolic compounds were quantified by Folin–Ciocalteau method using gallic acid as standard [12,13]. While, total flavonoids content was estimated using rutin as reference [14].

Chromatographic techniques

Aliquot of 5 µl of Alc. Ext and Eth. Ac. Ext was applied, separately, to two thin-layer chromatography (TLC) with the available authentic samples of phenolic compounds. TLC was performed on silica gel GF_{254} pre-coated plates (Merck, Darmstadt, Germany). The chromatograms were visualized under UV light at 254 and 366 nm before and after exposure to ammonia vapor, as well as spraying with $AlCl_3$ or *p*-anisaldehyde/sulfuric acid reagent using the solvent systems: S_1, benzene/ethyl acetate (9:2, v/v) and S_2, hexane/ethyl acetate (1.5:8.5, v/v). Paper chromatography (descending) Whatman no. 1 column chromatography (CC) papers, using solvent systems (v/v): S_3, acetic acid/H_2O (1.5:8.5, v/v) and S4, *n*-butanol/acetic acid/H_2O (4:1:5, upper layer) were applied. Spray reagents used were: R1,

sulphuric acid/methanol (30%), followed by heating at 105°C for 1–2 min for terpenes and R2, 1 g powder of $AlCl_3$ in 100 ml of ethanol for flavonoids. CC was carried out using silica gel (Si) 60 (E. Merck) and Sephadex LH-20 (Pharmacia Fine Chemicals AB, Uppsala, Sweden).

Extraction and isolation

In order to identify the active components in the effective fraction, part of the ethylacetate extract (14 g) was preliminary subjected to a polyamide column (150 g, 120 × 5 cm) using a step gradient of H_2O/MeOH (100:0–0:100 v/v) for elution to give 44 fractions. The eluted fractions were grouped into four main fractions (Fc I–Fc IV) based on paper chromatography (PC) examination. They were collected, monitored by TLC (solvent systems S1 and S2) and Comp-PC (solvent systems S3 and S4) using Whatman no. 1 paper sheets. Fraction I (Fc. I, 150 mg) was subjected to repeated column chromatography on microcrystalline cellulose using *n*-BuOH/*iso*-propanol/H2O [butanol:isopropanol: water ratio (BIW), 4:1:5 v/v/v, top layer] as an eluent, followed by repeated cellulose column chromatography for each major subfraction using MeOH/BIW (50%) to give pure samples of 1 (18 mg) and 2 (20 mg). Fraction II (300 mg) was chromatographed on a Sephadex column chromatography (10% aqueous MeOH as an eluent) to give pure sample of 3 (31 mg). Fc III (150 mg) was separated on a Sephadex column eluted with MeOH to give pure sample of 4 (9 mg). Fc IV (0.8 g) was subjected to column chromatography (2 × 9 × 40 cm, 150 g) using Si 60 and elution with benzene/ethyl acetate (9:1) provided compound 5 (15 mg) after crystallization from chloroform. Subfraction Fc IV-2 was applied on Si CC (100 × 2 cm, 30 g) and elution was made with toluene/ethyl acetate (7:3) and the yellow precipitate obtained was subjected to Sephadex LH-20 (ethanol) affording compound 6 (28 mg) and 7.

Animals

This study was performed on 70 adult male albino of the Wistar strain rats weighing 160 ± 10 g, supplied from the animal house of the NRC (Dokki, Giza, Egypt). The study was approved by the Medical Research Ethics Committee of the NRC approval number (12-017) following the Guide for the Care and Use of Laboratory Animals_(Eighth Edition, 2011, published by The National Academies Press, 2101 Constitution Ave. NW, Washington, DC 20055) [15]. Animals were kept for 2 weeks to accommodate to laboratory conditions and were allowed *ad libitum* to chow and water.

Induction of T2DM and experimental design

Induction of type 2 diabetes was performed through feeding on high-fat chow, which was produced by addition of (lard fat to standard normal chow to raise the ratio of total fat to 20%) [16] as well as oral administration of cholesterol at a dose of 30 mg/0.3 ml olive oil/kg of animal body weight (BW) five times a week for the whole experiment period [17]. Table 1 shows constituents of the normal chow and HFD chow.

After 12 weeks of dietary manipulation, rats were subjected to a single intra-peritoneal STZ injection (40 mg/kg BW, dissolved in 0.01 M citrate buffer, pH 4.5) [16], after which rats were given 5% glucose solution 2 hours following STZ injection to avoid hypoglycaemic shock.

Animals were divided into seven groups (10 rats each). Group I served as normal control and the animals were fed with standard normal chow and received a single intra-peritoneal of 0.01 M citrate buffer (pH 4.5). Groups II (Normal/Alc. Ext) and III (Normal/Eth. Ac Ext), separately, received orally normal chow and received a single intra-peritoneal of 0.01 M citrate buffer, pH 4.5 and given daily oral dose of Alc. Ext and Eth. Ac Ext of *A. fragrantissima* [500 mg/kg animal weight (AW)], respectively daily for three consecutive weeks [18]. Group IV (HFD-STZ control group) received high-fat chow and received a single intra-peritoneal STZ injection (40 mg/kg BW, dissolved in 0.01 M citrate buffer, pH 4.5) and did not receive any treatment. Groups V (HFD-STZ/Alc. Ext) and VI (HFD-STZ/Eth. Ac. Ext) were the diabetic rats that treated with daily oral dose (500 mg/kg AW) of Alc. Ext and Eth. Ac. Ext, respectively for three consecutive weeks [18]. Group VII (HFD-STZ/reference drugs) received a daily oral dose of glibenclamide (10 mg/kg BW) [18]. along with fenofibrate (50 mg/kg BW) [19].

Table 1. The constituents of the normal chow and HFD chow.

Constituents	Regular chow (%)	High-fat chow (%)
Carbohydrates	72.2	62.4
Fats	3.4	20.2
Proteins	19.8	13.2
Cellulose	3.6	3.2
Vitamins and minerals	0.5	0.5
Salts	0.5	0.5

Sample preparation

Three weeks of the respective diet, the animals were fasted overnight, each rat was anaesthetized with diethyl ether and its blood samples were collected. The serum was prepared by centrifugation and used for biochemical investigation. The rats were then sacrificed by cervical dislocation, the livers rapidly removed, washed in saline, dried on filter paper, weighed, and homogenized in 50 mM phosphate buffer, pH 7.4 using an Ultra-Turrax homogenizer where resultant homogenate (20% w/v) then centrifuged at 3,000 rpm for 15 minutes at 4°C. The resulted supernatant was stored at −80°C for further investigation.

Biochemical investigations

Estimation of BW, blood glucose levels, and liver glycogen content

A weekly body weighting using digital scale, as well as, determination of serum fasting blood glucose levels using enzymatic colorimetric Biodiagnostic kits, Catalog no. GL1320 [20] using rats' tail vein by a portable glucometer (Accu-Chek Active, Roche Diagnostics Ltd., Mannheim, Germany) periodically at day 0, 3, 7, 14, and 21 of diabetes induction were performed. Liver glycogen content was estimated according to the method of Carroll et al. [21] where one gram of liver tissue was boiled in 5 ml 30% potassium hydroxide for 15 minutes, then addition of 5 ml of absolute ethyl alcohol and centrifuged at 3,000 rpm for 10 minutes, discarded the supernatant and dissolved the precipitate in 5 ml distilled water, then, developing color using anthrone reagent (0.05% anthrone, 1% thiourea, and 72% per volume H_2S0_4) measuring absorbance at 610 nm.

Estimation of lipid profiles parameters

Lipid profile assessments were conducted through estimation of serum total cholesterol (TC), low-density lipoprotein (LDL-C), high-density lipoprotein (HDL-C), TG, total lipids (TL), and phospholipids (PL) according to the method of Richmond [22], Wieland and Seidel [23], Burstein et al. [24] and Lopez-Virella et al. [25], Fossati and Prencipe [26], Zöllner and Kirsch [27], Zilversmit et al. [28] and Connerty et al. [29], respectively using kits supplied from Biodiagnostics (Egypt).

Estimation of hepatic functions markers

Investigation of serum aspartate and alanine aminotransferase (AST and ALT), alkaline phosphatase (ALP), and total bilirubin were proceeded according to the method of Reitman and Frankel [30], Bowers et al. [31], and Walters and Gerarde [32], respectively using kits provided from Biodiagnostics (Egypt). Whereas, γ-Glutamyltransferase (GGT) was estimated kinetically according to the method of Persijn and van der Slik [33,34] using kits provided from the Egyptian Company of Biotechnology, Spectrum, Egypt.

Estimation of kidney functions markers

Serum urea was determined colorimetrically according to the method of Fawcett and Scott [35] using kits purchased from Biodiagnostics (Egypt). While, serum creatinine was determined by kinetic analysis according to the method of Bartels et al. [36] using kits purchased from Biodiagnostics, Egypt.

Estimation of oxidative stress markers

The oxidative stress markers hepatic malondialdehyde (MDA), hepatic superoxide dismutase (SOD), and hepatic glutathione peroxidase (GPx) were estimated according to the method of Satoh [37], Nishikimi et al. [38], Paglia and Valentine [39], respectively using kits purchased from Biodiagnostics (Egypt).

