A Possible Hypoglycemic and Antioxidant Effect of Herbal Mixture Extraction in Diabetic Rats

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ABSTRACT

Back ground: The use of medicinal plants for management of diabetes mellitus is an old practice which has become even more relevant in modern perspective. The present work was designed to evaluate the effect of a mixture of five medicinal plants which used in folk medicine in Egypt especially in Sinai as hypoglycemic agents on the adult male diabetic rats. These plants are *Cleome droserifolia* (Samwah), *Ambrosia maritima* (Damsissa),*Artemisia judaica* (Shih kharasani), *Chiliadenus montanus* or *Jasonia Montana* (Neheda) and *Artemisia annua (Kaysom)*.**Material and Methods:** Thirty male albino rats were divided equally into three groups including control, diabetic and diabetic treated with a mixture of aqueous extract. A single dose of alloxan (120 mg/kg body weight) was used to induce diabetes in rats. Diabetic rats were given plant mixture extract orally twice daily for 30 days (28.5 mg/kg body weight (b. wt.) twice/day). **Results**: there was a marked decline (p<0.01) in levels of serum insulin, body weight (4.98 %), total proteins, albumin, globulin and high density lipoproteins (HDL) accompanied with marked elevation (p<0.01) in levels of fasting blood glucose, homeostasis model assessment of insulin resistance (HOMA_IR), aspartate aminotransferase(ASAT), alanine aminotransferase (ALAT), gamma-glutamyltransferase (GGT), urea, creatinine, uric acid, serum total lipids(TL), total cholesterol (TC), triglycerides TG, low density lipoproteins (LDL), and very low density lipoproteins(VLDL),risk ratios of TC/HDL and LDL/HDL in diabetic rats as compared to the control rats. While administration of *aqueous extract* of plant mixture to alloxan diabetic rats significantly ameliorated the disturbed biochemical parameters.
Conclusion: These results proved that this plant mixture extract has a significant hypoglycemic effect and ameliorating metabolic abnormalities induced by diabetes mellitus.
Key words: Diabetes mellitus, Insulin, Hypoglycemic, Alloxan, medicinal plants

INTRODUCTION

Diabetes mellitus (DM) is highly recognized as the most common metabolic and endocrine disorder worldwide. It is linked to disturbances in carbohydrate, fat, and protein metabolism.[1] At least 250 million individuals worldwide suffer from diabetes and this number will double by 2030. Lifestyle and food habit changes are known to be the major causes of diabetes. Increases in complications will undeniably follow increasing diabetes incidence rates. More than 80% of diabetes deaths take place in low- and middle-income countries[2] .

Plant products are known to be rich in phenolic compounds, flavonoids, terpenoids, coumarins and other constituents which reduce blood glucose levels.[3]Sinai deserts in Egypt have hundreds of medicinal plants, that were traditionally used in the treatment of diabetes and passed down from the ancient Egyptians through the generations and till now. Some people in the desert of Sinai have great experiences of treating different diseases by using such plants.

One of these plants was *Ambrosia maritima* (Damsissa plant) is considered as a very important herb which used in folkloric treatments, widely grown in Egypt especially in Sinai.[4] Some researchers found that this medicinal plant exerted hypoglycemic effects and increased the level of serum insulin in alloxan-diabetic animals.[5]

Chiliadenus montanus or *Jasonia montana* (Neheda) (Family: Asteraceae), is common in the Sinai.[6] There were more evidences for their hypoglycemic, anticholestatic and antioxidant activities that have been recently investigated .[7]

The dried herb of *Cleome droserifolia* locally known as Samwah which is a plant of the Cleomaceae family. It is present in the deserts, especially the Eastern desert, Red Sea region, Sinai, Gebel .Its decoction of leaves and stems is widely used by the Bedouins of the southern Sinai for the treatment of diabetes. [8]

Artemisia judaica (Family: Asteraceae) known as "Shih kharasani" which grows in

Sinai has been known among herb experts in Egypt as a medicinal herb.[9] It is widely used in folk medicine and is recommended as a healer plant in traditional medicine by Bedouins there.

