

EVALUATION OF ANTIDIABETIC AND ANTI-OBESITY POTENTIAL AND SAFETY OF A POLYHERBAL REMEDY

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ABSTRACT

Objective: To evaluate the antidiabetic and anti-obesity potentials and the safety of a polyherbal remedy. **Method:** A mixture of polyherbal remedy containing *Gymnema sylvestre*, *Garcinia cambogia*, *Cleome droserifolia*, and *Nigella sativa* (100 or 200 mg/Kg, p.o.) was subjected to qualitative chemical identification for marker compounds on individual plant extracts using Q-TOF-LC-MS. In addition, the antidiabetic and anti-obesity potential were evaluated in streptozotocin-induced diabetic rats using metformin (100 mg/Kg, p.o.) and insulin (10 U/Kg, IP) as standards. The evaluation based on fasting blood glucose level (FBG), insulin level, and change in body weight gain of different treatment groups. Furthermore, the safety profile was assessed by the measurement of certain biochemical, hematological, and histopathological parameters. **Results:** A total of thirteen marker compounds, (Gymnemoside ND1, Myricetin, Hydroxycitric acid lactone, Garcinol, Garcimangosone D, 5,3`-Dihydroxy-3,6,7,4` ,5`-pentamethoxyflavone, 5-Hydroxy-3,6,7,3` ,4` ,5`-hexamethoxyflavone, Magnoflorine, Kaempferol glycoside, Sapindoside B, Alpha-hedrin, Tauroside H2, and Thymoquinone) were identified in the four plant extracts individually. The administration of polyherbal remedy extract (100 or 200 mg/Kg, p.o.)-, metformin-, and insulin-treated diabetic rats resulted in a significant decrease in the FBG by 62, 67, 44 and 73 % respectively, and significant increase in insulin level by 2161, 3433, 2400 and 5658 %, respectively, as compared to vehicle-treated control. Regarding the body weight change, polyherbal remedy extract (100 or 200 mg/Kg, p.o.)-, metformin-, and insulin-treated animals demonstrated a significant decrease by 45, 54, 72 and 65%, respectively, as compared to their initial weight. The results of polyherbal remedy (100 or 200 mg/Kg, p.o.)-treated groups showed a dose-independent potency between that obtained from insulin- and metformin-treated groups. Concerning the safety profile; treatment with remedy extracts markedly improved the streptozotocin-induced alteration in the hepatic biochemical, and hematological indices (liver enzymes, total protein, albumin, TC, TG, HDL, LDL, RBCs count, WBCs count, Hb concentration and platelet count) besides preservation of the normal histopathological features of liver, kidney, and pancreas. **Conclusion:** The polyherbal remedy can be used as an effective and safe treatment of obesity, diabetes, and its complications.

Keywords :Antidiabetic, anti-obesity, insulin, metformin, Q-TOF-LC-MS, remedy.

INTRODUCTION

mellitus (DM) is a heterogeneous group of metabolic disorders that have in common hyperglycemia as a consequence of failure of insulin action. The underlying cause of diabetes varies by type (Das et al., 1996). But, no matter what type of diabetes you have, it can lead to excess sugar in your blood which can lead to serious health problems. During the early stages of the disease, asymptomatic patients, especially those with type 2 DM, can present stupor, coma, and death due to ketoacidosis. The severity of the symptoms is related to the type and duration of diabetes (Kharroubi & Darwish, 2015). The International Diabetes Federation listed Egypt among the world top 10 countries in the number of patients with diabetes. It is expected that the number of patients with diabetes in the Middle East and North Africa region to grow by 96 % from year 2013 to 2035 or from 34.6 million to 67.9 million (Hegazi et al., 2015). Obesity generally described as a condition of abnormal body weight resulting from an accumulation of extra body fat, basically in response to a state of positive energy balance that occurs when intake exceeds energy expenditure. It is a growing global health problem in the present era (Bennett et al., 1997). Obesity is associated with increasing the risk of other diseases and health problems, such as heart disease, diabetes, high blood pressure and certain cancers (Srivastava et al., 2007). According to the World Health Organization (WHO), Egypt ranks 18th with the highest prevalence of obesity worldwide (Con.org., 2020). Deaths attributable to non-communicable diseases represent about 71% of the total mortality burden (Organization, 2021). Plants and natural products are long been recognized as an important source for the therapeutically effective medicines. The use of herbal medicines continues to expand rapidly across the world, with an estimated 80 % of the world's population using this type of medication, especially in developing countries (Bodeker & Ong, 2005; Ekor, 2014). Several studies have demonstrated the effect of medicinal plants that have shown to be promising as antidiabetic and anti-obesity. Among these plants, *Gymnema sylvestre*, *Garcinia cambogia*, *Cleome droserifolia*, and *Nigella sativa*. *Gymnema sylvestre*, family Apocynaceae, is widely used as a traditional therapy for different purposes. *Gymnema sylvestre* is known to have antioxidant, antibiotic, anti-inflammatory, antiviral, gastro and hepatoprotective, anticancer and lipid-lowering activities. Coumarins, gymnemic acid, and gymnema saponins are among the most effective phytoconstituents in *Gymnema sylvestre* (Shukla et al., 2020). *Garcinia cambogia*, family Clusiaceae, commonly known as “Malabar tamarind”, showed varied medicinal effects including anti-obesity, antiviral, antimicrobial, anti-inflammatory, and anticancer. Hydroxycitric acid (HCA), is the major organic acid in *Garcinia cambogia* (Jena et al., 2002). The major secondary metabolites in *Garcinia cambogia* are benzophenones followed by xanthenes and bioflavonoids (Jena et al., 2002). *Cleome droserifolia*, family: Capparaceae, locally known as “SAMWA” is widely used traditionally in Egypt to counteract hyperglycemia (Boulos, 1995). Phytochemical screening of the plant showed the presence of terpenoids, flavonoids, phenolics, and alkaloids, supporting use of *Cleome droserifolia* for culinary and therapeutical purposes (Marles & Farnsworth, 1995). It exhibited analgesic, anti-inflammatory, antifungal, antimicrobial, anti-diarrheal, anticancer, anti-arthritic, hepatoprotective, antinociceptive, wound healing, and psychopharmacological activities (Helal et al., 2002). *Nigella sativa*, family: Ranunculacea locally known as “black seed or black cumin” is a powerful antioxidant and medicinal plant with many therapeutic applications particularly in traditional

