

# **Smilax calophylla overcomes the effects of adrenalectomy on the testicular 11 $\beta$ -hydroxysteroid dehydrogenase activity and plasma levels of testosterone in rats**

**Author(s): Nuraliza AS, Khalid BAK, Hamid A, Morat PB, Nwe KHH**

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**Nuraliza AS<sup>1</sup>, Khalid BAK<sup>2</sup>, Hamid A<sup>3</sup>, Morat PB<sup>4</sup>, Nwe KHH<sup>3</sup>**

<sup>1</sup>Faculty of Medicine, University Technology MARA, 40450 Shah Alam, Selangor, Malaysia,

<sup>2</sup>School of Medicine and Health Sciences, Monash University Malaysia Campus, 46150 Petaling Jaya, Selangor, Malaysia,

<sup>3</sup>Faculty of Medicine and Health Sciences, University Putra Malaysia, 43400 Serdang, Selangor, Malaysia and

<sup>4</sup>Department of Biomedical Science, Faculty of Allied Health Sciences, Universiti Kebangsaan Malaysia, 50300 Kuala Lumpur, Malaysia

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## **Abstract**

Removal of adrenal gland (adrenalectomy) caused profound effects on male reproductive system. Adrenalectomy suppressed testicular 11 $\beta$ -hydroxysteroid dehydrogenase (11 $\beta$ -HSD) activity and plasma levels of testosterone (T) in rats. Our previous study documented that corticosterone-induced inhibition on testicular 11 $\beta$ -HSD activity and plasma levels of T could be reversed by Smilax calophylla, (Akar dawai) water extract (AD). The present study was therefore designed to determine whether the effects of adrenalectomy on the testicular 11 $\beta$ -HSD activity and plasma levels of T could be reversed by AD. Testicular 11 $\beta$ -HSD activity and plasma levels of T were found to be reduced significantly ( $p < 0.001$ ) following adrenalectomy. However, AD administration in the adrenalectomized rats was found to retain the testicular 11 $\beta$ -HSD activity and plasma levels of T as found in controls rats. The present study therefore suggests that AD is able to counteract the effects of adrenalectomy on the testicular 11 $\beta$ -HSD activity and plasma levels of T.

## **Introduction**

Long term glucocorticoid deficiency could eventually lead to disturbances in male reproductive function, where low plasma levels of testosterone (T) and delayed spermatogenesis are the subsequent consequences of reduced luteinizing hormone (LH) secretion [1]. Likewise, adrenalectomy evidently suppresses the 11 $\beta$ -hydroxysteroid dehydrogenase (11 $\beta$ -HSD) activity in the testis and liver [2,3] as well as plasma T levels [4].

11 $\beta$ -hydroxysteroid dehydrogenase a ubiquitous enzyme, which is known to be responsible in modulating the glucocorticoid levels in the tissues [5,6]. In the testis, 11 $\beta$ -HSD converts excess corticosterone (CORT) to the inactive form; thereby protects the testis from the adverse effect of CORT especially on T production [7]. Corticosterone, on the other hand has been reported to have a direct inhibitory effect on testicular steroidogenesis [8]. In certain treatments, the oxidative activity of 11 $\beta$ -HSD has been correlated with plasma levels of T [9]. Conversely, suppression of the 11 $\beta$ -HSD activity has been shown to be associated with low plasma levels of T or viceversa [10].

*Smilax calophylla* (Akar dawai) is found mainly in the northern part of Peninsular Malaysia. The local folks consume decoction of Akar dawai rhizome as a male sexual tonic. Indeed, the *Smilax* plant has been reported to be a potential aphrodisiac [11]. Studies on some other *Smilax* plants have documented the steroidal components of the plant rhizomes [12,13] as well as of the various effects of the *Smilax* plant extract [14,15]. Our previous study revealed that the water extract of Akar dawai rhizome (AD) could overcome the suppressive effect of exogenous CORT on testicular 11 $\beta$ -HSD activity and plasma levels of T in rats, and it is proposed that AD possibly acts by blocking glucocorticoid receptors [16].

## **Materials and methods**

### **Chemicals**

Chemicals used in the enzyme assay such as corticosterone, 11-dehydrocorticosterone, glucose, bovine serum albumin (BSA), and NADP were purchased from Sigma Chemical Co. St. Louis, USA. Bio-Rad Laboratory, CA, USA supplied the dye reagent used. The Coatcount commercial RIA kit for total testosterone was obtained from Diagnostic Products Corp., Los Angeles, CA. Akar dawai water extract was kindly supplied by Prof. Dr. Johari Mohd. Saad research group from Department of Biochemistry, Faculty of Medicine, University of Malaya, Kuala Lumpur.

