

Effects of *Smilax myosotiflora* on testicular 11 β -hydroxysteroid dehydrogenase oxidative activity and plasma hormone levels in rats.

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Abstract

Smilax myosotiflora, a popular local aphrodisiac is known to increase sexual libido. However, the aphrodisiac efficacy of *S. myosotiflora* has not yet been scientifically established. Present investigation describes the effects of *S. myosotiflora* on testicular 11 β -hydroxysteroid dehydrogenase (11 β -HSD) oxidative activity and the levels of plasma corticosterone and testosterone. Male Wistar rats (200-250g) were given either 8 mg/kg BW/day of *S. myosotiflora* or 120 μ g/kg BW/day of Mifepristone (RU486, a glucocorticoid receptor (GR) antagonist) or both together (RU486+*S. myosotiflora*), for seven consecutive days. Results between groups were analyzed using analysis of variance (ANOVA) and Student's *t* test. Differences were considered significant at $P < 0.05$. Rats given *S. myosotiflora* showed no significant changes in 11 β -HSD activity but had increased corticosterone ($P < 0.01$) and decreased testosterone ($P < 0.01$) levels compared to controls. Administration of RU486 alone decreased 11 β -HSD activity ($P < 0.001$), but increased testosterone levels ($P < 0.05$) compared to controls. Conversely, rats with RU486+*S. myosotiflora* showed increased 11 β -HSD activity ($P < 0.001$) but decreased testosterone levels ($P < 0.05$) compared to RU486-treated rats. In the rats treated with RU486+*S. myosotiflora*, none of the parameters differed significantly from controls. Plasma corticosterone levels were found to be lowered in rats treated with RU486+*S. myosotiflora* compared to *S. myosotiflora*-treated rats ($P < 0.05$) towards control values. In this study, *S. myosotiflora* at a dose of 8 mg/kg BW/day was found to increase corticosterone with a corresponding decrease in testosterone levels. Previous studies show that RU486 acts through GR in affecting 11 β -HSD activity and hormone levels. Since RU486+*S. myosotiflora* rats show opposite effects from RU486 rats, we therefore suggest that the actions of *S. myosotiflora* on these parameters are possibly mediated through the GR. *S. myosotiflora* and RU486 probably competitively inhibit each other at the GR level.

Key words: *Smilax myosotiflora*, Testicular 11 β -HSD, Plasma corticosterone, Plasma testosterone

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Introduction

Smilax myosotiflora is a slender herbaceous creeper from the family Smilacaceae. Found throughout the tropics and in the northern warm temperate regions, *S. myosotiflora* generally grows in the lowlands and foothills of Peninsular Malaysia, Java (Indonesia) and Southern Thailand [1, 2]. It is known by several names locally, such as Ubi Jaga (most common), Ubi Besi, Itah Besi, Akar Ali, Akar Ding, Akar Tanding, Akar Restong, Akar Kerating, Keleh, Manto and Similax [1, 3, 4, 5].

In Malay traditional medicine, *S. myosotiflora* is used for several purposes. The leaves and fruit of the *S. myosotiflora* are used for treating syphilis and rheumatism [5, 6, 7]. Externally, *S. myosotiflora* is used in the treatment of skin ailments including wounds, inflammations, boils and ulcers [8].

S. myosotiflora is popular as an aphrodisiac amongst the Malays and aborigines [5, 7, 9]. *S. myosotiflora* rhizomes are used as a sexual tonic to improve the male libido [5]. In fact, *S. myosotiflora* has even a greater reputation

among the Malays as a sexual tonic than *S. callophylla* (Itah Tembaga) [4]. However, the aphrodisiac property of *S. myosotiflora* has not yet been scientifically established.

