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Physiological effect of Ephedra ViridisAs Antibacterial and Antidiabetic in Normal and Diabetic Rats

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ABSTRACT
The **purpose** of this study was to determine the effects of Ephedra viridis in normal and diabetic rats with antibacterial activities.
Methods 40 male albino rats weighing between 200 and 240 gm were included in this study streptozotocin induced diabetic rats, randomly divided into 4 groups. Group 1 Normal control group,Group 2 Normal rats treated with Ephedra viridis,Group 3 Diabetic rats and Group 4 Diabetic rats treated with Ephedra viridis.. These groups tested for oral glucose tolerance test, HbA1C and lipids profile. The
Results were significant in the group 4 for oral glucose tolerance test and HbA1C also showed significant difference in lipids profile. Also Antibacterial Activities showed inhibited both Gram negative and Gram-positive bacteria.
Conclusion the Ephedra viridis showed significant effect in diabetic hyperlipidemia rats and antibacterial activities
KEYWORDS— Antibacterial, Ephedra viridis, HbA1C, lipid. (OGTT), Streptozoto

INTRODUCTION

Diabetic illness can be identified by performance one of three different types of blood tests, the oral glucose tolerance test, the fasting plasma glucose test and the hemoglobin A1C test. Modern research displays that more than 470 million people around the world will be affected by diabetes in 2030 [25]. Patients are struggle to create the essential lifestyle changes to control blood sugar levels, the researchers have often recommended the use of natural herbal to

treatment diabetes rather than depend on drugs. Therefore, herbs may offer a new choice for organization blood sugar levels, either alone or in grouping with other treatments [10].
Diabetes mellitus is a universal metabolic disease considered by hyperlipidemia, hyperglycemia, hyperaminoacidemia, and hypo insulinaemia it clues to decline in both insulin excretion and insulin action [11, 12]. Diabetes mellitus treating involves the use of different drugs depending on the stage of the disease, a lot of patients try to change methods of treatment, depend on medicinal plants. Nowadays, there has been a positive movement about the use of the herbal medicine in treating of chronic diseases, however this therapeutic attitude has been the only choice in the unused countries of the world, Ephedra viridis is completed with many compact groups of erect bright green branches. They might yellow rather with age [23].
The effect of obesity on many kinds of chronic disease for example abnormalities in hypertension, hyperinsulinemia, lipid metabolism, glucose intolerance and type 2 diabetes disease affected by the overall or comparative absence of insulin, the common categorized of diabetes as either type 1 or type 2 diabetes, type 1 diabetes arises from the autoimmune damage of insulin-secreting pancreatic β-cells, causing in following hyperglycemia and insulin absence type 2 diabetes is described by abnormal insulin excretion, the indicators of diabetes exciting hunger, very arid skin, extreme thirst and frequent urination [27].Ephedraceae are the family of the Ephedra one of the medicinal plants. Green Ephedra is scraggly tree only just more than 60 cm to 150 cm high. The nude seeds are the features, which places of Ephedra with the typical group of the gymnosperms. Ephedra found in dry regions of the tropics and subtropics [2]. Numerous reports have expected the antimicrobial activities of the genus of Ephedra against several microorganisms species, including Bacterial species such as; Bacillus anthracis, Pseudomonas aeruginosa, Staphylococcus aureus, Lactobacillus acidophilus, and Lactobacillus casei; Fungal species such as Saccharomyces cerevisiae, Aspergillus parasiticus, Candida utilis, Candida albicans; virus-related species such as avian influenza virus [24],[26].The main residents of Ephedra species are, flavonoids and alkaloids [9]. Ephedrine alkaloids, which are also identified as amphetamine analogs, phenylpropylamino alkaloids, or substituted amphetamines. These complexes compound have a phenethylamine backbone through a methyl group set at the α-position relation to the nitrogen[17].However, Ephedra revealed varied range of pharmacological activities with antimicrobial, antioxidant, anticancer, cardiovascular, central nervous, respiratory, hypolipidemic, anti-inflammatory, antipyretic immunological, and many other pharmacological properties[18-1].The present study was scheduled to supervise the effects of Ephedra viridi aqueous extract on gram positive and gram negative bacteria, and antidiabetic effects of aqueous extract on OGTT, HbA1c, and lipids profile in normal and streptozotocin-induced diabetic rats.