### **Animals and treatment**

Male Wistar rats of 200-250 g body weight (BW) were purchased from the Animal Unit, Institute of Medical Research, Malaysia. Rats were randomly assigned into three groups: control group, adrenalectomized (ADX) control group and ADX rats treated with AD water extract (8mg/kg BW). Six to twelve rats were assigned into each group. Control

rats were given normal saline orally. Water extract of AD was gavaged (0.5ml) daily for seven consecutive days. From the time dependant graph of this herb, we found that AD (8mg/kg BW) for three consecutive days in normal rats could increase plasma T levels [16]. Therefore, in addition to the three experimental groups, six identical rats were subjected to bilateral adrenalectomy to compare the enzyme and hormonal parameters.

Bilateral adrenalectomy was performed as per the reported method [17]. Its completeness was inspected and verified at the time of killing. The rats were housed together in a temperature controlled environment (27-29°C) with 12:12 hour light-dark cycle. Normal rats were given tap water as drinking water whereas 0.9% normal saline was substituted to ADX rats. Animal were sacrificed 24 hours after the last treatment schedule between 8.30 and 9.00 a.m.

The Medical Research and Ethics Committee of the Universiti Kebangsaan Malaysia (UKM) had approved methodology used in the present study.

### **Assay of 11 $\beta$ -HSD enzyme activities**

Testes were removed immediately from the sacrificed animals, dissected and homogenized with Krebs solution containing glucose. The enzyme assay was performed as previously described [9] by incubating 250  $\mu$ l tissue homogenate with 12nM 3H-B and 200  $\mu$ M NADP at 37°C for 10 minutes (all steps were carried out on ice unless stated otherwise). The reaction was stopped and steroids were extracted by addition of ethyl acetate. Steroids were then separated by thin layer chromatography (TLC) and identified under ultraviolet light. The radioactivity was determined in  $\beta$ -liquid scintillation counter. 11 $\beta$ -HSD oxidative activity was expressed as percentage conversion of corticosterone to 11-dehydrocorticosterone.

### **Radioimmunoassay (RIA) of plasma testosterone**

Animals were anesthetized by diethyl ether and 3-4 ml blood was collected in heparinized tube. Ether was used to minimize marked fluctuation of plasma level of T [18]. Hormone levels in the plasma were analyzed as previously reported [4,7]. Plasma T levels were measured using commercial RIA kit (Coat-a-count, Diagnostic Products Corp. CA). The intra- and interassay variation coefficients of the total T was within 10%.

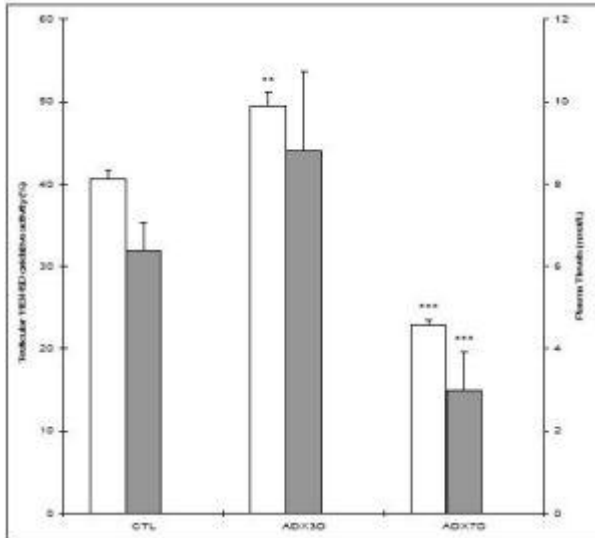
### **Statistical analysis**

Data were analyzed using Statistix Programme. The data for 11 $\beta$ -HSD oxidative activities were expressed as mean  $\pm$  standard error of mean (SEM) while for plasma T levels were expressed as mean  $\pm$  95% confidence interval (CI). Differences between groups were analyzed by analysis of variance (ANOVA) and student t-test. The differences were considered significant at  $p < 0.05$ . Statistical analysis was made in accordance with the previous report [19].