Traditionally, *S. myosotiflora* rhizomes are either chewed (with betel) or made into a decoction as a sex-tonic [6, 7]. Habitual betel quid chewing is said to give euphoria, short-term increase in heart rate and blood circulation as well as an overall feeling of well-being [5]. Traditional preparation of the sexual tonic involves either boiling the *S. myosotiflora* rhizome alone or together with Tongkat Ali roots and other herbs (such as horny goat weed etc.) to enhance the efficacy of the decoction [5].

Testicular 11 β -hydroxysteroid dehydrogenase (11 β -HSD) is believed to regulate intracellular glucocorticoid receptor concentration and prevent glucocorticoid-associated inhibition of luteinizing hormone (LH)-induced steroidogenesis [10]. Profound stressful conditions increase plasma corticosterone (B) levels, decrease testicular 11 β -HSD oxidative activity and plasma testosterone levels in rats [11].

The present investigation describes the effects of *S. myosotiflora* on testicular concentrations of 11 β -HSD and the levels of plasma corticosterone and testosterone in rats. The possible mechanism of action of *S. myosotiflora* on the parameters studied is also explored.

Material and Methods

Plant Material

Smilax myosotiflora rhizomes were collected from Gua Musang, Kelantan, Malaysia and a voucher specimen was deposited in the herbarium at the Forest Research Institute of Malaysia (FRIM). The *S. myosotiflora* rhizomes were washed, cleaned, then air-dried under shade at room temperature, and finally the whole rhizome was ground to a fine powder. The *S. myosotiflora* rhizomes used in this investigation was supplied in a fine powder form by FRIM and stored in tinted glass bottles under refrigeration (4°C) at our laboratory.

To make 8 mg/kg BW/day of *S. myosotiflora*, 160 mg of its rhizome powder was reconstituted at room temperature (just prior to treatment) in 10 ml of 0.9% saline. The mixture was then vortexed until well-mixed and sonicated at room temperature for an hour. The suspension of *S. myosotiflora* in 0.9% saline was given (gavaged) to the experimental rats.

Chemicals

Corticosterone (B), 11-dehydrocorticosterone (A), bovine serum albumin (BSA) and Mifepristone (RU486) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Bio Rad-protein assay standard II (1mg/ml) and

dye reagent concentrate were purchased from Bio-Rad Laboratories (Hercules, California, USA).

Animals and Treatments

Adult male Wistar rats (200-250g BW) were used in this study. Animals were allowed free access to rat chow and drinking water and kept under controlled temperature (27-29°C) and lighting (12:12 hours light-dark cycle) schedule.

The rats were randomly divided into 3 groups, A, B and C. Animals were treated daily for seven consecutive days. Rats in Group A were gavaged with 0.5 ml of 8 mg/kg body weight/day *S. myosotiflora*. Rats in Group B were given 0.1 ml of 120 μ g/kg BW/day Mifepristone (RU486) as intramuscular (i.m.) injections. RU486 at a dosage of 120 μ g/kg BW/day was prepared by mixing 3 mg of Mifepristone in 1 ml ethanol and 9 ml olive oil.

The animals in Group C received both 0.5 ml of *S. myosotiflora* orally and 0.1ml of RU486 i.m. The control group for Group A was gavaged with 0.5 ml of 0.9% saline as the vehicle, while the control animals for Group B were given 0.1 ml of olive oil i.m. The controls in Group C received both 0.5 ml of 0.9% saline orally as well as 0.1 ml olive oil given i.m. Rats were sacrificed twenty four hours after the last dose of treatment.

