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Ferula harmonis 'zallouh' and enhancing erectile function in rats: efficacy and toxicity study

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***Ferula harmonis*, which is locally called 'zallouh' in the Middle East, is used as an aphrodisiac as it is reputed to enhance male sexual behavior, however, there is no scientific verification. In this study, the oil extracted from the seeds of *Ferula harmonis* was tested for its efficacy in enhancing erectile function and toxicity in male rats. The sexual activities assessed by penile erection index were dose dependent. The ED_{50} (12.03 mg/kg) was 880 times less than the LD_{50} (10.6 g/kg). However, when doses ranging from 0.05, 0.5 to 2 g/kg were given daily for 28 days, acute and subacute toxicity were observed. There was a decrease in total body weight, hepatomegaly, atrophic testis, significant decrease in hemoglobin and red blood cell count. In addition, there was a significant decrease in cholesterol level. All the above indicate that the crude oil from the plant *Ferula harmonis* can enhance erectile function, however, it becomes toxic if it is used for a long period of time. Further studies are underway to isolate and identify the active ingredients and their exact mechanisms of action.** *International Journal of Impotence Research* (2001) **13,** 247–251.

Keywords: *Ferula harmonis*; zallouh oil; erectile function

Introduction

Erectile dysfunction is a common condition in men, which presents a major health problem. It can cause considerable distress, unhappiness and relationship problems. The search has therefore continued from time immemorial to find an effective, safe, and easy to administer treatment for erectile problems.[1–4]

Herbal remedies are the oldest modality used for enhancing erectile function. One of the most frequently used is the shrub-like plant, *Ferula harmonis*, it is known locally as 'Zallouh Root'. Zallouh Root is grown above 6000 feet in the mountains of eastern Lebanon and is harvested from August to October before the snow falls. The medicinal value has always been found in the root, especially when the root is spit and the oil oozes out. Even though this plant is widely used for the enhancing erectile function in the Middle East, there is no scientific verification of its efficacy and toxicity.

We tested the crude oil extracted from the roots and the seeds of zallouh. Our preliminary experiments found that the oil from the seeds showed better erection indexes than from the root itself (unpublished observation). In this study, the efficacy and the toxicity of the zallouh oil extracted from the seeds is studied. In addition, the mode of action is discussed, based on the known chemistry of the plant *Ferula*.

Materials and methods

Animals

Young Wistar, 8–10 week-old, male rats were acclimatized to laboratory conditions for 5 days prior to experiments. The rats were housed in groups of 10 in standard metal cages, under conventional laboratory conditions at a room temperature of 22°C. They received standardized pellet food and tap water as required.

Drug dosage form for ED_{50} and parameters

Crude oil was extracted from the seeds of *Ferula harmonis* by mincing the seeds and then adding boiling water. The oil starts to emerge within the hot water and forms a layer on the surface, which was

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then extracted. The extracted oil (1 g/ml) was diluted with Tween-80 either to 6.25 mg/ml or to 100 mg/ml for administration. Fifty rats were divided into five groups (10 rats/group) and each group was given 0 (only Tween-80), 15.6, 31.2, 62.5, or 125 mg/kg orally through a specialized needle. The volume that was given to the rats ranged from 0.60 to 0.96, 1.1 to 1.65, 2.4 to 3.6, from the 6.25 mg/ml stock, for the 15.6, 31.2, and 62.5 mg/ dose-groups, respectively, and 0.95 – 1.4 ml from the 100 mg/kg for the 125 mg/kg dose-group. The animals were observed for 3 h while having free access to food and water.

The parameters for determining the effective dose were the number of episodes of erection, sucking and mounting observed during the 3 h period. The penile erection index (PEI)[5,6] was calculated from the following formula:

$$\text{PEI} = (\text{mean episodes per group of rats}) \times (\text{\% of rats showed at least one episode of activity})$$

Drug dosage form for LD_{50}

Fifty rats were divided into five groups (10 rats/ group) and each group was given either 0 (only Tween-80), 0.5, 2, 4, 8, 10 or 15 g of oil/kg extracted as above. The volume that was given to the rats ranged from 0.95 to 1.55, from a 100 mg/ml stock, for the 0.5 g/kg dose-group, 0.8 – 1.03, 1.9 – 2.5, and 3.0 – 4.5 ml, from a 660 mg/ml stock, for the 2, 4, and 8 g/kg dose-groups, respectively, and 2.6 – 3.7 and 4.1 – 5.4 ml were given as oil without dilution for the 10 and 15 g/kg dose-groups, respectively.