medicine for respiratory, gastrointestinal, rheumatic, and inflammatory disorders, as well as cancer. *Nigella sativa* seeds and oil have a long history of folklore usage in various systems of medicines and food. In Islamic literature, it is considered as one of the greatest forms of healing medicine. *Nigella sativa* has many different chemical ingredients including thymoquinone, flavonoids, anthocyanins, alkaloids and essential fatty acids, particularly linoleic and oleic acid (Ahmad et al., 2013; Tembhurne et al., 2014). Nowadays, the best authentication protocols are based on analytical chemistry. Plant chemical markers is a valuable tool for quality control of herbal medicines and authentication of the genuine species. These techniques allow for the discrimination of chemotypes within species, as well as maturity stages and origins (Drouet et al., 2018). The selected markers should be therapeutic, characteristic, correlative, synergistic, and/or toxic components (Li et al., 2008).

The objective of this work was to evaluate for the first time the antidiabetic and anti-obesity potentials of a polyherbal remedy consisted of the four methanolic extracts of *Gymnema sylvestre*, *Garcinia cambogia*, *Cleome droserifolia*, and *Nigella sativa* in a ratio of 1:1:1:1 (hereafter called AMAN). The herbal remedy was authenticated chemically and evaluated for its safety biochemically and histopathologically.

1. Experimental Section

1.1. Plant material

Leaves of *Gymnema sylvestre*, fruits of *Garcinia cambogia*, leaves of *Cleome droserifolia* and seeds of *Nigella sativa* were purchased from a local shop, Cairo, Egypt. The plant materials were kindly authenticated by Prof. Dr. Abdu Mareey, Professor of plant taxonomy, Faculty of Science (Boys), Al-Azhar University, Cairo, Egypt. The samples were preserved and deposited in repository of Faculty of Pharmacy (Girls), Al-Azhar University, Cairo, Egypt. The voucher specimen numbers are GS2016, GC2016, CD2016 and NS2016. The powdered plant materials were weighed to afford (500 gm *Gymnema sylvestre*, 570 gm *Garcinia cambogia*, 2000 gm *Cleome droserifolia* and 1650 gm *Nigella sativa*). Methanolic extracts of plant materials were prepared separately. Soxhlet apparatus using 1L of 70% methanol for each plant was used for extraction process followed by evaporation under vacuum by rotatory evaporator. The extracts obtained were weighed to afford (78, 88.5, 310 and 256 gm) for *Gymnema sylvestre*, *Garcinia cambogia*, *Cleome droserifolia* and *Nigella sativa*, respectively. Polyherbal remedy consisted of the four methanolic extracts in a ratio of 1:1:1:1 and collectively named AMAN. Polyherbal remedy used at a dose of (100 mg/Kg, p.o.) as a single daily dose and (200 mg/Kg, p.o.) divided into two daily doses.

1.2. Chemical authentication of plant materials

polyherbal remedy (AMAN) was subjected to qualitative chemical identification of marker compounds on individual plant extracts using 6530 Q-TOF-LC-MS (Agilent Technologies) at Faculty of Pharmacy, Fayoum University. The device equipped with an autosampler (G7129A), a quaternary pump (G7104C), and a column compartment (G7116A). The analytes were injected in a volume of 2 μ L and separated on a Zorbax RP-18 column (Agilent Technologies) with dimensions (150 mm \times 3 mm \times 2.7 μ m) by

gradient elution demonstrated in table 1 with a flow rate of 0.2 mL/min. The solvent composition was:

Mobile phase A: Deionized water which contains 0.1 % Formic acid (Sigma-Aldrich Co. Louis St., MO, USA) LC-MS grade.

Mobile phase B: Acetonitrile which contains 0.1 % Formic acid (Sigma-Aldrich Co. Louis St., MO, USA) LC-MS grade.

Mass spectra were acquired using ESI in positive ionization modes with a capillary voltage of 4000 V. The mass spectra were recorded in the m/z range of 50 to 1500. The gas temperature and drying gas flow were 300 °C and 10 L/min, respectively. The skimmer and fragmentator voltages were set at 65 and 130 V, respectively and collision energy was 10 V. The nebulization pressure was 35 psig. ESI-Low Concentration Tuning mix was used to test the instrument performance and producing result with confidence.

Table 1: Q-TOF-LC-MS elution timetable

Time	0	10	15	25	30	32
Solvent A	70	55	35	20	10	70
Solvent B	30	45	65	80	90	30

1.3. Experimental design

Adult Sprague Dawley male rats weighing 190–200 g and aging 120 days were used in the current study. The animals were obtained from the breeding colony maintained at the animal house of the El-Nile Company for Pharmaceuticals, Cairo, Egypt. They were housed in the animal facility of Faculty of Pharmacy, Al-Azhar University in plastic cages with stainless steel wire lids and mesh floor with 5 animals per cage. They were kept at 23±2 °C, 55% relative humidity with 12:12-h light: dark cycle. All animals' procedures were performed in accordance with the Ethics Committees of the Faculty of Pharmacy, Al-Azhar University, Cairo, Egypt. Animals were first divided into two main groups.