Artemisia annua (Kaysom) (Asteraceae), commonly known as sweet Annie, grown as a pharmaceutical crop in China, Vietnam, Egypt and north and east Africa.[10] It has been used in folk medicine for the treatment of diabetes mellitus.[11] *Artemisia annua* aqueous extract seem to have hypoglycemic effect on rats.[12]

Therefore the purpose of the present work was to evaluate the hypoglycemic efficacy and antioxidant effect of mixture of five medicinal plants *Cleome droserifolia* (Samwah), *Ambrosia maritima* (Damsissa), *Artemisia judaica* (Shih kharasani), *Chiliadenus montanus* or *Jasonia montana* (Neheda) and *Artemisia annua (*Kaysom) which commonly used in Egyptian folk medicine for diabetes treatment.

Material and Methods
Plant material
Aerial parts of plants were bought from plant market in Southern Sinai, Egypt.

Preparation of extract
Equal weights of each plant were grinded and mixed. The aqueous extract of this mixture was prepared by boiling 2 g of mixture with 200 ml of tap water for 15 minutes, left to cool at room temperature then filtered. The resultant extract was stored refrigerated in a glass container. The mixture extract was freshly prepared each two days.

Animals
Thirty adult male albino rats weighing around 100–110 g, at the age of 8-10 weeks purchased from Theodore Bilharz Research Institute, Giza, Egypt. They were kept under observation for about 15 days before the onset of the experiment for adaptation.

Induction of diabetes
Diabetes mellitus was induced in animals by a single intra peritoneal injection of alloxan dissolved in saline solution at a single dose level of 120 mg/kg body weight. Rats were deprived of food for 16 hrs before induction of diabetes. After three days of alloxan injection, rats were deprived of food overnight and they were then given glucose (3 g/kg body weight) by gastric intubation. After 2 hrs of oral glucose administration, blood samples were taken from tail vein and the fasting blood glucose (FBG) concentration was determined by means of one touch ultra glucometer and compatible blood glucose strips. Rats with fasting blood glucose levels ≥300 mg/dl were considered as mild diabetic animals and included in the experiment.

Experimental design
Experimental animals were divided into three groups ten for each, as follows:

- Group I (Control group): Non-diabetic rats.
- Group II (Diabetic group): Rats were injected intraperitoneal with a single dose of alloxan (120 mg/kg dissolved in saline solution).
- Group III: Diabetic rats treated orally with mixture extract (28.5 mg/kg b.wt. twice /day) for 30 days.

Blood sample collection
At the end of the experiment and under diethyl ether anaethesia, blood samples were taken from the retro-orbital plexus of the overnight fasted animals (12 hrs). Blood was centrifuged at 3000 rpm for 10 min. The clear supernatant sera were quickly removed and immediately stored at -20°C till used for further analysis of biochemical parameters.

Biochemical analyses:
Serum glucose was estimated using a commercially the method according to Tietz.[13] Serum insulin level was measured according to Reeves. [14] While values of HOMA-IR[15] were calculated using the following equation:

$$HOMA\text{-}IR = \frac{\text{fasting serum glucose (mg/dl)} \times \text{fasting serum insulin}(\mu U/L)}{405}$$

Creatinine and urea concentrations were determined colorimetrically as described by Tietz.[13] Serum uric acid was determined using the uricase-PAP enzymatic colorimetric method.[13]

Aspartate aminotransferase (ASAT), alanineamino-transferase (ALAT), gamma glutamyltransferase (γGT), albumin and total protein concentrations were determined colorimetrically.[16,17,18] Serum globulin was calculated by subtracting albumin from total protein.[13]

Serum total lipids (TL), total cholesterol (TC), triglycerides (TG) and HDL-cholesterol content were analysed by the enzymatic colorimetric method.[13,19,20,21]

LDL-C was calculated using the Friedwald's formula when the values of TG were less than 400 mg%.[22] VLDL may be calculated using the Friedewald's equation. [22]

Friedewald's equation: LDL (mg/dl) = TC-HDL - [TG/5].
VLDL = TG/5
Risk 1 = TC / HDL
Risk 2 = LDL / HDL

Statistical analysis: The results were expressed as Mean ± SE of 10 rats per group and the statistical significance was evaluated by one way analysis of variance (ANOVA) test using the SPSS/17.0 software. Values were considered statistically significant at $P < 0.05$.

