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Phytochemical screening and pharmacological activities of various fractions derived from *Ephedra foeminea Forssk* aerial parts

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Abstract

Obesity is a major risk of chronic diseases such as metabolic syndrome, cardiovascular diseases and type-2 diabetes mellitus. These diseases are the leading causes of death worldwide. Therefore, it is necessary to search for anti-obesity therapeutics. This study aimed to investigate, in vitro, the anti-obesity effect of Ephedra foeminea Forssk. for the first time. To achieve our aim, acetone, hexane, aqueous and methanol fractions were derived from E. foeminea. It appeared that the four fractions had strong inhibitory effect against the activity of α-amylase enzyme with IC50 value range 13-15 µg/mL. Methanol fraction possessed the strongest inhibitory effect on glucosidase (IC50 117.5 µg/mL) and lipase enzymes activities (IC50 24.5 µg/mL). Furthermore, methanol fraction possessed the strongest antioxidant activity (IC50 14.5 µg/mL) and had no cytotoxic effect. This indicates that methanol fraction is a potential and safe source of therapeutically active compounds with anti-obesity and anti-diabetic potency.

Keywords: *Ephedra foeminea Forssk*. Antioxidant. Anti-α-amylase. Anti-α-glucosidase. Anti-lipase. Anti-obesity. Anti-diabetes.

Introduction

Obesity is escalating at an alarming rate worldwide. More than 1.9 billion adults (~39%) were overweight worldwide, of these, over 600 million were obese (~13%) **[1]**. Obesity results from complex interactions of genetic, behavioral and environmental factors correlating with economic, social status and lifestyles. The development of obesity is mainly caused by an imbalance between energy intake and expenditure **[2-4]**. Obesity is a major risk to health. It has been shown to be responsible for an estimated 216,000 deaths accounting for about 1 in 10 deaths in US adults **[5]**. This is because obesity increases the risk of chronic diseases such as metabolic syndrome, insulin resistance syndrome, cardiovascular diseases, type-2 diabetes mellitus and some cancers **[6]**.

These diseases are the leading causes of death worldwide. Obesity is not just a critical health concern; it is also a threat to the global economy. The worldwide economic impact of obesity amounts to approximately USD 2 trillion annually (McKinsey Global Institute, 2014). A considerable interest has recently aroused toward the potential of natural products as anti-obesity therapeutics and consequently anti-diabetic and other related diseases **[3, 4]**.

Ephedra is a genus of non-flowering seed plants belonging to the Ephedraceae family, which includes approximately 67 species, mainly in the desert areas of Asia, America, Europe and North Africa **[7]**. *Ephedra foeminea* Forssk, is a wild light green densely branched, monogenic, small and perennial climber shrub, the twigs appear leafless and the leaves reduced to small scales, cones sessile shaped, clustered in the axils or at branch tips, the red fruits are fully matured upon October. *E. foeminea* grows in the Mediterranean region, it is distributed in different geographical levels in Palestinian lands **[8]**.

E. foeminea has been addressed in traditional Arab medicine as a stimulant in treatment of anxiety and skin rash, also as a deobstruent, to treat kidney, bronchi, circular system, digestive system disorders and to relieve asthma attacks. The dried stems are the used parts of the plant in traditional medicine, they are used as hot drink decoction **[9-13]**.

Anti-obesity and related diseases affect of *E. foeminea* have never been investigated. As a consequence, this study aimed to 1) evaluate in vitro the phytochemical active constituents of *E. foeminea* aerial parts 2) to investigate the pharmacological action of *E. foeminea* against some digestive enzymes like lipase, α-amylase and α-glucosidase 3) to determine the antioxidant effect and 4) to evaluate the cytotoxicity effect.

Materials and Methods

Collection and preparation of Plant material

Aerial parts of *E. foeminea* were collected in May 2019 from different parts of Palestine. Its identification was completely done at the Pharmacognosy Laboratory at An-Najah National University. Aerial parts of *E. foeminea* were washed by distilled water and then dried in the shade at room temperature (25°C). After drying, the plant material grounded using a mechanical grinder in the laboratory to obtain a fine powder then kept in airtight containers with proper labeling for future use.