Estimation of pro-inflammatory markers

Serum tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) levels were estimated by an enzyme-linked immunoassay (ELISA) kit purchased from Quantikine®, R & D systems (MN, USA).

Estimation of serum adhesion molecules

Serum soluble intercellular adhesion molecule-1 (sICAM-1) and vascular cell adhesion molecule-1 (sVCAM-1) levels were measured by ELISA kit purchased from Quantikine®, R and D systems (MN, USA) and EIAab® (USA), respectively.

Histopathological investigation

Liver and kidney samples were preserved in 10% formalin, while pancreas samples were preserved in Bouin's solution. Samples were molded into paraffin bees wax tissue blocks and cut into 4-μm thickness sections and stained by hematoxylin and eosin stain for routine examination through the light electric microscope, as well as, Masson'strichrome stain for detection of fibrosis and collagen [40].

Statistical analysis

Data were expressed as mean ± standard deviation (SD). Results were analyzed statistically by one-way analysis of variance (ANOVA) followed by post-hoc

Tukey's procedure for comparison of therapeutic group's means. Differences were considered significant at $P < 0.05$ using SPSS (SPSS for windows 7, version 20, Chicago, IL) software.

Results

Phytochemical results

Quantitative estimation of total polyphenolic and flavonoid contents

Total polyphenolics content (expressed as milligram of gallic acid equivalent per milligram of plant extract) was higher for Eth. Ac. Ext (25.73 ± 0.39) compared to Alc. Ext (17.52 ± 0.19). Similar observations were made with total flavonoids content (mg/g extract), in rutin equivalents and found to be 1.09 ± 0.02 for Eth. Ac. Ext and 0.79 ± 0.01 for the Alc. Ext.

Phytochemical identification of the ethyl acetate extract fractions

Seven phenolic compounds were isolated from the Eth. Ac. Ext of *A. fragrantissima*. Three were the flavonoid glycosides apigenin-6-*C*-glucoside, quercetin-3-*O*-galactoside, and apigenin-7-glucoside [41]. Four flavonoid aglycones, luteolin, jaceidin, and scutellarein 6,7-dimethyl ether [41], along with a triterpene, taraxerol acetate, were identified. The compounds were identified on the basis of spectral data (UV, ^{1}H-, and ^{13}C-NMR) and the analytical data were in agreement with those reported in the literature.

Biochemical results

Weight monitoring assessment

After 12 weeks of dietary manipulation (HFD and oral cholesterol), rats showed a significant BW increase when compared to normally fed control group with an increase in weight by 85% of their initial weight, while rats feeding on standard chow only increased by 14.32% of their initial weight. Meanwhile, introduction of Alc. Ext and Eth. Ac. Ext of *A. fragrantissima* along with HFD caused significant reduction when compared with diabetic control feeding on the same high-fat chow mix as shown in Table 2.

Fasting blood glucose (FBG) and liver glucogen content assessment

Table 3 represents FBG levels, where HFD-STZ diabetic rats demonstrated a significant increase in FBG reaching 549.74% when compared to normal control group. Treatment with Alc. Ext and Eth. Ac. Ext of *A. fragrantissima* and combination of glibenclamide and fenofibrate for 1 week caused significant decrease by 36.62%, 30.24%, and 32.66%, respectively when compared to untreated diabetic rats. While, treatment for 2 weeks by Alc. Ext and Eth. Ac. Ext of *A. fragrantissima* as well as combination of glibenclamide and fenofibrate exhibited significant decrease in blood glucose level by percentage of improvement 75.06%, 40.59%, and 31.62%, respectively when compared to untreated diabetic group. Wheras, after three weeks of treatment by Alc. Ext and Eth. Ac. Ext, as well as the combination of (glibenclamide and fenofibrate) caused significant reductions in fasting blood glucose level by 78.02%, 68.84%, and 70.69%, respectively when compared to untreated diabetic rats. Furthermore, treatment by Alc. Ext and Eth. Ac. Ext caused FBG levels to reach normal value range.

Regarding liver glycogen content, HFD-STZ diabetic rats exhibited a significant decrease in liver glycogen content reaching 51% when compared with normal control. Treatment with Alc. Ext and Eth. Ac. Ext caused significant increase in liver

Table 2. Effect of alcoholic and ethyl acetate extracts of *A. fragrantissima* on BW of HFD-STZ diabetic rats and different therapeutic groups.

Groups	Initial wt.	HFD 12 weeks	Week of treatment		
			The first	The second	The third
Normal control	158.7 ± 3.302	183.25 ± 10.59^{b}	189.38 ± 4.44^{b}	188.12 ± 7.84^{b}	188.13 ± 7.85^{b}
Normal/Alc. Ext	161.30 ± 3.093	183.00 ± 18.85^{b}	181.00 ± 11.30^{b}	186.13 ± 25.73^{b}	187.13 ± 25.73^{b}
Normal/Eth. Ac. Ext	160.90 ± 3.247	183.00 ± 7.01^{b}	187.17 ± 5.60^{b}	185.00 ± 11.34^{b}	182.0 ± 11.34^{b}
HFD-STZ control	159.60 ± 1.265	292.13 ± 38.57^{a}	290.13 ± 38.57^{a}	291.13 ± 38.56^{a}	296.13 ± 38.57^{a}
HFD-STZ/Alc. Ext	160.40 ± 2.951	299.67 ± 7.79^{a}	190.00 ± 40.2^{b}	184.34 ± 38.7^{b}	180.34 ± 38.7^{b}
HFD-STZ/Eth. Ac. Ext	159.45 ± 2.252	290.71 ± 30.77^{a}	226.57 ± 20.88$^{a/b}$	196.861 ± 26.1^{b}	196.87 ± 26.07^{b}
HFD-STZ/Reference drugs	159.78 ± 3.153	293.43 ± 17.57^{a}	202.43 ± 4.58^{b}	197.43 ± 5.01^{b}	195.43 ± 5.01^{b}

Values are expressed as mean ± SD, (n = 10). P-Value < 0.05, Groups superscripted by (a) symbol are significantly different from normal control group, and those superscripted by (b) are significantly different from HFD-STZ control group.

Table 3. Effect of alcoholic and ethyl acetate extracts of *A. Fragrantissima* on fasting blood glucose of HFD-STZ diabetic rats and different therapeutic groups.

Groups	72 hours after STZ injection	Week of treatment		
		The first	The second	The third
Normal control	68.29 ± 2.55[b]	62.29 ± 3.22[b]	67.43 ± 1.94[b]	76.29 ± 3.51[b]
Normal/Alc.Ext	67.15 ± 4.08[b]	69.15 ± 4.08[b]	67.14 ± 5.81[b]	84.57 ± 6.61[b]
Normal/Eth.Ac Ext	59.86 ± 2.62[b]	65.86 ± 4.27[b]	63.86 ± 4.59[b]	68.86 ± 2.84[b]
HFD-STZ control	443.71 ± 18.65[a]	396.7 ± 18.35[a]	410.71 ± 15.18[a]	395.73 ± 16.34[a]
HFD-STZ/Alc. Ext	432.0 ± 25.22[a]	251.43 ± 16.4[a/b]	102.43 ± 14.11[b]	87.00 ± 4.46[b]
HFD-STZ/Eth. Ac. Ext	465.29 ± 7.63[a]	276.72 ± 21.9[a/b]	244.0 ± 17.05[a/b]	123.29 ± 17.67[a/b]
HFD-STZ/Reference drugs	444.57 ± 18.58[a]	267.15 ± 30.18[a/b]	280.86 ± 7.17[a/b]	116.0 ± 5.33[b]

Values are expressed as mean ± SD, (n = 10). P-Value < 0.05 ,Groups superscripted by (a) symbol are significantly different from normal control group, and those superscripted by (b) are significantly different from HFD-STZ control group.

Table 4. Effect of alcoholic and ethyl acetate extracts of *A. fragrantissima* on liver glycogen content of HFD-STZ diabetic rats and different therapeutic groups.

Groups	Glycogen (mg/g tissue)
Normal control	8.76 ± 0.45[b]
Normal/Alc. Ext	7.54 ± 1.35[b]
Normal/Eth. Ac. Ext	7.95 ± 1.06[b]
HFD-STZ control	4.27 ± 0.14[a]
HFD-STZ/Alc. Ext	6.18 ± 0.37[a/b]
HFD-STZ/Eth. Ac. Ext	6.19 ± 0.13[a/b]
HFD-STZ/Reference drugs	5.35 ± 1.22[a]

Values are expressed as mean ± SD, (n = 10). P-Value < 0.05,Groups superscripted by (a) symbol are significantly different from normal control group, and those superscripted by (b) are significantly different from HFD-STZ control group.

glycogen content with 44.76% and 44.96%, respectively when compared with untreated diabetic group. Meanwhile, combination of glibenclamide and fenofibrate caused insignificant increase in liver glycogen content as illustrated in Table 4.