Control group: 10 rats did not receive any drugs or injected with streptozotocin (STZ).

Model group: 100 rats were injected with STZ 58 mg/Kg body weight, intraperitoneal (IP) single injection. The criterion of modelling success was that the fasting blood glucose (FBG) of rats between 200-300 mg/dl using Aqua-check apparatus after 72 hrs. 50 rats were insured to be diabetic and were then randomly divided into the following five subgroups (each group including 10 rats):

STZ group (Diabetic non-treated group): Diabetic rats which did not receive any drug for treatment, only normal saline per oral (p.o.), once daily for 4 consecutive weeks.

Metformin group: Diabetic rats treated with metformin (100 mg/Kg, p.o.), once daily for 4 consecutive weeks.

Insulin group: Diabetic rats treated with insulin (10 U/Kg, IP) once daily for 4 consecutive weeks.

AMAN-100 group: Diabetic rats treated with AMAN (100 mg/Kg, p.o.), once daily for 4 consecutive weeks.

AMAN-200 group: Diabetic rats treated with AMAN (200 mg/Kg, p.o.), divided into two doses for 4 consecutive weeks.

The weights of rats were observed once a week and the dose of insulin, metformin and AMAN were adjusted with the change in weights.

1.4. Safety and efficacy evaluation

The efficacy evaluation was based on FBG and insulin levels measurements in addition to change in weight of different groups using metformin (100 mg/Kg, p.o.) and insulin (10 U/Kg, IP) as comparison standards. The safety evaluation was based on biochemical, hematological, and histopathological changes (Li et al., 2019). At the end of administration for the four weeks, rats were weighted, after fasting for 12 hr, and then, the blood samples were withdrawn from the retro-orbital plexus using heparinized micro-capillaries (Optilab, Berlin, Germany) under anesthesia (thiopental sodium 50 mg/kg, IP) (Gazdhar et al., 2013). Serum was separated by blood centrifugation at 4000 rpm for 10 min at -4°C (Heraeus Biofuge, Berlin, Germany). FBG was measured using Aqua-check apparatus, while insulin level was estimated by radioimmunoassay. Red blood cells (RBCs) count, white blood cells (WBCs) count, hemoglobin (Hb) concentration and platelets count were determined using hematology analyzer (Mindray-BC-28000). Serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), Alkaline phosphatase (ALP), total cholesterol (TC), triglyceride (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), total protein and albumin levels were determined spectrophotometrically using standard kits (Biodiagnostic, Giza, Egypt) according to Reitman and Frankel (1957) method (Reitman & Frankel, 1957). Animals were euthanized by cervical dislocation, and livers, pancreases and kidneys were dissected out, washed with normal saline, and blotted dry on filter paper. The organs were preserved in 10% formol saline using Hartz Technique (1947) (Hartz, 1947), and used thereafter for histopathological examinations by Hematoxylin and Eosin (H&E) staining. The histopathological examination was done through a light electric microscope according to previously published method (Bancroft & Gamble, 2008) at Pathology Department, Faculty of Medicine, Al-Azhar University, Cairo, Egypt.

Statistical analysis: Results were expressed as mean \pm standard deviation (SD). ANOVA was carried out when multiple comparisons were evaluated. Differences between groups were considered as statistically significant at ($P \leq 0.05$). All statistical analyses were performed using Statistical Product and Service Solutions software (version 20.0).

2. Results

2.1. Authentication of plant materials

A total of thirteen marker compounds were identified in the four plant extracts individually using Q-TOF-LC-MS principal component analysis (PCA) of non-targeted metabolites. The marker compounds with a predefined significant correlation with each plant were tabulated in table 2. Two, three, two, and six marker compounds were identified in *Gymnema sylvestre*, *Garcinia cambogia*, *Cleome droserifolia* and *Nigella sativa*, respectively.

Table 2: Marker compounds for each plant extract

No.	Plant extract	R _t (min)	M/z	Error (ppm)	Proposed compound	Identification reference
1	<i>Gymnema sylvestre</i>	30.489	827.4054 [M+H] ⁺	4.5	Gymnemoside ND1	(Jamadagni et al., 2021)
2	<i>Gymnema sylvestre</i>	35.808	319.1761 [M+H] ⁺	0	Myricetin	(Parveen et al., 2019)
3	<i>Garcinia cambogia</i>	7.568	191.0379 [M+H] ⁺	0	Hydroxycitric acid lactone	(Aravind et al., 2016)
4	<i>Garcinia cambogia</i>	25.283	603.3488 [M+H] ⁺	0	Garcinol (Camboginol)	(Chattopadhyay & Kumar, 2007)
5	<i>Garcinia cambogia</i>	30.107	392.1108 [M] ⁺	0	Garcimangosone D	(Olatunde et al., 2018)
6	<i>Cleome droserifolia</i>	8.564	405.1931 [M+H] ^{c+}	0.02	5,3'-Dihydroxy- 3,6,7,4',5'- pentamethoxyflavone	(Zhou et al., 2009)
7	<i>Cleome droserifolia</i>	35.379	419.1337 [M+H] ⁺	0	5-Hydroxy-3,6,7,3',4',5'- hexamethoxyflavone	(Zhou et al., 2009)
8	<i>Nigella sativa</i>	6.961	342.1702 [M] ⁺	0	Magnoflorine	(Avula et al., 2010; Farag et al., 2014)
9	<i>Nigella sativa</i>	8.501	773.2140 [M+H] ⁺	0	Kaempferol 3-glucosyl- (1->2)- galactosyl-(1->2)- glucoside	(Avula et al., 2010; Harborne & Williams, 1995)
10	<i>Nigella sativa</i>	15.899	883.5046 [M+H] ⁺	0	Sapindoside B	(Avula et al., 2010)
11	<i>Nigella sativa</i>	16.637	751.4624 [M+H] ⁺	0	alpha-hedrin	(Alagawany et al., 2021)
12	<i>Nigella sativa</i>	16.672	1243.6068 [M+Na] ⁺	0	Tauroside H2	(Avula et al., 2010)
13	<i>Nigella sativa</i>	38.878	164.0834 [M] ⁺	0	Thymoquinone	(Avula et al., 2010)