Chemicals and reagents

Millon's reagent, Benedict's reagent, Ninhydrin solution Iodine solution, H2SO4, HCl, Chloroform and Molisch's were obtained from Alfa Agar (England). Methanol, NaOH, n-hexane, and acetone were purchased from LobaChemie (India). Magnesium ribbon, acetic acid, FeCl3 and DMSO (Dimethyl sulfoxide) were obtained from Riedeldehan (Germany). 2, 2-Diphenyl-1-picrylhydrazyl (DPPH), Trolox ((s)-(-)-6 hydroxy -2, 5, 7, 8-tetramethychroman- 2-carboxylic acid), α-amylase, DNSA (3,5-dinitrosalicylic acid) reagent Acarbose, p-nitrophenyl butyrate, Orlistat, tris-HCl buffer, porcine pancreatic lipase type II, α-glucosidase (Baker's Yeast alpha) and *P*hpg were purchased from Sigma-Aldrich (USA).

Experimental preparation of *Ephedra foeminea* aerial parts four fractions

25 g of fine powder from aerial parts of *E. foeminea* was extracted by fractional method by adding 500 mL of solvents differ in their polarities; start with hexane (non-polar organic solvent) then acetone (weak polar organic solvent), methanol (strong polar organic solvent) and final step with distilled water (inorganic polar solvent) and placed in a shaker device at 100 rounds per minute for 72 hours at room temperature. After that, each organic fraction was filtered and concentrated under vacuum on a rotator evaporator device. While aqueous fraction dried using freeze dryer. Finally, all dried fractions stored at 4ºC in the refrigerator for project applications **[14]**.

Preliminary phytochemical laboratory tests

Acetone, hexane, aqueous and methanol fractions derived from aerial parts of *E. foeminea* were phytochemically evaluated to determine the presence of alkaloids, flavonoids, phenols, saponins, terpenoids, steroids, tannins, anthraquinones according to standard methods, which described by Mabry and Harborne, 1998 **[9,10]**. Mayers and Wagners, Shinodas, Benedicts and Molisch's, Keller-Killani, Froth, Lead acetate, Salkowski, Ninhydrin and Biuret and Ammonia tests to identify Alkaloids, flavonoids, carbohydrates, cardiac glycosides, saponins, tannins, terpenoids, protein and anthraquinone contents respectively in water, methanol, acetone, and n-hexane fractions of aerial parts.

Antioxidant activity

A stock solution of a concentration of 1 mg/mL in methanol initially prepared for each extract fraction and Trolox which was utilized as a positive control. Stock solutions used to prepare working solutions of 2, 5, 10, 20, 50 and 100 µg/mL concentrations in methanol. At the same time, a freshly prepared (0.002% w/v) DPPH solution mixed with methanol as well as with each of the used working concentrations in a ratio of 1:1:1 using methanol as the blank solution. All solutions incubated in a dark place at room temperature for 30 minutes. Then, their absorbance values evaluated by using the spectrophotometer at a wave length of 517 nm **[11]**. The percentage of antioxidant activity of each plant fraction and the Trolox calculated by using the following formula:

DPPH radical scavenging activity = ((A0 – A1) / A0)) × 100 %, where, A0 and A1 are the optical densities of Trolox standard and the working solution at 30 min, respectively. The antioxidant activity of a substance is expressed as IC50 (µg/mL), which represents the concentration of that substance that causes a 50 % decrease in the optical density at 517 nm.

α-Amylase inhibitory assay

With some modifications, the adopted procedure for anti-amylase evaluation was according to McCue and Shetty **[12]**. A total of 200 µL of each plant fraction (10, 50 , 70, 100, 500 µg/mL) placed in a tube and 200 µL of 0.02 M sodium phosphate buffer (pH 6.9) containing α-amylase solution (2 units/mL) added. This solution pre-incubated at 25°C for 10 min, after which 200 µL of 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9) added at timed intervals and then further incubated at 25°C for 10 min. After that the reaction was stopped by adding 200 µL of dinitrosalicylic acid (DNS) reagent. The tubes then incubated in boiling water for 5 min and cooled to room temperature. The reaction mixture diluted with 5 mL distilled water and the absorbance measured at 540 nm using spectrophotometer. A control prepared using the same procedure replacing the plant fraction with distilled water. The α-amylase inhibitory activity calculated as percentage inhibition.

α– amylase Inhibition (%) = (B-S/B) ×100%

B: the absorbance without enzyme inhibitor

S: the absorbance in the presence of the enzyme inhibitor

Concentrations of plant fractions resulting in 50% inhibition of enzyme activity (IC50) determined graphically. The same procedure was repeated for the used positive control of α–amylase inhibitory activity which was Acarbose.

