Feminine is a proprietary combination of *Ferula assa-foetida* L. and *Capparis spinosa* L. to enhance sexual functioning in women.

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Summary

A concentrated dry extract of Ferula assa-foetida L. roots and Capparis spinosa L. buds, was prepared into so called Feminine tablets and assessed for safety and efficacy in enhancing female libido. Experimental studies on rats disclosed a high level of safety of this extract with an LD50 of 15 g/kg. Cultured human fibroblasts incubated with increasing concentrations of the extract did not exhibit any sign of cellular toxicity as evidenced by LDH-release. Antioxidant properties were demonstrated using the lipid peroxidation method and were substantial at very low concentrations of this extract when incubated with rat liver cells and ferrosulphate. Derived from Sprague Dawley rats, arterial rings with and without their endothelial tissue were contracted under controlled conditions and during the addition of different extract concentrations. These experiments revealed the Feminine extract to be a potent vasodilator due to an endothelial-mediated effect rather than a direct effect on arterial smooth muscle cells. Two groups of married and healthy females were followed for 6 months while consuming one Feminine tablet daily. Feminine was well tolerated by all females and no side effect was reported. The one group (n = 32) was studied due to difficulties in their sexual activity and the other (n = 28) was studied due to infertility that could not be helped further by medical evaluations or treatment. Twenty seven ladies of the first group (84%) reported significant improvements in their sexual difficulties and their libido while the remaining 5 women reported no remarkable change. Twenty one women of the second group (75%) reported that their libido was improved within one month of Feminine consumption and 11 women got pregnant. The results indicate that Feminine is a safe sexual tonic enhancing the female sexual functioning.
Introduction

The roots of *Ferula assa-foetida* L. “Devil’s Dunk” produce the well known spice asafoetida used to flavour foods in India and all over the world (1, 2). The plant is approved for food use in both the EU and USA (3) and ingestion of 15 grams has not produced any adverse effect (4). The herbal use of *Ferula assa-foetida* L. seems thus to be without safety concerns and this was confirmed by two clinical studies in India (5) and in Germany (6). Both studies disclosed no side effects whatsoever in patients treated by this plant extract that proved to be a useful treatment of hyperlipidemia (5) and of irritable bowel syndrome (6). Supportive action of asafoetida on the digestive process was also evidenced in animal studies indicating that the spice is a carminative which is able to stimulate pancreatic digestive enzymes (7).

*Capparis spinosa* L. “Capers” is also classed as a food in the EU and all over the world (3, 4, 8, and 9). The plant buds extract has been given to healthy human volunteers in a study revealing significant antioxidant and photo-protective effects of the extract (10). There were no reported side effects. Flowers or buds of capers contain mainly phenolic compounds such as glucosinolates or glucocapperin (11) reported to possess significant antioxidant (12), liver-protective (13) and hypoglycaemic (14) effects in animal studies.

Possible aphrodisiac effects of both asafoetida and capers has been experienced through generations as indicated by ethnopharmacological studies in the Middle East (8, 9). The present study used extracts from asafoetida roots and caper flowers and aimed at investigating their safety, possible cytoprotective properties as well as their sexual tonic effects in animal models and in human female volunteers. The study was undertaken in accord with legal and ethical requirements as well as current scientific standards as indicated by European Good Clinical Practice Guidelines and the Declaration of Helsinki.
Material and Methods

The roots of *Ferula assa-foetida* L. and flowers of *Capparis spinosa* L. were collected, dried under shade and powdered to a fine grade. Extracts were prepared by Antaki Ltd.-laboratories, Kfar Cana, Israel. Vitamin E (4.1 mg) and calcium (150 mg) were added during preparations at Karmat Micro Encapsulation laboratories, Kibbutz Ramot Menashe, Israel. Feminine tablets each of 510 mg net weight were thus produced.

Experimental Investigations

Animal- and cell-culture-experimental-studies were carried out at the pharmacology department laboratories, Technion University, Haifa, Israel.