2.2. Efficacy evaluation

2.2.1. FBG and insulin levels

As indicated in table 3, the control group showed normal FBG and insulin levels. The STZ group showed a significant increase in the FBG by 551.3% and a significant decrease in the insulin level by 97 % when compared with their corresponding control values. The AMAN-100-, AMAN-200-, metformin-, and insulin-treated groups showed a significant decrease in the FBG by 62, 67, 44 and 73 % respectively, while regarding the insulin level a significant increase by 2161, 3433, 2400 and 5658 %, respectively were observed when compared with their corresponding values in the STZ group.

2.2.2. Body weight

The result of the four weeks treatment with AMAN, metformin, or insulin on the body weight percentage changes are reported in table 3. The control group showed an increase in the animal's body weight by 12 %, while STZ group showed a decrease by 17 % at the end of the study experiment as compared to their initial weights. The AMAN-100-, AMAN-200-, metformin-, and insulin-treated animals demonstrated a significant decrease by 45, 54, 72 and 65%, respectively, as compared to their initial weight.

2.3. Safety evaluation

2.3.1. Liver enzymes (ALT, AST, ALP)

To evaluate the safety of the AMAN remedy, the liver function was assessed in the different treatment groups after four-week treatment. As demonstrated in table 3, the STZ group showed a significant increase in the ALT serum level by 102.3 %, as compared with control group. AMAN-100-, AMAN-200-, metformin-, and insulin-treated groups showed a significant decrease by 27, 32, 27, 36 % respectively when compared with STZ group without significant change from control group. The STZ group showed a significant increase in the AST serum level by 49 %, when compared with control group. Non-significant changes in the AST serum level were observed in AMAN-100-, and metformin-treated groups while AMAN-200-, and insulin-treated groups showed a significant decrease by 17 and 25 % respectively when compared with STZ group without significant change from control group. The STZ group showed a significant increase in the ALP serum level by 308 %, when compared with control group. AMAN-200-, and insulin-treated groups showed a significant decrease in the ALP serum level by 28 and 30 % respectively, while no change were observed regarding AMAN-100-, and metformin-treated groups when compared with STZ group.

2.3.2. Total protein and albumin

Given the fact that the liver is the main site for protein biosynthesis, therefore we assessed serum protein level as a major for liver toxicity. Our results demonstrated that the STZ group showed a non-significant decrease in the total protein serum level, and a significant decrease in the albumin serum level by 42 % when compared with control values. Interestingly, there is no significant difference between control group and AMAN-100-, AMAN-200-, metformin-, and insulin-treated groups at ($P \leq 0.05$); regarding total protein and albumin serum levels as shown in table 3.

2.3.3. Lipid profile assessment

As known, lipid profile assessment is indeed associated with liver function, therefore lipid profile was essential for AMAN remedy safety evaluation. The data presented in table 3 revealed that in the STZ group the TC and LDL were significantly increased by 84 and 71 %, respectively, as compared to control group. However, the TG level wasn't significantly different from control group. There were no changes in AMAN-100-, AMAN-200-, metformin-, and insulin-treated groups in TC, LDL, and TG when compared with STZ group except the significant decrease of TC by 29 % in insulin group. AMAN-100-, AMAN-200-, metformin-, and insulin-treated groups weren't significantly different from control group regarding TC, LDL, and TG except the significant increase of TC by 58 % in metformin group. Regarding HDL level, the STZ group demonstrated a significant decrease by 65 % when compared with control group. AMAN-100-, AMAN-200-, and metformin-treated groups weren't significantly different from STZ group except the significant increase by 153 % in insulin group. AMAN-200-, metformin-, and insulin-treated groups weren't significantly different from control group while AMAN-100-treated group decreased HDL level significantly by 62 %.

2.3.4. Hematological analysis

Diabetes is a predominant factor for hematological changes; therefore, hematological analysis of our remedy AMAN was essential for its safety evaluation. Presented data in table 3 showed that RBC_S count significantly decreased in STZ group by 47 % when compared with control group. The AMAN-100-, AMAN-200-, metformin-, and insulin-treated groups exhibited a significant increase by 92, 114, 98 and 84 % respectively when compared with STZ group. Presented data in table 3 showed that WBC_S count significantly decreased in STZ group by 46 % when compared with control group. There weren't significant changes in the WBC_S count regarding AMAN-100- or AMAN-200-treated groups when compared to either control or STZ groups. The metformin-, and insulin-treated groups showed a significant increase in the WBC_S count by 118 and 95 %, respectively when compared with STZ group. Regarding Hb concentration, the STZ group showed a non-significant change when compared with control group. The treated groups with AMAN-100, AMAN-200, metformin, or insulin exhibited a non-significant change when compared with STZ group. The STZ group showed a significant decrease in platelet count by 37 % when compared with control group. There weren't significant changes in the platelet count regarding AMAN-100-, and metformin-treated groups when compared with STZ group while, AMAN-200-, and insulin-treated groups exhibited a significant increase by 45 and 55 % respectively.