α-Glucosidase inhibitory activity

With some modification α-glucosidase inhibitory activity of each extract fraction was carried out using the standard protocol **[15-19]**. 10 µL α-glucosidase (1 U/mL), and 20 µL of varying concentrations of extract and fractions (100, 200, 300, 400 and 500 mg/mL). In each working test-tube a reaction mixture contained 0.1 mL α-glucosidase solution mixed with 0.2 mL from each extract dilution and 0.5mL phosphate buffer (100 mM, pH = 6.8). Incubation was carried out at nearly 37°C for 15 min. After this incubation 0.2 mL of (5 mM) PNPG (the used substrate) was added to the reaction mixture and again incubated further at 37°C for 20 min. The reaction was terminated by adding Na_2CO_3 (0.1M). The absorbance was recorded at 405 nm wavelength for all samples. Acarbose was used as appositive control at the same concentrations as plant extract the results were expressed as percentage inhibition according to the following equation:

A-Glucosidase Inhibition (%) = (B– S/B) ×100%

B: is the absorbance without enzyme inhibitor

S: is the absorbance in the presence of the enzyme inhibitor

Antilipase activity

The porcine pancreatic lipase inhibitory method was followed in this research. In general, in the following steps this procedure was abbreviated. A stock solution of 500 µg/mL from each plant fraction in 10% DMSO from which five different solutions prepared with the following concentrations 50, 100, 200, 300 and 400 µg/mL. 1 mg/mL stock solution of porcine pancreatic lipase enzyme was freshly prepared before use which should be dispersed in tris-HCl buffer. For the used substrate; p-nitrophenyl butyrate (PNPB) was prepared by dissolving 20.9 mg in 2.0 mL of acetonitrile. For each working test tube, 0.1mL of porcine pancreatic lipase (1.0 mg/mL) was mixed with 0.2 mL plant fraction from each diluted solution series for each plant fraction. The resulting mixture then completed to 1.0 mL by adding Tri-HCL solution and incubated at 37°C for 15 minutes. After the previous incubation, 0.1 mL of p-nitrophenyl butyrate solution was added to each test-tube. The mixture again incubated for 30 minutes at 37°C. Pancreatic lipase activity determined by measuring the hydrolysis of *p*NPB compound into p-nitrophenolate ions at 410 nm using a UV-spectrophotometer. The same procedure repeated for Orlistat which used as a standard reference compound.

Lipase inhibition % = (B-S)/B ×100%

B: is the recorded absorbance of the blank solution

S: is the recorded absorbance of the tested sample solution.

Cell culture and cytotoxicity assay

HeLa cervical adenocarcinoma and colo205 colon cancer cells were cultured in RPMI-1640 media and supplemented with 10% fetal bovine serum, 1% Penicillin/Streptomycin antibiotics and 1% l-glutamine. Cells were grown in a humidified atmosphere with 5% CO_2 at 37°C. Cells were seeded at 2.6 x 10^4 cells/well in a 96-well plate. After 48 h cells were incubated with various concentrations of the tested compounds for 24 hr. Cell viability was assessed by CellTilter 96® Aqueous One Solution Cell Proliferation (MTS) Assay according to the manufacturer's instructions (Promega Corporation, Madison, WI). Briefly, at the end of the treatment, 20 µL of MTS solution per 100 µL of media

was added to each well and incubated at 37°C for 2 hours. Absorbance was measured at 490 nm.

Results

Phytochemical characteristics for aerial parts of *E. foeminea*

Phytochemical characteristics of Aqueous, methanol, acetone and hexane fractions of the aerial parts of *E. foeminea* were investigated for the detection of various phytochemical compounds such as alkaloids, proteins, polysaccharides, reducing sugars, phenols, tannin, steroids, flavonoid, cardiac glycosides, saponin glycoside and volatile oil.

As shown in Table 1 Polysaccharides, alkaloids, phenols, flavonoids and cardiac glycosides were appeared in the methanol fraction of the aerial part for *E. foeminea.* Aqueous fraction contained polysaccharides, sugars, flavonoid and cardiac glycosides. Hexane fraction contained alkaloids, polysaccharides, phenols, steroids, flavonoids, cardiac glycosides and volatile oil. Alkaloids and polysaccharides have appeared in the acetone fraction. Saponin compounds, proteins and tannins were absent in all fractions.

Table1. Phytochemical screening assessment of for aerial parts of *E. foeminea* four solvents fractions.