RESULTS:

Diabetic rats showed significant decreased ($P<0.01$) in body weight as compared to control rats. Mixture treatment showed a significant increasing in body weight of treated group in comparison with diabetic rats (Table 1).

Table (2) showed marked decline ($p<0.01$) in the level of serum insulin accompanied with marked elevation in the level of fasting blood glucose and value of HOMA-IR ($P<0.05$) in diabetic rats when compared with control group. While after mixture administration serum insulin and fasting blood glucose levels as well as value of HOMA-IR were returned back approximately to normal value.

Moreover, our results showed a significant increase ($p<0.01$) in serum ALAT, ASAT and γGT activities in diabetic group. On the other hand, treatment of the diabetic rats with this mixture significantly decreased these activities (Table 3). In addition, diabetic animals showed marked decline in serum total proteins and albumin relative to the corresponding controls. Globulin and A/G ratio values showed non-significant changes in diabetic rats treated with this mixture. These results showed modulation of the measured serum protein profile parameters compared to the normal rats (Table 4).

On the other hand, the recorded results of biochemical parameters of renal function including urea, uric acid and creatinine showed significant increase ($p<0.01$) in diabetic group in comparison with the control group. While treatment of diabetic rats with this mixture extract returned these parameters towards normal values (Table 5). Diabetic animals showed marked significant elevation in TL,TC, TG, LDL, VLDL and ratios of TC/HDL (risk ratio 1) and LDL/HDL (risk ratio 2) accompanied with marked decline in HDL relative to the corresponding controls. Treatment of diabetic rats with the aqueous extract of this mixture showed significant reduction in the values of TC, TG, LDL, VLDL and ratios of TC/HDL and LDL/ HDL with marked elevation of HDL (Table 6).

DISCUSSION

Diabetes mellitus is characterized by disturbances of carbohydrate, fat, and protein metabolism resulting from defects in insulin secretion, insulin action, or both.[23]

Since centuries, medicinal plants provide a natural source of potent anti-diabetic drugs. In the Egyptian regions especially in Sinai, there are many types of plants herbs are known to be used in folk medicine and are considered to be of benefit in this context. Among which a mixture including *Cleome droserifolia* (Samwah), *Ambrosia maritima* (Damsissa), *Artemisia judaica* (Shih kharasani), *Chiliadenus montanus* or *Jasonia Montana* (Neheda) and *Artemisia annua (*Kaysom).

The aim of our research is to study the possible antidiabetic action and antioxidant effect of a mixture of these medicinal plants which used in Egypt in folk medicine and if they can ameliorate the metabolic abnormalities accompanied to alloxan-induced diabetic albino rats.

The present results showed that the alloxan diabetic rats exhibited severe hyperglycemia and significant hypoinsulinemia. This may be due to diabetogenic action of alloxan which causes selective destruction of β-cells and induces hyperglycemia resulting in a marked hypo-secretion of insulin by pancreatic β-cells led to elevated blood glucose and impaired glucose tolerance. [24]

Moreover, HOMA-IR recorded highly significant increase in diabetic rats. This is attributed to high glucose concentration cause the development of insulin resistance in peripheral tissues owing to impairment of both insulin secretion and insulin sensitivity, as HOMA-IR has proved to be a robust tool for

the surrogate assessment of insulin resistance. [25]

These findings are in agreement with Krishnapuran *et al*. [26]who recorded that, insulin-resistant states in obesity or diabetes are often associated with a decrease in the binding of insulin to insulin receptors, and/or a decrease in insulin-stimulated activation of insulin Receptor Substrate(IRS), which may diminish the effectiveness of anti-diabetic agents dependent on this signaling. [26]