Lipid profile assessment

Serum lipids profile investigation as shown in Table 5 revealed that HFD-STZ diabetic group exhibited significant elevation in serum TC, LDL-C, TG, TL, PL reaching 169.22%, 761.09%, 239.00%, 177.35%, and 157.53%, respectively and significant reduction by a value of 49.97% in serum HDL-C when compared to the normal control group. Treatment of HFD-STZ diabetic rats with Alc. Ext and Eth. Ac. Ext caused significant decrease in values of serum TC, LDL-C, TG, TL, and PL by 50.13%, 63.78%, 63.37%, 50.32%, and 50.00%, respectively for Alc. Ext; 47.10%, 63.61%, 64.67%, 52.03%, and 46.81%, respectively for Eth. Ac. Ext and when compared to untreated diabetic group. Concerning HDL, treatment by Eth. Ac Ext caused significant increase by the value 42.43%; while treatment with alcoholic extract of *A. fragrantissima* and reference drug combination (glibenclamide and fenofibrate) caused insignificant increase in serum HDL when compared to untreated diabetic group.

Liver functions assessment

Regarding liver functions assessment, as shown in Table 6, it is shown that HFD-STZ induced diabetic rats exhibited significant elevation in hepatic functions parameters ALT, AST, ALP, GGT, and total bilirubin reaching to 171.48%, 76.69%, 145.22%, 435.75%, and 65.62%, respectively when compared to the normal control levels. Treatment with Alc. Ext and Eth. Ac. Ext deduced significant reduction in ALT,

Table 5. Effect of alcoholic and ethyl acetate extracts of *A. fragrantissima*on lipid profile parameters of HFD-STZ diabetic rats and different therapeutic groups.

Groups	TC (mg/dl)	LDL-C (mg/dl)	HDL-C (mg/dl)	TG (mg/dl)	TL (mg/dl)	PL (mg/dl)
Normal control	88.10 ± 5.01[b]	12.31 ± 1.89[b]	75.38 ± 6.3[b]	31.38 ± 6.79[b]	209.13 ± 18[b]	91.25 ± 5.72[b]
Normal/Alc. Ext	87.13 ± 1.33[b]	17.90 ± 1.18[b]	64.75 ± 6.15[b]	24.67 ± 1.79[b]	195.03 ± 12.3[b]	86.67 ± 4.08[b]
Normal/Eth. Ac. Ext	85.50 ± 1.52[b]	18.96 ± 2.49[b]	62.59 ± 3.20[b]	24.67 ± 1.79[b]	195.03 ± 12.3[b]	85.07 ± 3.08[b]
HFD-STZ control	237.19 ± 4.76[a]	106. 0 ± 9.86[a]	37.72 ± 2.55[a]	106.38 ± 6.36[a]	580 ± 49.14[a]	235.0 ± 11.95[a]
HFD-STZ/Alc. Ext	118.28 ± 11.8[a/b]	62.06 ± 5.73[a/b]	43.40 ± 2.0[a]	38.97 ± 8.69[b]	288.13 ± 24[a/b]	117.5 ± 3.89[a/b]
HFD-STZ/Eth. Ac. Ext	125.47 ± 14.7[a/b]	62.35 ± 3.99[a/b]	53.52 ± 2.53[a/b]	37.59 ± 6.29[b]	278.3 ± 14.3[a/b]	125.00 ± 4.56[a/b]
HFD-STZ/Reference drugs	94.69 ± 11.97[b]	39.92 ± 4.02[a/b]	46.05 ± 2.32[a]	60.90 ± 6.93[a/b]	265.63 ± 22.1[a/b]	100.0 ± 5.98[b]

Values are expressed as mean ± SD, (n = 10). P-Value < 0.05,Groups superscripted by (a) symbol are significantly different from normal control group, and those superscripted by (b) are significantly different from HFD-STZ control group.

Table 6. Effect of alcoholic and ethyl acetate extracts of *A. fragrantissima* on liver functions of HFD-STZ diabetic rats and different therapeutic groups.

	AST (U/L)	ALT (U/L)	ALP (U/L)	GGT (U/L)	Total bilirubin (mg/dl)
Normal control	35.10 ± 1.99[b]	34.71 ± 3.65[b]	32.81 ± 2.62[b]	8.97 ± 1.21[b]	0.63 ± 0.07[b]
Normal/Alc. Ext	42.40 ± 4.02[b]	41.54 ± 2.85[b]	32.11 ± 6.0[b]	12.84 ± 2.3[b]	0.63 ± 0.069[b]
Normal/Eth. Ac. Ext	41.28 ± 9.1[b]	37.64 ± 1.39[b]	33.91 ± 2.21[b]	12.1 ± 2.12[b]	0.65 ± 0.03[b]
HFD-STZ control	95.27 ± 7.89[a]	61.34 ± 5.37[a]	80.46 ± 4.09[a]	48.05 ± 7.07[a]	1.05 ± 0.17[a]
HFD-STZ/Alc. Ext	65.46 ± 1.99[a/b]	49.33 ± 5.89[a/b]	41.74 ± 3.28[a/b]	28.44 ± 2.47[a/b]	0.73 ± 0.15[b]
HFD-STZ/Eth. Ac. Ext	77.82 ± 1.92[a/b]	50.95 ± 6.20[a/b]	43.73 ± 2.40[a/b]	31.85 ± 3.095[a/b]	0.80 ± 0.04[a/b]
HFD-STZ/Reference drugs	76.14 ± 2.95[a/b]	54.53 ± 1.96[a/b]	47.32 ± 2.79[a/b]	27.50 ± 6.62[a/b]	0.84 ± 0.11[a/b]

Values are expressed as mean ± SD, (n = 10). P-Value < 0.05, Groups superscripted by (a) symbol are significantly different from normal control group, and those superscripted by (b) are significantly different from HFD-STZ control group.

AST, ALP, GGT, and total bilirubin values by 31.28%, 19.51%, 48.13%, 40.83%, and 30.33%, respectively for treatment with Alc. Ext and 18.30%, 16.86%, 45.65%, 33.74%, 24.00%, and 21.63%, respectively for Eth. Ac. Ext group when compared with untreated diabetic group.

Kidney functions assessment

HFD-STZ induced diabetic rats suffered a significant elevation in serum urea and creatinine levels as shown in Table 7 which reached 182.24% and 124.25% respectively, when compared with normal negative control. Treatment with Alc. Ext and Eth. Ac. Ext caused significant decrease in serum urea and creatinine values, this decrease reached 48.34% and 32.85%, respectively for Alc. Ext and 48.34% and 45.76%, respectively for Eth. Ac. Ext when compared with untreated diabetic control group.

Oxidative stress assessment

Untreated diabetic rats suffered a significant increase in hepatic MDA this increase reached a percentage of 202.08%. However, a significant reduction in both hepatic GPx and SOD occurred by percentage of 28.26% and 80.76%, respectively when compared with normal control. Treatment of HFD-STZ induced diabetic rats with Alc. Ext and Eth. Ac. Ext caused a significant decrease in MDA by the value of 47.50% and 44.41%, respectively as shown in Figure 2. Furthermore, as demonstrated upon treatment, Alc. Ext and Eth. Ac. Ext shows significant elevation in GPx levels occurred by value of 21.07% and 32.2%, respectively and a significant increase in SOD levels by 156.87% and 135.30%, respectively when compared to untreated diabetic group as shown in Figures 1–3.

Pro-inflammatory markers assessment

HFD-STZ diabetic control rats illustrated significant increase in serum TNF-α and IL-6; with a percentage 86.77% and 34.18%, respectively when compared with normal control values. Treatment of HFD-STZ diabetic by Alc. Ext and Eth. Ac. Ext caused

Table 7. Effect of alcoholic and ethyl acetate extracts of *A. fragrantissima* on kidney functions of HFD-STZ diabetic rats and different therapeutic groups.

Groups	Serum urea (mg/dl)	Serum creatinine (mg/dl)
Normal control	35.09 ± 3.7[b]	0.54 ± 0.03[b]
Normal/Alc. Ext	36.52 ± 1.62[b]	0.53 ± 0.03[b]
Normal/Eth. Ac. Ext	36.26 ± 2.52[b]	0.50 ± 0.07[b]
HFD-STZ control	99.12 ± 3.45[a]	1.21 ± 0.34[a]
HFD-STZ/Alc. Ext	51.21 ± 4.16[a/b]	0.81 ± 0.07[a/b]
HFD-STZ/Eth. Ac. Ext	51.75 ± 4.45[a/b]	0.66 ± 0.10[b]
HFD-STZ/Reference drugs	48.03 ± 6.74[a/b]	0.63 ± 0.07[b]

Values are expressed as mean ± SD, (n = 10). P-Value < 0.05 is considered significant using one way ANOVA with post-hoc Tukey's procedure. Groups denoted by (a) symbol are significantly different from normal control group, while those denoted by (b) are significantly different from HFD-STZ control group.