Phytochemical Compounds	Aqueous extract	Methanol extract	Acetone extract	Hexane extract
Alkaloids	-	+	+	+
Proteins& Amino acids	-	-	-	-
Complex polysaccharides	+	+	+	+
Reducing sugars	+	+	-	-
Phenols	-	+	-	+
Tannin	-	-	-	-
Steroids	-	-	-	+
Flavonoid	+	+	-	+
Cardiac glycosides	+	+	-	+
Saponin glycoside	-	-	-	-
Volatile oil	-	-	-	+

Source: Own authorship.

Antioxidant activity for aerial parts of *E. foeminea*

Antioxidant activities possessed by aerial parts of *E. foeminea* fractions were investigated employing free radical scavenging method (DPPH). The scavenging property of all studied fractions and Trolox standard compound showed a concentration dependence property (Figure 1). IC50 of antioxidant activities possessed by acetone, hexane, aqueous and methanol fractions and Trolox were 154.88±0.39 µg/mL, 21.87±0.45 µg/mL, 41.68±0.46 µg/mL, 14.45±0.24 µg/mL and 2.22±1.57 µg/mL respectively (Table 2).

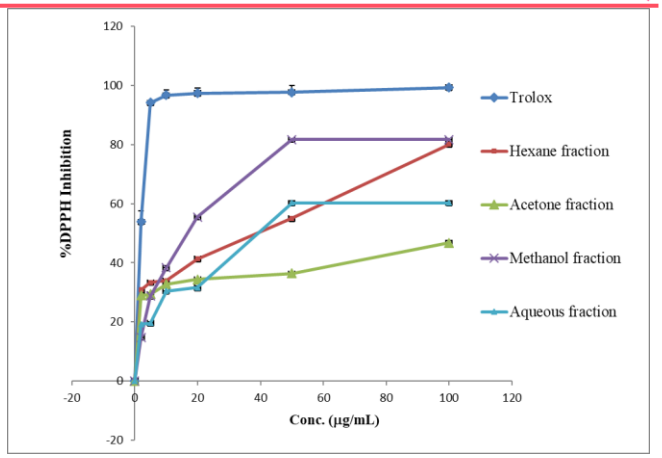

Figure 1. Percentage of radical scavenging activity inhibition by aqueous, methanol, acetone and hexane extracts derived from *E. foeminea*. Trolox was used as the reference standard. Source: Own authorship.

Table 2. Antioxidant effect of *E. foeminea.* Values of IC_{50} for aerial parts of *E. foeminea* extract fractions compared to Trolox.

IC50 (µg/mL)	Trolox (standard)	Hexane fraction	Acetone fraction	Methanol fraction	Aqueous fraction
E. foeminea	2.22±1.57	21.87±0.45	154.88±0.39	14.45±0.24	41.68±0.46

Source: Own authorship.

α-Amylase inhibit activity

As demonstrated in Figure 2, hexane, acetone, methanol and aqueous fractions obtained from the aerial parts of *E. foeminea* possessed *in vitro* inhibitory activities against porcine pancreatic α-amylase activity in a concentration-dependent manner. As shown in Table 3, IC50 values of acetone, hexane, aqueous and methanol fractions were 13.8±0.67 µg/mL, 15.13±1.73 µg/mL, 14.79±1.31 µg/mL and 12.88±0.4 µg/mL respectively. The IC50 of Acarbose standard reference was 28.18±1.22 µg/mL.

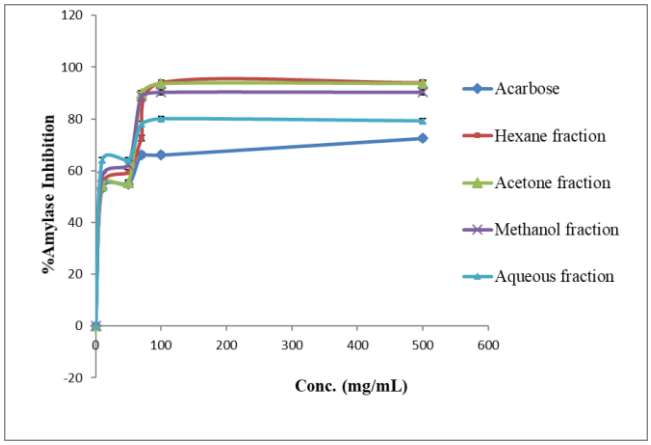

Figure 2. α-amylase inhibition activity by acarbose and aerial parts of *E. foeminea* extract fractions. Source: Own authorship.