2.3.5. Histopathological examinations Liver

Liver histopathological examination showed normal healthy features in high power view of control group as showed in figure 1. The main features included portal tract with average portal vein (PV), average bile ducts, average hepatocytes in periportal area and average central vein (CV) with hepatocytes arranged in single cell cords with intervening blood sinusoids. The livers of diabetic rats treated with AMAN-100, metformin, and insulin showed average portal tracts with average PV, bile ducts, CV, and scattered apoptotic hepatocytes in peri-portal and peri-venular areas. In some rats, AMAN-100-treated group showed markedly dilated congested central veins with detached lining and mildly dilated blood sinusoids while in insulin group mild portal inflammatory infiltrate was observed. Similarly, AMAN-200-treated group showed portal tracts with mildly dilated congested PV and average bile ducts and CV with scattered apoptotic hepatocytes in peri-portal and peri-venular areas.

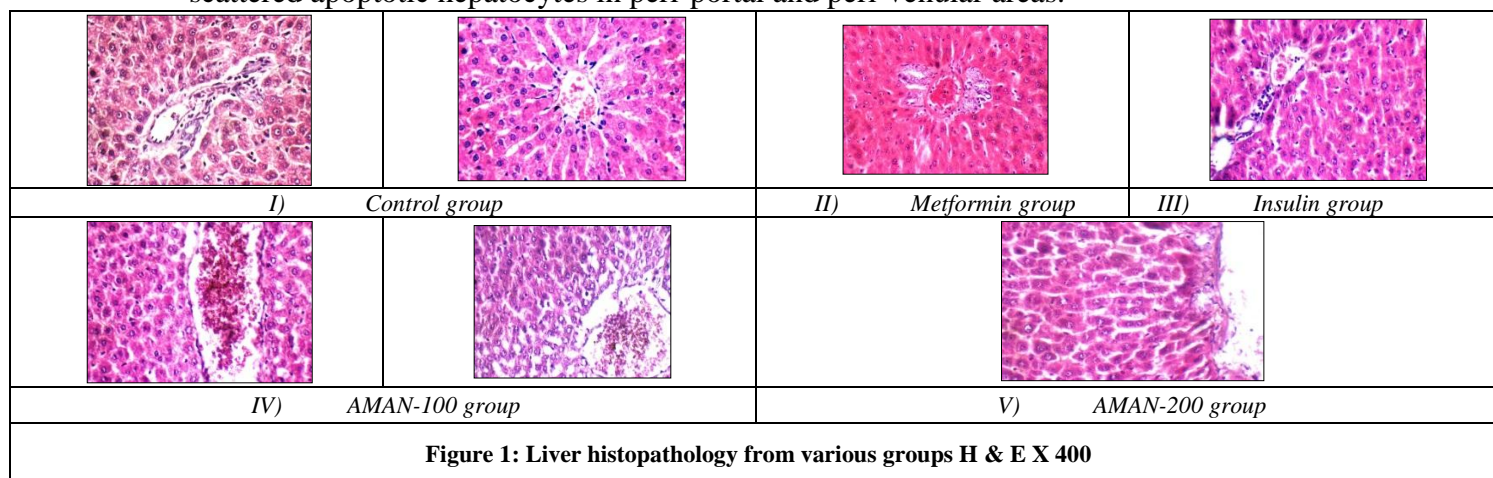


Figure 1: Liver histopathology from various groups H & E X 400

Pancreas

Pancreas histopathological examination showed normal healthy features in high power view of control group as showed in figure 2. The features included average-sized normocellular islets with predominating beta cells and less frequently alpha cells in the periphery, average intervening blood capillaries, and average exocrine areas. STZ induced a marked damage of pancreas as detected in high power view of STZ group. That is shown in the spread of markedly apoptotic beta cells and increase in the number of alpha cells along with mildly dilated intervening blood capillaries. Pancreas of diabetic rats treated with metformin kept the healthy histological features of pancreas to a great extent showing average-sized islets of Langerhans and did not show any damage. Pancreas of rats treated with insulin showed relatively small-sized islets of Langerhans with average beta cells, average alpha cells, average intervening blood capillaries, and average exocrine areas. The treatment with AMAN either (100 or 200 mg/Kg, p.o.), for four weeks caused decrease in the size of islets showing small sized hypocellular islets, mild dilatation of intervening blood capillaries.