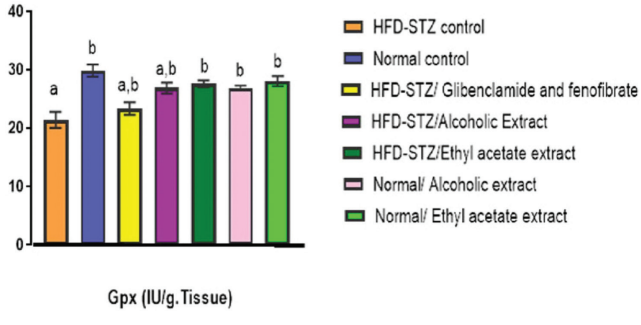

Figure 1. Therapeutic effects of *A. fragrantissima* extracts on Glutathione peroxidase levels. Values are expressed as Mean ± SD, (n = 10). P-value < 0.05 is considered significant using using one way ANOVA with post hoc tukey's procedure. Groups denoted by (a) symbol are significantly different from normal control group, while those denoted by (b) are significantly different from HFD-STZ control group.

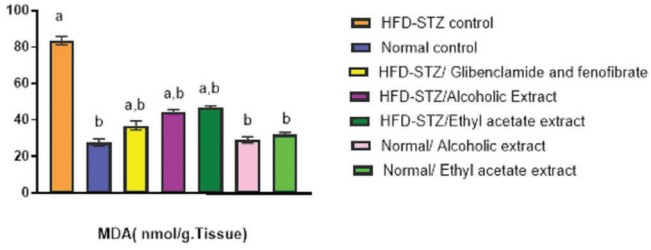

Figure 2. Therapeutic effects of *A. fragrantissima* extracts on Malondialdehyde levels. Values are expressed as Mean ± SD, (n = 10). P-value < 0.05 is considered significant using using one way ANOVA with post hoc tukey's procedure. Groups denoted by (a) symbol are significantly different from normal control group, while those denoted by (b) are significantly different from HFD-STZ control group.

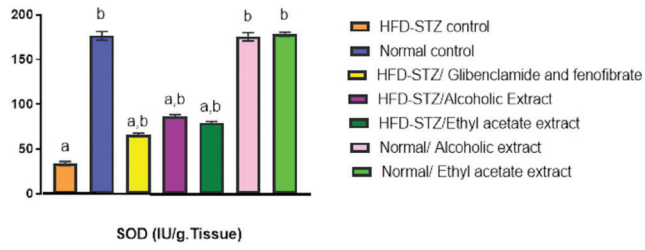

Figure 3. Therapeutic effects of *A. fragrantissima* extracts on superoxide dismutase levels. Values are expressed as Mean ± SD, (n = 10). P-value < 0.05 is considered significant using using one way ANOVA with post hoc tukey's procedure. Groups denoted by (a) symbol are significantly different from normal control group, while those denoted by (b) are significantly different from HFD-STZ control group.

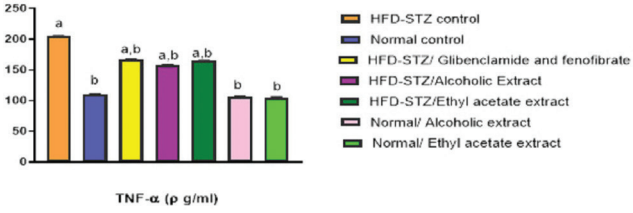

Figure 4. Therapeutic effects of *A. fragrantissima* extracts on Tumor necrosis factor alpha levels. Values are expressed as Mean ± SD, (n = 10). P-Value < 0.05 is considered significant using using one way ANOVA with post hoc tukey's procedure. Groups denoted by (a) symbol are significantly different from normal control group, while those denoted by (b) are significantly different from HFD-STZ control group.

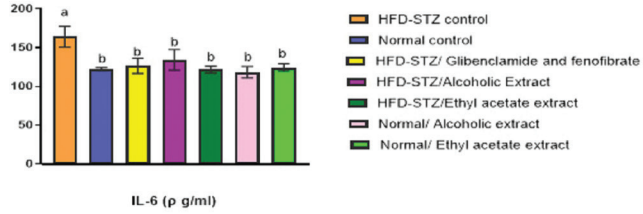

Figure 5. Therapeutic effects of *A. fragrantissima* extracts on IL 6 levels. Values are expressed as Mean ± SD, (n = 10). P-Value < 0.05 is considered significant using using one way ANOVA with post hoc tukey's procedure. Groups denoted by (a) symbol are significantly different from normal control group, while those denoted by (b) are significantly different from HFD-STZ control group.

significant reduction in serum TNF-α and IL-6, with percent change of 23.32% and 17.66%, respectively for alcoholic extract and 19.66% and 25.80%, respectively for Eth. Ac. Ext when compared to HFD-STZ induced diabetic control rat as recorded in Figures 4 and 5.

Atherosclerosis indicators assessment

HFD-STZ diabetic control group rats suffered a significant increase in sICAM-1 and sVCAM-1 as demonstrated in Figures 6 and 7, this increase reached 205.09%, 133.96%, and 36.63%, respectively when compared with normal control levels. Eth. Ac. Ext. treatment caused a significant reduction in sICAM-1 and sVCAM-1 levels by a percentage of 43.37% and 19.71%, respectively, while treatment by alcoholic extract of *A. fragrantissiman* recorded a significant reduction in VCAM-1 by 5.26%, while an insignificant decrease in sICAM-1 when compared with untreated HFD-STZ induced diabetic control.

Histopathological results

Pancreatic sections of normally fed non-diabetic rats when given Alc. Ext and Eth. Ac. Ext showed no histopathological alteration as recorded in Figure 8B and C in comparison to normal control group that showed no histological alteration and the normal histological structure of the islets of Langerhans cells as the endocrine portion as well as the acini of the exocrine portion as shown in Figure 8A. Meanwhile, HFD-STZ induced diabetic rat's pancreas sections showed degeneration and atrophy in the islets of Langerhans cells (Fig. 8D). However, HFD-STZ diabetic rats treated by alcoholic extract of *A. fragrantissima* demonstrated atrophy in the islets of Langerhans cells (Fig. 8E). Fortunately, HFD-STZ diabetic rats treated by Eth. Ac. Ext of *A. fragrantissima* showed some improvement, as it was found that the islets of Langerhans cells showed moderate degeneration and atrophy with congestion in the blood vessels (Fig. 8F). While, pancreas sections of HFD-STZ induced diabetic rats treated by the drug

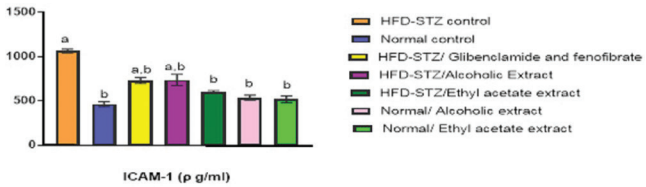

Figure 6. Therapeutic effects of *A. fragrantissima* extracts on Intercellular Adhesion Molecule 1 levels. Values are expressed as Mean ± SD, (n = 10). P-value < 0.05 is considered significant using using one way ANOVA with post hoc tukey's procedure. Groups denoted by (a) symbol are significantly different from normal control group, while those denoted by (b) are significantly different from HFD-STZ control group.

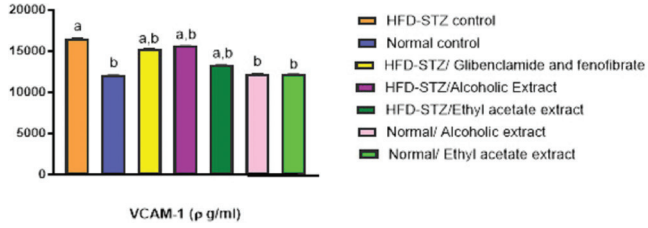

Figure 7. Therapeutic effects of *A. fragrantissima* extracts on vascular cell adhesion molecule 1 levels. Values are expressed as Mean ± SD, (n = 10). P-Value < 0.05 is considered significant using using one way ANOVA with post hoc tukey's procedure. Groups denoted by (a) symbol are significantly different from normal control group, while those denoted by (b) are significantly different from HFD-STZ control group.

