Microsc. Microanal. Res. 2018, 31(1) 9-17

Microscopy and Microanalysis Research
- The Journal of The Microscopy Society of Thailand

Journal Home Page: https://www.tci-thaijo.org/index.php/mmres/

Microsc. Microanal. Res.

Effects of *Uvaria rufa* Blume on the Histology of Male Reproductive Organs of Testosterone Induced Alopecia Rats

Supaporn Pamok[1], Kanokporn Saenphet[2], Wararut Buncharoen[2,*]

[1] *Biology Program, Faculty of Science, Buriram Rajabhat University, Buriram Province, 31000, Thailand*
[2] *Department of Biology, Faculty of Science, Chiang Mai University, Chiang Mai, 50200, Thailand*

*Corresponding author's e-mail address: *applepaleel@gmail.com (W. Buncharoen)*

ARTICLE INFO

Article history
Submitted: 10 December 2017
Revised: 7 April 2018
Accepted: 25 June 2018
Available online: 30 June 2018

Keywords:
Androgenic alopecia
Histology
Reproductive organs
Testosterone;
Uvaria rufa Blume

ABSTRACT

Reports on the male reprotoxicity of some medicinal plants have been documented. This study was undertaken to evaluate the effects of ethyl acetate extract from the stems of *Uvaria rufa* Blume (UREtA) on the male reproductive organs of alopecia-induced rats. Androgenic alopecia (AP) was induced by testosterone propionate (0.07%). After hormonal injection, the rats were topically given vehicle (AP model), minoxidil, and 2% and 4% UREtA for 21 days. The normal control rats were given olive oil injections and received distilled water. The relative weights of the testes, epididymis, prostate glands, and epididymis, and histopathological alterations were examined. The results showed that testosterone injection reduced the relative weights of all organs when compared to those of the normal control, while significant decreases (P>0.05) were found only in epididymis and prostate glands. The increases in relative weights of epididymis observed in all treated groups, also testes and prostate glands observed in 4% UREtA and minoxidil, including seminal vesicles observed in 2% UREtA were clearly noted when compared to those of AP models. Vacuolizations were observed in testicular tissues treated with both concentrations of UREtA, while germ cell death in the lumens of the seminiferous tubules was seen in the minoxidil treated group. Dead spermatozoa in the lumens of the epididymis were markedly noted in all groups. Moreover, AP-induced rats treated with minoxidil revealed leucocyte infiltrations and blood congestions in their prostatic tissues. Therefore, it can be concluded that 2% and 4% UREtA has some adverse effects on male reproductive organs.

INTRODUCTION

Androgenic alopecia (AR) is an imbalance condition between hair regrowth and hair loss which occurs in both males and females. Miniaturization of hair follicles, genetics, stress, drugs, toxic chemicals and ultraviolet radiation contribute to AR development. In addition, two sex hormones, dihydrotestosterone (DHT) and testosterone, are the androgen-dependent factors leading to the pathogenesis of AR [1]. Although DHT is responsible for growth of hair follicles [2], an excessive level of this hormone can miniaturize hair follicles and produces hair loss [3]. There are two synthetic drugs currently used to treat AR, minoxidil and finasteride. These drugs can remedy AR by different mechanisms. Minoxidil stimulates hair regrowth by dilating blood vessels and increasing blood circulation in hair roots to supply oxygen and nutrients [4]. Finasteride inhibits a function of 5-alpha reductase (5αR) in a conversion of testosterone into DHT [5]. This decreases the DHT level and consequently reduces hair loss. However, the adverse effects of minoxidil (skin irritation, itching, and redness), and finasteride (reduced sperm concentrations, reduced reproductive organ weights, and male infertility) have been documented [6].

Medicinal plants provide an alternative for the management of AR or ameliorating hair loss, while reducing the problems above. A number of medicinal plants such as *Emblica officinalis* Linn [7,8], *Bacopa monieri* Linn. [9], *Trigonella foenum-graecum* Linn. [10], *Citrullus colocynthis* (L.) [11], *Nardostachys jatamansi* [12], *Allium cepa* L. [13] and *Capsicum annum* Linn [14] have traditionally been used throughout the world to control hair loss. Among them, *T. foenum-graecum*, *C. colocynthis*, *N. jatamansi* and *A. cepa* have been demonstrated as anti-fertility agents in laboratory animals [15]. Male rabbits fed with a diet containing 30% of *T. foenum-graecum* seeds had low concentrations of androgens and low sperm numbers. Furthermore, damage in the seminiferous tubules and the interstitial tissues were seen in their testicular tissues [16]. In addition, the antifertility of ethanolic extract of *T. foenum-graecum* was recorded in male rats [17]. Alcoholic extract of *C. colocynthis* has been proven to impair male reproductive functions by decreasing sex hormones, testicular weights, sperm concentrations and the numbers of spermatogonia. Spermatogenesis was also disrupted by this plant extract [18]. As shown by the literature surveys above, new plant

materials which have anti-hair loss efficacies and have no adverse effects on the male reproductive system are much needed.

Uvaria rufa Blume belongs to the Annonaceae family, known by local Thai people as *pee puan noi*. It has traditionally been used as a medicine for different kinds of ailments such as tuberculosis [19], diabetes [20] and prostate enlargement and inflammation [21,22]. Bioactive compounds including flavonoids, alkaloids [23], flavonols, kaempferol, quercitrin [19], rutin and isoquercitrin [24] have been found in various extracts from different parts of *U. rufa*. A previous study reported the inhibitory activities of the ethyl acetate extract from the stems of *U. rufa* against 5αR in both *in vitro* and *in vivo* experiments [22]. Thus, this plant may act as an effective anti-hair loss agent through the same mechanism. However, due to the inhibitory effects of *U. rufa* on normal production of androgens *via* the inhibition of 5αR, alterations of reproductive organs secondary to androgen depletion may occur. To determine the adverse effects of ethyl acetate extract from the stems of *U. rufa* on the male reproductive system, histological examinations of male rats' reproductive organs were carried out in alopecia-induced rats. The results obtained from this study may provide guidelines for the safe use of this plant as an anti-hair loss agent.