Bioassay of 11 β -Hydroxysteroid Dehydrogenase Oxidative Activity

At sacrifice, the testes were rapidly removed then homogenized in Krebs-Ringer bicarbonate buffer solution containing glucose (Sigma Chemical Co., St. Louis, MO, USA) on ice. Subsequently, 250 μ l of tissue homogenate was incubated at 37°C in a shaking water bath with 12nM (1,2,6,7-³H)-corticosterone (specific activity: 24 Ci/mmol) (Amersham Life Science, Buckinghamshire, England) as the substrate, in the presence of 200 μ M NADP (Sigma Chemical Co., St. Louis, MO, USA). The amount of protein incubated was such that it would provide a 40-50% conversion of (³H)-corticosterone to (³H)11-dehydrocorticosterone in control rats during 10 minutes of incubation, which has been reported as 200 μ g/ml for the testis [12, 13]. The reaction was terminated and steroids extracted by adding ethyl acetate. The steroids were then separated chromatographically on thin layer plates (Merck, Darmstadt, Germany), following which the steroid bands were identified under ultraviolet light and radioactivity was measured with a Liquid Scintillation Counter Wallac 1409 (Wallac Oy, Turku, Finland) counting ³H-B activity. 11 β -HSD oxidative activity was determined as the percentage conversion of (³H)-corticosterone to (³H)11-dehydrocorticosterone [14].

Hormone Assay

Under anaesthesia, blood samples were collected from the heart using heparinized syringes and centrifuged at 4°C.

The plasma was removed and kept frozen at -20°C until used. Plasma total testosterone and corticosterone levels were assayed by radioimmunoassay (RIA) using commercially-available kits (Diagnostic Products Corporation, LA, California, USA). The intra- and inter assay variation coefficients for total testosterone were within 10%.

Statistical Analysis

Data were analyzed using the Statistical Package for Social Sciences (SPSS Inc., Chicago, Illinois, USA). 11β-HSD activity was expressed as mean ± standard error of mean (SEM) while plasma testosterone levels were stated as mean ± 95% confidence interval (CI). Plasma corticosterone levels were stated as mean ± range. For enzyme activity and plasma testosterone levels, differences between experimental and control groups were evaluated by analysis of variance (ANOVA) and Student’s t-test. Mann Whitney test was employed for plasma corticosterone levels. Differences were considered significant at P<0.05.

Ethical Matters

This research was approved by the Medical Research and Ethics Committee of the Faculty of Medicine, UKM.

Results

Effects of Smilax myosotiflora on testicular 11β-HSD activity and plasma hormone levels

Rats receiving 8mg/kg BW of *S. myosotiflora* for seven consecutive days did not show any significant changes in testicular 11β-HSD oxidative activity compared to controls (Table 1). However, in this group of rats, plasma corticosterone levels were increased (P<0.01) with a corresponding decrease in plasma testosterone (P<0.01) as compared to controls (Table 1).

This increase in corticosterone with a corresponding decrease in testosterone could possibly be mediated through the glucocorticoid receptors. RU486 (Mifepristone), an established anti-glucocorticoid [15, 16], was therefore

administered concurrently with *S. myosotiflora* to see whether RU486 could effectively block the testicular endocrine profile caused by *S. myosotiflora*.

Effect of RU486 on testicular 11β-HSD activity and plasma hormone levels

Rats treated with RU486 alone had reduced testicular 11β-HSD oxidative activity (P<0.001) with an increase in plasma testosterone (P<0.05) compared to control (Table 2). However, no change in plasma corticosterone was detected (Table 2). The reduction in testicular 11β-HSD activity as observed in these animals could possibly be due to glucocorticoid receptor affinity of RU486.

Moreover, by blocking the effect of corticosterone at the receptor level, RU486 possibly augmented plasma testosterone levels to almost double in concentration.

Effects of Smilax myosotiflora and RU486 on testicular 11β-HSD activity and plasma hormone levels

Testicular 11β-HSD oxidative activity in rats receiving *S. myosotiflora* concurrently with RU 486 was increased (P<0.001) compared to RU486 alone (Table 3), to that of control levels (Table 3). Thus, it seems that the effect of RU486 on 11β-HSD oxidative activity could be minimized by *S. myosotiflora*.

Animals receiving 8mg/kg BW of *S. myosotiflora* and RU486 had corticosterone levels that were lower (P<0.05) than that of *S. myosotiflora* alone (Table 4), and which did not significantly differ from control levels (Table 4). Thus, it seems that RU486 could reverse the effect of *S. myosotiflora* on corticosterone levels.