On the other hand, after mixture administration, diabetic rats showed increasing in insulin levels accompanied with decrease in blood glucose levels. In addition, the level of HOMA_IR decreased in treated rats and returned to the normal values. These findings are in consistent with previous studies reported that the mixture of these plants exhibited antihyperglycemic properties and enhanced insulin release in alloxan-induced diabetic rats. [27,28]

These results indicated that the plant mixture possesses a significant hypoglycemic and anti-hypoinsulinemic effects, due to the presence of hypoglycemic compounds which are sources of antidiabeticand antioxidant agents such as flavonoids, terpenoids, alkaloids, carbohydrates, glycosides, steroids, peptides and amino acids, lipids, phenolics and glycopeptides as well as the presence of damsin, ambrosin and hymenin which are considerd as an effective hypoglycemic agents. [29,30]

In addition, artemisinin and its derivatives(artesunate, amodiaquine, artesunate-amosdiaquine combination and quinine) have been clinically used to treat diabetes. [31]

A variety of phytochemicals have been reported for *C. droserifolia*, including; alkaloids, tannins, saponins, coumarins, docosaniocacid, catechins, amino acids, cardenolides, hydrocarbons, sterols such as (β-sitosterol and stigmasterol), glucosinolates with sulfur aglycones such as glucocapparin, sesquiterpenes like (carotol and dihydrodihydroxycarotol), methoxylated flavones, four flavone aglycones and four flavone glycosides. Phytochemical analyses on *Ambrosia maritime* extract have identified the presence of some sesquiterpenes such as (neoambrosin, chloroambrosin, damsinic acid, damsin, ambrosin, tribromoambrosin, stamonin-b, parthenin, anhydrofarnserin,

farnserin and hymenin). Some researches reported the presence of some coumarins as scopoletin, umbelliferoneisoprimpinellin, limettinesculetin, and isoscopoletin in *Ambrosia maritime* extract. In addition, tannin, alkaloid, saponins, resins and Hispidulin or (4', 5,7-trihydroxy-6-methoxy flavone) were isolated from the *Ambrosia maritime.* Other constituents were found in *Artemisia annua* including sesquiterpenoids, flavonoids and coumarins, together with proteins (such as β-galactosidase, β-glucosidase), steroids (e.g. B-sitosterol and stigmasterol) have significant pharmacological activities such as antitumor and antioxidant activities as well as have direct radical scavenging activity that contribute to the therapeutic effects of the herb. The main chemical constituents of *Artemisia annua*are sesquiterpenoids, including artemisinin, artemisinin I, artemisinin II, artemisinin III, artemisinin IV, artemisinin V, artemisic acid, artemisilactone, artemisinol and epoxyarteannuinic acid.[31]

Moreover, *Jasonia montana* contains high levels of phenolic compounds such as flavonols [quercetinglucuronide**,** syringentingalactoside or glucoside, kaempferol 3-O-acetyl-glucoside, coumarins, cinnamic acids and caffeic acids.[31]

Cleome droserifolia extract is also rich in bioactive compounds as flavonoids such as kaempferol-3,7-dirhamnoside, isorharmnetin-3-gluco-7-rhamnoside,kaempferol-3-gluco-7-rhamnoside,quercetin-3-gluco-7-hamnoside, kaempferol, artemitin, quercetin-3`-methoxy-3-*O*-(4``-acetylrhamnoside)-7-*O*-α-rhamnosidekaempferol-4`-methoxy-3,7-*O*-dirhamnoside. Also flavonoids such as apigenin; coumarins; sterols: ß-sitosterol were found in damsissa. Phytochemical analysis shows *Artemisia judaica* is a rich source of flavonoids including apigenin, cirsimaritin. Also, seventeen flavonoid glycosides and 12 a glycones were isolated and identified from *A. judaica*. Also, many reports confirmed that *Jasonia montana*is rich in flavonoidsuch as 3,6,7,3`,4`-pentamethoxy quercetin (artemitin), 3,6,7,3`-tetramethoxy quercetin (chrysosplenetin), 3,6,3`,4`-tetramethoxy quercetin, 3,6,7- trimethoxykaempferol, 3,6,3`-trimethoxy quercetin (jaceidin), 3,6,4`-trimethoxy quercetin (centaureidin), which may be responsible for free radical activity.[13]