METHODOLOGY

Plant materials and extractions

The stems of *U. rufa* were collected from Buriram Province, Thailand in March, 2016. The plant materials were botanically identified and a voucher specimen (QGB 78882) was deposited at Queen Sirikit Botanic Garden, Chiang Mai Province, Thailand. The stems were thoroughly cleaned with tap water, dried at 60 ºC and ground into fine powder. One hundred grams of the plant powder were extracted with one liter of 95% ethanol using Soxhlet extractor. The crude ethanolic extract was defatted by petroleum ether and re-extracted using ethyl acetate. The resultant ethyl acetate extract was filtered through Whatman No. 1. The ethyl acetate was eliminated using a rotary evaporator to obtain the crude ethyl acetate extract of *U. rufa* (UREtA).

Experimental animals

Male Wistar rats (7-8 weeks age, 200-220 g) were purchased from the National Laboratory Animal Center, Thailand. All animals were housed in a controlled environmental laboratory (12 h light/dark cycles, 25 ± 1 ºC) for one week to acclimatize. Standard pellet diets and water were provided *ad libitum*. The Institutional Animal Care and Use Committee of the Biology Department, Faculty of Science, Chiang Mai University approved the animal experimentation (Re. 004/13).

Testosterone injection and treatment of androgenic alopecia

The experimental rats were randomly divided into five groups of six rats each. Group I was subcutaneously injected with olive oil and served as a normal control group. Groups II-V were subcutaneously injected with testosterone propionate (TP, 0.07%) to induce AP. After 30 min of hormonal injection, group II or AP model group was topically given 0.2 ml of vehicle consisting of propylene glycol: ethanol: water (1:8:1) while groups III-V were topically given minoxidil (2%), UREtA at 2% and 4% respectively. AP induction and treatment were done for 21 consecutive days [25]. The body weight (BW) of each rat was measured every week. After 14 days of AP induction, all rats in the AP model group showed signs of hair loss on their heads. After the experimental period, all rats were sacrificed and reproductive organs including testes, epididymis, seminal vesicle and prostate glands were quickly dissected. The connective tissues and excessive fats were removed.

Relative weights of reproductive organs

Reproductive organs including testes, epididymis, prostate glands and seminal vesicles were weighed. The organ weights (OW) were used to calculate the relative weights using the following equation:

$$\text{Relative weight (g/100 g BW)} = [\text{OW (g)} / \text{BW (g)}] \times 100 \quad (1)$$

Histological examination

The organ samples of each rat, testes, epididymis, seminal vesicles and prostate glands, were fixed in bouin's solution for at least 24 h. The fixed tissues were dehydrated through ascending grades of ethyl alcohol. After dehydration, xylene was used as a clearing solution. All tissues were embedded in paraplast wax. Six micrometers of tissue sections were prepared prior to staining with hematoxylin and eosin (H & E). All sections were examined for their histological changes using a light microscope (Olympus BX41).

Phytochemical screening

Plant secondary metabolites including phenolics, flavonoids, alkaloids, saponins, terpenoids, anthraquinones, glycosides, tannins and reducing sugars were screened in the UREtA using the standard protocols previously described [26,27].

Statistical analysis

All data are expressed as mean ± standard deviation (mean ± SD). One-way ANOVA followed by Duncan's post hoc test for multiple comparisons (SPSS version 17) was used to analyze the data. A statistically significant difference was considered if P value was less than 0.05.

RESULTS

Weights of reproductive organs

Table 1 shows the relative weights of the male reproductive organs of the rats. An administration of TP for 21 consecutive days significantly decreased (P<0.05) the relative weights of the epididymis and prostate glands of the AP model rats when compared to the normal control rats. Although the relative weights of the testes and seminal vesicles of the AP models decreased, there were no significant differences (P>0.05) between those of the AP models and the normal controls. Minoxidil and both concentrations of UREtA elevated the

Table 1 Relative weights of reproductive organs of AP-induced rats treated with UREtAat 2 and 4% and minoxidil for 21 consecutive days.

Experimental group	Relative weights (g/100 g BW)			
	Testes	Epididymis	Seminal vesicle	Prostate gland
Normal control	0.43 ± 0.04^{ab}	0.13 ± 0.01^{a}	0.29 ± 0.06^{a}	0.22 ± 0.06^{a}
AP model	0.40 ± 0.07^{a}	0.06 ± 0.01^{c}	0.23 ± 0.03^{ab}	0.15 ± 0.02^{b}
AP + minoxidil	0.48 ± 0.04^{b}	0.08 ± 0.01^{ab}	0.20 ± 0.02^{b}	0.19 ± 0.04^{ab}
AP + 2% UREtA	0.39 ± 0.09^{a}	0.08 ± 0.01^{ab}	0.24 ± 0.04^{ab}	0.15 ± 0.03^{b}
AP + 4% UREtA	0.46 ± 0.06^{ab}	0.08 ± 0.05^{b}	0.21 ± 0.03^{b}	0.19 ± 0.03^{ab}

Values are exhibited as mean ± SD of six rats. The different superscript letters indicated statistically significant differences between the experimental groups (P<0.05, One way ANOVA followed by Duncan's multiple comparison test).

relative weights of the epididymis of AP-induced rats when compared to AP model rats but only the 4% UREtA led to a significant elevation of the relative epididymal weight. Treatment with 4% UREtA or minoxidil also increased the relative weights of the testes and prostate glands of the AP-induced rats when compared to the AP model rats. However, there were no significant differences (P>0.05) among these treated groups and the AP model groups, except for the testicular weights of minoxidil-treated rats. In addition, only UREtA at 2% significantly increased the relative seminal weight of the AP-induced group when compared to the AP model group.