Table 3. α-Amylase inhibitory activities of *E. foeminea*. Values of IC_{50} for aerial parts of *E. foeminea* fractions compared to acarbose.

IC50 (µg/mL)	Acarbose (standrd)	Hexane fraction	Acetone fraction	Methanol fraction	Aqueous fraction
E. foeminea	28.18±1.22	15.13±1.73	13.8±0.67	12.88±0.4	14.79±1.31

Source: Own authorship.

α-Glucosidase inhibition assessment

Figure 3 and Table 4 depict the α-glucosidase inhibitory activity of hexane, acetone, methanol and aqueous fractions obtained from the aerial parts of *E. foeminea* in a dose-dependent manner. The values of IC50 of α-glucosidase inhibitory activities of acetone, hexane, aqueous and methanol fractions were 251.18±0.44 µg/mL, 194.98±0.21 µg/mL, 416.86±0.78 µg/mL and 117.48±0.35 µg/mL respectively. The IC50 of Acarbose standard reference was 41.68±0.34 µg/mL.

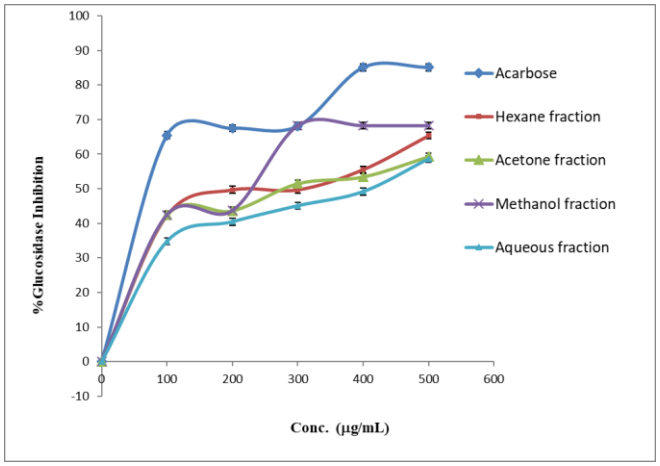

Figure 3. α-glucosidase inhibitory activity of *E. foeminea*. α-glucosidase inhibition activity by acarbose and aerial parts of *E. foeminea* extract fractions. Source: Own authorship.

Table 4. α-Gucosidase inhibitory activity of *E. foeminea.* Values of IC_{50} for aerial parts of *E. foeminea* fractions compared to Acarbose

IC50 (µg/mL)	Acarbose (standard)	Hexane fraction	Acetone fraction	Methanol fraction	Aqueous fraction
E. foeminea	41.68±0.34	194.98±0.21	251.18±0.44	117.48±0.35	416.86±0.78

Source: Own authorship.

Lipase enzyme inhibition

In vitro assessment of lipase enzyme inhibitory activity using orlistat (anti-obesity medicine) as a positive control was conducted on the four fractions derived from the aerial parts of *E. foeminea* (Figure 4). As described in Table 5 the values of IC50 of lipase inhibitory activity for aqueous, methanol, acetone and hexane fractions were 165.95±1.28, 24.54±0.49, 177.83±1.49 and 104.7±1.74 µg/mL respectively. The IC50 of the anti-obesity medicine (orlistat) was 41.68±0.34 µg/mL.

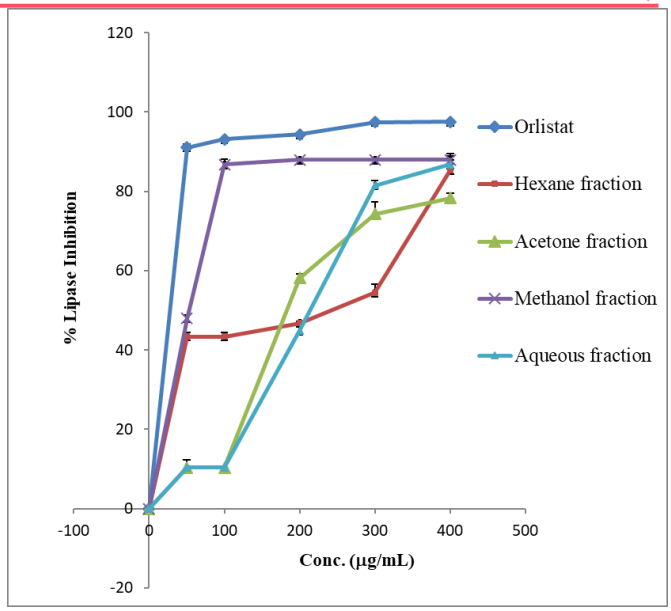

Figure 4. Porcine lipase inhibition activity by Orlistat and aerial parts of *E. foeminea* extract fractions. Source: Own authorship.