In addition, phytochemical screening of *Cleome droserifolia* indicated the presence of volatile oil, which consist of 3-butenylisothiocyanate, 2-methyl butenylisothiocyanate, benzylisothio-cyanate, α, *β*,andγ-caryophyllene and2-naphthyl-n-propyl ether.[13,14]

Moreover, three terpenoidal compounds such as (buchariol, new diacetyltriterpene lactone, drosericarpone and stigmasterolglucoside) and dolabellanediterpenes like isorhamnetin-3-*O-β*-D-glucosidewere isolated from *Cleome droserifolia*. Essential oils (volatile oils) like (carvone, camphor, caryophyllene, cineole) were extracted from damsissa. Moreover, a number of essential oil chemical constituents from the aerial parts of *A. judaica* have been identified, namely, artemisyl-oil, apiperitone-oil, piperitone and trans-ethylcinnamate. *Artemisia Annuais* considered an important medicinal plant species with high content of essential oils which consist of camphene, β-camphene, isoartemisia ketone, 1-camphor, β-caryophyllene and β-pinene. In addition, other minor ingredients, such as artemisia ketone, 1, 8-cineole, camphene hydrate, and cuminal. The oils from Artemisia genus have shown a moderate antioxidant activity.(32)

The presence of these constituents may explain the hypoglycemic activity of these herbs. Furthermore, the mechanisms of medicinal plants for glucose control in diabetes include the inhibition of glucose absorption, improvement of insulin sensitivity, protection of β-cell damage, increase of insulin release, enhancement of antioxidant defense , attenuation of inflammation, modulation of carbohydrate metabolism pathway and regulation of insulin signaling pathways.[32]

Otherwise, the present results revealed a significant decrease in body weight gain in diabetic rats. These results may be associated with induction of diabetes by alloxan which caused catabolic effect on protein metabolism by retarding protein synthesis and stimulating protein degradation.[33]

This may be attributed to oxidative stress impair glucose uptake in muscle and adipose tissue in diabetic condition and decreases insulin secretion from pancreatic B cells. [34]

This results to a hyperglycemic environment which may impair radical scavenging activity and hence exposing proteins and lipids to peroxidation.[35]

Treatment with plant mixture causes significant enhancement of body weight. This may be attributed to enhance glucose uptake by adipose tissues or muscle and glucose production from liver. [36]

These results indicated that this plant mixture may exert antioxidant activities and protect the tissues from lipid peroxidation due to secondary metabolites such as alkaloids, tannins, flavanoids, polysaccharides, Saponins, coumarins, cinnamic acids and caffeic acids which are well known for its proven and demonstrated chemopreventive potential against ROS overproduction though direct and indirect antioxidant mechanisms.[37]Alkaloids can inhibit α-glucosidase and decrease glucose transport through the intestinal epithelium. Furthermore, polysaccharides increase the level of serum insulin, reduce the blood glucose level and enhance tolerance to glucose. Also, saponins stimulate the release of insulin and block the formation of glucose in the bloodstream. [38]

The present study showed a significant increase in ASAT, ALAT and GGT activities when compared with the control group. The elevation in the activities of these enzymes in diabetic rats indicated to hepatic injury. It may be due to leaking out of enzymes from the tissues and migrating into the circulation due to hepatotoxic effect of alloxan.[38]

This lesion may be attributed to the oxidative stress which can cause liver damage and can also produce an inflammatory reaction through cell injury. [40]