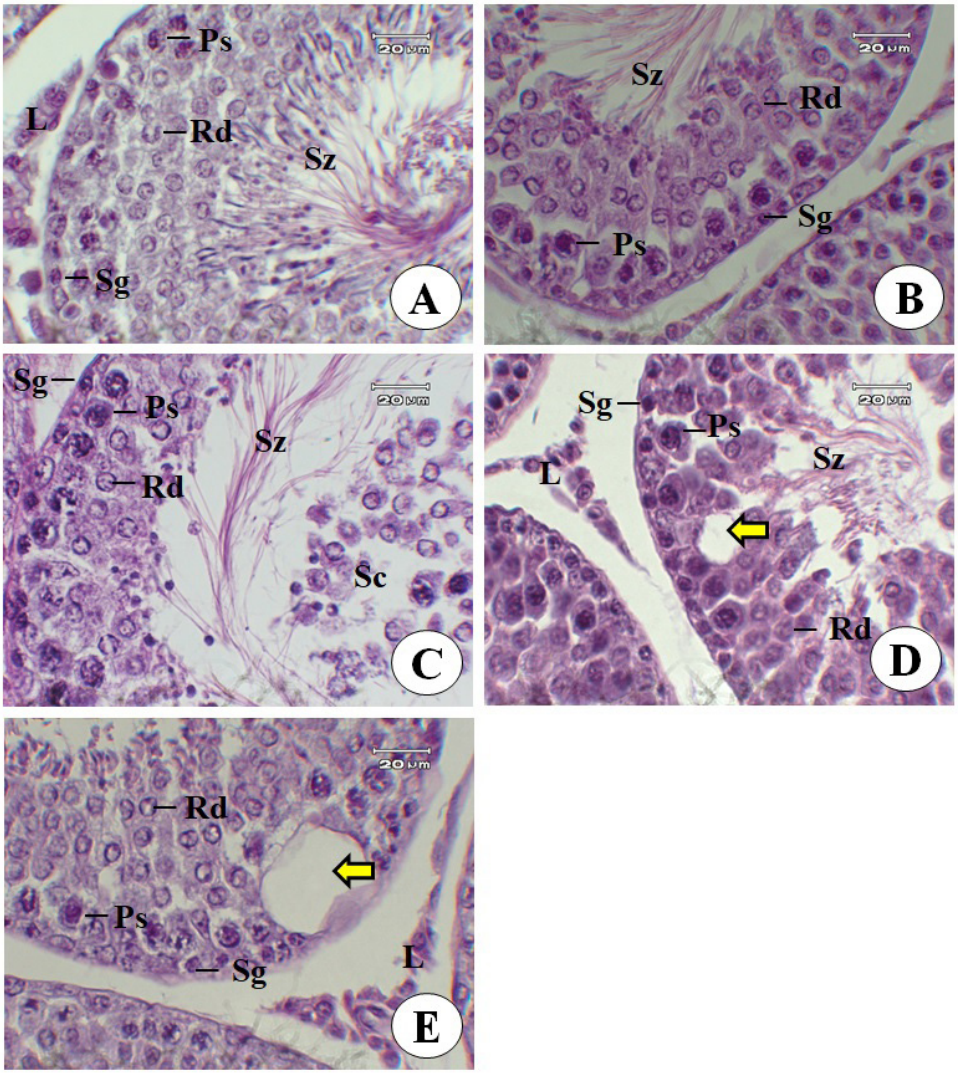

Figure 1 Microscopic photographs show testicular tissues of AP-induced rats. Normal control rat (A), AP model rat (B), AP-induced rat received with minoxidil (C) and UREtA at 2% and 4% (D and E). H&E stain, 40x. Spermatogonia (Sg), spermatocyte (Sc), primary spermatocyte (Ps), round spermatid (Rd), spermatozoa (Sz), Leydig's cell (L), vacuolizations (yellow arrow).

Histopathological examination

Testes

Examination of the testicular histology of normal and AP model rats under a light microscope showed normal histological features. The testicular tissues consisted of regular sizes of seminiferous tubules. The tubules contained germinal epithelium with normal arrangement of germ cells including spermatogonia, spermatocyte, round and elongate spermatid, and spermatozoa (Fig. 1A and Fig. 1B). The seminiferous tubules were separated by the interstitial connective tissues in which Leydig's cells were embedded. Histopathological observations found vacuolizations in testicular tissues of AP-induced rats treated with both concentrations of UREtA (Fig. 1C and Fig. 1D). However, the vacuolization was located in the cytoplasm of the Sertoli cell and it was only spotted in some areas in the seminiferous tubule. Thus, the vacuole may limit a number of differentiating spermatids to become spermatozoa, but not in entire tubule. Moreover, sloughing germ cells were clearly noted in the tubular lumens of seminiferous tubule in minoxidil-treated rats (Fig. 1E).

Epididymis

All rats in the normal control group had the normal histological structures in their cauda epididymis. A regular arrangement of epididymal tubules with abundant sperm in the lumens and ciliated columnar epithelium were found in the normal control group (Fig. 2A). The epididymal walls of all UREtA-treated rats and minoxidil-treated rats were also lined by ciliated columnar epithelium, but the abundances of connective tissue stroma were noted (Fig. 2C-E). Dead spermatozoa in the lumens of the cauda epididymis were markedly seen in all treated groups and the AP model group (Fig. 2B-E).

