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Effects of a local commercial herbal cleanser on hair growth in Wistar rats

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In this present study, we evaluated the hair growth promoting effect of a local commercial herbal cleanser which contains three Chinese herbs namely, *Camellia oleifera* Abel., Radix *Angelica sinensis* and *Polygonum multiflorum* Thunb. *In vitro* toxicology testing on the human fibroblast cell line, CCD-118Sk using the tetrazolium dye (MTT) assay showed that the herbal cleanser exhibited mild cytotoxic activity. An *in vivo* study was conducted whereby the shaved dorsal skin of male and female Wistar rats were treated daily with topical application of 10 and 20% solutions of the herbal cleanser for 30 days. Histological examination of skin biopsies taken from the treated areas at the end of the study period showed that the topical application of the herbal cleanser did not cause any apparent toxicity on the rats' skin. In fact, a significant increase in hair follicles count was observed and more anagen phase hair follicles induced as compared to the control group. These findings suggest that the herbal cleanser has hair growth promoting potential.

Key words: Herbal cleanser, hair follicles, hair growth.

INTRODUCTION

Use of herbal extracts to ameliorate dermatological problems and promote hair growth has become fairly widespread in the hair care industry. There are numerous products in the market made available as hair tonics, herbal cleansers or hair growth promoters (Saraf et al., 1991). The ingredients in these products are usually a combination of several herbs. Among the more commonly used Chinese herbs are *Camellia oleifera* Abel., Radix *Angelica sinensis* and *Polygonum multiflorum* Thunb. *C. oleifera* Abel, from the family of Theaceae, is cultivated mostly for its seeds and the seed oil serves as a raw material in the manufacturing of soap, margarine, hair oil, lubricants, paint as well as the synthesis of other high-molecular weight-compounds and rustproof oil (Ruter, 2002). The root of Radix *A. sinensis* (Oliv.) Diels or Dang Gui, a well-known oriental herb belonging to the Umbelliferae family, has been used for thousand of years in traditional Chinese medicinal prescriptions (Lao et al., 2004). In traditional Chinese medicine, Radix *A. sinensis* extract is said to improve blood circulation (Xiangcai, 2001).

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An *in vitro* study undertaken by Ryu et al. (2002) found that *P. multiflorum* Thunb has antioxidant properties. This was subsequently supported by Chiu et al. (2002) who also reported that the delayed aging response *in vivo* was believed to be mediated through the oxidative stress mechanism. Several other authors (Chen and Li, 1993; Huang, 1993; Xiangcai, 2001 and Um et al., 2006) also reported on the use of these roots as a tonic and for maintaining the blackness of hair. The other uses of the root in traditional Chinese practice include treatment for blood deficiency (term used in traditional Chinese medicine) and early greying of hair (Xiangcai, 2001). With the increasing interest and promotion on the use of herbal hair care products to treat hair loss or thinning, it is important to investigate the scientific basis for these purported claims. In this study, a herbal cleanser containing mixtures of *C. oleifera* Abel., Radix *A. sinensis* and *P. multiflorum* Thunb was investigated for its potential in promoting hair growth.

MATERIALS AND METHODS

Herbal cleanser preparation

The raw materials (herbs) were supplied by Caryn Personal Healthcare Sdn. Bhd. The herbal cleanser preparation was conducted according to the manufacturer's instructions. Briefly, the roots of Radix *A. sinenis*, *P. multiflorum* Thunb and leaves of *C. oleifera* Abel. were cleaned, ground and dried in oven. Cold extraction using dH_2O was performed on equivalent weight of the three herbs for 72 h. They were then refluxed in hot dH_2O 80°C for 5 min. The mixture constituted 67.69% of the total chemical composition of the herbal cleanser as the remaining chemicals were sodium laureth sulfate (16%), cocamide DEA (2%), polyquaternium-7 (6%), cocamidopropyl betaine (7%), methylchloroisothiazolinone and methylisothiazolinone (0.0015%), citric acid (0.01%), and sodium choride (1.3%). The pH of the herbal cleanser was tested using a pH meter and the viscosity was measured using a viscometer (Brookfield Digital Viscometer LV-E (V02).