Subacute toxicity

In this study, the extracted crude oil was formulated by mixing it with Arabic gum and water. Fifty rats were divided into five groups (10 rats/group). Each group was given the oil suspension orally through a specialized needle once daily for 28 days at doses of 0 (only suspension without oil, placebo), 0.05, 0.5, or 2 g/kg, and the fifth group of rats were kept with no treatment (untreated control).

At the end of the study period and from each rat, a blood sample was withdrawn from the jugular vein for complete blood count and differential analysis. In addition, alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase (ALP), total protein, albumin, globulin, prothrombin time (PT), partial thromboplastin time (PTT), cholesterol, glucose, urea, creatinine, sodium, potassium and chloride levels were performed. Animals were then killed and the brain, heart, lung, liver, kidney, spleen, submandibular salivary glands and the testes were removed and weighed.

ED_{50}, LD_{50} calculations and statistical analysis

ED_{50} and LD_{50} were calculated using a probit analysis program. The program assumed the proportion of control groups responding is zero. For statistical analysis, the frequency of rats showing erection and sucking activities was tested using χ^2 analysis. One way ANOVA was used to test for significant differences between rats' weights in each treated group. In addition, a non-parametric Wilcoxon-rank sum test was used to test for significance between means of all parameters and Pearson correlation test was applied when stated.

Results

Effective dose

The percentage of rats showing erection and sucking activities following oral administration of different doses of zallouh oil is shown in Figure 1. These activities started to appear 1 h after administration of the oil. The ED_{50} was calculated to be 12.03 mg/kg of crude oil. Results showed that at the lowest dose used (15.6 mg/kg), a statistically significant difference ($P = 0.05$) was found in the percentage of rats that had erection and sucking activities when compared to control rats. However, the percentage of rats showing activity plateaud at 80% following a dose of 62.5 mg/kg (Figure 1). On the other hand the PEI was shown to be increased significantly with the administered dose ($r = 0.95$, $P < 0.02$), which indicates that recurrence of erection and sucking is dose-dependent (Figure 1).

Acute toxicity

The LD_{50} value of zallouh oil following oral administration was calculated to be 10.602 g/kg. All deaths occurred within 72 h (Table 1). The rest of the animals in each group survived for the rest of the observation period (14 days), without any obvious or serious complication.

Subacute toxicity

All rats at the doses of 0.05 g/kg and 0.5 g/kg survived for 28 days without any drug-related

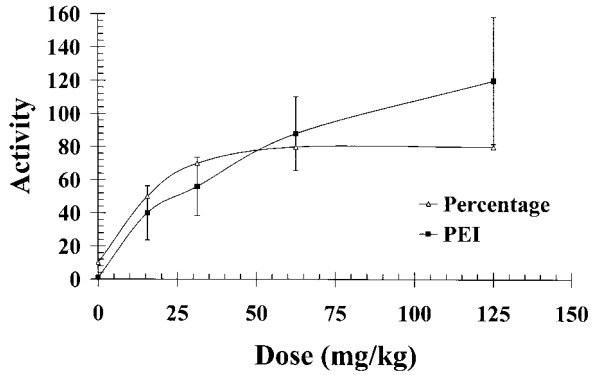

Figure 1 The activity of rats interms of percentage of rats that showed erection or sucking and penile erection index (PEI ± s.e.m.) following oral administration of different doses of zallouh oil.

Table 1 Number of rats that died following an oral administration of zallouh oil

Dose (g/kg)	*% Death* (n = *10*)
0	0
0.5	0
2	0
4	0
8	20%
10	50%
15	90%

n is the number of rats/group.

deaths. One death did happen in the 0.5 g/kg group but was not drug related. In the last group (2 g/kg) seven deaths were recorded within the 28-day study period. Out of these deaths, five were drug related.

