A new herbal combination, Etana, for enhancing erectile function: an efficacy and safety study in animals

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We present herein a new herbal combination called Etana that is composed of five herbal extracts including *Panax quinquelotius* (Ginseng), *Eurycoma longifolia* (Tongkat Ali), *Epimedium grandiflorum* (Horny goat weed), *Centella asiatica* (Gotu Kola) and flower pollen extracts. Most of the above-mentioned extracts have been a long historical and traditional use for erectile dysfunction (ED). On the basis of the mechanism of action of each of the above, a combination is introduced to overcome several physiological or induced factors of ED. This study was conducted to show an enhancement of erectile function in male rats. The animals were observed for 3 h after each administration for penile erection, genital grooming and copulation mounting, and the penile erection index (PEI) was calculated. The maximum response was observed at the concentration of 7.5 mg kg^{-1} of Etana. At a 7.5 mg kg^{-1} single dose, the percentage of responding rats was 53 ± 7 with a PEI of 337 ± 72 compared with 17 ± 6 with a PEI of 30 ± 10 for control animals. This PEI was significantly ($P<0.001$) higher than each single component and than the sum of any two herbal components of Etana. When compared with sildenafil citrate, Etana induced more pronounced PEI than 0.36 mg kg^{-1}, but similar to 0.71 mg kg^{-1} of sildenafil. Furthermore, full acute and sub-acute toxicity studies showed no toxic effects of Etana. In conclusion, this study describes a new and safe combination of herbal components that enhance erectile function in male rats. Clinical studies are warranted for evaluating Etana's significance in ED.

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Introduction

Erectile dysfunction (ED) affects 50% of men aged between 40 and 70 years and therefore is considered to be an important health problem.[1] As men age, several physiological or induced factors arise that contribute to ED, such as a decline in the testicular production of testosterone, vascular functionality, levels and responsiveness to vasoactive amines and neurotransmitters, diseases (for example, cardiovascular, hypertension, diabetes mellitus, chronic prostatitis) and certain drugs.[1, 2] A number of pharmacological agents are introduced to correct ED transiently, such as the orally consumed phosphodiesterase type 5 inhibitors, testosterone therapy, or vasoactive agents inserted intraurethrally or injected intracavernosally.[1-3]

Some natural products such as *Panax quinquelotius, Eurycoma longifolia and Epimedium grandiflorum* have the ability to act as an aphrodisiac and to help restore ED. The

medicinal activity of *Panax quinquelotius* (Ginseng) has improved penile rigidity, libido and patient satisfaction in men with ED,[4, 5] whereas using a *Eurycoma longifolia* extract (Tongkat Ali) and *Epimedium grandiflorum* (Horny Goat Weed) in animals increased sexual arousal, motivation and frequency of sexual activity.[6-10] Furthermore, there are some natural products that could play a role in improving circulation to the prostate and penis such as *Centella asiatica* (Gotu Kola) and flower pollen.[11-13] Therefore, it was our hypothesis that the development of a herbal combination of the above five plant extracts, called Etana,[14] could work on several age-induced causes of ED. On the basis of the mechanism of action of each component, this herbal combination could have an additive or synergistic effect to restore erectile function.

To introduce Etana as an enhancer of male erectile function, this study examines the efficacy of Etana in relation to each of its components, to its dose-response effect, in comparison to sildenafil as a known drug to restore erectile function and to different herbal combinations. In addition, acute and subacute toxicity studies of Etana were carried out to establish the safety of this herbal combination.

Materials and Methods

Herbal Extracts

Panax quinquelotius, Eurycoma longifolia and Epimedium grandiflorum extracts were purchased from Hongjiu Ginseng, the Active Ingredients Group and from the Chengdu Wagott Pharmaceutical Co., Chengdu, China, respective. *Centella asiatica* (Gotu Kola) and flower pollen extracts were purchased from Graminex, USA and Ennagram, France, respectively. Sildenafil citrate was obtained from JPM, Jordan.