Antiproliferation assay

Human fibroblast cell line namely CCD-1118Sk was obtained from the American Type Culture Collection. CCD-1118Sk cells were maintained in Dulbecco's modified eagle's medium supplemented with 10% heat-inactivated fetal calf serum in a humidified atmosphere of 5% CO_2 in air at 37°C. Cells were kept in the logarithmic growth phase by routine passage every 2 to 3 days using 0.025% trypsin-ethylenediaminetetraacetic acid (EDTA) treatment. The antiproliferative activity was evaluated using the MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, a tetrazole) method as previously described (Cheah et al., 2006). Cells were seeded 24 h prior to treatment in a 96-well plate at 1×10^4 cells/well in order to obtain semi-confluent cultures. Before addition to the culture media, the herbal cleanser was dissolved in water by 2× serial dilution for 8 points ranging from 100 to 0.78%. The mixtures in the 96-well plate were incubated for 72 h and at the end of incubation, 50 µl MTT solution (2 mg/ml MTT in plain culture medium) was added into each well. The plate was then further incubated for 4 h and at the end of it, the remaining MTT solution was removed and the purple formazan crystals formed at the bottom of the wells were dissolved with 200 µl dimethyl sulphoxide (DMSO) for 20 min. The absorbance at 570 nm was read on a spectrophotometric plate reader and the proportion of surviving cells was calculated (OD of drug-treated sample - OD of blank) / (OD of control - OD of blank) × 100%. Dose-response curves were constructed to obtain the EC_{50} values. All the data were derived from at least 3 independent repeated experiments.

Animal test

Three weeks-old male and female Wistar rats were housed individually and allowed to adapt to their new environment for one week, and given food and water *ad libitium*. They were subsequently divided into 3 groups (n = 4) namely, vehicle control, 10% herbal cleanser treated, and 20% herbal cleanser treated for male and female, respectively. The dorsal part (2 cm × 4 cm) of each rat was shaved with a clipper at four weeks of age in which all the hair follicles were synchronized at the telogen phase. On the following day (day 1), 2 ml of 10 or 20% solution of the herbal cleanser in water was applied topically onto the shaved area of the rats daily for 30 days. The control rats received topical application of 2 ml of dH_2O. A day after the final treatment, all the rats were sacrificed and skin biopsies from the treatment side obtained (shaved dorsal side of all the rats) and subjected for histological examination.

Histological examination

The skin biopsies were fixed in 10% buffered formalin, dehydrated in a graded ethanol series, cleared in xylene, embedded in paraffin wax and subsequently sectioned into uniform thickness of 5 µm. The sections were then stained with haematoxylin and eosin. The number of hair follicles per millimeter of the skin and the percentage ratio of different cyclic (telogen-to-anagen) of hair follicles were determined using a microscope fitted with a camera.

Statistical analysis

All data were expressed as mean ± standard deviation. The statistical differences were analyzed using one-way analysis of variance (ANOVA) followed by a Tukey honestly significantly different (HSD) test. Values of $p < 0.05$ were considered significant.

RESULTS

Effect of herbal cleanser on cell viability *in vitro*

Cytotoxicity of herbal cleanser on human skin fibroblast was determined with the EC_{50} value from dose-response curve as in Figure 1. Treatment with increasing concentrations of herbal cleanser showed moderate cytotoxicity on human fibroblast as the viability of CCD-1118Sk was reduced by half at 33.67 ± 0.26% solution of herbal cleanser after 72 h incubation.