## **Results**

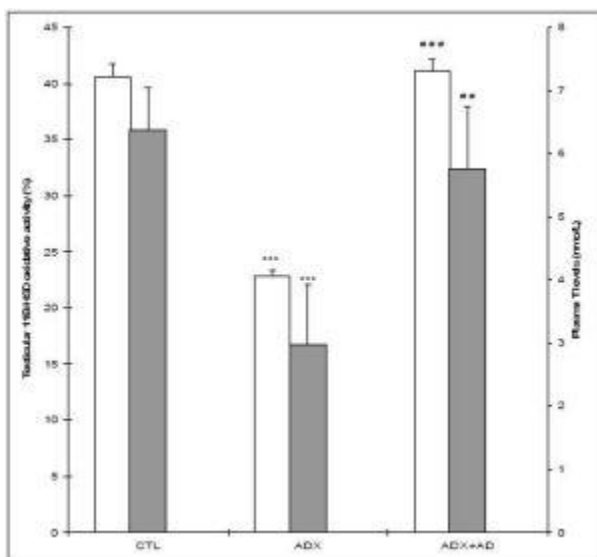
The  $11\beta$ -HSD activity was significantly higher three days following adrenalectomy (ADX3D), ( $p < 0.01$ ). However, the plasma T levels remained unchanged compared to that of the controls (Fig. 1). Moreover, a significant reduction in  $11\beta$ -HSD activity ( $p < 0.001$ ) as well as plasma T levels ( $p < 0.001$ ) was recorded following seven days of adrenalectomy compared to that of the control value (Fig. 1).

Administration of Akar dawai water extract (ADX+AD), on the other hand, increased the  $11\beta$ -HSD activities ( $p < 0.001$ ) and plasma T levels ( $p < 0.01$ ) compared to ADX rats (Fig. 2).



(For larger image of graph, click [here](#))

**Fig. 1:** Showing testicular  $11\beta$ -hydroxysteroid dehydrogenase ( $11\beta$ -HSD) activity (grey) and plasma testosterone (T) levels (white) following bilateral adrenalectomy. Rats were divided into two treatment groups: three days following adrenalectomy. The enzyme activity is expressed as mean  $\pm$  SEM while plasma T levels are expressed as mean  $\pm$  95% CI. \*\*  $p < 0.01$ ; \*\*\*  $p < 0.001$  compared to control (CTL).



(For larger image of graph, click [here](#))

**Fig. 2:** Showing testicular 11β-hydroxysteroid dehydrogenase (11β-HSD) activity (white) and plasma testosterone (T) levels (grey) following bilateral adrenalectomy. Rats were divided into two treatment groups: seven days following adrenalectomy. The enzyme activity is expressed as mean ± SEM while plasma T levels are expressed as mean ± 95% CI. \*\*\* p<0.001 compared to control (CTL). ##p<0.01; ###p<0.001 compared to ADX

## Discussion

Adrenalectomy has been reported to reduce luteinizing hormone levels (LH) as well as the levels of plasma T in male rats [1]. We have observed that administration of AD (8mg/kg BW) in rats for three consecutive days elevates plasma T levels, without altering the testicular 11β-HSD activities. On the other hand, administration of AD (8mg/kg BW) for seven days, although significantly decreases the 11β-HSD activity, yet the plasma T levels remain unchanged [16]. Results of our experiment show that three days following bilateral adrenalectomy the 11β-HSD activity has been elevated, however the plasma levels of T remain unaltered. It has been reported that seven day after bilateral adrenalectomy, both the parameters are reduced significantly [4,10].

Treatment of the water extract of AD to adrenalectomized rats has been found to maintain the activity of 11β-HSD and plasma levels of T almost identical to controls. Treatment of AD for seven consecutive days in normal rats has similarly been found to counteract the inhibitory effect of exogenous CORT, on the 11β-HSD activity and plasma levels of T. Infact, AD is proposed to act via glucocorticoid receptor in affecting both parameters [16]. It has been suggested that 11β-HSD activity and plasma T levels regulate each other in certain conditions [9,10]. In conclusion, our finding suggests that water extract of AD is possibly able to overcome the effects of bilateral adrenalectomy on testicular function.

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Correspondence:

**Nuraliza AS**

Faculty of Medicine

University Technology MARA,

40450 Shah Alam, Selangor, Malaysia

e-mail: nuraliza064 ( at ) salam.uitm.edu.my