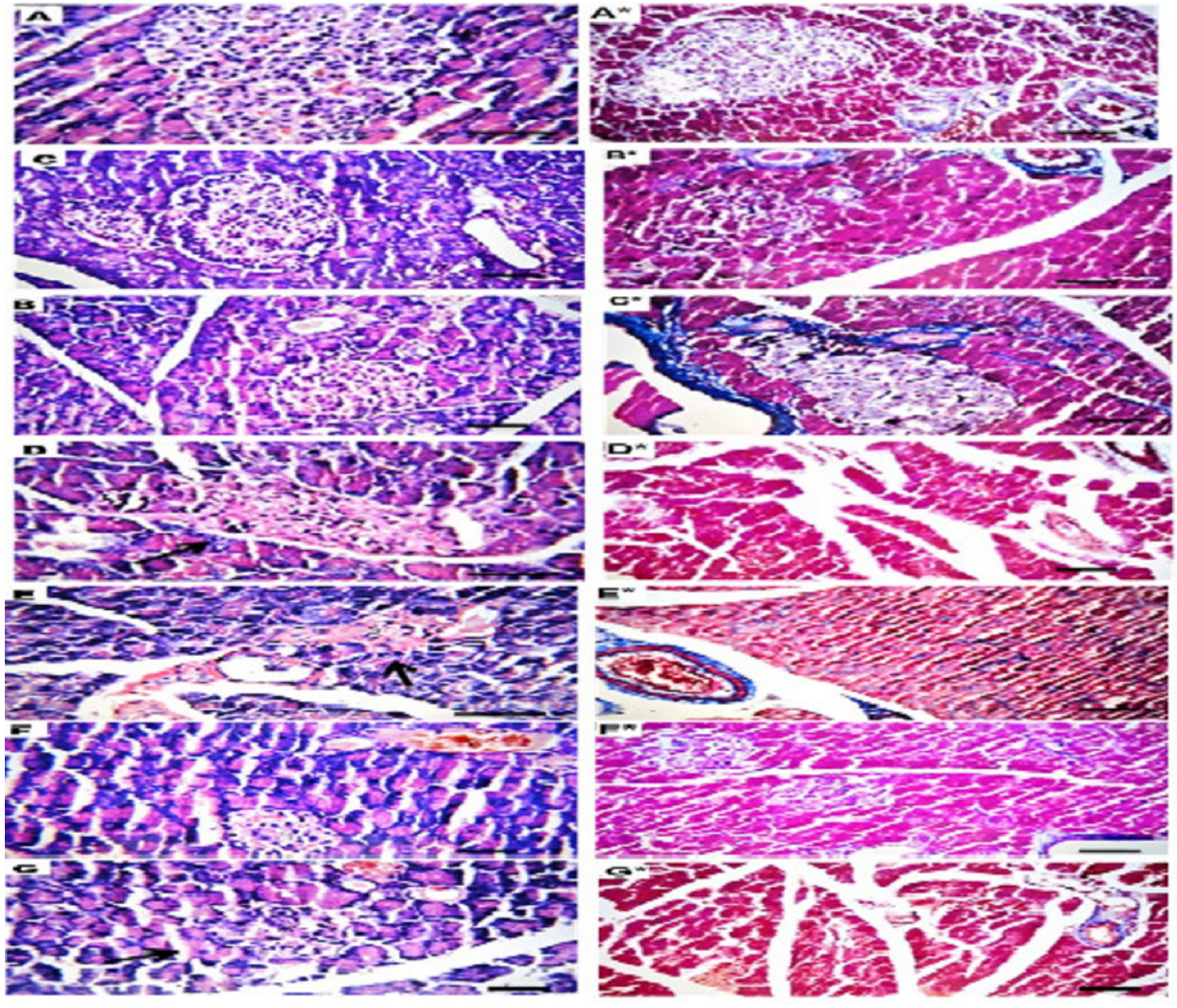

Figure 8. Effect of *A. fragrantissima* extracts on pancreatic tissue histology in HFD-STZ induced diabetic rats and other therapeutic group. (A) Normal control, (B) normal/alcoholic extract, (C) normal/ethyl acetate extract, (D) HFD-STZ induced diabetic, (E) HFD-STZ/alcoholic extract, (F) HFD-STZ/ethyl acetate extract, and (G) HFD-STZ/glibenclamide and fenofibrate; Where each group denoted with a letter only for (H & E) stained slides and those stained with Masson's trichome are denoted with a letter accompanied with (*), scale bar = 200 µm.

combination of fenofibrate and glibenclamide showed atrophy in the islets of Langerhans cells (Fig. 8G).

Liver sections in normal control group showed no histological alteration and normal histological structure of the central vein and surrounding hepatocytes were recorded in (H & E) stained slides as shown in Figure 9A and Masson'strichome stained slides (Fig. 9A*); as well as, liver sections of normally fed non-diabetic rats given Alc. Ext and Eth. Ac. Ext showed no histological alterations as recorded in Figure 9B and B* and Figure 9C and C*. HFD-STZ diabetic rats liver sections demonstrated sever dilatation and congestion in the central vein as well as the portal veins associated with degeneration in the hepatocytes surrounding and adjacent the first one, while the inflammatory cells infiltration was detected in the portal area (Fig. 9D); while in the slides stained with Masson'strichome stained increased collagen depositions as it developed mild fibrosis in the portal area (Fig. 9D*). Treatment of diabetic rats by alcoholic extract of *A. fragrantissima* deduced slight improvement in liver sections, where it less congestion in the portal veins associated with less oedema in the portal area (Fig. 9E and E*). While treatment of diabetic rats by ethyl extract of *A. fragrantissima* caused a noticeable improvement in liver sections was recorded in Figure 9F, where less dilatation and congestion of the portal veins and less deposition of collagen as shown in Figure 9F*. Treatment by drug combination of glibenclamide and fenofibrate caused diminished improvement in liver sections, where there was congestion in the central and portal veins associated with oedema and mild fibrosis in the portal area (Fig. 9G and G*).

HFD-STZ induced diabetic kidney sections demonstrated degeneration in the lining epithelium in some of cortical tubules and necrosis with congestion in the glomeruli. Focal hemorrhage was noticed in between the degenerated tubules at the corticomedullary portion Figure 10D. HFD-STZ induced diabetic rats treated by alcoholic extract of *A. fragrantissima* deduced minor improvement in kidney sections, where it still showed degenerative change and necrosis in the lining epithelium of some few tubules at the cortex Figure 10E. While treatment of HFD-STZ induced diabetic rats by ethyl extract of *A. fragrantissima* demonstrated improvement in kidney sections, where no histopathological alteration was detected when compared to normal control sections as in Figure 10F. Kidney sections of HFD-STZ induced diabetic rats treated by drug combination of glibenclamide and fenofibrate showed congestion in the cortical blood vessels and glomeruli Figure 10G.

Discussion

Obesity and hyperlipidemia are considered to be one of the epidemics due to processed foods consumption and non-active life style [42]. The current study simulated metabolic syndrome through association between hyperlipidemic state accompanied with hyperglycemia (T2DM) propagated due to dietary manipulation through HFD followed by moderate destruction of β-cells by inflammation due to low dose of STZ, which was in agreement with the findings of Hwang et al. [43]. After treatment for three weeks with Alc. Ext and Eth. Ac. Ext of *A. fragrantissima,* FBG levels were significantly reduced and even surpassed the hypoglycemic effect recorded after treatment by glibenclamide. Even though, it was recorded that fenofibrate enhances glibenclamide's hypoglycemic effect due to inhibition of cytochrome *p*-450 isoforms responsible for the metabolism of glibenclamide [11].

A present data indicated that the Eth. Ac. Ext has more activity against oxidative stress parameters than the Alc. Ext. The activity may be related to the presence of various classes of phenolic compounds of flavones and flavonols classes that were present in the two extracts, but in different amounts. In our present work, three flavones derivatives luteolin, apigenin-6-C-glucoside, and cosmosiin (apigenin 7-glucoside) were isolated and identified from the Eth. Ac Ext of *A. fragrantissima*. Numerous reports showed that apigenin and other flavones exhibited different properties, such as anti-inflammatory, anti-oxidative, and antihepatotoxic properties and the hypolipidemic and antidiabetic activities of these flavones are likely due to their properties [44]. Structure–activity relationship study has revealed that the specific structure of flavones with the presence of the 3′- and 4′-OH groups in the B-ring and a double bond between C-2 and C-3 are important factors for their recognition and binding by glycogen phosphorylase (GP). Flavones inhibited dephosphorylated GP, and they could have the potential to contribute to the protection or improvement of control of T2DM [13]. Quercetin was found to inhibit hepatic cholesterol biosynthesis *in vitro* and to have a hypocholesterolemic effect *in vivo* [44].

The susceptibility to free radical attack and harmful effect on liver function might be done with the excessive storage of fat in the liver and the diabetic