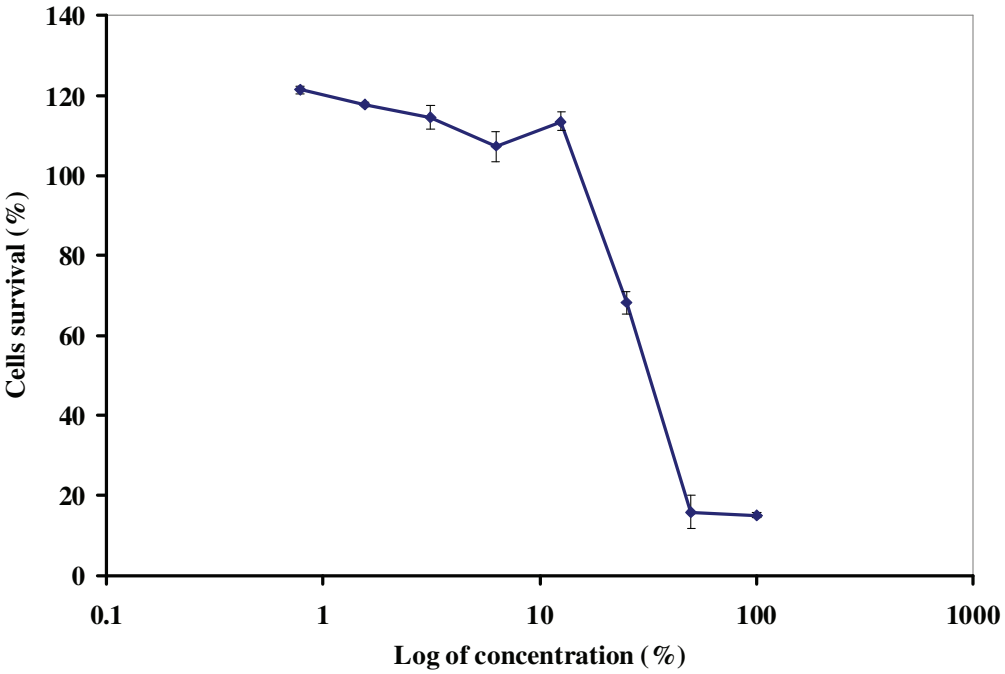

Figure 1. *In vitro* cytotoxicity of herbal cleanser on human skin fibroblast cells, CCD-1118Sk. From the graph, there is a decrease in cells survival after the treatment with high concentration of herbal cleanser. Therefore, lower concentrations of herbal cleanser were used in the *in vivo* study.

Histology of skin biopsy of the rats treated with topical application of the herbal cleanser

Our result showed that topical treatment with 10 and 20% solutions of herbal cleanser on the dorsal skin of female and male rats, respectively did not alter their normal skin histological appearance. From the images (Figure 2), it is clear that the treated rats did not exhibit any distinct erosion or inflammation of the epidermis layer of the skin section as compared to the control. The epidermis of rats treated with 10 and 20% herbal cleanser showed a stratified squamous epithelium with normal thickness comparable to the control, and the border between the epidermis and dermis was clearly demarcated. The thickness of the epidermis for both untreated and treated groups was analyzed quantitatively and is as shown in Figure 3.

Numbers of hair follicles multiply after 4 weeks treatment with herbal cleanser

Examination of the vertical sections of skin biopsies showed a significant difference ($p < 0.05$) in the number of hair follicles per mm^2 skin of the both female and male treated rats as compared to the control group (Figure 4). In female rats, the number of hair follicles increased from 2.96 ± 0.15 hair follicles per mm^2 in untreated rats to 4.52 ± 0.23 and 8.91 ± 0.49 hair follicles per mm^2 for 10% herbal cleanser treated-rats and 20% herbal cleanser treated-rats, respectively. In the treated male rats, the number of hair follicles per mm^2 was in the range of 2.84 to 10.14. It accounted for a significant increase of 1.38 and 3.57-fold, respectively in 10% herbal cleanser treated-rats and 20% herbal cleanser treated-rats as compared to the control (Figure 4).

Examination for hair follicle growth phase

From the histological sections, the increased numbers of hair follicles were clearly seen. At the 4th week, hair follicles of both female and male control rats had entered the anagen phase. This was similarly observed in the treated rats which in addition also showed the growth of many new hair plugs at the base of the old follicles (Figure 5). Both female and male treated rats also demonstrated hair plug canalization which is an indication of the growing stage of hair sheath. The hair follicles of the control female rats marked 70.45 ± 4.38% anagen hairs compared to the treated group which were 79.01 ± 7.59 and 90.05 ± 4.61% ($p < 0.05$) after induction with 10

