The Efficacy of HDDPiW-jSB Solution on Docetaxel-Induced Alopecia of Rats

Bilgin Demir (bilgin287@hotmail.com)
Adnan Menderes University
Buket Demirci
Adnan Menderes University
Canten Tataroglu
Adnan Menderes University
Sabri Barutca
Adnan Menderes University
Duygu Barutca
İzmir University of Economics

Research Article

Keywords: Chemotherapy induced alopecia, Docetaxel, Hair loss, Oncology, Animal

Posted Date: May 24th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-2959814/v1

Abstract

Objective: Chemotherapy induced alopecia (CIA) is one of the most common side effects in cancer patients, however; it doesn't have an effective pharmacological treatment yet. In this study we aimed to research the protective effect of HDDPiW-jSB solution on docetaxel (DTX) -induced rat alopecia model.

Material and Methods: Docetaxel (10 mg/kg/week) was administered to the 6-8 months old rats for three weeks. HDDPiW-jSB solution was applied once or twice a week for 4 weeks beginning prior to one week before DTX. Rat hair follicles were evaluated with hematoxylin-eosin and immune-histochemical staining.

Results: In the first stage of this study, alopecia was successfully developed by DTX application. In the second stage, application of HDDPiW-jSB solution without DTX, didn't change the study parameters significantly. In DTX-induced alopecic rat groups' skin samples, the solution ameliorated the anagen hair follicle count and Bcl-2 values, especially when used as twice a week. Additionally, level of Caspase 3 was decreased. HDDPiW-jSB solution was safe when applied on the skin.

Conclusion: Topical HDDPiW-jSB solution could be effective and safe for the protection of DTX-induced alopecia in rat models.

Introduction

Alopecia is the partial or total loss of scalp hair that can be seen due to genetic factors as well as in many clinical conditions such as, endocrinologic, immunologic-rheumatologic and oncologic diseases and drug treatments (1,2). Alopecia is also one of the most common side effects of cancer treatments. Chemotherapy induced alopecia (CIA) can be seen in approximately 65% of the patients and it affects all body hair such as the eyelashes and eyebrows besides the scalp. It has been frequently reported after the use of common chemotherapy (CT) agents (busulfan, cyclophosphamide, thiotepa, melphalan, etoposide, carboplatin, docetaxel, and paclitaxel) as well as other cancer treatment modalities (2,3). Alopecia has a negative impact on physical appearance, body perception and self-esteem though it is usually reversible. Sometimes alopecia is described as the most traumatizing and painful experience, especially by women with cancer (4,5).

Docetaxel (DTX) is a member of taxane family, microtubule antagonist CT agents widely used in oncology practice (6). It has been reported that patients who had received taxane-based treatment regimens had more likely experienced CIA (3).

HDDPiW-jSB is a vitamin-based product, developed to protect the patient from CIA. It was experienced clinically by the two authors of this study on themselves before. One author used the solution while under DTX and cabazitaxel-induced alopecia for prostate cancer with complete success and the other under the treatment of combination CT regimen of epirubicin plus cyclophosphamide followed by trastuzumab plus weekly paclitaxel for breast cancer with partial success on scalp, with complete protection of eyebrows and eyelashes. This encouraged the authors for the presented study.

Up to now, the best and only FDA accepted treatment is scalp hypothermia to reduce and/or prevent CIA, unfortunately, there is no effective pharmacological treatment option (3,7). Therefore, we aimed to study the efficacy of HDDPiW-jSB solution on DTX-induced rat alopecia model.

Material and Methods

Animals and Experimental Design

Wistar rats, 6-8 months old albino male, were obtained from Animal Care and Research Unit of University and all experiments were performed according to the principles and guidelines of Animal Ethical Committee's approval (HADYEK 64583101/2020/075). A total number of 44 rats were used in this study.