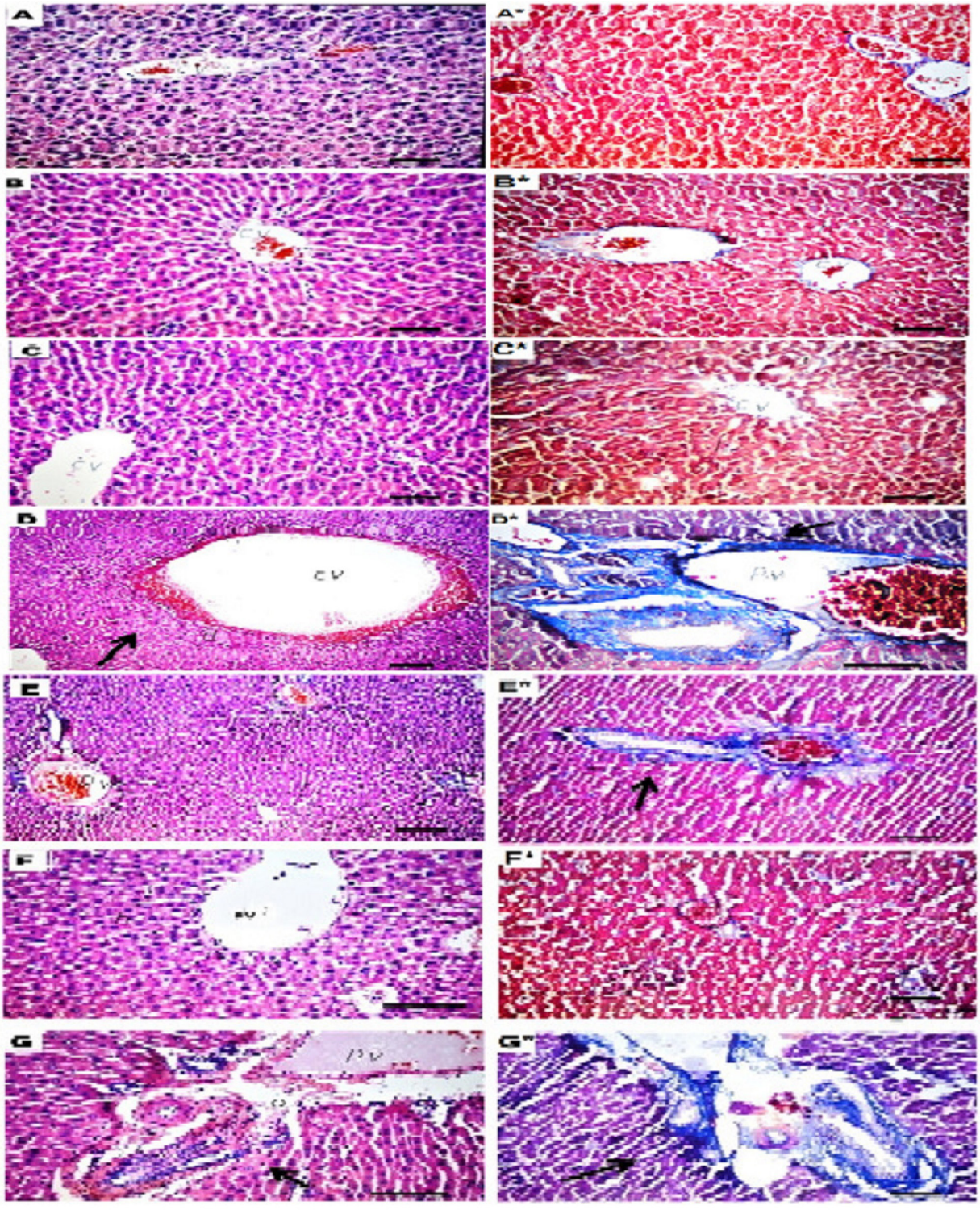

Figure 9. Effect of *A. fragrantissima* extracts on hepatic tissue histology in HFD-STZ induced diabetic rats and other therapeutic group. (A) Normal control, (B) normal/alcoholic extract, (C) normal/ethyl acetate extract, (D) HFD-STZ induced diabetic, (E) HFD-STZ/alcoholic extract, (F) HFD-STZ/ethyl acetate extract, and (G) HFD-STZ/glibenclamide and fenofibrate; Where each group denoted with a letter only for (H & E) stained slides and those stained with Masson's trichome are denoted with a letter accompanied with (*), scale bar = 200 μm.

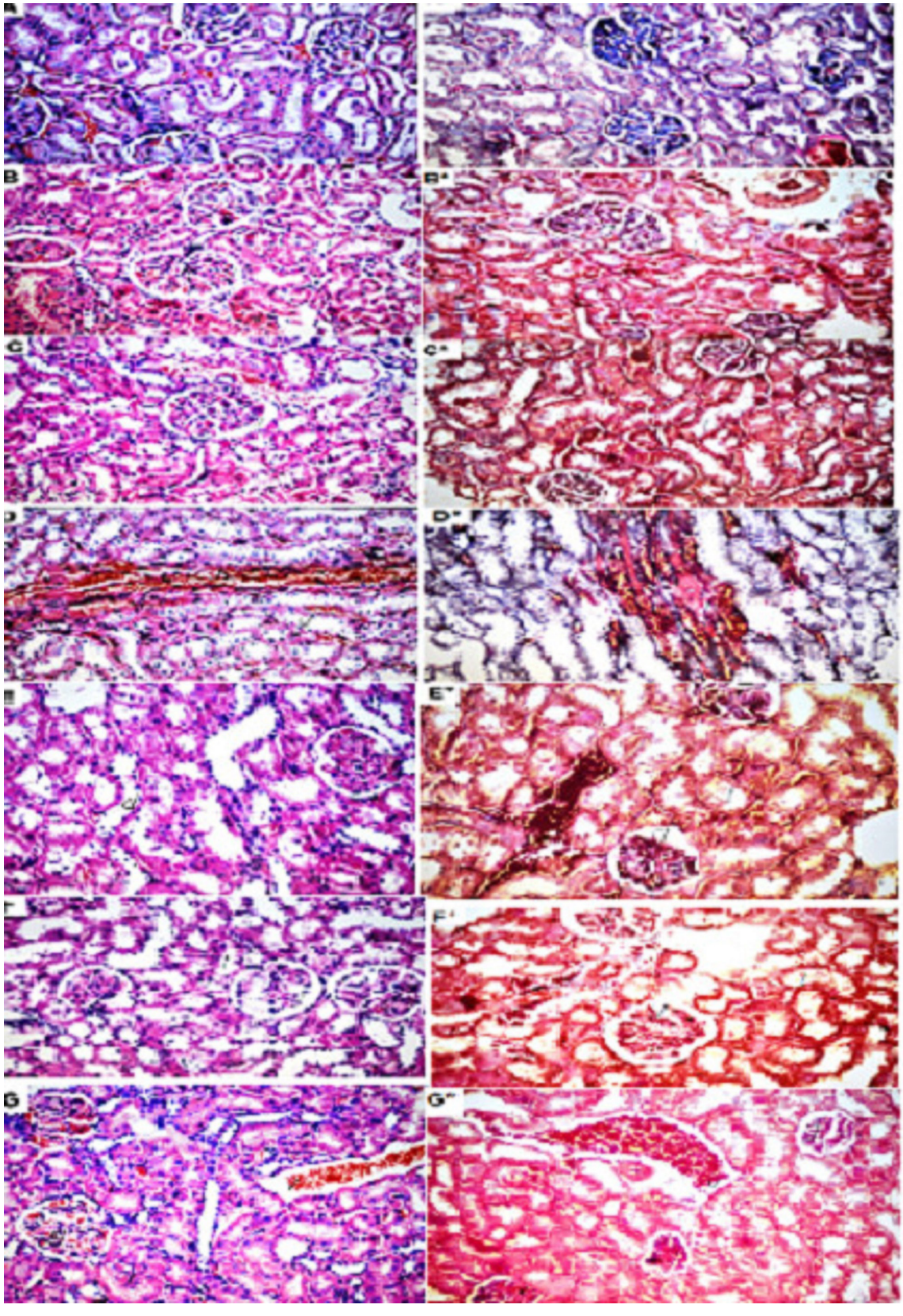

Figure 10. Effect of *A. fragrantissima* extracts on kidney tissue histology in HFD-STZ induced diabetic rats and other therapeutic group. (A) Normal control, (B) normal/alcoholic extract, (C) normal/ethyl acetate extract, (D) HFD-STZ induced diabetic, (E) HFD-STZ/alcoholic extract, (F) HFD-STZ/ethyl acetate extract, and (G) HFD-STZ/glibenclamide and fenofibrate; Where each group denoted with a letter only for (H & E) stained slides and those stained with Masson's trichome are denoted with a letter accompanied with (*), scale bar = 200 μm.

state. Treatment with the Eth. Ac. Ext of *A. fragrantissima*, containing the flavones aglycone luteolin and the glycosides apigenin-6-C-glucoside and cosmosiin, may prevent oxidative damage by detoxifying reactive oxygen species (ROS); thus, reducing hyperlipidemia [45] with a concomitant decrease in ALT, AST, and ALP enzyme activities. The decreased ALT and AST enzyme activities in serum, as a result of the treatment with the Alc. Ext and Eth. Ac. Ext, might be ascribed to the ability of phenolic compounds to maintain membrane integrity extract. Other flavonoids were also reported, such as afroside, cirsimartin, chrysoplenol, and cirsiliol [3]. *A. fragrantissima* tannin content reaches 8% such as resorcin, phloroglucin, methyl phloroglucin, and pyrocatechol. High percentage of tannins may have reduced lipid peroxidation by acting as antioxidants, and hence, aiding the endogenous antioxidant enzymes involved in the scavenging inactivation of the ROS or redox metal ions before lipid peroxidation takes place.

Also, *A. fragrantissima* showed a significant α-glucosidase inhibitory activity due to the presence of phenolic compounds which were previously isolated from *Achillea* species. This activity may related to these compounds that have the potential of reducing the postprandial glucose and insulin peaks to reach normoglycaemia and delay the absorption of ingested carbohydrates [7], as this pathway may be differed from the regular mechanisms of acarbose and miglitol α-glucosidase inhibitors [46].

A significant reduction in liver glycogen content level was showed in STZ-induced diabetic rats. These results were in consistence with the findings of Mandour [18]. However, treatment by Alc. Ext and Eth. Ac. Ext caused increase in liver glycogen content suggesting increased gluconeogenesis and a decreased glycogenolysis [47]. Structure–activity relationship study has revealed that the present flavones posses special structure concerning the presence of the 3′- and 4′-OH groups in the B-ring and a double bond between C-2 and C-3, which was an important factor for their recognition, binding, and inhibition to GP [48].