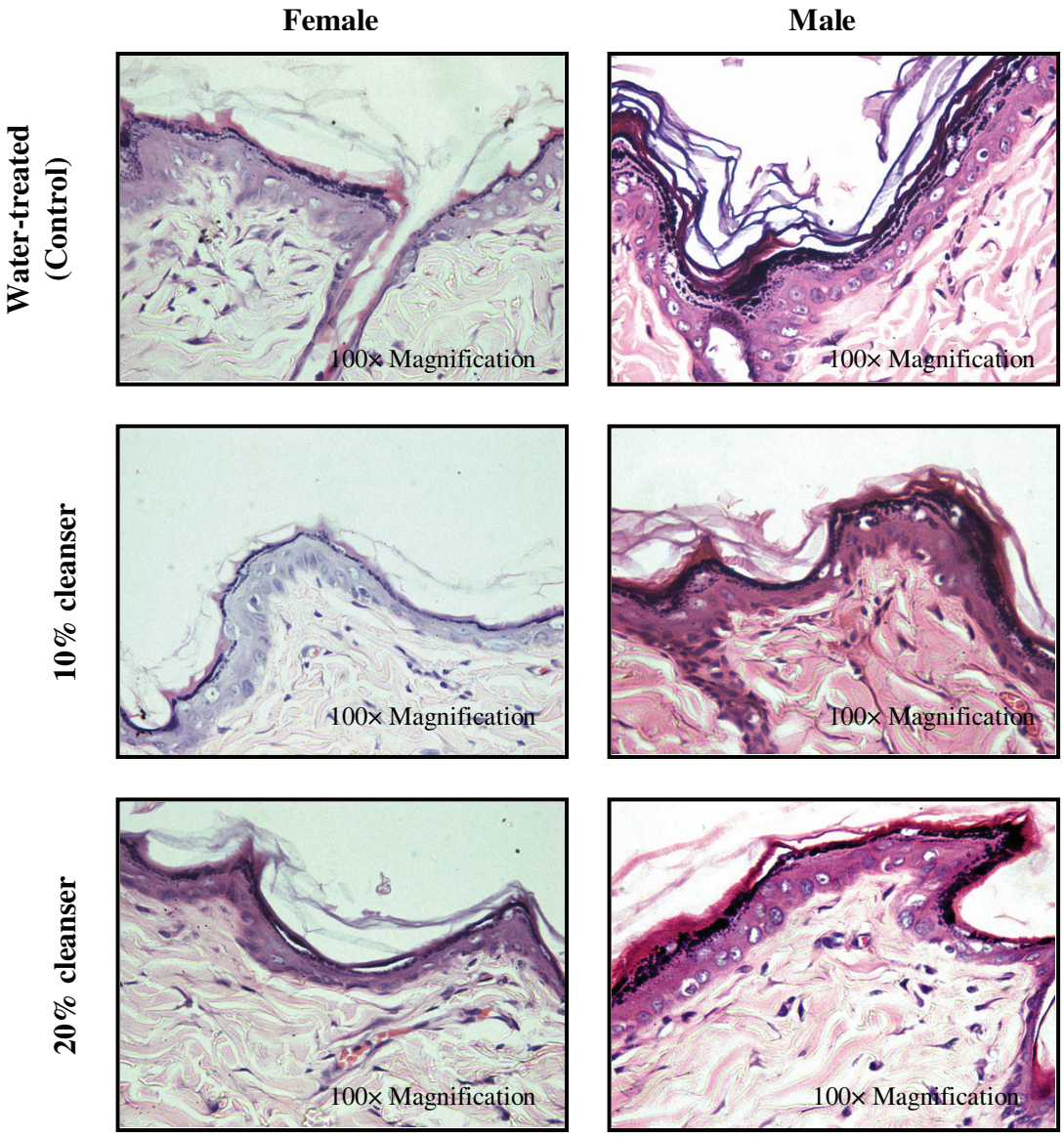

Figure 2. Histology study of skin epidermis from both female and male Wistar rats treated with graded concentration of cleanser for 1 month. No abnormal histological patterns were observed in the epidermis sections.

and 20% solutions of herbal cleanser, respectively (Figure 6a). For the male rats, the control group marked 75.97 ± 7.68% anagen hairs whereas in the groups treated with 10 and 20% herbal cleanser, the percentage of anagen hair was 81.86 ± 5.69 and 90.45 ± 4.32% ($p < 0.05$), respectively (Figure 6b).