Table 5. Values of IC_{50} for aerial parts of *E. foeminea* extract fractions compared to orlistat.

E.foeminea	12.3±0.35	104.7±1.74	177.83±1.49	24.54±0.49	165.95±1.28

Source: Own authorship.

Cytotoxic effect of extracts derived from *Ephedra foemenia*

As shown in Figure 1, treatment of colo205 cells with 80 and 40 mg/mL of methanol extracts derived from *Ephedra foemenia* significantly induced cytotoxicity ($p \leq 0.0001$) by approximately 80% and 70% respectively, while 20, 10 and 5 mg/mL did not have any significant effect. As described in Figure 2, treatment of colo205 cells with 80, 40, 20, 10, 5 and 2.5 mg/mL of water extract induced cytotoxicity significantly ($p \leq 0.0001$) by approximately 95%, 97%, 93%, 78% and 84% respectively, while 1.25 and 0.625 mg/mL did not have a significant effect. 5, 2.5, 1.25 and 0.625 mg/mL of acetone and hexane extracts derived from *E. foeminia* (Figures 3 and 4) significantly induced cytotoxicity in colo205 cells ($p \leq 0.0001$) by approximately 95%, 0.3125 mg/mL had approximately 80% induction of cytotoxicity, while 0.15625 mg/mL did not have a significant effect.

As demonstrated in Figure 5 treatment of hela cells with 80 and 40 mg/mL of methanol extracts derived from *E. foemenia* significantly induced cytotoxicity ($p \leq 0.0001$) by approximately 99% and 48% respectively, while 20, 10 and 5 mg/mL did not have any significant effect. As described in Figure 6, treatment of hela cells with 80, 40, 20, 10 and 5 mg/mL of water extract induced cytotoxicity significantly ($p \leq 0.0001$) by approximately 93%, 81%, 76%, 53% and 24% respectively, while 2.5, 1.25 and

0.625 mg/mL did not have a significant effect. 5, 2.5, 1.25 and 0.625 mg/mL of acetone and hexane extracts derived from *Ephedra foeminia* induced cytotoxicity in colo205 cells significantly ($p \leq 0.0001$) by approximately 95%, 0.3125 mg/mL of hexane and acetone extract had approximately 74% and 44% induction of cytotoxicity, while 0.15625 mg/mL from both extracts did not have a significant effect.

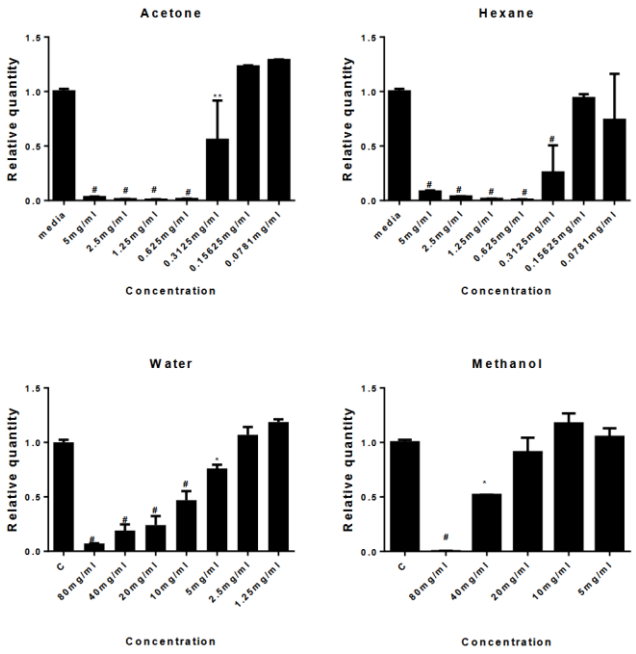

Figure 5. Effect of 4 fractions derived from Ephedra foeminea on the cytotoxicity of colo205 cells. Colo205 cells were treated with 80, 40, 20, 10, 5, 2.5, 1.25 and 0.625 mg/mL of methanol and water fractions, while they were treated with 5, 2.5, 1.25, 0.625, 0.3125, 0.15625, 0.078 and 0.039 mg/mL obtained from *E. foeminea* and incubated for 24 h. Cytotoxicity was determined by MTS assay. Results were depicted as relative quantities (RQs) compared to the control (with only media; C). #p<0.0001. Error bars represent SD. Source: Own authorship.