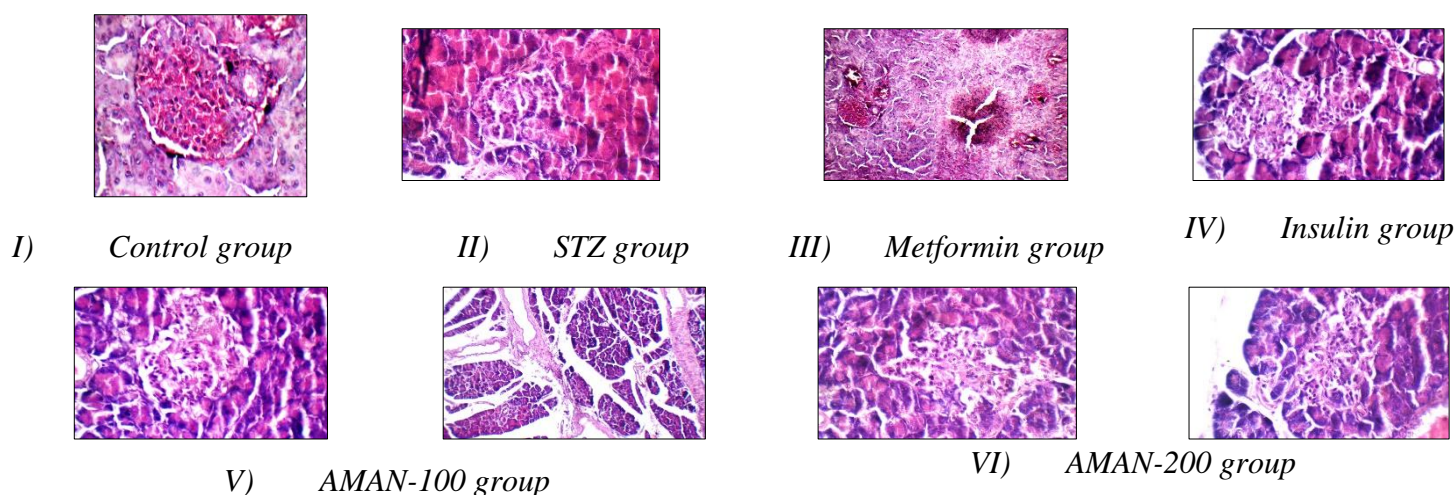


Figure 2: Pancreas histopathology from various groups H & E X 400

2.3.5.1. Kidney

Figure 3 demonstrated the noted change in the histopathological structure of kidneys in STZ group. Kidneys showed scattered small sized congested glomeruli with narrowing of the Bowman's spaces, marked change in the proximal tubules, and apoptotic epithelial lining with marked congestion. The dilated interstitial blood vessels of the distal tubules showed areas of interstitial hemorrhage and the collecting tubules of the renal medulla showed apoptotic epithelial lining. Kidneys of insulin-treated group rats also showed a marked changes including proximal tubules with apoptotic epithelial lining and partial loss of brush borders. Glomeruli and Bowman's spaces were average-sized. It also showed a markedly dilated congested blood vessels with areas of hemorrhage. The renal medulla showed collecting tubules with apoptotic epithelial lining and intra-tubular hyaline casts. The metformin-treated group was observed to maintain the normal structure of the kidney to a great extent where the kidneys showed average renal capsule, and average glomeruli with average Bowman's spaces. The

proximal tubules were seen with apoptotic epithelial lining and partial loss of brush borders. The renal medulla showed collecting tubules with average epithelial lining and average interstitium. It was obviously noticed that kidneys of rats treated with AMAN-100, showed scattered relatively small-sized and distorted hypocellular glomeruli with average Bowman's spaces. The proximal tubules were with apoptotic epithelial lining and partial loss of brush borders. The distal tubules were average sized with dilated thick-walled blood vessels and mild interstitial inflammatory infiltrate. The renal medulla showed collecting tubules with mildly edematous or apoptotic epithelial lining with average interstitium and in some cases with intra-tubular hyaline casts. Kidneys of AMAN-200-treated group rats showed average renal capsule, and average glomeruli with average Bowman's spaces. The proximal tubules were seen with scattered apoptotic epithelial lining and partial loss of brush borders. Kidneys were with average sized distal tubules. The renal medulla showed collecting tubules with average epithelial lining and mildly congested peri-tubular capillaries.

