RESEARCHS / INVESTIGACIÓN

Study the effect of herbal mixture plants extract on blood sugar level in normal and experimentally diabetic mice.

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Abstract: The use of medicinal plants for the management of diabetes mellitus is an old practice that has become even more relevant in a modern perspective. The present work was designed to evaluate the effect of a mixture of three medicinal plants which used in folk medicine in Iraq, These plants are (*Aloe vera*, *Artemisia herba alba*, and *Teucrium polium*) on the levels of blood glucose in normal and alloxan-induced diabetic mice, The aqueous extract of the herbal mixture was prepared and chemical detection of phenols, flavonoids, tannins, terpenes, steroids, glycosides, and saponins was carried out. Results revealed that the aqueous extract contains phenols, flavonoids, tannins, terpenes, glycosides, and saponins compounds. Evaluations of the parameters of our study were carried out on both standard and alloxan-induced diabetic mice. Thirty male mice were randomly divided into six equal groups: Group I (control): was kept as control negative mice treated with only distilled water. Group II: normal mice treated with aqueous extract of an herbal mixture at a dose (500 mg/kg/day).Group III: normal mice treated with aqueous extract of an herbal blend at a dose (250 mg/kg/day). The other 3 groups were subcutaneously administered a single dose (100 mg/kg) of alloxan to induce experimental diabetes. Groups IV (Diabetic): was kept as control positive, alloxan-induced diabetic mice treated with only distilled water. Groups V: alloxan-induced diabetic mice treated with aqueous extract of an herbal mixture at a dose (500 mg/kg/day). Group VI: alloxan-induced diabetic mice treated with aqueous extract of an herbal blend at a dose (250 mg/kg/day), respectively, for ten days. Results showed that normal mice treated with aqueous extract has no significant change in body weights and blood glucose level except those treated with a high dose of aqueous extract since they exhibited a significant decrease ($P \leq 0.05$) in blood glucose level. The results indicated a significant reduction in glucose level in diabetic mice after treatment with a high dose of aqueous extract of the herbal mixture. In conclusion, our results support that the aqueous extract of these plant exhibits anti-diabetic as compared with each plant alone, where we tested each of these plants in previous studies.

KeyWords: Herbal Mixture, Chemical Composition, Hypoglycemic Activity, Alloxan Induced Diabetic Mice.

Introduction

Diabetes mellitus, or only diabetes, is a group of diseases characterized by high blood glucose levels that result from defects in the body's ability to produce and/or use insulin. It is a condition primarily defined by the level of hyperglycemia, giving rise to risk of microvascular damage (retinopathy and neuropathy[1]. It is associated with reduced life expectancy, significant morbidity due to specific diabetes-related microvascular complications, increased risk of macrovascular complications (ischaemic heart disease, stroke, and peripheral vascular disease), and diminished quality of life[2]. Several pathogenetic processes are involved in the development of diabetes. These include operations, which destroy the beta cells of the pancreas with consequent insulin deficiency, and others that result in resistance to insulin action. The abnormalities of carbohydrate, fat and protein metabolism are due to the deficient effect of insulin on target tissues resulting from insensitivity or lack of insulin[3].

Herbal medicine is a growing area of health care that demands attention. Plants have played a significant role in maintaining human health and improving the quality of human life for thousands of years, and have served humans as valuable components of medicines[4]. World Health Organization (WHO) estimated that around 80% of the earth's inhabitants rely on traditional medicine for their primary health care needs, and most of this therapy involves the use of plant extracts or their active components[5]. This is reasoned by the fact that medicinal plants have advantages (low cost and fewer side effects) over the conventionally used drugs, which are expensive and known to have harmful side effects. Diabetes mellitus is a metabolic disorder featured by hyperglycemia and alteration in carbohydrate, fat, and protein metabolism associated with an absolute or relative deficiency of insulin secretion and/or insulin action. Although numerous oral hypoglycemic drugs exist alongside taking insulin, still there is no promising therapy to cure diabetes[6]. Diabetes mellitus is now taking place as a serious health care problem in the 21st century. The number of people who have diabetes is expecting to increase from 150 million actually to 220 million in 2010 and 300 million in 2030. This explosive increase has already imposed a significant burden on health-care systems, and this will continue to increase in the future[7]. Over the last few decades, the reputation of herbal remedies has increased globally due to its therapeutic efficacy and safety. In recent years, numerous traditional medicinal plants were tested for their antidiabetic potential in the experimental animals. There are more than 1200 plant species worldwide that are used in the treatment of diabetes mellitus, and a substantial number of plants have shown productive hypoglycemic activity after laboratory tests. The medicinal plants provide a useful source of oral hypoglycemic compounds for the development of new pharmaceutical leads as well as a dietary supplement to existing therapies. Of these traditional hypoglycemic herbs are Teucrium polium, Aloe Vera, and Artemisia herba Alba. The aqueous extract of the dried aerial parts of these herbal