In the present study, dietary manipulation caused significant increase in BW associated with hyperlipidemia which was harmony with the findings of Shalaby et al. [12]. Extracts of *A. fragrantissima* caused a significant reduction in BW as well as serum lipid profile parameters (TC, LDL-C, TG, TL, and PL). Additionally, treatment by Eth. Ac. Ext caused significant increase in HDL-C values, this could be attributed to the quercetin which was found to inhibit hepatic cholesterol biosynthesis *in vitro* and to have a hypocholesterolemic effect *in vivo* [44].

Approximately, 70%–80% of T2DM patients suffer from non-alcoholic fatty liver [49], which accounts for 13% probability of hepatocellular carcinoma (HCC) [49]. In the present study, significant increase in ALT, AST, ALP, GGT, and total bilirubin in HFD-STZ diabetic control, while treatment with Alc. Ext and Eth. Ac. Ext caused significant decrease when compared to untreated diabetic rats; this reduction might be ascribed to the ability of phenolic compounds to maintain membrane integrity as well as flavonoids such as afroside, cirsimartin, chrysoplenol, and cirsiliol [50].

Diabetic nephropathy also known as diabetic kidney disease (DKD) is one of the most serious complication of diabetes [51]. Regarding kidney clearance performance and functions in the present work, an elevation in serum urea and creatinine levels was recorded in serum of untreated diabetic group. Treatment with Alc. Ext and Eth. Ac. Ext of *A. fragrantissima* caused significant reduction in serum urea and creatinine levels. This results were in accordance with the results of Mandour [18] who studied the acute and long-term safety evaluation of *A. fragrantissima* consumption.

Meanwhile, concerning oxidative stress state, results of the present study elucidated that the induction of diabetes by HFD and low dose of STZ caused a significant elevation in hepatic MDA. These results were in agreement with the findings of Zheng et al. [52] and Mohamed et al. [53]. A significant decrease in hepatic GPx and SOD suggesting an increase in the surge of ROS produced by CYP2E1 which is an imperative cause of insulin resistance in diabetes and related non-alcoholic fatty liver [54]. Additionally, treatment with Alc. Ext and Eth. Ac. Ext induced significant reduction in hepatic MDA and significant elevation in GPx and SOD levels, predicting the retrieval of oxidative equilibrium state which can be correlated with the essential oil of *Achillea* species, as it can suppress the inflammatory responses of lipopolysaccharides (LPS)-stimulated RAW 264.7 macrophages. This improvement including decreased levels of cellular nitric oxide (NO) and superoxide anion production, lipid peroxidation, and glutathione (GSH) concentration as existing monoterpenes are the most representative metabolites [50].

Insulin resistance and adipose tissue hyperplasia are considered as inflammatory status which

is accompanied with increased pro-inflammatory mediators and cytokines [9]. In the current study, a significant elevation in serum TNF-α and IL-6 levels was recorded in untreated HFD-STZ diabetic group. Meanwhile, treatment with Alc. Ext and Eth. Ac. Ext of *A. fragrantissima* reverted these changes claiming that this plant extract possess an anti-inflammatory activity which have been attributed to its flavonoid content as well as highly oxygenated terpenoids [6,7]. These results came in agreement with another study reported other *Achillea* species has showed anti-inflammatory activities as they inhibit lipopolysaccharide-induced NO production in RAW264.7 macrophage cells [55].

Cardiovascular complications are known to be lethal consequences of T2DM [56]. In the current work, untreated diabetic group exhibited increased adhesion molecule attributed to increased phospholipase activity due to increased vasoconstrictive eicosanoids. These results were consistent with Varga et al. [57]. However, treatment of HFD-STZ induced diabetic rats by Alc. Ext and Eth. Ac. Ext caused reduction in sICAM-1 and sVCAM-1 values, suggesting that this plant extracts could have counteracted to some extent the cardiovascular repercussion evolved due to metabolic disorder.

Regarding histopathological examination, HFD-STZ diabetic pancreatic tissue sections showed degeneration and atrophy in the islets of Langerhans cells and β-cells accompanied with congestion in the blood vessels. These observations were in agreement with the findings of Ding et al. [58]. Meanwhile, treatment with alcoholic extract of *A. fragrantissima* and combination drugs of glibenclamide and fenofibrate caused moderate improvement, while treatment with Eth. Ac. Ext caused remarkable amelioration. Moreover, examination of liver tissue showed that HFD-STZ untreated diabetic group suffered severe dilatation and congestion of portal and central vein as well as showing mild fibrosis in the portal area which was clearly observed in slides stained by Masson'strichome accompanied with loss of the hepatic lobular architecture. These findings were in agreement with the results of Shalaby et al. [48]. Furthermore, treatment by *A. fragrantissima* Alc. Ext and Eth. Ac. Ext caused reverting of some of these changes. Also, the histopathological examination of untreated diabetic kidney tissue showed degeneration in lining epithelium in some of cortical tubules and necrosis with congestion in the glomeruli accompanied with hemorrhage in the corticomedullary portion. This observation was in harmony with observations of Suman et al. [59]. However, treatment with alcoholic and ethyl acetate extracts of *A. fragrantissima* showed minor degeneration in epithelium of some cortical tubules in alcoholic extract group and even a nearly similar to normal renal architecture in Eth. Ac. Ext treated rats.

Conclusion

Results of current study suggest that Alc. Ext and Eth. Ac. Ext of *A. fragrantissima* showed a very promising anti-diabetic, hypolipidemic, anti-inflammatory, and antioxidant activity. Both extracts reduced and may even diminished most of the changes deduced through simulation of T2DM and metabolic syndrome pathological condition which exhibited altered blood glucose level, serum lipid profile, liver, and kidney functions parameters as well as inflammatory, oxidative stress, and vasoconstriction biomarkers. These findings were in agreement with the results of histopathological examinations of pancreas, liver, and kidney tissues. These findings have confirmed the traditional uses of the plant under investigation. However, further clinical investigations should be carried out to encourage using *A. fragrantissima* as a candidate nutraceutical for ameliorating metabolic disturbances associated with diabetes.

Funding

This work was funded by the National Research Center, Cairo, Egypt.

References

[1] Kaur J. A comprehensive review on metabolic syndrome. Cardiol Res Pract 2014; 2014:943162.

[2] IDF Diabetes Atlas. IDF diabetes atlas. 7th edition. Brussels, Belgium: International Diabetes Federattion, 2015.

[3] Mustafa Eh, Abu Zarga M, Abdalla S. Effects of cirsiliol, a flavone isolated from *Achillea Fragrantissima*, on rat isolated ileum. Gen Pharmacol 1992; 23:555–60.

[4] Ali-Shtayeh Ms, Yaniv Z, Mahajna J. Ethnobotanical survey in the Palestinian area: a classification of the healing potential of medicinal plants. J Ethnopharmacol 2000; 73:221–32.

[5] Eissa TA, Palomino OM, Carretero ME, Gómez-Serranillos MP. Ethnopharmacological study of medicinal plants used in the treatment of CNS disorders in Sinai Peninsula, Egypt. J Ethnopharmacol 2014; 151:317–32.

[6] Daur I. Chemical composition of selected Saudi medicinal plants. Arab J Chem 2015; 8:329–32.