MATERIALS AND METHODS

Study area:

This study was carried out in the period from June 2023 to October 2023 at the Department of Biological Sciences, Al al-Bayt University, Jordan

Plant Materials:

Ephedra viridisaerial parts were collected in May 2022 from the Almafraq district, east of Jordan. The plant was identified using Flora Palaestina, Part 3 as a guide, and further confirmed by the botanist Prof. ImadAlhashmi. The collected plant materials were firstly washed under running tap water to remove external debris of dust particles or epiphytic hosts found normally on the surface, followed by washing with

sterilized distilled water. The plant was further air-dried at room temperature on filter paper and then grinded with sterilized pestle and mortar under aseptic condition to get fine powder.

Ephedra viridis extraction: Aqueous Extract

The aqueous extract was prepared by using 150 gm of Ephedra viridis powder soaked in 500 ml hot water (80°C) in shaking water bath for 6hrsand then filtered by capronsilic cloth 150 µ. The filtrate was further centrifuged at 5000 rpm for 10 minutes and the supernatant was completely evaporated to dry by rotary evaporator. Collected powder was kept in refrigerator until the desired extract concentration been prepared.

Antibacterial effect

Bacteria strains

Different types of gram positive and gram negative bacteria were used in this work, Gram positive bacteria; *Bacillus subtilis* (skin clinical isolate) and *Staphylococcus aureus* (ATCC 25923), Gram negative bacteria; *Pseudomonas aeruginosa* (ATCC 27853) and *Escherichia coli* (ATCC 25922), and urinary clinical sample). Bacteria were cultured in fresh nutrient broth at 37 C incubator for 24 hr. Inoculum size for susceptibility test was estimated by OD 680 at nearly 0.8- 0.9.

Agar well diffusion method

Basically, agar well diffusion method was used to assess the potential antibacterial activity of the extract. Briefly, 0.1 ml of bacterial broth culture were spread by hooky glass rod at the surface of Muller Hinton agar petri dishes Holes with a diameter of 6 mm in solid agar are cut aseptically by a sterile cork borer. Then a volume 100 µL of different concentrations of plant extract (50,100,150 and 200) mg/ml were added in each hole. Inhibition zones were measured after 24 hours incubation at 37°C and compared to 5 µg/ml tetracycline as positive control antibiotic.

Evaluation of Minimal inhibitory concentration (MIC)

Serial dilution method was adopted depending on the result by agar well diffusion method where a stock solution of 5 mg/ml was serially diluted in 96-well microtiter plate with Mueller-Hinton broth to obtain a concentration ranging from 8.0 µg/mL to 2000 µg/ml. Results were visualized after 24 hr incubation at 37 C . Further investigation were done to estimate the minimal bactericidal concentration (MBC) by using petri dishes containing nutrient agar culture media.

Animals

Forty male albino rats, each weighing between 200 and 240 grams, were obtained from the Department of Medical Technology at Alahlya Amman University, Jordan. The rats were maintained in a well-ventilated environment with a 12-hour light-dark cycle. The animals were divided into four groups:

Group 1 Normal rats (control group).

Group 2 Normal rats treated with Ephedra viridis

Group 3 Diabetic rats Rats treated with streptozotocin (45 mg/kg intraperitoneally) to induce diabetes.

Group 4 Diabetic rats treated with Ephedra viridis.

Groups 1 and 4 were orally administered Ephedra viridis for eight weeks.

Blood Sampling

Blood samples were collected from the rats under different conditions:

From normal rats and three days after streptozotocin treatment (considered zero time).After administration of 0.5 mL of Ephedra viridis extract, blood was drawn from the eye at intervals of

30 minutes, 1 hour, and 2 hours for an oral glucose tolerance test (OGTT).From diabetic rats at zero time and after two months of treatment.Blood samples for HbA1c analysis were taken from the normal and diabetic rats treated with Ephedra viridis at zero time and after two months.
Lipid profiles were obtained from the diabetic rats treated with Ephedra viridis at zero time and after one month.

Statistical Analysis

Collected data were tabulated and needed statistical analyses were done using descriptive statistic, means, and standard deviation (SD) of the means were calculated utilizing the computer data processing (SPSS, version 15). A probability value (P) of <0.05 was considered to be statistically significant.