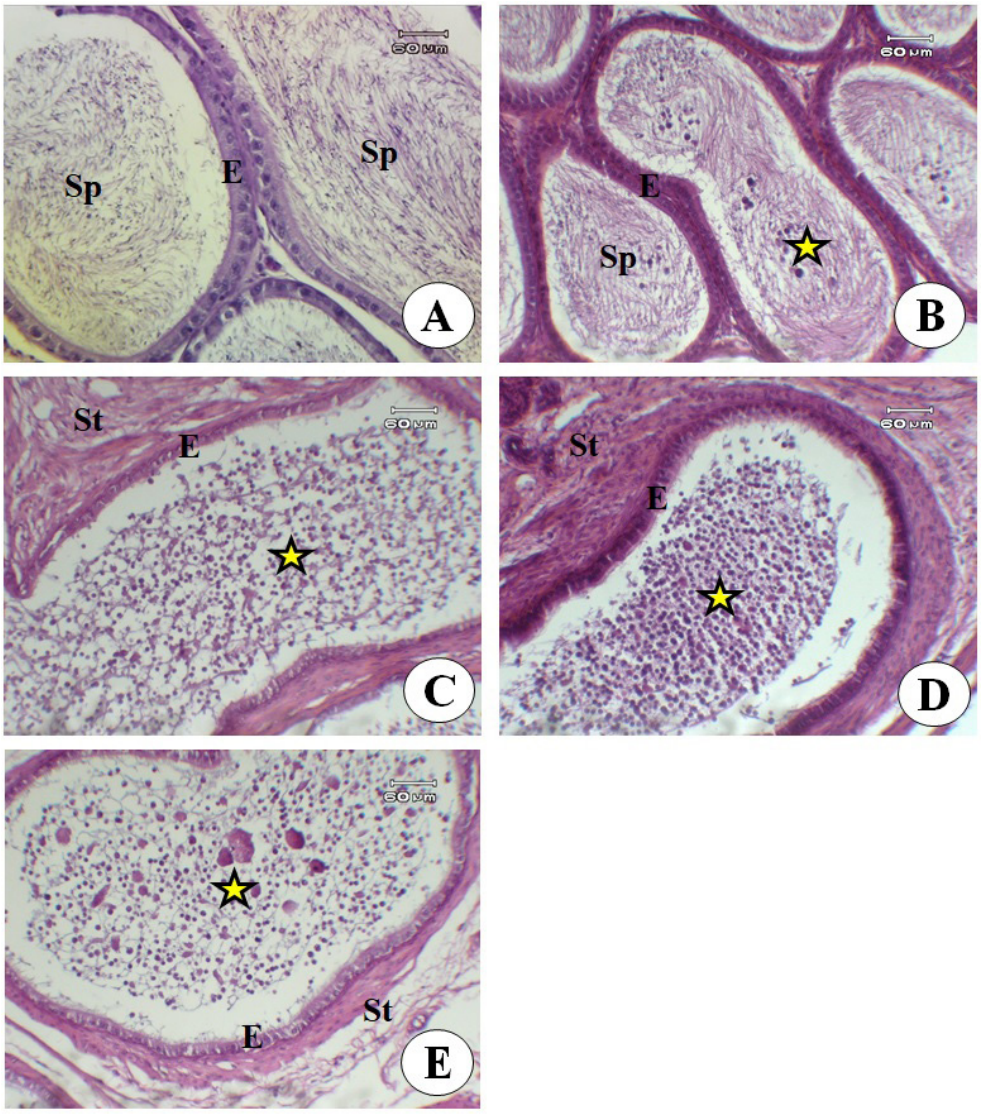

Figure 2 Microscopic photographs of epididymal tissues of AP-induced rats. Normal control rat (A), AP model rat (B), AP-induced rats received with minoxidil (C) and UREtA at 2% and 4% (D and E). H&E stain, 10x. Epididymal epithelium (E), epididymal sperm (Sp), stroma (St), dead spermatozoa (yellow star).

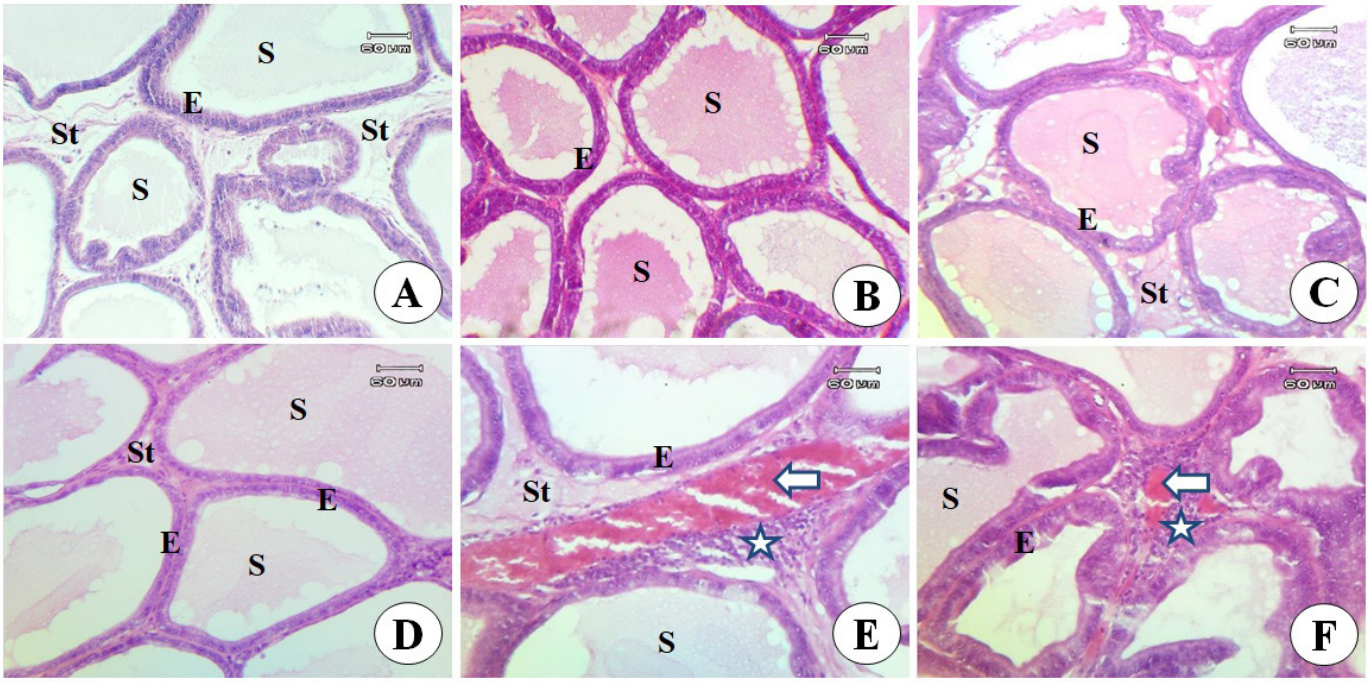

Figure 3 Histology of prostatic tissues of AP-induced rats. Normal control rat (A), AP model rat (B), AP-induced rats received with UREtA at 2% and 4% (C and D) and minoxidil (E and F). H&E stain, 10x. Prostatic epithelium (E), prostatic secretion (S), stroma (St), blood congestions (white arrow), leucocyte infiltrations (white star).

Prostate glands

Investigations of prostatic histology showed that the rats in the control group had normal histological structures in their prostates. The glandular epithelia were cuboidal and they were located on the basement membrane. Each gland contained orange-red elements of a prostatic secretion. An interstitial compartment or prostatic stroma consisted of connective tissue fibers, fibroblast and blood vessels (Fig. 3A). TP administration as well as treatments with any dose of UREtA did not alter the prostatic histology (Fig. 3B-D). However, the AP-induced rats treated with minoxidil exhibited leucocyte infiltrations and blood congestions in their prostatic tissues (Fig. 3E and Fig. 3F).