The study was conducted in two stages. In the first stage, the application dose of DTX, which was chosen in accordance with the purpose of the study, to cause hair follicle loss without causing lethal toxicity was determined. While establishing the experimental model, we aimed to mimic the clinical condition of DTX (Doxitax® sol, Kocak Farma, Istanbul, Turkey) treatment, well known for the side effect on hair growth. Dykes et al. stated that maximum tolerable dosage of DTX was 33mg/kg and lethal dosage was 50 mg/kg on mice (8). These investigators administered DTX once weekly to the rats for three times. Another research that was performed by Bissery et al. showed that non-lethal maximum dose of DTX was 32.2 mg/kg (9). In another study, DTX produced oxidative stress at 10 mg/kg dosages (10). Based on these previous studies, we have planned the first stage of experimental study groups as 10 mg/kg (low dose) and 30 mg/kg (high dose) weekly DTX, for three weeks, to assess the hair tissue response.

Taking this data as the basis, three groups were created for the first stage.

- Control Group (n=6): Serum physiologic (%09) was administered intra-peritoneal (IP) to the rats in this group once a week for three weeks, it was planned to sacrifice the animals at the 22^{nd}
- DTX10 Group (n=8): DTX 10 mg/kg was administered IP to the rats in this group once a week (days 0, 8 and 15) of the study; it was planned to sacrifice the animals at the 22^{nd}
- DTX30 Group (n=8): DTX 30 mg/kg was administered IP to the rats in this group once a week (days 0, 8 and 15) of the study; it was planned to sacrifice the animals at the 22^{nd}

Using the findings obtained in the first stage of the study, the second stage was started. At this stage, three groups were created with the aim of determining the effectiveness and safety of the solution to be applied experimentally. Considering the results obtained in the first stage, the application dose for DTX was taken as 10 mg.

On the second stage, HDDPiW-jSB solution was applied on the one cm^2 interscapular region of hairy area, starting one week before the administration of DTX in all groups. HDDPiW-jSB is a vitamin-based product (a mixture of multi-vitamin in specifically adjusted concentrations, developed by the authors S. & D. Barutca, depending on their clinical and patient experiences), developed to protect the patient from CIA.

Each rat was individually caged to avoid licking of the solution. The second stage of experimental groups was as follows:

- HDDPiW-jSB Group (n=8): The solution was applied to the previously described dorsal area of the rats (no-DTX administration), twice a week (on Mondays and Thursdays), for four weeks, to watch the safety of the solution. It was planned to sacrifice the animals at the 28th
- DTX10 + HDDPiW-jSB I Group (n=7): The solution was applied once a week (on Mondays), for four weeks (days 0, 8,15 and 21); meanwhile IP DTX 10 mg/kg was administered on days 8, 15, 21 of the study; it was planned to sacrifice the animals at the 28th
- DTX10 + HDDPiW-jSB II Group (n=7): The solution was applied twice a week (on Mondays and Thursdays), for four weeks; meanwhile IP DTX 10 mg/kg was administered on days 8, 15, 21 of the study; it was planned to sacrifice the animals at the 28th

Ketamine and Xylasine (50 mg/kg and 5 mg/kg, respectively) were used for the scarification process.

Immuno-Histopathological Staining

The hair skin samples were fixed in 10% neutral-buffered formalin solution (Chempur, Poland), treated with graded alcohol, xylol series, and blocked-in paraffin (Histowax, Histo-Lab. Ltd, Goteborg, Sweden). Obtained 4 µm sections of skin from paraffin blocks were stained according to the hematoxylin-eosin (H&E) method. These sections were evaluated under the microscope and images were obtained (Olympus Stream Start BX 51, Olympus SP 350 digital camera, Japan).

On the first stage of study, the dose response of 10 mg/kg and 30 mg/kg DTX on hair follicles were evaluated. Then, the study was continued with 10 mg/kg DTX in order to reveal and interpret the possible protective effects of HDDPiW-jSB solution. Tissues were stained with H&E and follicles counted as anagen, catagen, telogen and terminal hair follicles in five areas (x40) of each sample. Next, immuno-histopathological staining of CD34 (Clone QBEnd10, Dako, Denmark), Bcl-2 oncoprotein (b cell leukemia protein-2) (Clone 124, Dako, Denmark), apoptosis-related cysteine protease (caspase 3) (mouse monoclonal antibody, Vision Biosystems Novocastra, Newcastle, United Kingdom) were performed in three areas of each sample (x400). Olympus Stream Start BX51 microscope and Image J software were used for the analyses.