RESULTS

Table 1 demonstrates Levels of oral glucose tolerance test (OGTT) before and after consumption of ephedra viridis changes in all the groups during the study. Our data showed group 3 returns to normal level after consumption of ephedra viridis. While group 2 shows significantly increased and group 1 not affected.
Table 2 exhibits HbA1C before consumption of ephedra viridis it was 9.6% on diabetic rats after consumption of ephedra viridis for two months the level of HbA1C decreased to 7.6% this means 2.0% reduction. While normal group reduction was 0.3%.
Table 3 demonstrates lipid profiles changes in all the groups during the study. remarkable elevation in triglyceride, cholesterol and LDL levels were noted in all the diabetic groups but after consumption ephedra viridis for one month significant decrease in triglyceride, cholesterol and LDL levels were observed, and HDL level was increased.
Table 4 indicated that the Ephedra viridis plant extract inhibited both Gram negative and Gram-positive bacteria. Plant extract made inhibition zones diameter ranging from 7±0.88 mm to 14±0.33 mm. However, the highest activity of the extract was found to be against Pseudomonas aeruginosa. Standard antibiotic (Tetracycline) and DMSO were evaluated for their antibacterial activity against the selected microorganisms where the zones of inhibition varied from 15 ±0.33mm to 18± 0.88mm at a concentration of 50 mg/ml, while DMSO didn't show any of growth inhibition for all tested microorganisms

Table (1) - Levels of oral glucose tolerance test (OGTT) before and after consumption of ephedra viridis on normal and diabetic rats.

Groups	Zero time mg/dL	After 1/2 hour mg/dL	After 1 hour mg/dL	After 2 hours mg/dL
Group 1	75	130	150	70
Group 3	250	300	350	340
Group 4	130	210	195	146*

*Significant different at P< 0.05 level.

Table (2): Levels of HbA1C before and after consumption of ephedra viridis on normal and diabetic rats.

Group	Zero time %	After two months Treated with Ephedra viridis %	% Reduction
NORMAL	4.8	4.5*	0.3
Diabetic	9.6	7.6*	2.0

*Significant different at P< 0.05 level.

Table (3): Levels of lipid profiles before and after consumption of Ephedra viridis on normal and diabetic rats presented as mean ± SE.

Test	Zero time from diabetic rats mg/dL	After one month diabetic rats treated with ephedra viridis mg/dL	% Reduction
Triglycerides	170.7±13.6	145.4±6.23*	15
Cholesterol	191.3± 11.20	158± 12.10*	18
LDL	127.7± 15.10	102.7± 10.70*	20
HDL	45.6± 3.2	57.3± 3.22*	21% increase

*Significant different at P< 0.05 level.

Table (4): Antibacterial activity of *Ephedraviridis*ethanolic extract against selected microorganisms.

Bacterial types	**Inhibition zone (mm)**				
Concentration (mg/ml)	**50**	**100**	**150**	**200**	**Tetra**
Escherichia coli	10 ± 0.33	11 ± 0.33	11 ±0.33	12 ±0.33	15 ±1.15
Bacillus subtilis	7± 0.88	8 ± 1.15	10 ± 0.57	10 ±1.15	18 ±0.88
Staphylococcus aureus	8 ± 1.15	9 ± 0.33	10 ± 0.33	11 ±1.15	15 ±0.33
Pseudomonas aeruginosa	10 ± 0.33	10 ± 0.88	12 ±1.15	14 ±0.33	16 ±1.15

Values are the diameter of the inhibition zone in (mm), Positive control, Tetracycline, (50mg/ml), Data are means of three replicate ± standard error.

DISCUSSION

Current study showed that Ephedra viridis pointedly reduced the levels of cholesterol, glucose, and increased the level of HDL in the serum. A huge number of people injured from diabetes all over the world, it is a universal chronic disease well-defined as protracted hyperglycemia and considered by metabolic disorders of carbohydrates, lipids and proteins (Assmann, 2003) [4]. Research indicate that ephedra non-alkaloids had the benefit of reduced the cholesterol and increased the level of HDL. The rising in the level of HDL might possibly contribute to anti-atherogenicity, inhibit LDL oxidation and repair endothelial cells from the cytotoxic effects [12]. These come to an agreement with our present results show that ephedra non-alkaloids may defend compared to cardiovascular disease by accumulative the level of HDL.

(Punithain, 2005) [21] investigated that lipid droplets were less demanding and lesser in ephedra non-alkaloid groups which recommended that ephedra extractions decrease the fat absorption which reduction the levels of triglyceride moreover dropped the lipid levels, these support our study.