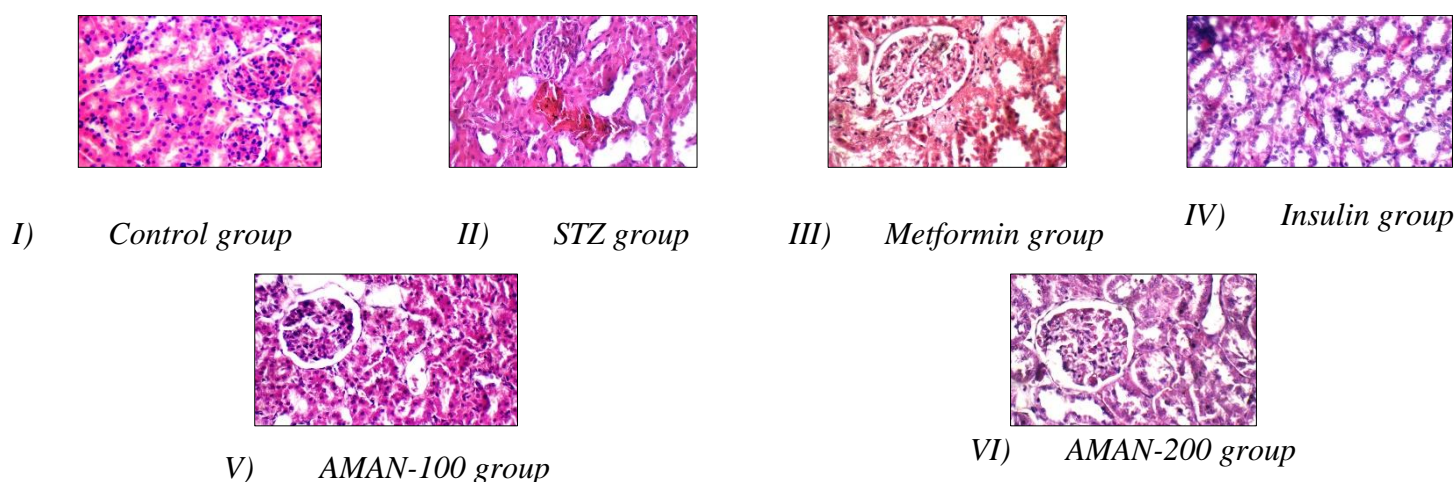


Figure 3: Kidney histopathology from various groups H & E X 400

3. Discussion and conclusion

DM is a disease characterized by inherited or acquired deficiencies in insulin secretion and/or action. DM is a rapidly growing disease worldwide that is estimated to be present in 6.6 % of the international population and projected to be increased by 7.8 % in 2030 (El-Komy & Serag, 2017). Reduction of the FBG was previously reported upon the intake of *Gymnema sylvestre*, *Garcinia cambogia*, *Cleome droserifolia* and *Nigella sativa* individually. The saponin components responsible for the antihyperglycemic effect of *Gymnema sylvestre* are gymnemosides and gymnemic acid upon glucose uptake inhibition (Shenoy et al., 2018). Adaramoye and Adeyemi, 2006 reported that the reduction of the glucose concentration associated with the intake of *Garcinia cambogia* could be caused by bioflavonoids, which promote the entry of glucose into the cells, stimulate glycogenic enzymes and glycolytic enzymes or inhibit the glucose 6-phosphatease in the liver and logically reduce the release of glucose in blood in addition to their lipid lowering effect by inhibiting hepatic HMG-CoA reductase (Adaramoye & Adeyemi, 2006). The presence of three flavonoids (quercetin, kaempferol, and isorhamnetin) together with three phenolic acids (sinapinic acid, ferulic

acid and 4-coumaric acid) explain the antidiabetic and antioxidative properties of *Cleome droserifolia* (El Naggari et al., 2005). The magnitude of *Nigella sativa* on the management of DM and its complications via various therapeutic strategies was previously reported and the best dosage was 2 g daily powder for at least 12 weeks (Mahmoodi & Mohammadzadeh, 2020). Interestingly, results indicated the effects on FBG level, insulin level, and body weight of AMAN treatment either (100 or 200 mg/Kg, p.o.), was comparable to that of optimal doses of insulin or metformin (10 U/kg, or 100 mg/Kg, respectively). Safety study results revealed that the oral administration of AMAN can maintain the normal values of liver enzymes, total protein, albumin, TC, TG, HDL, LDL, and hematological parameters. Besides, noticeable improvements were observed on these parameters' values affected by STZ. Finally, metformin maintained the normal healthy histological features of the liver and so did insulin and AMAN groups with only mild changes includes portal inflammatory infiltrate, dilated congested PV and scattered apoptotic hepatocytes in peri-portal and peri-venular areas. Metformin and insulin did not much affect the healthy histological features of pancreas while AMAN increased the blood supply and dilated ducts. Kidneys of diabetic rats treated with AMAN were negatively affected as they showed scattered smaller sized and distorted hypocellular glomeruli. The apoptotic epithelial lining of the proximal tubules was observed with partial loss of brush borders. The blood vessels of the distal tubules were dilated thick walled. The combination caused inflammation of the kidney seen in the interstitial inflammatory infiltrate. The renal medulla showed intratubular hyaline casts. Kidneys of diabetic rats given AMAN-200 showed further congestion of the peritubular capillaries. Metformin maintained the normal histological structure of the kidney to a great extent while insulin showed change in the histological structure of the kidney.

Table 3: The effects of metformin, insulin and AMAN on different biochemical parameters and body weight change in normal and diabetic rats for 4 weeks.

Parameters Groups	FBG (mg/dl)	Blood insulin level (ng/dl)	ALT (U/ml)	AST (U/ml)	ALP (U/ml)	Total protein (gm/dl)	Albumin (gm/dl)	Cholesterol (mg/dl)	Triglyceride (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	RBCs count ($10^6/\text{mm}^3$)	WBCs count ($10^3/\text{mm}^3$)	Hb conc. (g/dl)	Platelet count ($10^3/\text{mm}^3$)	Percentage change in body weight %
Control group	86.17 ± 1.99	1.1 ± 0.03	29.74 ± 2.8	99.3 ± 4.2	118.8 ± 1.7	9.4 ± 0.43	4.3 ± 0.07	61.2 ± 8.5	48.0 ± 4.4	41.8 ± 7.7	71.4 ± 13.6	7.183 ± 0.16	11.3 ± 0.14	15.8 ± 0.23	578 ± 20	11.83 ± 1.6
STZ group	561.3 ± 15.13 ^a	0.033 ± 0.007 ^a	60 ± 5.5 ^a	147.8 ± 7.5 ^a	484.1 ± 51.9 ^a	7.9 ± 0.40	2.5 ± 0.40 ^a	112.6 ± 12.3 ^a	68.3 ± 11.8	14.6 ± 2.5 ^a	122.4 ± 18.4 ^a	3.820 ± 0.003 ^a	6.1 ± 0.036 ^a	14.1 ± 0.03	362 ± 0.36 ^a	-16.83 ± 2.15
Metformin group	312.5 ± 33.74 ^{a,b}	0.825 ± 0.15 ^b	43.9 ± 3.6 ^b	125.8 ± 3.4 ^a	420.4 ± 6.8 ^a	8.6 ± 0.40	3.4 ± 0.11	96.5 ± 4.3 ^a	63.5 ± 9.0	28.5 ± 4.8	92.4 ± 12.5	7.580 ± 0.003 ^b	13.3 ± 2.1 ^b	14.3 ± 0.07	495 ± 16	-3.00 ± 4.29
Insulin group	151.8 ± 9.15 ^b	1.9 ± 0.02 ^{a,b,c}	38.2 ± 1.7 ^b	111.2 ± 5.4 ^b	339.9 ± 15.5 ^{a,b}	8.7 ± 0.60	3.6 ± 0.14 ^b	79.7 ± 3.6 ^b	54.0 ± 1.7	36.9 ± 4.5 ^b	75.7 ± 6.9	7.030 ± 0.14 ^b	11.9 ± 1.1 ^b	13.7 ± 0.24 ^a	560 ± 17 ^b	-3.66 ± 1.20
AMAN-100 group	214 ± 5.842 ^{a,b}	0.746 ± 0.08 ^{a,b}	43.8 ± 3.8 ^b	127.1 ± 4.7 ^a	423.1 ± 6.0 ^a	8.2 ± 0.30	3.4 ± 0.13	89.1 ± 5.1	68.3 ± 11.8	16.0 ± 1.5 ^{a,c}	95.5 ± 6.5	7.317 ± 0.43 ^b	9.3 ± 0.3	14.4 ± 0.78	443 ± 29	-5.83 ± 1.90
AMAN-200 group	185.3 ± 5.806 ^{a,b}	1.166 ± 0.07 ^{b,c}	40.7 ± 1.4 ^b	122.2 ± 4.4 ^b	348.5 ± 12.2 ^{a,b}	8.7 ± 0.20	3.7 ± 0.11 ^b	84.7 ± 3.2	64.6 ± 8.9	23.8 ± 5.2	91.3 ± 9.3	8.163 ± 0.20 ^b	10.4 ± 1.1	15.7 ± 0.62	525 ± 34 ^{a,b}	-4.83 ± 1.66