DISCUSSION

In vitro toxicology methods are also commonly used for screening chemicals and for risk assessment of natural products preparation (Eisenbrand et al., 2002). Viability or cytotoxicity test is one of the *in vitro* methods used for the study of irritant responses of chemicals towards skin (Welss et al., 2004). There is a close agreement between human skin patch test and the *in vitro* cytotoxicity endpoints assay using human skin fibroblast. Therefore, *in vitro* cytotoxicity tests on cultured human skin fibroblasts can be used to predict skin irritation (Lee et al., 2000). Our cytotoxicity results showed that the herbal cleanser had a moderate cytotoxic effect on the human

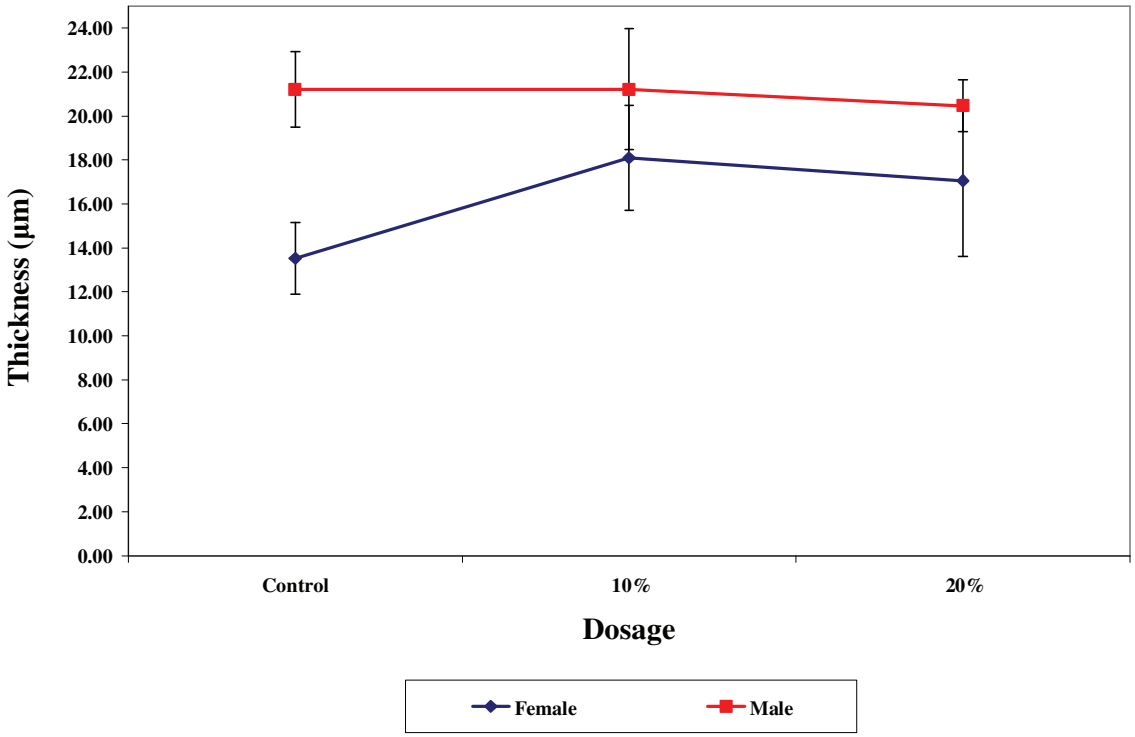

Figure 3. Thickness of stratified squamous epithelium after treatment with herbal cleanser 10% and 20%. According to the measurements, treatment of herbal cleanser did not induce thickness variations in treatment groups as compared to control.