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medicinal plants is used traditionally to treat diabetes in Southern Iran and some Middle East populations[8].

T. polium is a wild-growing flowering plant belonging to the family Labiatae and is found abundantly in southwestern Asia, Europe, and North Africa[9]. It is most common in Mediterranean climates and the Middle East. In Iraq, the plant is found all over the country, especially in the northern regions[10]. Polium is herbs, shrubs, or subshrubs. An unusual feature of this genus compared with other members of Labiatae is that the flowers lack the upper lip of the corolla entirely. Its flowers are small and range from pink to white. This plant is a dwarf, pubescent, aromatic shrub possessing oval leaves with enrolled margins and dense head of white flowers[11].

Popularly known in Iraq as "Sheh," is a well-known medicinal plant that has been used in the Middle East traditional medicine for treating various diseases. It is used by the local population of some countries as an anti-diabetic[12]. Herbal infusions from this species have been used as an analgesic, antibacterial, and hemostatic agents. It is used in Jordon in the form of a decoction against fever, menstrual, and nervous problems. The essential oil of this herb was found to be responsible for its therapeutic use as a disinfectant, anthelmintic and antispasmodic[13].

Aloe vera, commonly known as Barbados or Curaçao Aloe, is an herbal medicine with a long tradition of use by a variety of cultures. *Aloe vera* (syn.) has been used in traditional and folk medicines for thousands of years to treat and cure a variety of diseases[14]. Although the plant is native to the northern parts of Africa, it has rapidly spread across the world because its cultivation is easy. An important distinction has to be made between the strongly laxative and purgative latex derived from the bundle-sheath cells and the clear mucilaginous gel. The plant has been used by Egyptians, Assyrians, and Mediterranean civilizations, as well as in Biblical times. A variety of aloe species are still used in folk medicines of Africa and Asia. Hunters in the Congo reportedly rub their bodies in the clear mucilaginous gel to reduce perspiration; some African tribes apply the gel for chronic conjunctivitis; the gel is used in India for the treatment of asthma[15]. The present study was designed to evaluate the effects of aqueous extracts of an herbal mixture of three medicinal plants (*Aloe vera*, *Artemisia herba alba*, and *Teucrium polium*) on alloxan-induced diabetic as a hypoglycemic drug for treatment of diabetes mellitus using normal and experimental alloxan-induced diabetic mice as animal model for study.

Materials and methods

Plant Material

This research was conducted from January 2019 until April 2019. The plants (*Aloe vera*, *Artemisia herba alba* (*sheh*) and *Teucrium polium* (*Algeada*) were purchased from the local market in Iraq and identified in January 2019 at the College of Agriculture and Baghdad University - Iraq. The plants were left at room temperature (20-25ºC) until use.

Preparation of extract

Equal weights of each plant (*Aloe vera*, *Artemisia herba alba* (*sheh*) and *Teucrium polium* (*Algeada*) were ground and mixed. The aqueous extract of this mixture was prepared by boiling 2 g of the 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.

Laboratory Animals

Animal maintenance was performed at the Animal Hospital of Biotechnology Research Center at Al-Nahrin University. A total of 30 albino male mice (*Mus musculs*) were employed for experimenting with the study. They were supplied from (biotechnology research center / Al-Nahrin University), and their age at the start of operations was 2-4 months, and their weight was 25-35 grams. Mice were kept for one week for acclimatization before being used in the experiments. They were divided into groups, and each group was housed in separate transparent plastic cages with stainless steel cover lids. The animals were maintained at a temperature of 20-25°C, and they had free excess to food (standard pellets) and water throughout the experimental work.