Etana preparation and method of analysis

Etana is a mixture of Ginseng extract (100 mg), Tongkat Ali extracts (200 mg), Epimedium extract (50 mg), Gotu Kola extract (40 mg), and flower pollen extract (135 mg). The preparation was dissolved in distilled water and given to rats by oral gavage.

The method of analysis of Etana components is based on the HPLC method to assay a marker for each constituent (for example, icariin for *Epimedium grandiflorum* and malasiatic acid for *Centella asiatica*). The method is based on a solid stationary phase (C18 packed column), mobile phase, and separation by partition adsorption or ion exchange process. The gradient mixtures of acetonitrile: H_3PO_4 and the detection wavelengths were different for each component.

Animals

Male and female Wister rats (220-300 g) were obtained from the Yarmouk University animal house unit (Irbid, Jordan). The animals were housed at the animal facility in Petra University in a 12 h light or dark cycle at a constant temperature of 22° C. All animals were acclimatized for 10 days before the experiments with free access to a standard diet and drinking water. All animal experiments were carried out in compliance with relevant laws and institutional guidelines.

Sexual behavior and penile erection index

Each animal group consisted of 10 male rats weighing 200-300 g. Each test preparation was dissolved freshly in distilled water and doses were administered by oral gavage. Dosing of Etana was either as a single dose per day or as a triple dose per day, 3 h apart, to show any changes in the efficacy obtained from multiple administrations per day. Control animals were given the vehicle alone (distilled water). Rats were placed in glass cages, allowed free access to food and tap water and were observed for 3 h after each drug administration for penile erection, genital grooming and possible copulation mounting. The number of responding rats was recorded along with the number of sexual activity episodes (penile erection, genital

grooming or copulation mounting). Penile erection index (PEI) was calculated for each group by multiplying the percentage of active rats (responding rats) by the total number of activity episodes.[15-17]

Acute and subacute toxicity assessment

Acute toxicity for Etana was determined in rats (250-300 g) consisting of 10 rats per group (five males and five females). A single dose of 0, 7.5, 37.5, 75, 150, 225 and 300 mg kg^{-1} (that is, 1 x to 40 x of the human recommended daily dose based on 70 kg b.w.) was given by oral gavage to each animal per group. The animals were observed closely for any toxic or abnormal behavior in the first 2 h after dosing and were kept under further observation for 2 weeks.

A subacute toxicity study for 28 days was carried out according to ICH guidelines. A single dose of 0, 7.5, 15 and 75 mg kg^{-1} (that is, 1 x, 2 x, 10 x of the human recommended daily dose based on 70 kg b.w.) was given by oral gavage to each animal per group. Each test group consisted of five males and five females, and different sex animals were kept in separate cages to avoid pregnancy during the test period. Animals were monitored carefully and body weights were measured daily. At the end of 28 days, all animals were killed. Just before being killed, blood samples were taken from the jugular vein for a full blood and chemistry analysis. All internal organs were carefully removed, weighed and then fixed with 10% buffered formalin for histological examination.

Data analysis

All variables were analyzed using SPSS version 10 statistical package (SPSS Inc., Chicago, IL, USA) using different statistical tests. For sexual behavior and PEI analysis, Student's *t* test was carried out to compare the level of significance between groups. As for the toxicity study, statistics were generated for time interaction, gender effect and differences between each treatment group and the control group. The overall differences between the groups were analyzed using one-way ANOVA. In some cases, Turkey's post test was carried out after ANOVA to show the differences between selected groups. For all of the statistical comparisons, the level of significant difference was defined as $P<0.05$.

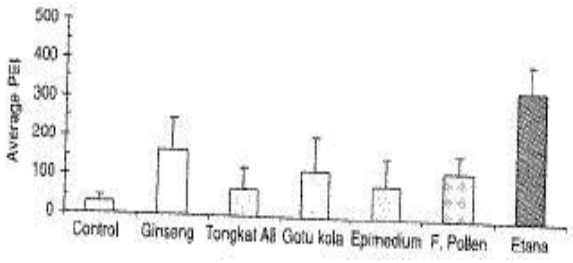

Figure 1. The PEI for different groups of rats administered either a single component of Etana, Etana or distilled water. The PEI was determined by multiplying the average scores reported during a 6-day treatment by the average percentage of responding rats. The rats were monitored for 3 h after a single administration. Each single group was administered the same amount (mg) as is present in Etana. Each bar point represents the mean of six experiments ± s.d.