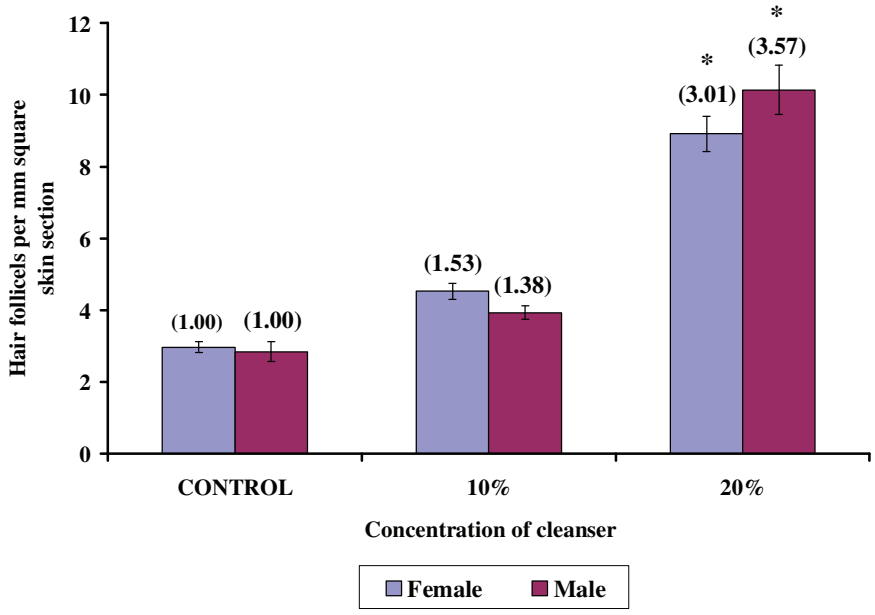

Figure 4. Number of follicles (per mm^2) in skin sections of Wistar rats treated with 10 and 20% cleanser as compared to control (water-treated). Number of follicles were found increased in both treatment groups as compared to control.