Similarly, plasma testosterone levels in rats receiving *S. myosotiflora* and RU486 were reduced (P<0.05) compared to that of rats given RU486 alone (Table 3) to control values (Table 3). Again here it seems that *S. myosotiflora* and RU486 cancelled out each other’s effect on testosterone levels, leading it towards normal values.

Table 1: Effect of Smilax myosotiflora on testicular 11β-HSD activity and plasma hormone levels

Treatment	Testicular 11β-HSD Activity (%)	Plasma Corticosterone Levels (ng/mL)	Plasma Testosterone Levels (nmol/L)
Control	40.61 ± 1.13 (n=30)	187.60 ± 11.41 (n=19)	6.37 ± 0.69 (n=25)
<i>Smilax myosotiflora</i>	40.18 ± 0.81 (n=7)	250.20 ± 17.88** (n=11)	2.94 ± 1.08** (n=18)

Table 2 . Effect of RU486 on testicular 11 β -HSD activity and plasma hormone levels

Treatment	Testicular 11 β -HSD Activity (%)	Plasma Corticosterone Levels (ng/mL)	Plasma Testosterone Levels (nmol/L)
Control	40.61 \pm 1.13 (n=30)	187.60 \pm 11.41 (n=19)	6.37 \pm 0.69 (n=25)
RU486	30.34 \pm 1.01 ^{***} (n=6)	173.70 \pm 12.90 (n=6)	11.78 \pm 1.58* (n=6)

*P<0.05; *** P<0.001

Table 3. Comparing the effects of RU486 vs. *Smilax myosotiflora* plus RU486

Treatment	Testicular 11 β -HSD Activity (%)	Plasma Corticosterone Levels (ng/mL)	Plasma Testosterone Levels (nmol/L)
Control	40.61 \pm 1.13 (n=30)	187.60 \pm 11.41 (n=19)	6.37 \pm 0.69 (n=25)
RU486	30.34 \pm 1.01 (n=6)	173.70 \pm 12.90 (n=6)	11.78 \pm 1.58 (n=6)
<i>Smilax myosotiflora</i> + RU486	39.65 \pm 0.88 ^{***} (n=8)	163.50 \pm 20.44 (n=10)	4.86 \pm 1.20* (n=9)

*P<0.05, ***P<0.001

Table 4. Comparing the effects of *Smilax myosotiflora* vs. *Smilax myosotiflora* plus RU486

Treatment	Testicular 11 β -HSD Activity (%)	Plasma Corticosterone Levels (ng/mL)	Plasma Testosterone Levels (nmol/L)
Control	40.61 \pm 1.13 (n=30)	187.60 \pm 11.41 (n=19)	6.37 \pm 0.69 (n=25)
<i>Smilax myosotiflora</i>	40.18 \pm 0.81 (n=7)	250.20 \pm 17.88 (n=11)	2.94 \pm 1.08 (n=18)
<i>Smilax myosotiflora</i> + RU486	39.65 \pm 0.88(n=8)	163.50 \pm 20.44* (n=10)	4.86 \pm 1.20 (n=9)

*P<0.05

Discussion

An aphrodisiac is a substance that is able to excite libido or aggravate sexual instinct [17]. Aphrodisiacs may exert its effect through three different modes of action i.e. by increasing libido or sexual desire, by increasing potency or effectiveness of erection and by increasing sexual pleasure [17]. Aphrodisiacs that enhance libido act at the level of the central nervous system by altering specific neurotransmitter or sex hormone concentrations [17]. Most of this type of aphrodisiac acts through an increase

in testosterone concentration and is, therefore, male-specific [17].