Statistical Analyses

Statistical analyses were performed using the NCSS 2007 program (Number Cruncher Statistical System, Kaysville, Utah, USA). Data are expressed with the descriptive statistics (mean, standart deviation, minimum value, maximum value, median, quartiles, frequency, percent). Shapiro-Wilk test and graphical evaluation were performed to assess the normality of quantitative data. If the variances were heterogeneous, between the groups Mann-Whitney U test; amongst the group Kruskal-Wallis test and Dunn-Bonferroni test were used to evaluate for significance. Fisher-Freeman-Halton exact test was used for qualitative data. In all cases, $p < 0.05$ was considered as significant.

Results

We started with two different dosages of DTX at the first stage of this study. In the DTX30 group, 3 out of 8 rats had died at the end of the second week due to toxicity. In the DTX10 group we could successfully produced alopecia and none of the rats had died. So, the DTX30 group was excluded from the study and 10 mg/kg of DTX was selected for the second stage.

Hair follicle counts and immuno-histochemical findings of the study groups are summarized in Table 1. Anagen hair follicle counts of DTX10 group was dramatically dropped to zero while catagen hair follicles were increased; increment of caspase 3 and decrement of Bcl-2 were significant as compared to the control group (Table 2, Figures 1 & 2). The difference of CD34 values were not significant in-between the study groups.

Application of HDDPiW-jSB solutions along with DTX10, significantly ameliorated the anagen hair follicle counts (Table 3). In the DTX10+HDDPiW-jSB II group, Bcl-2 values as an anti-apoptotic marker in skin samples were significantly higher as compared to both HDDPiW-jSB and DTX10+HDDPiW-jSB I groups (Table 3). Additionally, Bcl-2 staining of the follicular epithelium was higher in the DTX10+HDDPiW-jSB II group (Figure 3). Also, the caspase 3 values as an apoptotic enzyme activity marker were lower in all solution groups as compared to the DTX10 group (Table 3).

When all groups are considered, we determined that catagen hair follicle numbers were the highest in DTX10 group (Figure-3). In contrary, in the HDDPiW-jSB solution groups catagen hair follicle numbers were decreased.

Interestingly, aforementioned values (hair follicle counts and Bcl-2, caspase 3, and CD34 values) of the HDDPiW-jSB Group (without DTX) were not statistically different from the Control Group ($p \geq 0{,}05$). Also, no skin toxicity was observed secondary to HDDPiW-jSB solution in our study.

Discussion

It was stated that rat hair follicles could be a suitable model for human hair tissue studies (11). In our study of rat experimental alopecia model, weekly 10 mg/kg DTX for three weeks successfully achieved alopecia.

Hair follicles are composed of epithelial and mesenchymal tissues, which evolve from the stem cells. The key stages of hair follicles are anagen, catagen, telogen and, exogen phases. Normally, the majority (90%) of the hair follicles are in anagen phase (growth), 1–2% in catagen phase (regression), and 8–9% are in telogen phase (resting, no mitotic activity, quiescence) (1).

Under the influence of CT agents, massive apoptosis occurs in the proximal bulb and the percentage of hair follicles in the anagen phase decrease (3). These hair follicles transform to dystrophic catagen phase instantly, hence; CIA known as anagen effluvium (12).

The acute toxicity of DTX to skin and skin annexes have been described in previous clinical trials (13). Alopecia was reported in 74.2% of 965 breast cancer (BC) patients treated with DTX (14). In a previous study on BC patients (n = 744) under TAC CT regimen (DTX plus doxorubicin and cyclophosphamide), alopecia incidence (all grades) was 97.8%. Nabholtz et al. described, for the first time, four patients with persistent alopecia (> 2 years) following TAC regimen for metastatic BC (15). Similar to previous papers, DTX administration decreased the numbers of anagen hair follicles and increased the catagen follicles in the first stage of our study.