Chemical Detection of Plant Extracts

Phenols: Equal quantity of aqueous ferric chloride (1%) was mixed with potassium iron cyanide (1%). The equal amount of the reagent and water or alcoholic plant extract was mixed. The appearance of blue-green color indicates a positive result[16].

Flavonoids: The detecting solution was prepared by mixing 10 ml of ethanol (50%) with 10 ml of potassium hydroxide (50%), and then 5 ml of this solution was added to 5 ml of the plant extract. The appearance of yellow color was an indicator of the presence of flavonoids[17].

Tannins: The procedure of Harbone (1984)[17] was used for the detection of tannins. In this procedure, 50 ml of each extract was equally divided into two conical flasks. For the first one, lead acetate solution (CH_3COOPb) (1%; w/v) was added and the appearance of jelly pellet was considered a positive reaction, while for the second flask, ferric chloride solution (FeCl2) (1%; w/v) was added and the appearance of blue color was an indicator for the presence of tannins.

Glycosides: This method was done according to the method described by Harbone (1984)[17] .

Non hydrolyzed extract: An equal amount of the plant extract was mixed with Fehling reagent in the test tube and then boiled in the water bath for about 10 minutes. The development of red precipitate indicates a positive result.

Hydrolyzed extract: Few drops of dilute HCl was added to 5ml of the aqueous extract of the plant, then left in a boiling water bath for 20 minutes, the acidity was neutralized by NaOH solution, an equal volume of Fehling reagent was added. The development of red precipitate indicates a positive result.

v. Saponins: This method was done according to the method described by Harbone (1984)[17]. Two methods detected saponins:

- A solution of plant powder was shaken vigorously in a test tube. The formation of foams standing for a time indicates a positive result.

- Five ml solution of plant powder was added to 1-3 ml of 3% ferric chloride solution. The development of white precipitate shows a positive effect.

vi. Terpenes and steroids: one ml of a solution of plant powder participated in a few drops of chloroform, and then a drop of acetic anhydride and a reduction of concentrated sulphuric acid were added, brown precipitate appeared which represents the presence of terpenes. The appearance of a dark blue color after few minutes indicates the presence of steroids[18].

Experimental Design

The experiment was designed to assess the effects of two doses (250,500 mg/kg) of aqueous extract of a herbal mixture on the investigated parameters in normal and alloxan-induced diabetic mice. The plant extracts were given orally using gavage needle as a single dose (0.2 ml) per day and for 10 days, and then the mice were sacrificed in day 8 for laboratory assessments. Thirty male mice were used in this study and divided into six groups (five mice for each group):

Group I (control): mice treated with only distilled water.

Group II: normal mice treated with aqueous extract of an herbal mixture at a dose (500 mg/kg/day).

Group III: normal mice treated with aqueous extract of an herbal mixture at a dose (250 mg/kg /day).

Groups IV (Diabetic): alloxan-induced diabetic mice treated with only distilled water.

Groups V: alloxan-induced diabetic mice treated with aqueous extract of an herbal mixture at a dose (500 mg/kg/day).

Group VI: alloxan-induced diabetic mice treated with aqueous extract of an herbal mixture at a dose (250 mg/kg/day).

Blood Glucose Level

Blood glucose level was measured with commercially dextrose measurement strips read by Accu-Chek active system.

Sample required and testing time: Accu-Chek active meter requires 1-2 µL blood per test, and the testing time is about 5 seconds.

Test Principle: On each test strip, there is a test area containing sensitive chemicals. When blood is applied to this area, a chemical reaction takes place (glucose dye oxidoreductase mediator reaction) causing the color of the test area to change. The meter registers this color change and converts the signal obtained into a blood glucose result.

Results and Discussion

Diabetes mellitus (DM) is a group of metabolic disorders that result in hyperglycemia as a result of a relative or absolute lack of insulin, or the actions of insulin on its target tissues or both[19]. It is the most common endocrine disorder, and by the year 2010, it is estimated that more than 200 million people worldwide will have DM, and 300 million will subsequently have the disease by 2025. Regulation of blood glucose concentration plays a vital role in diabetic patients. For centuries, medicinal plants provide a natural source of potent anti-diabetic drugs. In Iraqi regions, there are many types of plant herbs are these contexts. Among which an Herbal mixture including *Teucrium polium*, *Aloe vera*, and *Artemisia herba alba*. Our research aims to study the possible anti-diabetic effect of a mix of these medicinal plants used in folk medicine in Iraq and if they can improve the metabolic abnormalities accompanied to alloxan-induced diabetic albino mice.