Data are presented as means ± SEM, n = 6

^aSignificantly different from control at $P \leq 0.05$;

^bSignificantly different from STZ at $P \leq 0.05$; using one way ANOVA, followed by Tukey-Kramer for multiple comparison.

^cSignificantly different from metformin at $P \leq 0.05$; using one way ANOVA, followed by Tukey-Kramer for multiple comparison.

Ethical Approval:

This article does not contain any studies with human participants performed by any of the authors. All experimental procedures were approved by the Ethics Committees of the Faculty of Pharmacy (Girls) - Al-Azhar University - Cairo - Egypt and encoded (334).

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تقييم التأثير المضاد لمرض السكر والسمنة ودراسة الأمان لوصفة متعددة الأعشاب

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الهدف: إظهار الإمكانات المضادة لمرض السكر والسمنة لوصفة متعددة الأعشاب بالإضافة إلى تقييمها من حيث الأمان.

الطريقة: تم عمل تحديد كيميائي نوعي للمركبات المميزة للوصفة النباتية لكل مستخلص نباتي على حده باستخدام الكروماتوغرافيا السائلة مع قياس الطيف الكتلي. كما استند تقييم الفعالية المضادة لمرض السكر الناجم عن الستربتوزوتوسين والسمنة للوصفة متعددة الأعشاب بتركيز ١٠٠ أو ٢٠٠ مجم لكل كجم بالفم يوميا لمدة أربعة أسابيع إلى قياسات مستويات السكر والأنسولين في الدم بالإضافة إلى التغيير في وزن المجموعات المختلفة. وذلك باستخدام الميتفورمين (١٠٠ مجم لكل كجم) والأنسولين (١٠ وحدات لكل كجم) كمعايير مقارنة. بينما استند تقييم الأمان على التغيرات الحيوية الكيميائية ومكونات الدم وفحص الأنسجة. **النتائج:** تم تحديد إجمالي ثلاثة عشر مركباً محددًا في المستخلصات النباتية الأربعة بشكل فردي. أدى العلاج بالوصفة متعددة الأعشاب عن طريق الفم بتركيز ١٠٠ أو ٢٠٠ مجم لكل كجم بالفم يوميا لمدة أربعة أسابيع أو الأنسولين أو الميتفورمين انخفاضا ملحوظا في نسبة السكر الصائم في الدم بنسبة (٦٢، ٦٧، ٤٤ و ٧٣ ٪ على التوالي)، بينما زاد مستوى الأنسولين بنسبة (٢١٦١، ٣٤٣٣، ٢٤٠٠ و ٥٦٥٨ ٪ على التوالي) مقارنةً بالمجموعة الغير مصابة بداء السكري. فيما يتعلق بوزن الجسم أظهرت الحيوانات المعالجة بالوصفة متعددة الأعشاب عن طريق الفم بتركيز ١٠٠ أو ٢٠٠ مجم لكل كجم بالفم يوميا لمدة أربعة أسابيع أو الأنسولين أو الميتفورمين انخفاضا ملحوظا بنسبة ٤٥ و ٥٤ و ٧٢ و ٦٥ ٪ على التوالي، مقارنة بوزنها الأول. وبذلك يتضح من تلك النتائج أن فاعلية الوصفة متعددة الأعشاب تقع بين النتائج التي تم الحصول عليها من الفئران المصابة بداء السكري والتي عولجت بالأنسولين وتلك التي عولجت بالميتفورمين. أما بخصوص تقييم الأمان؛ فقد أدى العلاج بالمستخلص إلى تحسن ملحوظ في التغيير الناجم عن الستربتوزوتوسين في المؤشرات الكيميائية الحيوية والكبدية و الدموية والتي شملت إنزيمات الكبد، والبروتين الكلي، والألبومين، و الكوليستيرول، و الجلوسريدات الثلاثية، و البروتين الدهني المفيد عالي الكثافة، و الضار منخفض الكثافة، وعدد كرات الدم الحمراء، وعدد كرات الدم البيضاء، وتركيز الهيموغلوبين وعدد الصفائح الدموية إلى جانب الحفاظ على السمات النسيجية الطبيعية للكبد والكلى والبنكرياس. **الخلاصة:** يمكن استخدام العلاج متعدد الأعشاب كعلاج فعال وآمن للسمنة ومرض السكري ومضاعفاته.

الكلمات المفتاحية: مضاد لداء السكر، مضاد للسمنة، الأنسولين، عقار الميتفورمين، Q-TOF-LC-MS، وصفة عشبية