Otherwise, our results showed significant reduction in the serum total protein and albumin in alloxan diabetic rats. This may be ascribed to liver damage due to the decreased amino acid uptake greatly decreased concentration of a variety of essential amino acids and an increased conversion rate of glycogenic amino acid to CO_2 and H_2O. [41]

Furthermore, Colen recorded a new hormone called Betatrophin which secreted by liver and adipose tissues prompts beta cells in the pancreas to multiply and produce more insulin. [42]

However, the oral administration of water extract of plant mixture to diabetic rats returned liver enzymes and protein markers nearly to the normal levels. Therefore, our results are agree with previous studies which

indicated that other plant mixtures act as a hepatoprotective and antioxidant agent against the biochemical alterations induced by alloxan administration by inhibiting the liver damage leading to improvement in the liver function accompanied with regeneration of hepatocytes.[43,44]

These effects could be attributed to the anti-inflammatory and antioxidant influence of flavonoids and phenolic compounds which may be responsible for free radical activity. Flavonoids (bioflavonoids) are natural products, they are capable of modulating the activity of enzymes and affecting the behavior of many cell systems and they possess significant antihepatotoxic effect. [45]

The hepatoprotective activity of flavonoids was possibly due to its antioxidant properties, acting as scavengers of reactive oxygen species (ROS). Flavonoids were not only able to suppress the elevation of ALAT and ASAT but also reduce the damage of hepatocytes.[46]

Also, the essential oils (Camphor, borneol, bornyl acetate, chrysanthemol, intermediol, and 1,8-cineole artemisyl-oil-apiperitone-oil) which are excellent antioxidant agent exhibit anti-hypoinsulinemic effect may be attributed to its protective effect against hepatocyte damage and was shown to have a modulatory effect on the values of HOMA-IR.[47]

This may be due to the components of essential oils might be stimulating normal beta cells for insulin production enhanced peripheral uptake of glucose and restored protein concentration to the control level where insulin increased significantly and caused regeneration of hepatocytes.[48] These hepatocytes produced more β-trophin enhancing insulin production by β-cells of pancreas and enhancing body weight.

The present study reveals high prevalence of hypercholesterolemia, hypertriglyceridemia, high LDL and low HDL levels in diabetic rats. This increases the risk factors for cardiovascular diseases. [49]

Diabetes mellitus is associated with an increased risk of cardiovascular diseases mediated via oxidative stress. [50]

Hyperglycaemia-induced oxidative stress, it promotes reactive oxygen species (ROS) accumulation, accelerates cellular damage and significantly contributes to the cardiovascular dysfunction development in diabetes mellitus. [51]

This may be attributed to insulin deficiency which leads to a decrease in the activity of lipoprotein lipase and an increase in the metabolism of free fatty acids from peripheral fat depots. [52]

In addition, destruction of beta cells lead to depletion of plasma insulin, which resulted in hyperlipidemia and hypercholestermia caused derangement of metabolic abnormalities. [53]

So that, there is increasing evidence to support the idea that the antioxidant activity of both plant successive extracts can play a very important role in the treatment of hyperlipidemia–hyperglycemia case in rats.

Moreover, the present results showed significant amelioration of the lipid profiles by reducing the values of TL,TC, TG, LDL, VLDL and ratios of TC/HDL and LDL/HDLand elevating HDL levels in treated group.

These results are in consistent with other studies using different other hypoglycemic plants found plants play an important role in decreasing lipid peroxidation and have anti-atherosclerotic and anticholestatic activities. [54]

These may be explained by the presence of some phytochemical compositions which responsible for the hypolipidemic and hypoglycemic effects. Antioxidants remain as the medical choice strategy for protection against this unbalanced oxidant-antioxidant status.(53)

Flavonoids (artemitin, chrysosplenetin, jaceidinand centaureidin) are one of the most diverse and widespread group of natural compounds and are probably the most important natural phenolics as they possess radical scavenging properties.(53) Also, flavonoids act as reducers of hyperglycaemia. [55]