1. Safety and toxicity studies (15):

Thirty two rats were given a progressive overdose of the product to determine the lethal dose sufficient to kill half of them (LD50). Toxicity of the product was estimated by the LDH-release-assay. Release of the intracellular enzyme LDH is the consequence of necrotic or toxic cell-membrane-rupture. Integrity of the cell membrane was determined by measuring LDH-activity released into the culture medium. LDH-activity was monitored following the oxidation of NADH as the decrease in absorbance at 334 nm. The reaction was carried out in a potassium phosphate buffer (40 mM K$_2$HPO$_4$, 10 mM KH$_2$PO$_4$, pH 7.5), containing 0.24 mM NADH and 0.62 mM pyruvate. The percentage of LDH released was defined as the ratio of LDH activity in the supernatant compared to the sum of LDH amount released plus LDH activity measured in the cell lysate. Human fibroblasts were incubated with various concentrations of the product extract and LDH activity was measured in the medium at 24, 48 and 72 hours of incubation.

2. In-vitro efficacy studies:

a. Cytoprotective effects (16, 17):

Antioxidant properties of the product extract were studied in rat liver cells. Oxidative stress leads to generation of reactive oxygen species (ROS) which play an important pathogenetic role in different disease-states. Lipid peroxidation has damaging effects on cell membranes. The extent of lipid peroxidation was measured using a technique based on a thiobarbituric acid reactive substance
(TBARS) assay that detects malondialdehyde (MDA), an end product of peroxidative decomposition of polyeonic fatty acids in in-vitro systems. To accurately quantify TBARS in the analytical procedure, the protein was precipitated before the addition of thiobarbituric acid to the reaction, while the antioxidant butylated hydroxytoluene was added before heating of samples. Rat liver homogenates were incubated with 100 μM of FeSO₄ as ROS generating system and with various concentrations of the product.

b. Effects on arterial smooth muscle tonus (15, 18, 19):
Arterial rings were harvested from Sprague Dawley rats that were anesthetized with 10% Chlroral hydrate. Thoracic aorta was dissected and rapidly immersed in Krebs-Henseleit solution. All conjunctive and adipose tissues were removed. Two arterial rings (0.4 cm in diameter each) were obtained and the endothelium was mechanically removed from one of the two rings. Each ring was suspended by a fine steel wire and connected to an isometric tension transducer (MyographF60). This transducer was connected to Narco Trace 40 polygraph (Narco-Bio-Systems Inc., Texas, USA). The rings were maintained in 15 ml Krebs-Henseleit solution at 37°C. A tension of 500 mg was maintained during an equilibration period of 60 minutes during which the bathing medium was changed every 20 minutes. The arterial rings were incubated with noradrenaline 10⁻⁷ M, a concentration that induces 60-70% of the maximal contraction and the developed tension was measured. The same incubate was exposed to progressively increased concentrations of the product and the developed tension was measured at each concentration.
Clinical Investigations
Selection of female volunteers and clinical protocol:

Two groups of young and married women were studied. One group (n = 32) was recruited from 5 general-physician-clinics in Galili and selected on the basis of having difficulties in the female sexual activity with their husbands. The other group (n= 28) was recruited from two fertility clinics (“New Medical Center” run by Dr. F. Nahhas and “Lyn Medical Center” run by Dr. B. Laver). They were selected on the basis of infertility that needed no further medical evaluations and the offered treatment (in-vitro insemination), was refused by these subjects. All 60 females were otherwise healthy and were motivated to take herbs. After a thorough review of the herbal components of Feminine, an informed consent was obtained from each lady who was asked to continue her daily activities and habits unchanged and to remember to take one tablet of Feminine daily. They were followed at regular intervals of 4 weeks during the study period of 6 months. At baseline and each monthly control, both the female and her husband were interviewed. A review of sexual activities included expressions like: libido, pain or healthy lubrication during intercourse. A review of well being and side effects was as well undertaken.