Seminal vesicles

Microscopic examinations showed that all rats in the control and treatment groups had normal histological structures in their seminal tissues. The folded mucosa consisted of simple columnar epithelium or pseudostratified columnar epithelium and lamina propria. The nuclei of the epithelium were spherical or oblate. The red seminal fluids produced by seminal epithelium were located in the seminal lumens (Fig. 4A and 4B).

Phytochemical screening

Screening of the secondary metabolites in the UREtA showed the presence of flavonoids, phenolics, alkaloids, tannins, terpenoids, anthraquinones and reducing sugars in the UREtA, while other compounds – saponins, glycosides and phlobatannins – were not present in the extract.

DISCUSSION

In developing countries, the use of medicinal plants as sources of natural drugs for primary health care has become popular. Although people believe the use of medicinal plants is safe, careless handling, usage over long time periods, or high doses can produce various symptoms of toxicity [28]. In recent years, there has been growing concern about the adverse effects of various medicinal plants used for a wide range of medicinal purposes, especially their effects on the male reproductive system.

In the present study, we found that injection of TP for 21 days reduced the relative weights of testes, epididymis, prostate glands and seminal vesicles when compared to normal rats (Table 1). We speculate that the weight reductions found in this study illustrate a negative feedback of androgen concentrations. A previous study showed a reduction in testicular and epididymal weights of rats after administration of exogenous testosterone at 100 mg/kg BW for 36 days. Moreover, the concentrations of serum and testicular testosterones were decreased [29]. Therefore, it appears that injection of exogenous testosterone in a high dose negatively affected testosterone production in the hypothalamo-pituitary-testicular axis and adversely affected androgen-dependent organs. Additionally, there is evidence that high doses of testosterone can damage testicular and prostatic tissues by inducing oxidative stress. A high level of malondialdehyde, a lipid peroxidation end product, was found in rats treated with testosterone isobutyrate (5 mg/kg) [30]. Similarly, exogenous injection of testosterone caused oxidative stress by increasing lipid peroxidation end products and lowering antioxidant indices including catalase, superoxide dismutase, glutathione reductase and glutathione-*S*-transferase in prostatic tissues [31]. The elevations of relative organ weights in UREtA- or minoxidil-treated rats may be linked to the androgenic roles of these agents. There was a report

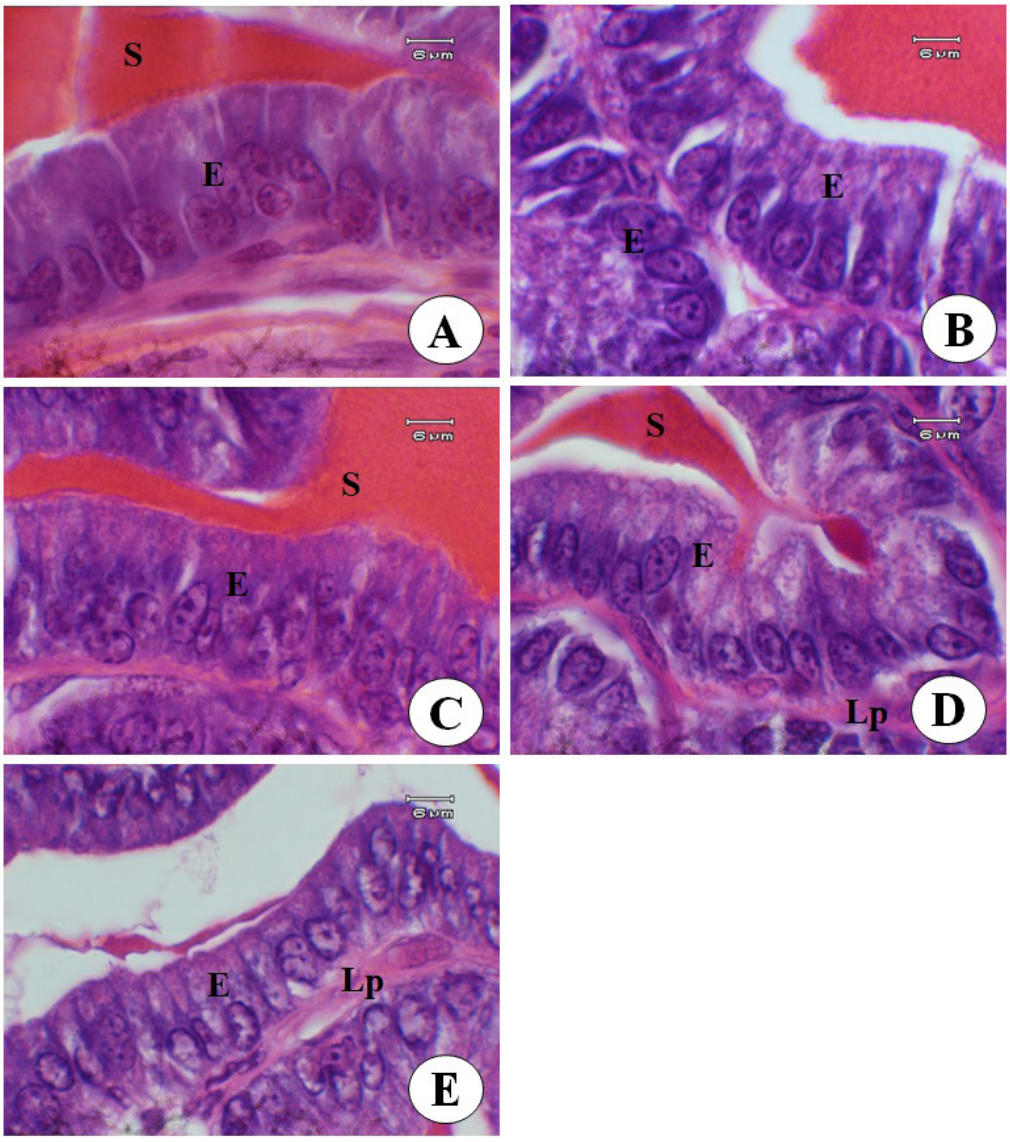

Figure 4 Microscopic photographs show seminal tissues of AP-induced rats. Normal control rat (A), AP model rat (B), AP-induced rats received with minoxidil (C), UREtA at 2% and 4% (D and E). H&E stain, 100x. Seminal epithelium (E), seminal fluid (S), lamina propria (Lp).

demonstrating that experimental rats treated with *Sesamum radiatum* leaf extract at 28 mg/kg BW had higher testosterone levels and higher epididymal weights than normal rats. This suggests that *S. radiatum* extract contains substances like androgens or phytoandrogens and consequently enhanced the testosterone levels and the epididymal weights [32]. Therefore, UREtA may affect reproductive organ weights through an androgenic action similar to that of *S. radiatum*. On the other hand, minoxidil increased testicular, epididymal and prostatic relative weights of AP-induced rats by increasing androgenic levels in these organs via dilating blood vessels.