Results

Etana versus each single component on male rat sexual behavior

The PEI after the administration of each single component of Etana in comparison with Etana is presented in Figure 1. In all treated animals, PEI increased significantly ($P<0.001$) when compared with control animals. In addition, Etana-treated rats showed significantly higher ($P<0.001$) PEI than did each single Etana component-treated rats (Figure 1). In addition, the PEI of Etana is higher than the sum of any two herbal components. Furthermore, the number of responding rats after a single administration of Etana was significantly higher ($P<0.001$) than each single Etana component-treated rat.

Dose response of Etana on male rat sexual behavior

The dose response of Etana showed a significantly higher ($P<0.001$) PEI at a dose of 7.5 mg kg^{-1} of Etana when compared with 2.5, 15 mg kg^{-1} and controls (Figure 2). In addition,

when Etana was administered thrice a day, 3 h apart to the same rats, the PEI was significantly higher ($P<0.001$) at 7.5 mg kg^{-1} dose when compared with that in the other doses and control, and the cumulative PEI did not change after the second or third dose to the same rats (Figure 2).

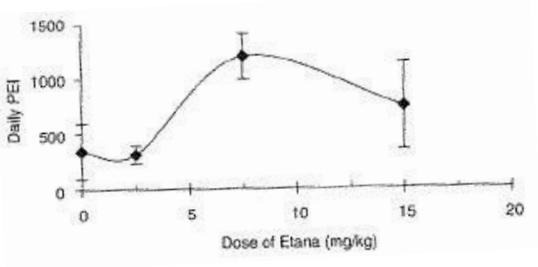

Figure 2. The PEI for different groups of rats administered different doses of Etana. Etana was administered thrice a day, and PEI was determined by multiplying the average scores reported during a 3-day treatment by the average percentage of responding rats. Each bar point represents the mean of three experiments ± s.d.

Etana versus Sildenafil on rat sexual behavior

In this set of experiments, the effect of Etana 7.5 mg kg^{-1} was compared with the effect of two therapeutic doses (0.36 and 0.71 mg kg^{-1}, based on 70 Kg b.w.) of sildenafil citrate. The PEI after Etana (7.5 mg kg^{-1}) administration as a single or triple dose per day was similar to that of 0.71 mg kg^{-1} of sildenafil and was significantly higher ($P<0.001$) than that of 0.36 mg kg^{-1} of sildenafil and the control group (Figure 3).

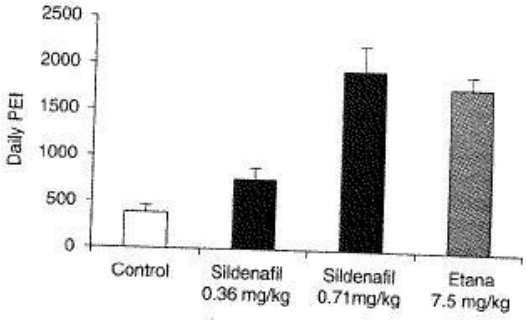

Figure 3. The PEI for different groups of rats administered two doses of sildenafil and Etana. Doses were administered thrice a day, 3 h apart, and PEI was determined by multiplying the average scores reported during a 3-day treatment by the average percentage of responding rats. Each bar point represents the mean of three experiments ± s.d.

Etana versus a different mixture of herbal components on male rat sexual behavior

The PEI after administration of different mixture components, group 1 (*Centella asiatica, Eurycoma longifolia, Epimedium grandiflorum, flower pollen extract and Gingko)* and group 2 (Ginseng, *Eurycoma longifolia* and *Epimedium grandiflorum*), is shown in Figure 4. The cumulative PEI and the percentage of responding rats after the administration of Etana thrice a day were significantly higher ($P>0.001$) than the PEI and percentage of responding rats of group1 and 2 (Figure 4). In group 1, Gingko was added instead of Ginseng because of its known function as an aphrodisiac. A single administration of Gingko (0.86 mg kg^{-1}) showed PEI and percentage of responding rats at 80 and 22%, respectively.