Flavonoids suppress the glucose level, reduce plasma cholesterol and triglycerides significantly and increase hepatic glucokinase activity probably by enhancing the insulin release from pancreatic islets.(53)

Otherwise, quercetin (quercetin-3-O-β-glucuronide and quercetin-3-O- β-glucoside) inhibits LDL oxidation through its antioxidant action which can arise from direct scavenging of free radicals. [56]

Flavonoid glycosides (apigenin-7-*O*-(2″-*O*-β-Dglucopyranosyl)-β-D-glucopyranoside and 6-methoxy qurecetin-7-*O*-β-D-glucopyranoside) these compounds may be responsible for antihyperlipidemic and hypoglycemic effect. Also, saponins are known to inhibit pancreatic lipase, leading to greater fat excretion due to reduced intestinal absorption of dietary fats. [57]

In addition, essential oils (trans-pinocarveol, camphor, kaempferolcan reduce levels of cholesterol and triglycerides significantly through its antioxidant activity by free radical scavenging assay. [58]

The current study showed increasing in levels of serum creatinine, urea and uric acid in diabetic rats as compare to the normal control rats. Increased concentrations of these renal markers indicated kidney dysfunction in diabetic rats may be due to high activities of xanthine oxidase, lipid peroxidation, and increased triglycerides and cholesterol levels, as well as impairment of the urea cycle enzyme activities. [59]

On the other hand, administration of aqueous extract of plant mixture to diabetic rats induced significant improvements in kidney functions. Otherwise, the reduction in creatinine, urea and uric acid concentrations in treated rats may be reflected the effect of mixture on improved kidney functions and its protection against oxidative damage. The possible mode of action of kidney serum parameters-lowering level could be explained by the following process. Polyphenols improved the kidney weight and serum levels of urea, nitrogen, creatinine and creatinine clearance as well as increased the activity of superoxide dismutase in the kidney. [46]Also, flavanone produced significant protection of renal function by significant reduction in serum urea and creatinine concentrations, decreased polyuria and reduction in body weight loss, marked reduction in urinary fractional sodium excretion as well as protected kidney tissues. [60]Finally, flavonoids lowered plasma creatinine and urea concentration. [61] The most powerful effect of the mixture may be attributed to the presence of synergistic effect of components of plants leading to ameliorate the hyperglycemia. This may be due to the interacting of one or more active ingredients of plant with each other and increasing the hypoglycemic effect of these mixture.

In conclusion,our results support that the aqueous extract of these plant mixture exhibit dantidiabetic and antihyperlipaedemic activity as well as strong antioxidant and free radical scavenging activity than each plant alone, where we tested each of these plants in a previous works.

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Table (1): Body weight changes in the control, diabetic and mixture extract treated rats

Groups	% Change of BW
Control	9.92 ± 7.41
Diabetic	4.98 ± 3.54^a
Diabetic + Mixture	10.0 ± 0.18^c

Values represent mean ±S.E. (n=10 rats).Values with different superscripts differ from each other significantly (P<0.01).[a]P<0.01 significant decrease than control. [c]p<0.01 significant increase than diabetic.

Table (2): Serum insulin and glucose levels in control, diabetic and mixture extract treated rats

Groups / Parameter	Control	Diabetic	Diabetic+ Mixture
Glucose(mg/dl)	87.44 ± 0.78	$350.20 \pm 0.85^{**}$	92.28 ± 0.55^a
Insulin (µU/l)	4.13 ± 0.45	2.54 ± 0.37^b	4.27 ± 0.85^c
HOMA-IR	0.89 ± 0.23	$2.20 \pm 0.36^*$	0.97 ± 0.08^a

Values represent mean ±S.E. (n=10 rats).Values with different superscripts differ from each other significantly (P<0.01). [**]p<0.01 increase than control; [a]p<0.01 significant decrease than diabetic; [b]p<0.01 significant decrease than control. [c]p<0.01 significant increase than diabetic. [*]P<0.05significant increase than control.