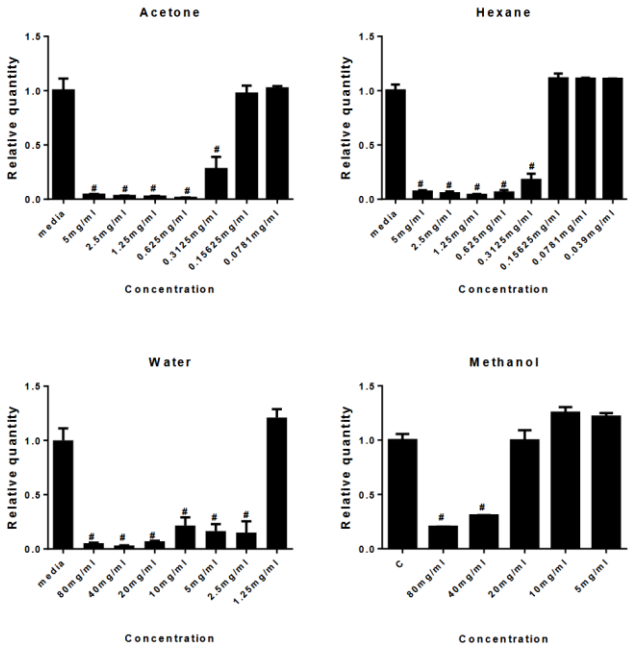

Figure 6. Effect of 4 fractions derived from ephedra foeminea on the cytotoxicity of Hela cells. Hela cells were treated with 80, 40, 20, 10, 5, 2.5, 1.25 and 0.625 mg/mL of methanol and water fractions, while they were treated with , 5, 2.5, 1.25, 0.625, 0.3125, 0.15625, 0.078 and 0.039 mg/mL obtained from *E. foeminea* and incubated for 24 h. Cytotoxicity was determined by MTS assay. Results were depicted as relative quantities (RQs) compared to the control (with only media; C). #P<0.0001, **p<0.01 and *p<0.05. Error bars represent SD. Source: Own authorship.

Discussion

Interventions to reduce weight based on reducing calories intake and/or increasing energy expenditure are often not successful on the long term **[14]**. On the other hand, synthetic medicines are expensive and possess potentially dangerous health side-effects, which have limited their use so far **[15]**. As a consequence, there is an enormous need to screen for and to develop safe, effective and cheap alternative medicines against obesity and its comorbidites such as diabetes and cardiovascular diseases. In the present study, we aim to investigate *in vitro*, for the first time, the anti-obesity and anti-diabetic properties of *E. foeminea*. To achieve our aim, aqueous, methanol, acetone and hexane fractions were derived from the aerial parts of *E. foeminea* and their inhibitory effects on α-amylase, α-glucosidase and lipase enzymes were investigated. It appeared that aqueous, methanol, acetone and hexane fractions had similar but strong inhibitory effect against the activity of α-amylase enzymes, with the following IC50 values, 14.79±1.31, 12.88±0.4, 13.8±0.67, 15.13±1.73 µg/mL respectively. The inhibitory effects of all fractions were even stronger than the pure antidiabetic medicine (acarbose), which was used as a positive control (IC50 28.2 µg/mL). The inhibitory effects of all fractions on the α-glucosidase activity were weaker than anti-α-amylase activity.

The strongest inhibitory effect against α-glucosidase was mediated by methanol fraction (IC50 117.5 µg/mL), followed by hexane (IC50 195.0 µg/mL), acetone (IC50 251.2 µg/mL) and finally by aqueous (IC50 416.9 µg/mL) fraction. These inhibitory effects were lower than the inhibitory effect of the antidiabetic drug acarbose, which had an IC50 value equal to 41.7 µg/mL. α-amylase hydrolyzes glycogen, while α-glucosidase hydrolyzes maltose to glucose units, which is the main source of energy and consequently obesity and postprandial hyperglycemia **[16]**. Therefore, the inhibitors of either enzyme alone or both enzymes are considered effective agents that can control postprandial hyperglycemia and may prevent obesity and control type-2 diabetes. Although

our data suggests that our fractions have a mild inhibitory effect on αglucosidase, they possess a very strong and powerful effect on α-amylase activity, suggesting a synergistic and very strong inhibition of hyperglycemia and glucose availability.