Acute and subacute toxicity of Etana

No deaths occurred after the administration of any of the single doses tested (7.5-300 mg kg^{-1}). After a 28-day administration of 7.5, 15 or 75 mg kg^{-1} (that is, 1 x, 2 x, 10 x of the effective dose), no deaths occurred, and the body weight did not show any significant changes in male or female rats. In addition, the weights of the internal organs did not show any changes after a 28-day administration of any of the doses tested.

The chemistry results after a 28-day administration of 1 x, 2 x, and 10 x dose of Etana showed no significant differences in triglycerides, ALT, AST, ALP, sodium, creatinine, calcium and phosphorus. However, a significant reduction in cholesterol, urea and potassium levels ($P<0.03$-0.001) was observed (Table 1). The reduction of cholesterol was dose dependent ($P<0.001$) as the percent reduction was 20, 26 and 34% for 7.5, 15 and 75 mg kg^{-1}, respectively, whereas the reduction of urea was dose dependent (15% for all doses; $P<0.03$) and the reduction of potassium was seen only at the

75 mg kg^{-1} dose (10%; P<0.015). On the other hand, the glucose level increased significantly only at 75 mg kg^{-1} dose (68%, P<0.025) (Table 1).

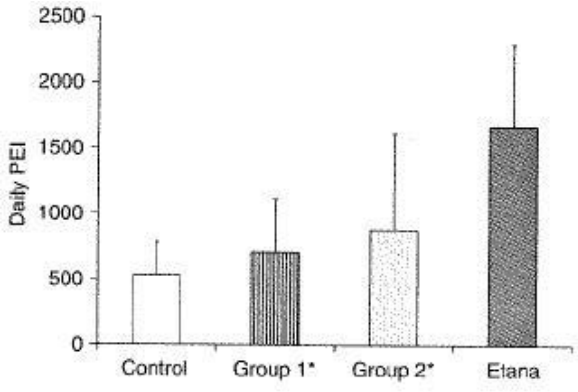

Figure 4. The PEI for different groups of rats administered different combinations: *Centella asiatica*, *Eurycoma longifolia, Epimedium grandiflorum,* flower pollen extract and Gingko for group 1: and Ginseng, *Eurycoma longifolia* and *Epimedium grandiflorum* for group 2 and Etana. Doses were administered thrice a day, 3 h apart, and PEI was determined by multiplying the average scores reported during a 3-day treatment by the average percentage of responding rats. Each single group was administered the same amount (mg) as is present in Etana. Each bar point represents the mean of three experiments ± s.d.

The hematological changes after a 28-day administration of 7.5, 15 and 75 mg kg^{-1} dose of Etana showed a significant increase in the percentage of lymphocytes, and a significant decrease in the percentage of neutrophils in peripheral blood at the doses of 15 and 75 mg kg^{-1} (P<0.05-0.001) (Table 1). However, the absolute number of the above cells in peripheral blood did not change because there was an apparent reduction in the total leukocytes count.

Discussion

This study describes a new and safe combination of herbal components that enhances erectile function in male rats. Most of the single constituents of Etana have been widely used for enhancing erectile function, and scientific evidence was reported to explain the mechanism of each component. The idea was to show the additive or synergistic effect of such combination. The results indicate that Etana showed a significantly higher percentage of responding rats and PEI. Furthermore, the Etana efficacy was dose dependent, showing higher activity at either single dose or triple dose of 7.5 mg kg^{-1} per day, and can be administered for a long period of time without any toxic effect.