Statistics

The Wilcoxon signed-rank was used. Comparison between groups was performed by the Wilcoxon rank-sum test. A 0.05 level of significance was set. Data obtained were expressed as mean ± standard error of mean (SEM).

Results

An extremely high dose of the product of 15 g/kg was necessary to obtain the LD50 in rats. LDH-release from cultured human fibroblasts is expressed in arbitrary units in Figure 1, at baseline (0, left column) and after incubating the fibroblasts with two concentrations of Feminine plant extracts: 180 and 360 mg/ml. A, B and C express the results at 24, 48 and 72 hours of incubation time respectively. Compared to baseline, no substantial change in LDH-release is noted in Figure 1 whether as a function of increasing the product concentrations or as a function of increasing the incubation period.
Lipid peroxidation induced by incubating rat liver homogenates with ferrosulphate is expressed in Figure 2 as the extent of MDA production. The addition of a very low dose of the product (0.01 mg) to the medium significantly reduces MDA-release from 0.60 ± 0.03 to 0.39 ± 0.02 nm/mg protein (p < 0.001). A higher concentration of the product (0.05 mg/ml) further reduces MDA production to 0.17 ± 0.02 nm/mg protein (p < 0.001), but no further antioxidative effect of the product is noted by augmenting its concentration from 0.05 to 0.1 mg/ml.

Figures 3 and 4 summarize the vasodilatating effects of the product extract as evidenced by the tension developed in arterial rings. During control conditions, the induced contraction (tension) measured in arterial rings is about 730 ± 50 mg and is kept almost unchanged during the experimental conditions (figures 3 and 4, upper curves). Such an induced tension in arterial rings with an intact endothelium (Figure 3) is significantly reduced to about 390 ± 60 mg (p < 0.001) by the addition of a low concentration of the product extract (0.2 mg/ml). Incremental but slight reductions in the developed tension are seen during increased product concentrations reaching a nadir value of 280 ± 40 mg at the 1 mg/ml concentration (Figure 3 lower curve). The same situation is expressed in Figure 4 but with those arterial rings deprived of their endothelium. The vasodilatating effects of the same concentrations of the product are substantially less pronounced when compared to those shown in Figure 3. However, the moderate vasodilatating effect observed in Figure 4 is therefore non-endothelial-mediated and may represent a direct effect on smooth muscle cells.

The 32 married women studied due to sexual reasons had a mean age of 33 (range 26-49) years. They all tolerated Feminine tablets well and no side effect was reported. Of them, 27 ladies (84%) reported remarkable improvements in libido such as: increased sexual activities, improved frequency of orgasms and more satisfaction with sex-life as 16 of these 27 ladies had less pain and more healthy lubrication during intercourse. These improvements were noted within 2-3 weeks of Feminine consumption. The remaining 5 of the 32 ladies reported no remarkable change. The 28 married women studied due to infertility reasons had a mean age of 30 (range 20-41) years. They had a history of infertility lasting 2-15 years. They all tolerated Feminine tablets well and no side effect was reported. Within the first month of Feminine consumption, 21 ladies (75%) reported remarkable improvements of their sexual activity and 11 of the 28 ladies (39%) got pregnant within a mean of 3.5 (range 2-6) months of Feminine consumption.
Discussion

The results disclose that Feminine is safe and well tolerated by all 60 young females and is therapeutically efficient as it remarkably enhanced libido in about 80% of these females.

A high safety level of Feminine was disclosed with very high concentrations of 15 g/kg to yield the LD50. Concentrations as high as 360 mg/ml did not show any sign of cellular toxicity as evidenced by LDH-release. Anti-toxic effects of both *Ferula assa-foetida* L. (20) and *Capparis spinosa* L. (13) have been disclosed in animal studies. A pregnancy interceptive effect of *Ferula assa-foetida* L. extract has been evidenced in rats, the extract was given at a very high dose of 400 mg/kg and was methanolic based (21). Each Feminine tablet contains an oily extract of asafoetida roots and its magnitude corresponds to a dose of 1.5 mg/kg assuming an average weight of 70 kg. Anti-fertility effects have been evidenced in rats with the use of *Ferula hermonis* at a high dose and for a long time (22). Such opposite effects seem to be a familiar situation with many herbs or drugs when used at the wrong dosage in the wrong way.