Malaysian plants such as *Smilax myosotiflora* (Ubi Jaga) and *Eurycoma longifolia* Jack (Tongkat Ali) are used in Malay traditional medicine for its reputed aphrodisiac properties [18] that increase testosterone levels and give added vigor to sexual health and strength [5]. *S. myosotiflora* rhizome is claimed to increase testosterone production in aging males, thereby improving spermatogenesis and sperm viscosity [5]. As such, it is traditionally be-

lieved that consumption of *E. longifolia* Jack and *S. myosotiflora* in combination gives a more pronounced aphrodisiac effect.

Studies show that *E. longifolia* Jack enhances the libido of sexually experienced male rats [19] and increases the sperm count and plasma testosterone levels in rats [20]. A potential phytoandrogen, i.e. a 4.3kDa bioactive peptide has been isolated from *E. longifolia* Jack and is reported to increase testosterone levels in rat [21]. A bioactive peptide similar to that of *E. longifolia* Jack has also been detected in the 50% ethanol extract of *S. myosotiflora* [17]. However, substantial scientific evidence documenting the purported aphrodisiac properties of *S. myosotiflora* remains lacking.

If *S. myosotiflora* does indeed cause an increase in sexual libido, then it is anticipated that *S. myosotiflora* consumption would lead to an increase in testosterone levels. However, in our present study, *S. myosotiflora* given in its crude form at a dose of 8 mg/kg BW/day for seven consecutive days caused a decrease in plasma testosterone levels. Instead, we found that *S. myosotiflora* at this dose elevated the levels of corticosterone. Circulating glucocorticoid levels rise sharply in response to stress, resulting in a significant drop in testosterone secretion, diminished libido and fertility [22]. Stress-induced depletion of plasma testosterone is due in part to a direct receptor-mediated effect of glucocorticoid in Leydig cells [23, 24, 25], which subsequently suppress testicular response to gonadotropins, LH and FSH [24].

On the other hand, the inhibitory effect of the elevated glucocorticoid on testosterone production could possibly be mediated by depressing testicular 11 β -HSD oxidative activity. In the rat testis, 11 β -HSD and glucocorticoid receptors are present in the rat Leydig cells [26]. Testosterone production is maintained in the presence of normal plasma concentration of corticosterone, and is inhibited when 11 β -HSD oxidative capacity is exceeded due to high levels of corticosterone [27]. Lowered 11 β -HSD oxidative activity increases glucocorticoid-dependent inhibition of testosterone production in Leydig cells [26]. However, in the rats given *S. myosotiflora*, testicular 11 β -HSD oxidative activity remained unaffected. It may be possible that *S. myosotiflora* acts through a different steroidogenic enzyme other than 11 β -HSD in affecting testosterone levels. Alternately, by acting through glucocorticoid receptors, *S. myosotiflora* possibly exerts its effect on plasma levels of testosterone. To test this hypothesis, we administered *S. myosotiflora* together with the glucocorticoid receptor blocker, RU486.

As reported previously, we have also recorded that RU486 treatment lowered 11 β -HSD oxidative activity but

increased testosterone levels in rats [28]. However, in the present study, RU486 prevented *S. myosotiflora*-induced corticosterone increase and thus the inhibition of plasma testosterone levels in rats receiving both *S. myosotiflora* and RU486. At the same time, *S. myosotiflora* prevented the RU486-induced reduction in 11 β -HSD activity as well as the increase in testosterone levels. It seems that both *S. myosotiflora* and RU486 competitively inhibit each other at the receptor sites to exert its effect on the parameters tested. Thus, we suggest that *S. myosotiflora* possibly acts through the same receptor as RU486 i.e. the glucocorticoid receptors. However, the sexual potency and fertility of the test animals are under investigation.

Conclusion

The local herb *S. myosotiflora* is well known for its aphrodisiac effects. The herb acts like a stressor by causing elevated corticosterone levels in male rats. It seems likely that *S. myosotiflora* and RU486 competitively inhibit each other at the glucocorticoid receptor sites. Further research is needed to evaluate the aphrodisiac mechanism of *S. myosotiflora*.

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