To confirm our hypothesis with regard to the efficacy of Etana combination versus other possibilities, it was compared with two other combinations. The choice of the two other combinations was based on the known mechanism of each component. Group 1 was a mixture of *Centella asiatica, Eurycoma longifolia, Epimedium grandiflorum,* pollen extract and Gingko versus Ginseng, Eurycoma longifolia and *Epimedium grandiflorum* (group 2) and Etana (Ginseng, *Eurycoma longifolia*, *Epimedium grandiflorum*, *and Centella asiatica* and flower pollen). Group 1 components are similar to Etana except that it contains Gingko instead of Ginseng. Gingko has also been used for aphrodisiac effects but it has a different mechanism of action from Ginseng.[18] Group 2 does not contain flower pollen and *Centella asiatica.* On the basis of the above, one of the mechanisms of action of Etana as a herbal combination to enhance blood flow is consistent with the synergistic effects observed by combining the individual components.[11-13]

Table 1. The significant hematological and biochemical findings of rats treated with different doses of Etana for 28 days

Group	*Cholesterol (mg dl^{-1})*	*Urea (mg dl^{-1})*	*Potassium (mmol^{-1})*	*Glucose (mg dl^{-1})*	*Lymphocytes %*	*Neutrophils %*
Control	58 ± 2	37 ± 2	6.5 ± 0.2	55 ± 9	73 ± 3	13 ± 2
7.5 mg kg^{-1}	46 ± 5*	31 ± 2*	6.7 ± 0.2	56 ± 5	70 ± 3	14 ± 2
15 mg kg^{-1}	41 ± 3**	32 ± 2*	6.8 ± 0.3	75 ± 3	80 ± 2*	8 ± 1*
75 mg kg^{-1}	38 ± 2**	33 ± 1*	5.8 ± 0.2*	92 ± 12*	87 ± 2*	5 ± 1*

*P<0.05 when compared with the control group, **P<0.001 when compared with the control group.

On the basis of the published scientific evidence of each Etana component, the mechanism of action of Etana can be fourfold. First, it has been shown that ginsenosides, which are extracted from Panax ginseng, increased the plasma levels of FSH, LH, testosterone (total and free forms) and spermatozoa concentration and motility.[4] This suggests that ginsenosides act on the hypothalamus and or pituitary to increase plasma FSH and LH, thus activating testes to increase testosterone levels and spermatozoa formation. [4, 19] Second, it was found that *Eurycoma longifolia* enhanced the testosterone effect by increasing the sexual performance of inexperienced castrated male rats. [6] Third, it has been shown that ginsenosides and icariin, isolated from *Epimedium grandiflorum*, promoted the release of nitric oxide (NO)from corpus cavernosum.[10, 20] The release of NO induces the relaxation of the smooth muscle and thus enhances erection. In addition, ginsenosides and icariin were found to increase intracavernosal pressure.[10, 20] Furthermore, icariin was found to be a cGMP-specific phosphodiesterase 5 inhibitor *in vitro*,[24] but not *in vivo*, after oral dosing for 4 weeks.[10] In this study, however, the dose response of Etana showed a bell-shaped curve (Figure 2), suggesting a phosphodiesterase inhibition. Fourth, the addition of flower pollen extract and *Centella asiatica* improves blood circulation to the prostate and penis, thereby enhancing the level of the other components (or their effects) of Etana to reach the genital tract.[11-13] Furthermore, it is known that one of the major problems that could result in ED is chronic prostatitis.[2, 13] Both *Centella asiatica* (Gotu Kola) and flower pollen have antioxidative activities that are important to reduce male infertility and help in managing chronic prostatitis.[11, 13]

In addition to enhancing erectile function, Etana has other benefits. It lowered the serum cholesterol level after 28 days of oral dosing in a dose-dependent manner. This cholesterol-lowering effect is mainly due to Panax ginseng and flower pollen.[21-23]

It has been shown that Panax ginseng lowers cholesterol and triglyceride levels by activating lipoprotein lipase in hyperlipidemic rats.[22] In this study, however, the rats were normal and 28 days of Etana administration did not cause any significant change in the triglyceride levels.

This paper describes a new combination of herbal extracts that enhances erectile function and is safe after a long day of use. In addition, this herbal combination could also help in reducing the serum cholesterol level and in managing chronic inflammation of the prostate.[11-13] Clinical studies are warranted for evaluating Etana's significance in ED and in men with chronic prostatitis.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgements

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