Pancreatic lipase digests dietary fats, which are considered a key source of excess energy intake. Therefore, pancreatic lipase can be targeted to develop anti-obesity, anti-diabetic and cardiovascular agents. Our data demonstrates again that methanol fraction possesses the strongest inhibitory effect on lipase enzyme activity (IC50 24.5 µg/mL) followed by hexane (IC50 104.7 µg/mL), aqueous (IC50 166.0 µg/mL) and acetone fraction (IC50 177.8 µg/mL). Methanol inhibitory effect is very close to the effect of the anti-obesity medicine (orlistat, IC50 12.3 µg/mL).

Obesity is associated with lower concentrations of specific antioxidants which play a role in the development of obesity-related diseases such as type-2 diabetes and cardiovascular disease **[17]**. Growing evidence also suggests that increased oxidative stress is associated with obesity and its comorbidities **[18]**. Consequently, we studied the antioxidant effect of *E. foeminea*. According to our results, methanol fraction again possessed the strongest antioxidant effect (IC50 14.5 µg/mL), followed by hexane (IC50 21.9 µg/mL), aqueous (IC50 41.7 µg/mL) and acetone fraction (IC50 154.9 µg/mL). Studies regarding antioxidant effect of *E. foeminea* are very little. In one study that methanol extract of *E. foeminea* exhibited DPPH scavenging effect 50% at 0.6 mg/mL **[19]**. This is weaker than what we observed; however, this could be due the difference in the experimental setup, such as extraction method used and the source of the extract (fruits vs. aerial parts).

To determine whether this herb is safe to use, we investigated the cytotoxicity effect *in vitro* on two different cell lines. Our results demonstrate that the cytotoxic effect of all extracts was weak. Hexane fraction had the strongest cytotoxic effect against both cell lines, followed by acetone and water and the lowest effect was mediated by methanol extract. Studies regarding cytotoxic effect of *E. foeminea* are scarce. Our findings show stronger effect than in earlier studies **[20-22]**. One of our tested cell lines is a colon cells and therefore our data suggest that extracts derived from *E. foeminea* most probably is save as the site of action is the digestive system.

The phytochemical active constituents of *E. foeminea* aerial parts were determined and it appeared that both methanol and hexane fractions were the richest fractions and contain the complex polysaccharides, alkaloids, phenols and flavonoids and cardiac glycosides. This indicates that this fraction was rich in phytochemicals that may be active in the observed pharmacological activities. Hexane fraction contained steroids, phenols, flavonoids, cardiac glycosides, volatile oil and polysaccharides. Saponin compounds, proteins and tannins were absent in all extract fractions. For example, the presence of phenolic, alkaloids and flavonoids content in *E. foeminea* were previously reported **[22,23]**.

Conclusion

In summary, our study revealed that methanol fraction of the aerial parts of *E. foeminea* contains a mixture of phytochemicals that possess the strongest antioxidant capacity and inhibitory activities against α-amylase, α-glucosidase and pancreatic lipase enzymes in comparison to the rest of the fractions and similar or even stronger than antidiabetic and antiobesity medicines. This indicates that methanol fraction is a potential and safe source of therapeutically active compounds with anti-obesity and anti-diabetic potency, which is of great pharmaceutical importance. Further investigations are necessary to determine the bioactive components and to prove its efficacy in *in-vivo* studies.

CRediT

Author contributions: Saad Al-Lahham: Conceptualization, Visualization, Writing – original draft, Writing – review & editing, Investigation, Formal analysis, Data curation, Methodology. Nidal Jaradat: Conceptualization, Writing – review & editing, Investigation, Formal analysis, Data curation, Methodology. Other authors: Investigation, Data curation, Formal analysis, Methodology, Writing – original draft, and Writing – review & editing and Data curation.

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Ethical Approval

Not applicable.

Informed Consent

Not applicable.

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Data sharing statement

All data is contained within the article.

Conflict of Interest

The authors declare no conflict of interest.

Similarity Check

It was applied by Ithenticate[@].

Application of Artificial Intelligence (AI)

Not applicable.

Peer Review Process

It was performed.

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