An alteration in organ weight is accepted as a toxicological index which can be directly determined after exposure to any toxic substance [33]. Previous investigations indicated that the reductions in weights of testes, epididymis and prostate glands were the results of the declines in populations of germ cells and Leydig's cells, testes atrophy, a decrease in numbers of epididymal sperms, and a decrease in the height of prostatic epithelium [34-37]. Therefore, organ weight alone is not sufficient to confirm the safety of natural products on the male reproductive system.

We also investigated the histopathological alterations within the reproductive organs of AP-induced rats after receiving UREtA. We found vacuolizations in the testicular tissues of AP-induced rats treated with any concentration of UREtA, and germ cell death in the lumens of the seminiferous tubules of AP-induced rats treated with minoxidil. Vacuolizations can occur within Sertoli cells or different parts of spermatogenic epithelium [38]. Although the etiology of testicular vacuolizations is unclear, a breakdown of Sertoli-germ cell junctions, gene expression abnormality and reproductive tract diseases [39-40] are the important causes of vacuolizations. Additionally, damage to the blood testes barrier or tight junctions between the Sertoli cells may produce germ cell degenerations. Since solanine, a plant alkaloid, has been reported to injure Sertoli cell junctions and induce germinal epithelium cell death in mice [41], vacuolizations found in this study may be caused by alkaloids present in the UREtA. There is evidence that minoxidil at 200 and 800 μM produces PC3 cell death by elevating concentrations of calcium ions. Accordingly, minoxidil

may induce germ cell death in the same way.

Epididymis is an accessory organ responsible for sperm maturation. In this study, we found sperm death in the lumens of the cauda epididymis of all experimental groups. This alteration indicates that administration of testosterone at a high dose (0.07%) might reduce male fertility. This finding concurs with previous reports that oligozoospermia and azoospermia were found in normal men after receiving either testosterone enanthate (100 mg for 6 months) or testosterone undecanoate (240 mg for 4 months) [42-43]. However, spermatozoa degenerations were markedly seen in the UREtA- or minoxidil-treated groups. We suggest that the UREtA or minoxidil negatively affected sperm viability through various mechanisms. The epididymal epithelium modulates various processes for supporting maturation such as protein synthesis, fluid resorption, hormonal activity and secretory activity [44]. Since epididymis is mainly controlled by DHT more than testosterone, inhibitions of DHT and 5αR results in DHT depletion and adversely affects fertility and organ atrophy. Thus, the histological alterations in epididymal tissues as well as the decreases in epididymal weights found in this study may result from the activities of alkaloids in UREtA or the toxicity of minoxidil. Alkaloids in UREtA may disrupt the production of DHT and disrupt the functions of epididymis for maintaining sperm maturation [45]. Nicotine alkaloids have previously been reported to alter the functions of epididymis and decrease the numbers of spermatozoa and motile sperm [46].

Investigations of prostatic histology revealed that minoxidil had adverse effects on the prostatic tissues of AP rats. All rats in the minoxidil-treated group possessed leukocyte infiltrations and blood congestions in their prostates. These alterations indicate inflammation in the prostatic tissues secondary to administration of testosterone at a high dose [47]. Furthermore, minoxidil was proven to induce inflammations and blood congestions in the cardiac tissues of experimental animals [48]. Since prostate glands are controlled by DHT, an inhibition of 5αR and a decrease in DHT caused by any compound lead to histopathogenesis in prostate glands. Toxic effects of some phytochemical compounds such as terpenoids, tannins, flavonoids, alkaloids and phenols in various plant materials have been documented [49,50]. Morphometric investigations in the prostatic tissues of rats treated with sesame seeds showed significant increases in the height and density of the prostatic epithelium when compared to normal control rats. This suggests that sesame seeds contain high concentrations of vitamin C and E. In addition, these two vitamins have been confirmed to have antioxidant properties in both *in vitro* and *in vivo* experiments. Thus, sesame seeds may enhance reproductive functions [51]. From the results of our study, UREtA may contain low amounts of the above compounds and high amounts of vitamins C or E, and thus it did not alter the rats' prostatic structures.

Seminal vesicles synthesize and secrete seminal fluids, which are essential for supporting viability of sperm in reproductive tracts and enhancing sperm quality. Seminal fluids mainly contain fructose, citric acid and nutrient substances, prostaglandin and fibrinogen [52]. Thus, injuries in seminal epithelium or disruptions in fluid synthesis may decrease sperm quality and produce infertility. Administration of *Piper betel* (Linn.) extract at 50 mg/kg for 15 consecutive days decreased the volume of seminal fluids and damaged seminal epithelium of laboratory rats [53]. From our study, histological examinations found that both UREtA and minoxidil did not alter the histology of seminal tissues of AP-induced rats. The occurrence of a bioactive compound in a small amount can act as an antioxidant and can show protective effects against any damage in reproductive organs [54]. Therefore, UREtA may contain a key bioactive constituent which protects against seminal tissue injuries via antioxidant mechanisms. Our previous investigation showed that the ethyl acetate extract of *U. rufa* contained high amounts of phenolics and flavonoids with the concentrations of 13.97 ± 0.43 mgGAE/g extract and 3.85 ± 0.25 QE/g extract, respectively [22]. This extract also contained saponins, tannins and alkaloids (3.27 ± 0.12, 2.31 ± 0.00 and 1.38 ± 0.00 mg/g extract, respectively) [55]. Although the presence of phytoconstituents such as phenolics and flavonoids in high amounts could act as antioxidants, the occurrence of alkaloids in a small amount could also produce adverse effects on male reproductive organs as per the reports described above. Based on the overall data gathered in this study, the applications of UREtA at 2% and 4% and minoxidil had some adverse effects on male reproductive organs. Consequently, further investigation is required for isolating the bioactive constituents in UREtA that have no adverse effects on male reproductive organs to support the safe use of *U. rufa* in treating AR or anti-hair loss.