[7] Ezzat SM, Salama MM. A new A-glucosidase inhibitor from *Achillea Fragrantissima* (Forssk.) Sch. Bip. growing in Egypt. Nat Prod Res 2014; 28:812–8.
[8] Reilly SM, Saltiel AR. Adapting to obesity with adipose tissue inflammation. Nat Rev Endocrinol 2017; 13:633–43.
[9] Capurso C, Capurso A. From excess adiposity to insulin resistance: the role of free fatty acids. Vascul Pharmacol 2012; 57:91–7.
[10] Srinivasan K, Viswanad B, Asrat L, Kaul CL, Ramarao P. Combination of high-fat diet-fed and low-dose streptozotocin-treated rat: a model for type 2 diabetes and pharmacological screening. Pharmacol Res 2005; 52:313–20.
[11] Candasamy M, Murthy TE, Gubiyappa K, Chellappan D, Gupta G. Alteration of glucose lowering effect of glibenclamide on single and multiple treatments with fenofibrate in experimental rats and rabbit models. J Basic Clin Pharm 2014; 5:62.
[12] Sellappan S, Akoh CC. Flavonoids and antioxidant capacity of Georgia-Grown Vidalia onions. J Agric Food Chem 2002; 50:5338–42.
[13] Alali FQ, Tawaha K, El-Elimat T, Syouf M, El-Fayad M, Abulaila K, et al. Antioxidant activity and total phenolic content of aqueous and methanolic extracts of jordanian plants: an ICBG project. Nat Prod Res 2007; 21:1121–31.
[14] Nikolova M. Screening of radical scavenging activity and polyphenol content of Bulgarian plant species. Pharmacogn Res 2011; 3:256–9.
[15] Council of National Research. Guide for the care and use of laboratory animals. Washington, DC: National Academies Press, 2010.
[16] Islam MS, Choi H, Loots DT. Effects of dietary onion (Allium cepa) in a high-fat diet streptozotocin-induced diabetes rodent model. Ann Nutr Metab 2008; 53:6–12.
[17] Rizk MZ, El-Sherbiny M, Borai IH, Ezz MK, Aly HF, Matloub AA, et al. Sulphated polysaccharides (SPS) from the green alga ulva fasciata extract modulates liver and kidney function in high fat diet-induced hypercholesterolemic rats. Int J Pharm Pharm Sci 2016; 8:43–55.
[18] Mandour MA, Al-Shami SA, Al-Eknah MM, Hussein YA, El-Ashmawy IM. The acute and long-term safety evaluation of aqueous, methanolic and ethanolic extracts of *Achillea Fragrantissima*. Afr J Pharm 2013; 7:2282–90.
[19] Sun B, Xie Y, Jiang J, Wang Y, Xu X, Zhao C, et al. Pleiotropic effects of fenofibrate therapy on rats with hypertriglycemia. Lipids Health Dis 2015; 14:27.
[20] Trinder P. Determination of blood glucose using an oxidase-peroxidase system with a non-carcinogenic chromogen. J Clin Pathol 1969; 22:158–61.
[21] Carroll NV, Longley RW, Roe JH. The determination of glycogen in liver and muscle by use of anthrone reagent. J Biol Chem 1956; 220:583–93.
[22] Richmond W. Preparation and properties of a cholesterol oxidase from Nocardia Sp. and its application to the enzymatic assay of total cholesterol in serum. Clin Chem 1973; 19:1350–6.
[23] Wieland H, Seidel D. A simple specific method for precipitation of low density lipoproteins. J Lipid Res 1983; 24:904–9.
[24] Burstein M, Scholnick HR, Morfin R. Rapid method for the isolation of lipoproteins from human serum by precipitation with polyanions. J Lipid Res 1970; 11:583–95.
[25] Lopes-Virella MF, Stone P, Ellis S, Colwell JA. Cholesterol determination in high-density lipoproteins separated by three different methods. Clin Chem 1977; 23:882–4.
[26] Fossati P, Prencipe L. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. Clin Chem 1982; 28:2077–80.
[27] Zöllner N, Kirsch K. Über die quantitative Bestimmung von Lipoiden (Mikromethode) mittels der vielen natürlichen Lipoiden (allen bekannten Plasmalipoiden) gemeinsamen Sulfophosphovanillin-Reaktion. Z Für Gesamte Exp Med 1962; 135:545–61.
[28] Zilversmit DB, Davis AK. Microdetermination of plasma phospholipids by trichloroacetic acid precipitation. J Lab Clin Med 1950; 35:155–60.
[29] Connerty HV, Briggs AR, Eaton EHJ. Simplified determination of the lipid components of blood serum. Clin Chem 1961; 7:37–53.
[30] Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am J Clin Pathol 1957; 28:56–63.
[31] Bowers GN Jr, Mccomb RB, Kelley ML. Measurement of total alkaline phosphatase activity in human serum. Sel Meth Clin Chem 1977; 8:31–9.
[32] Walters MI, Gerarde HW. An ultramicromethod for the determination of conjugated and total bilirubin in serum or plasma. Microchem J 1970; 15:231–43.
[33] Shaw LM, Stromme JH, London JL, Theodorsen L. International Federation of Clinical Chemistry. Scientific committee, analytical section. Expert panel on enzymes. IFCC methods for measurement of enzymes. Clin Chem 1983; 135:315f–38f.
[34] Persijn JP, van der Slik W. A new method for the determination of gamma-glutamyltransferase in serum. J Clin Chem Clin Biochem 1976; 14:421–7.
[35] Fawcett JK, Scott JE. A rapid and precise method for the determination of Urea. J Clin Pathol 1960; 13:156–9.
[36] Bartels H, Bohmer M, Heierli C. Serum creatinine determination without protein precipitation. Clin Chim Acta 1972; 37:193–7.
[37] Satoh K. Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. Clin Chim Acta 1978; 90:37–43.

[38] Nishikimi M, Appaji N, Yagi K. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen. Biochem Biophys Res Commun 1972; 46:849–54.
[39] Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med 1967; 70:158–69.
[40] Bancroft J, Stevens A. Theory and practice of histological techniques. 4th ed. Churchil Livingstone, Edinburgh, United Kingdom, 1996.
[41] Agrawal PK, Bansal MC (1989). 13C-NMR of Flavonoids. In: Agrawal PK, ed. Studies in Organic Chemistry 39, New York: Elsevier Science, 6:283-364.
[42] Gittelsohn J, Trude AC, Poirier L, Ross A, Ruggiero C, Schwendler T, et al. The impact of a multi-level multi-component childhood obesity prevention intervention on healthy food availability, sales, and purchasing in a low-income urban area. Int J Environ Res Public Health 2017; 14:1371.
[43] Hwang SH, Kang I-J, Lim SS. Antidiabetic effect of fresh Nopal (Opuntia ficus-indica) in low-dose streptozotocin-induced diabetic rats fed a high-fat diet. Evid Based Complement Altern Med 2017; 2017:4380721.
[44] Choo CY, Sulong NY, Man F, Wong TW. Vitexin and isovitexin from the leaves of ficus deltoidea with in-vivo alpha-glucosidase inhibition. J Ethnopharmacol 2012; 142:776–81.
[45] Yang J-P, He H, Lu Y-H. Four flavonoid compounds from *Phyllostachys edulis* leaf extract retard the digestion of starch and its working mechanisms. J Agric Food Chem 2014; 62:7760–70.
[46] Venditti A, Maggi F, Vittori S, Papa F, Serrilli AM, Di Cecco M, et al. Antioxidant and alpha-glucosidase inhibitory activities of Achillea tenorii. Pharm Biol 2015; 53:1505–10.
[47] Müller C, Assimacopoulos-Jeannet F, Mosimann F, Schneiter P, RiouJp, Pachiaudi C, et al. Endogenous glucose production, gluconeogenesis and liver glycogen concentration in obese non-diabetic patients. Diabetologia 1997; 40:463–8.
[48] Abduallah HFA. The anti-hypercholesterolemic effect of ulvan polysaccharide extracted from the green alga ulvafasciataon aged hypercholesterolemic rats. Asian J Pharm Clin Res 2016; 9:165–176.
[49] Targher G, Bertolini L, Padovani R, Rodella S, Tessari R, Zenari L, et al. Prevalence of nonalcoholic fatty liver disease and its association with cardiovascular disease among type 2 diabetic patients. Diabetes Care 2007; 30:1212–8.
[50] Ali SI, Gopalakrishnan B, Venkatesalu V. Pharmacognosy, phytochemistry and pharmacological properties of *Achillea millefolium* L.: a review. Phytother Res Ptr 2017; 31:1140–61.
[51] Papadopoulou-Marketou N, Kanaka-Gantenbein C, Marketos N, ChrousosGp, Papassotiriou I. Biomarkers of diabetic nephropathy: a 2017 update. Crit Rev Clin Lab Sci 2017; 54:326–42.
[52] Zheng X, Zhang L, Wang W, Wu Y, Zhang Q, Feng W. Anti-diabetic activity and potential mechanism of total flavonoids of *Selaginella tamariscina* (Beauv.) spring in rats induced by high fat diet and low dose STZ. J Ethnopharmacol 2011; 137:662–8.
[53] Mohamed AS, Soliman AM, Marie MAS. Mechanisms of echinochrome potency in modulating diabetic complications in liver. Life Sci 2016; 151:41–9.
[54] Abdelmegeed MA, Banerjee A, Yoo S-H, Jang S, Gonzalez FJ, Song B-J. Critical role of cytochrome P450 2E1 (CYP2E1) in the development of high fat-induced non-alcoholic steatohepatitis. J Hepatol 2012; 57:860–6.
[55] Mohamed AE-HH, Mohamed NS, Hamed AR, Hegazy M-EF. Anti-inflammatory activity of highly oxygenated terpenoids from *Achillea biebersteinii* Afan. Z Naturforschung C 2016; 71:429–32.
[56] Bachmann KN, Wang TJ. Biomarkers of cardiovascular disease: contributions to risk prediction in individuals with diabetes. Diabetologia 2017; (Epub ahead of print).
[57] Varga ZV, Giricz Z, Liaudet L, Haskó G, Ferdinandy P, Pacher P. Interplay of oxidative, nitrosative/nitrative stress, inflammation, cell death and autophagy in diabetic cardiomyopathy. Biochim Biophys Acta 2015; 1852:232–42.
[58] Ding Y, Zhang Z, Dai X, Jiang Y, Bao L, Li Y, et al. Grape seed proanthocyanidins ameliorate pancreatic beta-cell dysfunction and death in low-dose streptozotocin-and high-carbohydrate/high-fat diet-induced diabetic rats partially by regulating endoplasmic reticulum stress. Nutr Metab 2013; 10:1.
[59] Suman RK, Ray Mohanty I, Borde MK, Maheshwari U, Deshmukh YA. Development of an experimental model of diabetes co-existing with metabolic syndrome in rats. Adv Pharmacol Sci 2016; 2016:1–11.