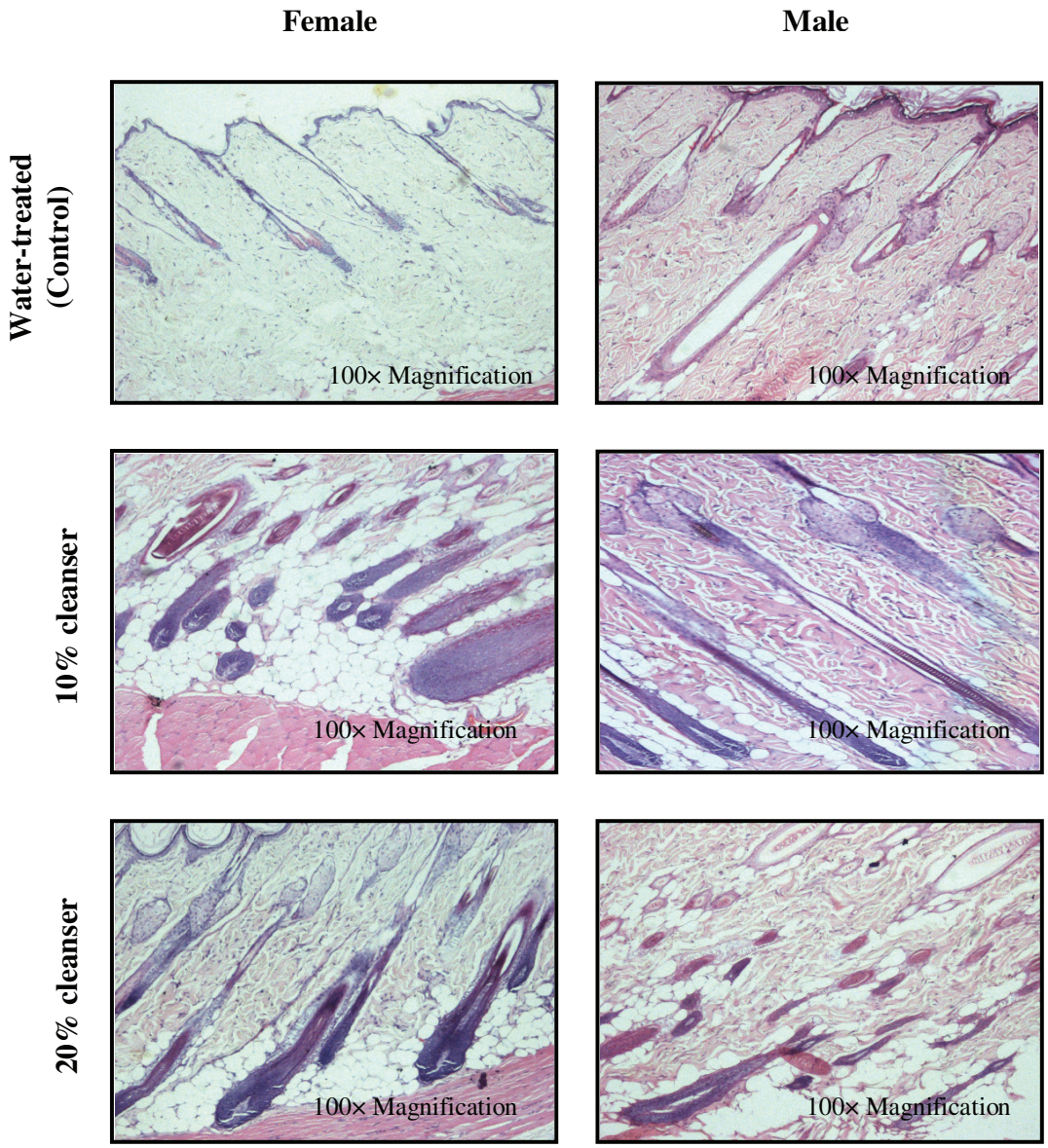

Figure 5. Histology study of skin sections from both female and male Wistar rats treated with graded concentration of cleanser for 1 month. No apparent histological abnormalities were observed.

fibloblast cell line CCD-1118Sk at 34% concentration level. Based on this finding, lower concentrations of 10 and 20% solutions of the herbal cleanser were used for topical application in the *in vivo* study.

After 30 days of treatment, skin biopsies were taken from the control and herbal cleanser-treated rats. They were first observed for potential inducing inflammation. Thickened epidermis is a common histological feature observed in inflammatory skin disease (Wakita et al., 2003; Lopez et al., 2004). Our experimental data showed that the thickness of the epidermis in treatment groups was not significantly different ($p > 0.05$) from the control groups for both female and male rats. In addition, skin erosion was not found as the early denuded area of the dorsal part of the treated rats was observed to have undergone full hair regrowth at the end of the experiments (data not shown). These data suggest that herbal cleanser treatment at 10 and 20% concentrations does not induce apparent skin toxicity.

In Wistar rats, the normal hair follicles undergo a cyclic process of development namely growth (anagen), regression (catagen) and rest (telogen). Histological