CONCLUSION

Based on the results of this study, topical applications of ethyl acetate extracts from the stems of *U. rufa* at 4% increased the relative weights of the testes, epididymis and prostate glands of androgenic alopecia-induced rats. The extracts at 2% and 4% showed slight reprotoxicity by inducing vacuolizations in the seminiferous tubules and causing death of epididymal sperm. However, they did not produce any change in prostatic and seminal tissues of androgenic alopecia-induced rats.

ACKNOWLEDGEMENTS

The authors would like to thank the Center of Excellence in Bioresources for Agriculture, Industry and Medicine, Faculty of Science, Chiang Mai University for their support. We also thank Mr. Chiramet Mankhetwit for his help.

REFERENCES

1. I. Urysiak-Czubatka, M.L. Kmieć, G. Broniarczyk-Dyla, Assessment of the usefulness of dihydrotestosterone in the diagnostics of patients with androgenetic alopecia, *Postepy Dermatol. Alergol*., 2014, 31, 207-215.
2. V.A. Randall, Androgen and human hair growth, *Clin. Endocrinol (Oxf)*., 2002, 40, 439-457.
3. K.D. Kaufman, Androgens and alopecia, *Mol. Cell Endocrinol*., 2002, 198, 89-95.
4. E.A. Olsen, F.E. Dunlap, T. Funicella, J.A. Koperski, J.M. Swinehart, E.H. Tschen, A randomized clinical trial of 5% topical minoxidil versus 2% topical minoxidil and placebo in the treatment of androgenetic alopecia in men. *J. Am. Acad. Dermatol*., 2002, 47, 377-385.
5. R. Tsuboi, S. Itami, S. Inui, R. Ueki, K. Katsuoka, S. Kurata, Guidelines for the management of androgenetic alopecia, *J.*