The role of nutrition and diet in the treatment of alopecia represents a dynamic and growing field of research. In many studies, vitamins and minerals such as vitamin A, vitamin B, vitamin C, vitamin D, vitamin E, iron, selenium and zinc have been investigated for the treatment of alopecia (16). Studies with topical calcitriol have produced conflicting data on the prevention of alopecia. Although no benefit was shown in a Phase I study involving 12 patients who were treated with anthracycline and cyclophosphamide CT regimen for BC, it was found to be reliable in another phase 1 study (17). In another animal experiment, it was shown that α-lipoic acid applied in addition to cooling treatment improved CIA in a cyclophosphamide-induced alopecia model. (18). In conclusion, vitamin based treatments have not been fully effective in the protection of CIA in previous studies. However; in the second stage of this study, topical application of vitamin-based HDDPiW-jSB solution decreased the transformation of hair follicles into the catagen phase.

It has been reported that morphogenesis and cycle of hair follicles are strictly controlled and characterized by proliferation, differentiation and apoptosis. Bcl-2, a well-known inhibitor of apoptosis, is expressed in hair follicles and facilitates their growth. Decreased Bcl-2 levels were reported to result with selective loss of stem cells and disruption of hair follicle regeneration (19). In our study HDDPiW-jSB solution was able to increase the Bcl-2 levels significantly, especially when it was applied twice a week along with DTX.

At the start of each new hair cycle, a cluster of stem cells at the base of the bulge become activated to proliferate (20). The presence of perifollicular stem cells was evaluated by CD34 staining and there was no significant difference in-between our study groups. Hence the increment of Bcl-2 was not enough to trigger stem cells and this result suggested that the HDDPiW-jSB solution was only protective.

Caspase 3 is a highly expressed apoptosis mediator both in human and rat hair follicles and it was suggested that apoptosis inhibitors could be used for the treatment of CIA (21, 22). In our study DTX has significantly increased Caspase 3 levels while HDDPiW-jSB solution was able to decrease the Caspase 3 levels significantly, along with DTX.

Conclusion

Chemotherapy induced alopecia is one of the most common side effects in cancer patients, however; it doesn't have an effective pharmacological treatment yet. The results of our study suggested that HDDPiW-jSB solution was safe in rats and protected the hair follicles by inhibiting apoptosis under DTX

treatment. It could prevent DTX induced transition of the hair follicles from anagen to catagen phase. Also the solution showed no significant effect without CT, so it was only protective. HDDPiW-jSB may be useful for the prevention CIA secondary to other CT agents.

Declarations

Declaration of Conflicting Interest

Author declares no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding: None

Approved by the following research ethics committee: Aydin Adnan Menderes University for Animal Experiments (HADYEK 64583101/2020/075).

Financial disclosure: None

Availability of Data and Material

The data sets and data analyzed in the study are available from the corresponding author on reasonable request.

Authors Contributions:

All authors have made substantial contributions to conception, design, acquisition of data; or analysis and interpretation of data; or have been involved in drafting the manuscript or revising it critically for important intellectual content.

ORCID ID

Bilgin Demir: 0000-0003-4380-9419, Buket Demirci: 0000-0002-3442-5061, Canten Tataroglu: 0000-0001-8063-4692, Sabri Barutca: 0000-0002-1941-053, Duygu Barutca: 0009-0004-5925-9268

References

1. Orăsan MS, Coneac A. Experimental Animal Models of Human Diseases - An Effective Therapeutic Strategy, Evaluation of Animal Models Suitable for Hair Research and Regeneration. British Library Cataloguing in Publication,2018. http://dx.doi.org/10.5772/intechopen.69698
2. Demir B, Barutca S. Cancer Treatments and Alopecia: A Systematic Review. Turkiye Klinikleri J Intern Med 2021; 6: 96–104.
3. Kang D, Kim IR, Choi EK, Im YH, Park YH, Ahn JS, Lee JE, Nam SJ, Lee HK, Park JH, Lee DY, Lacouture ME, Guallar E, Cho J. Permanent Chemotherapy-Induced Alopecia in Patients with Breast Cancer: A 3-

Year Prospective Cohort Study. Oncologist 2019; 24: 414–420.