We used asafoetida roots and caper buds in Feminine and the antioxidant properties of our extract were substantial at very low concentrations of 0.01 mg/ml. These cytoprotective effects were more significant at concentrations of 0.05 mg/ml while higher concentrations did not add to such properties of Feminine. Both components, *Ferula assa-foetida* L. (20) and *Capparis spinosa* L. (13) have disclosed antioxidant effects by augmenting antioxidant enzymes and detoxification in the liver of rats (13, 20).

We demonstrated potent vasodilatating effects of the Feminine extract upon intact arterial rings (Figure 3). A mild vasodilatating effect of the extract did remain on those arterial rings deprived of their endothelial tissue (Figure 4). These observations, taken together, indicate that the extract has mainly an endothel-mediated effect and a secondary direct effect on arterial smooth muscle cells. Nitric oxide (NO), is the principal product of vascular endothelial cells (23) that stimulate the production of cyclic guanosine monophosphate which relaxes vascular smooth muscle cells (24).

Our results therefore indicate that the main component of the vasodilatation evidenced in our extract can be attributed to activation of endothelial cells to release NO. The secondary mild component of this vasodilatation is a direct smooth muscle relaxing effect due probably to an effect on prostacycline synthesis. The former and principal effect
would increase blood supply to the genital area whereas the latter secondary effect would relax clitoral and vaginal smooth muscle cells. These observations would explain the sexual tonic properties and pro-fertility properties of Feminine in women.

Aphrodisiac effects of Feminine were experienced by 84% of the married females with sexual difficulties and by 75% of those with fertility problems. As experienced in good clinical practice, an optimal dose of a herb or a drug is the minimal dose that yields therapeutic efficacy with least side effects. This could explain the complete absence of side effects in the 60 studied females during 6 months of Feminine consumption. The antioxidant and the vasodilatating properties of Feminine were convincing at low concentrations of 0.05 and 0.2 mg/ml, respectively. These properties may have largely contributed to the observation that about one third of infertile females got pregnant within about 4 months of Feminine consumption.
References

Figure 1.
LDH-release in arbitrary units from cultured human fibroblasts at baseline (0) and during incubating the fibroblasts with two concentrations (180 and 360 mg/ml) of Feminine extract at 24, 48 and 72 hours of incubation time (A, B and C) respectively.

Figure 2.
Effects of different concentrations of Feminine extract upon malondialdehyde (MDA) released and produced by lipid peroxidation in rat liver cells incubated with 100 μM ferrosulphate.

Figure 3.
The tension (mg) developed during induced contraction of intact arterial rings at baseline (upper curve) and during the addition of different concentrations of Feminine extract (lower curve).

Figure 4.
The tension (mg) developed during induced contraction of arterial rings deprived of their endothelium at baseline (upper curve) and during the addition of different concentrations of Feminine extract (lower curve).
Figure 1

A

Feminine extract concentration [mg/ml]

LDH concentration [arbitrary units]

0 180 360

B

Feminine extract concentration [mg/ml]

LDH concentration [arbitrary units]

0 180 360

C

Feminine extract concentration [mg/ml]

LDH concentration [arbitrary units]

0 180 360
Figure 2

![Graph showing MDA release (nmol/mg) as a function of feminine extract concentration (mg/ml).]
Figure 3

![Graph showing the effect of feminine extract concentration on tension. The graph plots tension (in mg) against feminine extract concentration (in mg/ml). The control group is represented by a blue line, while the group with feminine extract is represented by a purple line. The graph indicates a decrease in tension as the concentration of feminine extract increases.]
Figure 4

Feminine extract concentration (mg/ml)

Control Group

With Feminine

Tension [mg]