Dermatol., 2012, 39, 113-120.
6. S. Varothai, W.F. Bergfeld, Androgenetic alopecia: an evidence-based treatment update, *Am. J. Clin. Dermatol*., 2014, 15, 217-230.
7. H. Ishida, Y. Inaoka, M. Okada, M. Fukushima, H. Fukazawa, K. Tsuji, Studies of the active substances in herbs used for hair treatment III isolation of hair-regrowth substances from *Polygara senega* var. latifolia TORR. Et GRAY., *Biol. Pharma. Bull*., 1999, 22, 1249-1250.
8. P.S. Banerjee, M. Sharma, R.K. Nema, Preparation, evaluation and hair growth stimulating activity of herbal hair oil, *J. Chem. Pharma. Res*., 2009, 1, 261-267.
9. C.S. Shah, J.S. Qudry, A text book of pharmacognosy, eleven ed., B.S. Shah Prakashan Publisher, Ahmadabad, 1996.
10. M. Semalty, A. Semalty, G.P. Joshi, M.S.M. Rawat, In vivo hair growth activity of herbal formulations, *Int. J. Pharmacol*., 2010, 6, 53-57.
11. R.K. Roy, M. Thakur, V.K. Dixit, Effect of *Citrullus colocynthis* on hair growth in albino rats, *Pharma. Biol*., 2007, 45, 739-744.
12. V.R. Gottumukkala, T. Annamalai, T. Mukhopadhyay, Phytochemical investigation and hair growth studies on the rhizomes of *Nardostachys jatamansi* DC, *Pharmacogn. Mag*. 2011, 26, 146-150.
13. K.E. Sharquie, H.K. Al-Obaidi, Onion juice (*Allium cepa* L.), a new topical treatment for alopecia areata, *J. Dermatol*., 2002, 29, 343-346.
14. N. Harada, K. Okajima, M. Arai, H. Kurihara, N. Nakagata, Administration of capsaicin and isoflavone promotes hair growth by increasing insulin-like growth factor-I production in mice and in humans with alopecia. *Growth Horm. IGF Res*., 2007, 17, 408-415.
15. V.N. Thakare, P.S. Kothavade, V.V. Dhote, A.D. Deshpande, Antifertility activity of ethanolic extract of *Allium cepa* Linn. in rats, *Int. J. PharmTech. Res*., 2009, 1, 73-78.
16. A. Kassem, A. Al-Aghbari, M. Al-Habori, M. Al-Mamary, Evaluation of the potential antifertility effect of fenugreek seeds in male and female rabbits, *Contraception*., 2006, 73, 301-306.
17. M.M. Mohammed, A.E. Mudathir, S.A.I. Shaddad, B. Elsharif, A.A.E.A. Algasem, Antifertility effects of *Trigonella foenum-graecum* (fenugreek) ethanolic extract in male rats & cocks, *J. Pharm. Biomed. Sci*., 2013, 32, 1299-1304.
18. A. Sharma, P. Sharma, M. Chaturvedi, S.C. Joshi, Contraceptive efficacy of *Citrullus colocynthis* methanolic extract in male rats, *Am. J. Phytomed. Clin. Therapeut*., 2014, 2, 229-241.
19. [19] E.M. Paragas, D. Gehle, K. Krohn, S.G. Franzblau, A.P.G. Macabeo, Anti-tubercular flavonol derivatives from *Uvaria rufa*, *Res. J. Pharm. Biol. Chem. Sci*., 2014, 5, 856-859.
20. I.O. Onoagbe, A. Esekheigbe, Studies on the anti-diabetic properties of *Uvaria chamae* in streptozotocin-induced diabetic rabbits, *Biokemistri*., 1999, 9, 79-84.
21. G.A. Asare, D. Afriyie, R.A. Ngala, H. Abutiate, D. Doku, S.A. Mohmood, H. Rahman, Antiproliferative activity of aqueous leaf extract of *Annona muricata* L. on the prostate, BPH-1 cells, and some target genes, *Integr. Cancer Ther*., 2015, 14, 65-74.
22. W. Buncharoen, K. Saenphet, S. Saenphet, C. Thitaram, *Uvaria rufa* Blume attenuates benign prostatic hyperplasia via inhibiting 5α-reductase and enhancing antioxidant status, *J. Ethnopharmacol*., 2016, 194, 483-494.
23. S. Tip-pyang, K. Payakarintarungkul, J. Sichaem, P. Phuwapraisirisan, Chemical constituents from the roots of *Uvaria rufa*, *Chem. Nat. Compd*., 2011, 47, 475-476.
24. K. Deepralard, K. Kawanishi, M. Moriyasu, T. Pengsuparp, R. Suttisi, Flavonoid glycosides from the leaves of *Uvaria rufa* with advanced glycation end-products inhibitory activity. *Thai J. Pharm. Sci*., 2009, 33, 84-90.
25. S.S. Roh, C.D. Kim, M.H. Lee, S.L. Hwang, M.J. Rang, Y.K. Yoon, The hair growth promoting effect of *Sophora flavescens* extract and its molecular regulation. *J. Dermatol. Sci*., 2002, 30, 43-49.
26. G.A. Ayoola, H.A.B. Coker, S.A. Adesegun, A.A. Adepoju-Bellol, K. Obaweyal, E.C. Ezennial, T.O. Atangbayilal, Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in Southwestern Nigeria, *Trop. J. Pharm. Res*., 2008, 7, 1019-1024.
27. H.O. Edeoga, D.E. Okwu, B.O. Mbaebie, Phytochemical constituents of some Nigerian medicinal plants. *Afr. J. Biotechnol*., 2005, 4, 685-688.
28. X.W. Chen, E.S. Serag, K.B. Sneed, S.F. Zhou, Herbal bioactivation, molecular targets and toxicity relevance, *Chem. Biol. Interact*., 2001, 192, 161-176.
29. Z.F. Dewan, I.D. Morris, R.G. Lendon, Administration of exogenous testosterone in the adult rat and its effects on reproductive organs, sex hormones and body weight, *Bangladesh Med. Res. Counc. Bull*., 2000, 26, 48-55.
30. L. Tóthová, P. Celec, D. Ostatníková, M. Okuliarová, M. Zeman, J. Hodosy, Effect of exogenous testosterone on oxidative status of the testes in adult male rats. *Andrologia*., 2013, 45, 417-423.
31. S. Prasad, N. Kalra, Y. Shukla, Modulatory effects of diallyl sulfide against testosterone-induced oxidative stress in Swiss albino mice. *Asian J. Androl*., 2006, 8, 719-723.
32. L.A.J. Shittu, M.A. Bankole, J.A. Oguntola, O. Ajala, R.K. Shittu, O.A. Ogundipe, M.N. Bankole, T. Ahmed, O.A. Ashiru, Sesame leaves intake improve and increase epididymal spermatocytes reserve in adults male Sprague Dawley rat, *Sci. Res. Essays*., 2007, 2, 319-324.
33. H. Black, Preparation of the report for a toxicology/pathology study, in: W.M. Haschek, C.G. Rousseaux, M.A. Wallig, (Eds.), Handbook of Toxicologic Pathology, first volume, Academic Press, San Diego, CA, 2002, pp. 419-433.
34. R. Mosbah, M.I. Yousef, A. Mantovani, Nicotine-induced reproductive toxicity, oxidative damage, histological changes and haematotoxicity in male rats: the protective effects of green tea extract, *Exp. Toxicol. Pathol*., 2015, 67, 253-259.
35. M.A. Rezvanfar, R.A. Sadikhanlou, A. Ahmadi, H. Shojaei-Sadee, M.A. Rezvanfar, A. Mohammadirad, A. Salehnia, M. Abdollahi, Protective of cyclophosphamide-induced toxicity in reproductive tract histology, sperm characteristics, and DNA damage by an herbal source; evidence for role of free-radical toxic stress, *Hum. Exp. Toxicol*., 2008, 27, 901-910.
36. M. Mohamed, S.A. Sulaiman, H. Jaafar, Histological changes in male accessory reproductive organs in rats exposed to cigarette smoke and the protective effect of honey supplementation, *Afr. J. Tradit. Complement. Altern. Med*., 2012, 9, 329-335.
37. A. Hentrich, M. Wolter, C. Szardening-Kirchner, G.H. Lüers, M.

Bergmann, S. Kliesch, L. Konrad, Reduced numbers of Sertoli, germ, and spermatogonial stem cells in impaired spermatogenesis, *Mod. Pathol*., 2001, 24, 1380-1389.
38. W. Liao, M. Cai, J. Chen, J. Huang, F. Liu, C. Jiang, Y. Gao, Hypobaric hypoxia causes deleterious effects on spermatogenesis in rats, *Reproduction*., 2010, 139, 1031-1038.
39. S. Chuma, M. Hosokawa, K. Kitamura, S. Kasai, M. Fujioka, M. Hiyoshi, K. Takamune, T. Noce, N. Nakatsuji, *Tdrd1/Mtr-1*, a *tudor*-related gene, is essential for male germ-cell differentiation and nuage/germinal granule formation in mice, *Proc. Natl. Acad. Sci. U.S.A*., 2006, 103, 15894-15899.
40. D.D. Seachrist, E. Johnson, C. Magee, C.M. Clay, J.K. Graham, D.N. Veeramachaneni, R.V. Keri, Overexpression of follistatin in the mouse epididymis disrupts fluid resorption and sperm transit in testicular excurrent ducts, *Biol. Reprod*., 2012, 87, 41.
41. Y. Ji, J. Sun, L. Lang, Analysis of testicular toxicity of salanine in mice, *Adv. Mater. Res*., 2010, 282-283, 195-200.
42. H. Kloer, H. Hoogen, E. Nieschlag, Trial of high-dose testosterone undecanoate in treatment of male infertility, *Int. J. Androl*., 1980, 3, 121-129.
43. A. M. Matsumoto, Is high dosage testosterone an effective male contraceptive agent?, *Fertil. Steril*., 1988, 50, 324-328.
44. J.D. Vidal, K.M. Whitney, Morphologic manifestations of testicular and epididymal toxicity, *Spermatogenesis*., 2014, 4, e979099.