Percentage of Extracts

Aqueous extraction resulted in 2.55g, representing 8.38 % per 25g of the raw plant material.

Characterization of chemical composition

Active compounds of an Herbal mixture revealed that flavonoids, phenols, tannins, saponins, and glycosides and terpenes were detected aqueous extracts were negative for steroids (Table 1). These results agreed with El-Shazly and Hussein (2004)[20] who demonstrated that Chemical detections in *T. Polium aqueous* extracts revealed that flavonoids, phenols, tannins, saponins and glycosides, and terpenes. On the other hand, active compounds of *Artemisia herba alba* is shown that a rich source of flavonoids such as hispidulin and cirsilineol. Flavonoids isolated from some medicinal plants have been proven to possess anti-inflammatory effect[21]. And *Aloe vera* is widely used in food supplements, beverages, pharmaceuticals, and cosmetics, Major proteins and mono- and polysaccharides were identified and analyzed from *Aloe vera* commercial extract.

Extraction test	Aqueous extract
Phenols	+
Tannins	+
Flavonoids	+
Terpenes	+
Saponins	+
Glycosides	+
Steroids	-

Table 1. Organic composition of the aqueous extract of an Herbal mixture.

General parameters and bodyweight of experimental mice

Normal mice treated with distilled water (control) showed no change in physiological activities and body weight during the period of treatment, suggesting that experimental conditions (nutrition, humidity, and light) did not affect body weight. However, mixture -treated diabetic mice showed signs of recovery in body weight gains at the end of the experiment as compared to non-treated diabetic mice. An herbal Mixture extract did not affect body weight in standard groups during the period of analysis (Table 2).

It is well known that alloxan, induces the formation of superoxide radicals which dismutate to hydrogen peroxide with the simultaneous massive increase in cytosolic calcium concentration resulting in rapid destruction of β pancreatic cells. This destroys a large number of β cells, resulting in a decrease in endogenous insulin release, which paves the way for the decreased use of glucose by the tissues[22] and leading to increased breakdown of stored carbohydrates, lipids, and proteins to compensate the deficiency of glucose. Using of carbohydrates, lipids, and proteins as a source of energy causing loss of body weight. Diabetic groups treated with extract of an Herbal mixture keep their loads from marked decreasing observed in the non-treated diabetic group. This might be attributed to flavonoid and/or terpenoid content in extract, which might be responsible for potentiating insulin action and increasing glucose consumption by tissues and finally reduce breakdown of stored carbohydrates, lipids, and proteins[23].

Blood glucose level

Regulation of blood glucose concentration plays a vital role in diabetic patients. The blood glucose levels (BGLs) of diabetic mice treated with aqueous extract of an Herbal mixture were reduced in comparison with those of the diabetic control group.

Treatment Groups		Dose (mg/kg)	Weight (g) ± SE Before treatment	Weight (g) ± SE After treatment
Normal + (distilled water)		0.0	27.2 ± 0.6	27.5 ± 0.8
Normal Mice	Aqueous (Mixture)	250	30.5 ± 0.6	30.8 ± 1.7
	Aqueous (Mixture)	500	30.1 ± 0.4	30.7 ± 0.6
Diabetic + (distilled water)		0.0	31.5 ± 0.5	29.2 ± 1.2
Diabetic Mice	Aqueous (Mixture)	250	30.8 ± 0.8	28.8 ± 3.3
	Aqueous (Mixture)	500	30.5 ± 1.5	26.0 ± 0.9*

**** Significant difference (P ≤ 0.05) between means before and after treatment for each group***

Table 2. Bodyweight of normal and alloxan-induced diabetic mice before and after treatment with a dose of aqueous extract of an Herbal mixture.