The mean body weights of treated and untreated animals over the 28 days are shown in Figure 2. There was a significant decrease ($P < 0.001$) in body weight over time in all rats, who received zallouh oil. This decrease was not related to the dose. This could be partly attributed to a lower consumption of

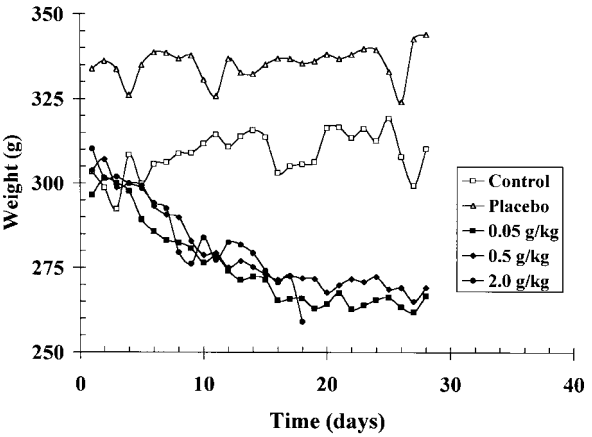

Figure 2 The change in mean body weight (± s.e.m.) of rats during oral administration of zallouh oil for 28 days.

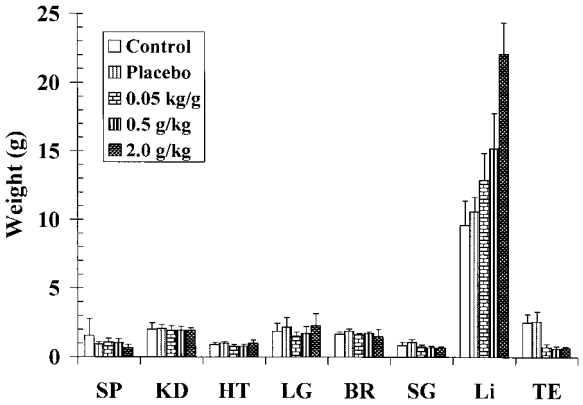

Figure 3 The mean of organ weights (± s.d.) of rats following oral administration of zallouh oil for 28 days. SP; spleen, KD; kidneys, HT; heart, LG; lungs, BR; brain, SG; submandibular gland, Li; liver and TE; testes.

food. It was noticed that food consumption was less in the 0.05, 0.5 and 2.0 g/kg groups when compared to placebo or control rats.

The mean weight of most organs did not change except for the liver and testes (Figure 3). An increase in liver weight was observed in the treated groups with zallouh oil which was statistically significant ($P = 0.012$) at doses of 0.5 and 2 g/kg (Figure 3). This increase in liver weight was correlated with the dose ($r = 0.981$, $P = 0.019$). The testes weight, however, showed a significant reduction ($P = 0.012$), but this reduction was not dose related.

The significant changes in hematology of rats treated daily for 28 days with 0 (control and placebo), 0.05 and 0.5 g/kg of zallouh oil are presented in Table 2. No statistically significant difference was observed in hematocrit (Hct), platelets, leukocyte counts, neutrophils, lymphocytes, monocytes and eosinophils between control, placebo 0.05 g/kg, and 0.5 g/kg groups. However, hemoglobin (Hb) and RBC values were significantly less in the 0.05 g/kg and 0.5 g/kg groups when compared with placebo and control groups (Table 2). These results might indicate that slight hemolysis occurred in rats following a long treatment with zallouh oil. This indication is supported by an increase in polychromasia in the 0.5 g/kg group and to lesser extent in the 0.05 g/kg group when compared with the control and placebo groups (data not shown).

The significant changes in clinical chemistry of rats treated as above with 0 (control and placebo), 0.05, 0.5, and 2 g/kg of zallouh oil are presented in Table 2. No statistically significant difference was observed in urea, creatinine, Na^+, K^+, CI^-, AST, ALP, PT, activated PTT (APTT), total protein and globulin between the treated, placebo and the control groups. However, elevation in ALT was observed in rats treated with zallouh oil and was significantly correlated with increase in liver weight (hepatomegaly) and to liver/body weight ratio

Table 2 The significant hematological and biochemical findings of rats treated orally with zallouh oil for 28 days