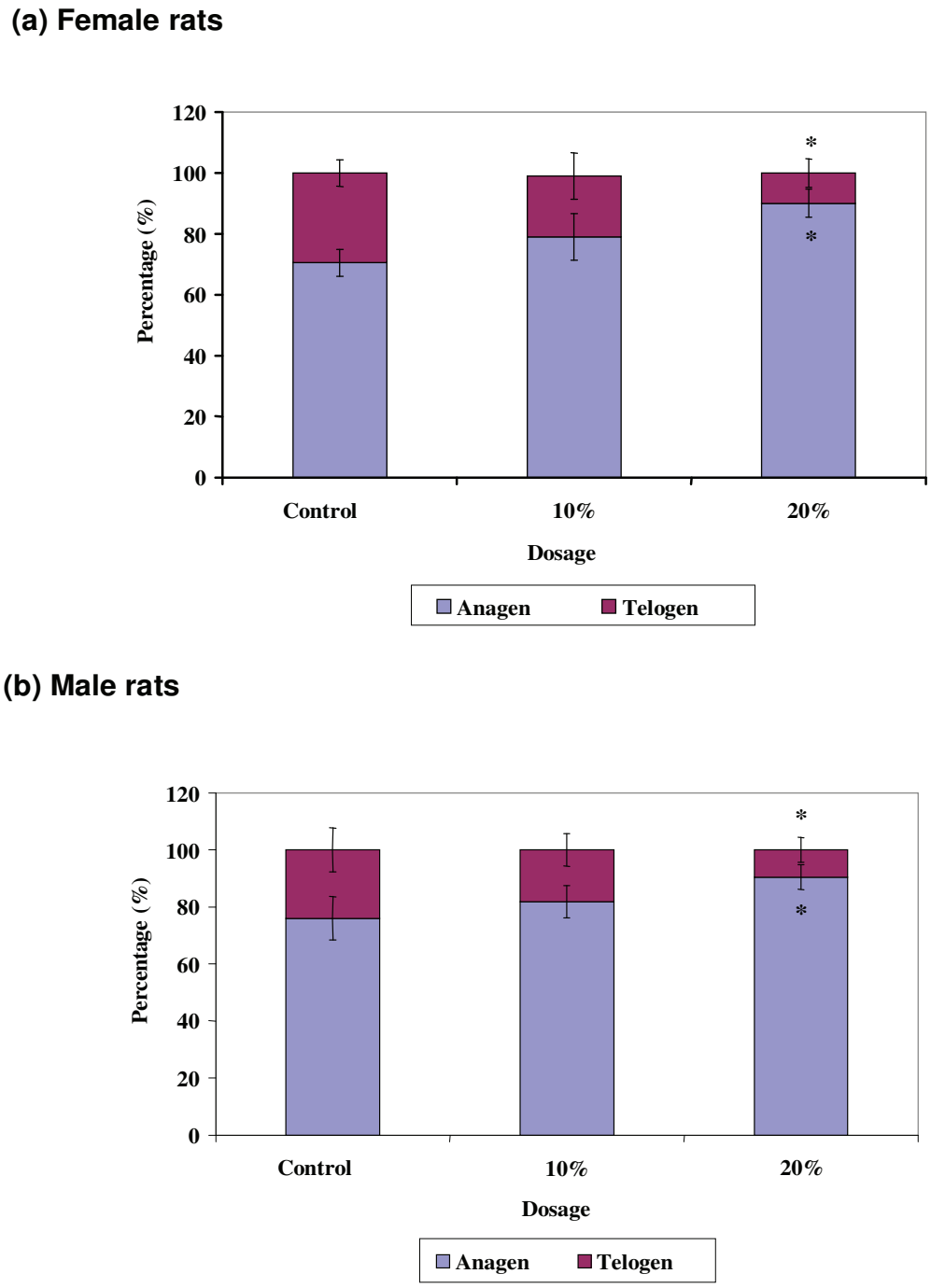

Figure 6. Anagen phase hair follicles induction after treatment with 10% and 20% cleanser as compared to control (dH_2O-treated).

observation had reported a period from 1 to 14 days that corresponded to follicular neogenesis (1st anagen) after which the hair follicles will enter the catagen-telogen phase at around 21 days. The second anagen will begin from about 4 weeks while the 2nd telogen phase will be at 10 weeks (Ishii et al., 1997). In the present study, we found that the number of follicles increased prominently in both herbal cleanser-treated female and male Wistar

rats. Our results showed that the increase in hair follicle counts was in correlation with the larger number of anagen hairs after treatment with herbal cleanser; indicating transition of telogen to anagen phase and the elevation of new anagen phase hairs. Similar effect was noted by Rho et al. (2005) where *Radix angelica* extract exerted hair growth promoting effect by inducing expression of vascular endothelial growth factor (VEGF) which may be involved in the induction of earlier telogen-to-anagen conversion. In addition to the above, our study showed that most of the hair plugs were undergoing canalization as indicated by the differentiation of hair matrix cells into hair and inner root sheaths. These are healthy validations of hair growth potential of herbal cleanser as reported in other study (Roy et al., 2007).

The findings from our experiments on Wistar rats showed that at doses where there is no apparent toxic effect on the skin (10 and 20% solutions of the herbal cleanser), the herbal cleanser was shown to promote hair growth *in vivo*. Further investigations to determine the mechanism of action and ability of this herbal cleanser to induce hair growth are needed and with that information, its potential in treating hair growth problems such as alopecia can be explored.

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