The blood glucose level in normal mice treated with distilled water (control) was observed during the period of treatment, which revealed that experimental conditions (nutrition, humidity, and light) did not affect the blood glucose level. A marked elevation in blood glucose levels was measured in the non-treated diabetic group. Diabetic mice treated with extracts exhibited a significant decrease in blood glucose levels as compared to non-treated diabetic groups. Only the high doses of aqueous extract showed a significant reduction in blood glucose levels in the normal mice during the period of experiment. The blood glucose levels are recorded in (Table 3). Depending on our results it is clear that oral administration of extracts of an Herbal mixture showed hypoglycemic effect on normal and diabetic mice. This result agreed with Iriadam *et al.*(2006), who reported the hypoglycemic action of *T. polium* and *Artemisia herba alba* extracts suggest more than possible mechanism of action.

A vera leaf pulp and gel extracts were ineffective in lowering the blood sugar level of ND rats. A. vera leaf pulp extract showed hypoglycaemic activity on IDDM and NIDDM rats, the effect being enhanced for type II diabetes in comparison with glibenclamide. On the contrary (24). Oral administration of the methanol extract of the aerial parts of Artemisia pallens Wall. (Used in Indian folk medicine for the treatment of diabetes mellitus) led to a significant blood glucose-lowering effect in glucose-fed hyperglycaemic and alloxan-induced diabetic rats.

A. vera leaf gel extract showed hyperglycaemic activity on NIDDM rats. It may, therefore, be concluded that the pulps of *Aloe vera* leaves devoid of the gel could be useful in the treatment of non-insulin dependent diabetes mellitus. One of which the potentiation of insulin action released from pancreatic β-cells. It had been found that the hypoglycemic effects of the aerial parts of *T. polium* may be due to its content of flavonoids and/or terpenoids[25]. Other researchers suggested that the hypoglycemic activity was due to the presence of several flavonoids in T. polium and Artemisia herba alba. One such flavonoid with hypoglycemic effects in diabetic animals was quercetin. They reported that quercetin might exert its effects on insulin release from rat islets of Langerhans via changes in Ca^{2+} metabolisms[26]. Similar findings were reported by Sulaiman *et al.*[27]who said that the flavonoids present in the aerial parts of *T. polium* and *Artemisia herba alba* might be respon-

Treatment Groups		Dose (mg/kg)	Glucose (mg/dl) Before treatment	Glucose (mg/dl) After treatment
Normal + (distilled water)		0.0	155.2 ± 6.0	158.2 ± 10.2
Normal Mice	Aqueous (Mixture)	250	190.0 ± 6.0	184.0 ± 10.8
	Aqueous (Mixture)	500	185.5 ± 10.4	165.1 ± 10.5*
Diabetic + (distilled water)		0.0	502.0 ± 12.2	530.2 ± 14.8
Diabetic Mice	Aqueous (Mixture)	250	510.5 ± 15.4	385 ± 13.1*
	Aqueous (Mixture)	500	420.2 ± 14.2	250±10.8*

**** Significant difference (P ≤ 0.05) between means before and after treatment for each group***

Table 3. Effect of aqueous extract of an Herbal mixture on Blood glucose level of normal and alloxan-induced diabetic mice.

sible for islet regeneration and possibly for β-cell regeneration and insulin release and/or they might have insulin-like properties. Other researchers reported that the therapeutic action of flavonoids is due to their antioxidant activity by various mechanisms, e.g., by scavenging or quenching free radicals, by chelating metal ions, or by inhibiting enzymatic systems responsible for free radical generation.

Statistical analysis

All observations were first recorded in a notebook and entered into PC and verified by another person for the accuracy of data entry. Values were expressed as mean ± SD (of 30 animals). The statistical analysis was performed using a Student's two tailed-test software program according to World Health Organisation(2015)[28]. P values less than 0.05 were considered statistically significant.

Conclusions

Different active compounds were detected in the aqueous extract of a mixture of three medicinal plants (*Aloe vera*, *Artemisia herba alba*, and *Teucrium polium*) include phenols, flavonoids, tannins, saponins, glycosides, and terpenes. Aqueous extract of an herbal mixture of three medicinal plants (*Aloe vera*, *Artemisia herba alba* and *Teucrium polium*) has hypoglycemic activity in both standard and alloxan-induced diabetic mice in a dose-dependent manner. Our results support that the aqueous extract of these plant mixture exhibits antidiabetic as compared with each plant alone, where we tested each of these plants in previous studies.

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