Group	*RBC* ($\times 10^6\,\mu l$)	*Hb* (*g/dl*)	*Cholesterol* (*mg/dl*)	*Glucose* (*mg/dl*)	*ALT* (*U/l*)	*AST* (*g/dl*)	*Albumin* (*g/dl*)
Control	6.4 ± 0.9	12.7 ± 1.9	52 ± 11	166 ± 69	76 ± 40	211 ± 76	3.6 ± 0.4
Placebo	6.9 ± 0.8	12.8 ± 1.2	48 ± 8	121 ± 54	61 ± 11	233 ± 139	4.1 ± 0.4
0.05 g/kg	5.4 ± 1.2^a	10.6 ± 1.9^c	37 ± 8^e	102 ± 35	68 ± 21	153 ± 84	3.7 ± 0.4
0.5 g/kg	5.1 ± 0.8^b	10.6 ± 1.5^d	34 ± 11^f	88 ± 27	108 ± 40	133 ± 26	4.4 ± 0.2^g
2.0 g/kg	ND	ND	46 ± 25	88 ± 28	218 ± 15	425 ± 123	3.8 ± 0.2

[a]$P = 0.025$ when compared to placebo group.
[b]$P = 0.036$ when compared to placebo group.
[c]$P = 0.035$ and 0.025 when compared to untreated control and placebo groups, respectively.
[d]$P = 0.017$ when compared to placebo group.
[e]$P = 0.007$ and 0.013 when compared to untreated control and placebo groups, respectively.
[f]$P = 0.013$ and 0.030 when compared to untreated control and placebo groups, respectively.
[g]$P = 0.017$ and 0.027 when compared to untreated control and placebo groups, respectively.

($r = 0.746$ and 0.703, $P < 0.01$, respectively). In addition, albumin levels in 0.5 g/kg group were significantly higher ($P = 0.017$ and 0.027) when compared to untreated control and placebo groups, respectively.

Cholesterol values reduced significantly following treatment with zallouh oil. Cholesterol values in groups 0.05 g/kg and 0.5 g/kg were less by 22.9% and 29.2% ($P = 0.013$ and 0.03), respectively, when compared to placebo group (Table 2). Furthermore, glucose values in the 0.05 g/kg and 0.5 g/kg groups were less, but not significantly different, by 15.7% and 27.2% ($P > 0.2$), respectively, when compared to the placebo group (Table 2).

Discussion

The present study demonstrated the efficacy of *Ferula harmonis* 'zallouh oil' in enhancing erectile function of rats. The PEI was dose dependent and the ED_{50} was 880 times less than LD_{50}. It was found earlier that the plants of the *Ferula* genus contain chemicals such as ferutinine, feroline and tenuferidine.[7,8] These chemicals were shown to have an estrogenic activity and structures resemble di-ethyl-stilbestrol, a synthetic estrogen, which therefore, has the ability to increase synthesis and release of testosterone through the pituitary-luteinizing hormone–testicles axis.[8,9] In addition, it was found that the root extract of one of the Ferula species, *F. sinaica*, inhibited contractions of the smooth muscles of the trachea, intestine, aorta and uterus of rabbits, rats and guinea pigs.[10,11] This inhibition of smooth muscle contraction might involve nitric oxide synthesis, which leads to penile erection,[12] Further studies are ongoing by us to explain the above mechanisms.

The toxic effects of zallouh oil were seen following a daily administration of 0.05, 0.5 and 2 g/kg doses. There was a decrease in total body weight, hepatomegaly, atrophic testes, hemoglobin reduction, a decrease in RBC count, and five drug-related deaths occurred in the 2.0 g/kg group. The latter could be explained by the slow absorption and clearance of the administered oil mixture, thus enabling it to accumulate in the body and become lethal. The deaths mainly occurred after the twentieth day of administration. Furthermore, testicular atrophy could be due to accumulation and prolonged administration of the estrogenic-like activity from *Ferula harmonis* causing exhaustion atrophy of the testes[9] and the ratio of free testosterone to estrogen reaches a critical point and the estrogenic gonadotropine suppressive effects predominate.[13,14] Furthermore, a report by Gunther in 1959, showed that some of the isolated compounds from *Ferula* genus have infertility activities.[15]

In addition, cholesterol and glucose values were reduced following treatment of zallouh oil. This could be attributed to first, a decrease in body wieght and lower food consumption, second, a decrease in gluconeogenesis in the liver, and third, the estrogenic activity (see earlier) of the Ferula, which decreases chloesterol synthesis.

Therefore, crude oil from *Ferula harmonis*, zallouh oil, can enhance erectile function in rats. However, it can also cause certain toxicities if it is used for a long period of time and even may become a male contraceptive agent. Therefore, the active ingredients should be separated, identified and tested for their activities and toxicities.

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