In hypercholesteremia, the free radicals levels was increased and the action of SOD gene is declined so Polyphenols and flavonoids may scavenge free radicals as well as hydroxyl and superoxide anions to inhibit lipid peroxidation and develop lipid profiles[8]. Ephedrine motivates

beta-1 receptors which stimulating heart rate receptors of beta-2, and beta-3 stimulating oxygen consumption, glucose insulin, and c-peptide [19]. Moreover, products containing of ephedrine were can't be used with heart disease by people, thyroid disease, enlarged prostate, hypertension, diabetes, anxiety and restlessness or glaucoma, people attractive inhibitors of monoamine oxidase or women who are lactating or pregnant. Ephedrine frequently survives in unapproved herbal arrangements that also contain caffeine these mixture products may supplement ephedrine's cardiac and CNS effects [14].

As a result, the side effect of hypoglycemic agent request for natural products had increased due to the great cost and deprived access to resolution medical therapy for most country population particularly in developing countries. The tradition of herbal medicine were used to advance diabetic complication [16]. However, our results showed prevented reduction of Langerhans islets. These data exposed that active components of ephedra extract could moderate oxidative stress, which was induced by STZ in the diabetic rats. It may also encourage restoration of the islets in the pancreas tissue. While many studies have been directed on the antidiabetc of many plants in the world, rare study has been finished in this case. Lately, a study was completed on this case of Ephedra on serum lipid levels in rats with the same result by way of the present study [5]. (Georgopapadakou and Walsh 1996) [10] found that ephedra extracts affected significant decrease in contents of total sterols, total lipids, total neutral lipids, and phospholipids. It has been recognized that lipid moieties are an imperative materials in biological membranes singing a critical role in their permeability. Similar modification has been documented in present study [22].

(Helal et al. 2007) [14] established in their study that the variation in fatty acids composition has been connected to mold resistance against the converse biotic stress to maintain the membrane fluidity of living cell, the finding explain our results in the reduction of lipids level [3].

In recent times, greatly attention has been directed on the way to plant extracts and biologically active compounds isolated from plants, Plants as a vital sources of effective drugs possess antimicrobial belongings due to the presence of a wide diversity of secondary metabolites, such as flavonoids, tannins, terpenoids, alkaloids [20]. The medical uses of Ephedra dates back to at least 2700 B.C., when the Chinese used Ma Huang ephedra sinicaStapf to treatment cough, asthma and bronchitis. Then, in developing countries people use medicinal plants against infective disease because they cannot afford exclusive drugs. In addition, due to increasing rate of drug-resistant diseases, there is an imperative need to distinguish novel antimicrobial compounds from medicinal plants [13]. Recently, the natural antioxidants have expressively increased in food, cosmetic and therapeutic products since they have multifacetedness in their mass and amount of activities and deliver giant scope in modifying imbalance [14]. Comparable study showed that the methanol extract of Ephedra sarcocarpa had exciting activity against both Gram negative and Gram-positive bacteria [6]. In another study, (Sumiko et al., 2016) [24] confident that the ephedrine alkaloids-free Ephedra Herb extract has significant antiviral activity against influenza virus and mentioned finding a authorization approval for the therapeutic use of ephedrine alkaloids-free Ephedra [4]. On the other hand, Phytochemistry of ephedra indicates also the presence of polyphenols and flavonoids which act as antioxidant compounds may scavenge free radicals as well as hydroxyl and superoxide anions to inhibit lipid peroxidation and develop lipid profiles[13]. In addition, Ephedrine- another constituent- motivates beta-1 receptors which stimulating heart rate receptors of beta-2, and beta-3 stimulating oxygen consumption, glucose insulin, and c-peptide[14,15].

CONCLUSION

The results of this study indicate significant reduction in oral glucose tolerance test, HbA1C and lipids profile by ephedra viridis extract.Furthermore antibacterial effects against Gram-positive and Gram-negative bacteria.

Ethics statement

The research protocol was approved by the Animal Care and Use Committee of the Al albayt University and conducted in compliance with institutional guidelines.

Author contributions

Abdulrahim Aljamal, Mahmoud Al Shawabkeh : Conceptualization and wrote the manuscript; TahaAlqadi performed the literature review and article writing and editing; SajedaAlbarri, Abdallah Ibrahim performed Methodology, All authors read and approved the final version of the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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