Table (3): Changes in the ALAT, ASAT and γGT (U/L) activities in control, diabetic and mixture extract treated rats

Groups / Parameters	Control	Diabetic	Diabetic+ Mixture
AST(U/L)	22.46 ± 0.56	$33.52 \pm 0.60^{**}$	31.58 ± 0.69^a
ALT(U/L)	37.02 ± 0.59	$53.24 \pm 1.08^{**}$	22.50 ± 0.96^a
γGT(U/L)	22.28 ± 0.12	$29.66 \pm 0.08^{**}$	23.18 ± 0.07^a

Values represent mean ±S.E. (n=10 rats). ASAT, aspartateaminotransferase; ALAT, alanineaminotransferase; GGT, gamma-glutamyltransferase Values with different superscripts differ from each other significantly(P<0.01). [**]p<0.01 increase than control; [a]p<0.01 significant decrease than diabetic.

Table (4): Changes in the serum proteins profile (g/dl) and A/G ratio in control, diabetic and mixture extract treated rats

Groups / Parameters	Control	Diabetic	Diabetic+ Mixture
Total protein(g/dl)	7.40 ± 0.08	$5.30 \pm 0.11^{**}$	7.36 ± 0.05^a
Albumin(g/dl)	4.68 ± 0.07	$3.12 \pm 0.06^{**}$	4.50 ± 0.08^a
Gobulin(g/dl)	2.72 ± 0.05	2.18 ± 0.07	2.86 ± 0.05
A/G ratio	1.72 ± 0.05	1.43 ± 0.04	1.57 ± 0.05

Values represent Mean ±S.E. (n=10 rats). Values with different superscripts differ from each other significantly (P<0.05). [**]p<0.05 significant decrease than control; [a]p<0.05significant increase than diabetic..

Table (5): Changes in creatinine, urea and uric acid levels ((mg/dl))in control, diabetic and mixture extract treated rats

Groups / Parameters	Control	Diabetic	Diabetic+Mixture
Creatinine (mg/dl)	0.91±0.005	1.66±0.08[**]	0.84±0.01[a]
Urea (mg/dl)	32.46±0.55	63.44±0.58[**]	33.28±0.95[a]
Uric acid (mg/dl)	3.26±0.04	7.24±0.09[**]	3.50±0.05[a]

Values represent Mean ±S.E. (n=10 rats). Values with different superscripts differ from each other significantly (P<0.01). [**]$p<0.01$ increase than control, [a]$p<0.01$ significant decrease than diabetic.

Table (6): Changes in total lipid(TL) , triglyceride (TG), total Cholesterol (TC), HDL cholesterol (HDL-C), LDL-cholesterol (LDLC) and vLDL- cholesterol (vLDLC) in control, diabetic and mixture extract treated rats

Groups / Parameters	Control	Diabetic	Diabetic+ Mixture
Total lipids (mg/dl)	474.00±8.12	1430.00±11.40[**]	486.60±0.68[a]
TC (mg/dl)	141.08±0.37	231.52±0.53[**]	143.70±0.58[a]
TG (mg/dl)	133.10±0.77	283.96±0.86[**]	135.38±0.40[a]
HDL (mg/dl)	47.88±0.49	38.08±0.39[b]	41.48±0.70[c]
LDL (mg/dl)	66.56±048[a]	136.40±0.59[**]	75.32±0.38[a]
VLDL (mg/dl)	26.62±0.16[a]	56.80±0.17[**]	27.12±0.05[a]
TC/HDL	3.00±0.00 [a]	6.08±0.04[**]	3.46±0.07[a]
LDL/HDL	1.38±0.02[a]	3.58±0.04[**]	1.80±0.04[a]

Values represent Mean ±S.E. (n=10 rats). Values with different superscripts differ from each other significantly (P<0.01). TC, total cholesterol; TG, triglycerides; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; VLDL, very low density lipoprotein cholesterol. [**]$p<0.01$ increase than control, [a]$p<0.01$ significant decrease than diabetic, [b]$p<0.01$significant decrease than control, [c]$p<0.01$ significant increase than diabetic.