4. Hansen HP. Hair loss induced by chemotherapy: An anthropological study of women, cancer and rehabilitation. Anthropol Med. 2007; 14: 15–26.
5. Kim IR, Cho J, Choi EK, Kwon IG, Sung YH, Lee JE, Nam SJ, Yang JH. Perception, attitudes, preparedness and experience of chemotherapy-induced alopecia among breast cancer patients: a qualitative study. Asian Pac J Cancer Prev. 2012; 13: 1383–1388.
6. Baker J, Ajani J, Scotté F, Winther D, Martin M, Aapro MS, von Minckwitz G. Docetaxel-related side effects and their management. Eur J Oncol Nurs 2009; 13: 49–59.
7. Breed WPM, Van Den Hurk CJG, Peerbooms M. Presentation, impact and prevention of chemotherapy-induced hair loss: Scalp cooling potentials and limitations. Expert Rev Dermatol. 2011; 6: 109–125.
8. Dykes DJ, Bissery MC, Harrison SD Jr, Waud WR. Response of human tumor xenografts in athymic nude mice to docetaxel (RP 56976, Taxotere). Invest New Drugs. 1995; 13: 1–11.
9. Bissery MC, Nohynek G, Sanderink G, Lavelle F. Docetaxel (Taxotere): a review of preclinical and clinical experience. Part I: preclinical experience. Anti-Cancer Drugs 1995; 6: 339–368.
10. Pieniążek A, Czepas J, Piasecka-Zelga J, Gwoździński K, Koceva-Chyła A. Oxidative stress induced in rat liver by anticancer drugs doxorubicin, paclitaxel and docetaxel. Advances in Medical Sciences 2013; 58: 104–111.
11. Müller-Röver S, Handjiski B, van der Veen C, Eichmüller S, Foitzik K, McKay IA, Stenn KS, Paus R. A Comprehensive Guide for the Accurate Classication of Murine Hair Follicles in Distinct Hair Cycle Stages. J Invest Dermatol 2001; 117: 3–15.
12. Paus R, Haslam IS, Sharov AA, Botchkarev VA. Pathobiology of chemotherapy-induced hair loss. Lancet Oncol. 2013; 14: 50–59.
13. Minisini AM, Tosti A, Sobrero AF, et al. Taxane-induced nail changes: incidence, clinical presentation and outcome. Ann Oncol. 2003; 14: 333–337.
14. Calhoun EA, Welshman E, Brown S. Taxane-induced alopecia: a historical review of patients with ovarian or breast cancer treated with paclitaxel, docetaxel or paclitaxel poliglumex. J Clin Oncol. 2005; 23: 8044–8044.
15. Nabholtz JM, Mackey JR, Smylie M. Phase II study of docetaxel, doxorubicin, and cyclophosphamide as first-line chemotherapy for metastatic breast cancer. J Clin Oncol. 2001; 19: 314–321.
16. Almohanna HM, Ahmed AA, Tsatalis JP, Tosti A. The Role of Vitamins and Minerals in Hair Loss: A Review. Dermatol Ther (Heidelb). 2019; 9: 51–70.
17. Hidalgo M, Rinaldi D, Medina G, Griffin T, Turner J, Von Hoff DD. A phase I trial of topical topitriol (calcitriol, 1,25-dihydroxyvitamin D3) to prevent chemotherapy-induced alopecia. Anticancer Drugs. 1999; 10: 393–395.
18. Aiba T, Kono Y, Etoh T, Kawano Y, Oshima Y, Inomata M. Efficacy of cooling therapy and α-lipoic acid derivative against chemotherapy-induced alopecia in an animal model. Cancer Sci. 2023; 114: 1007–

1014.

19. Geueke A, Mantellato G, Kuester F, Schettina P, Nelles M, Seeger JM, Kashkar H, Niemann C. The anti-apoptotic Bcl-2 protein regulates hair follicle stem cell function. EMBO Rep. 2021; 22: e52301.
20. Fuchs E. Skin stem cells: Rising to the surface. J Cell Biol. 2008; 180: 273–284.
21. Botchkareva NV, Ahluwalia G, Shander D. Apoptosis in the hair follicle. J Investig Dermatol. 2006; 126: 258–264.
22. Li J, Yuan J. Caspases in apoptosis and beyond. Oncogene. 2008; 27: 6194–6206.

Tables

Table 1. Hair follicle counts and immuno-histochemical findings of all study groups (X40 magnification)

	Control Group (n=6)	DTX10 Group (n=8)	HDDPiW-jSB Group (n=8)	DTX10 + HDDPiW-jSB I Group (n=7)	DTX10 + HDDPiW-jSB II Group (n=7)
Anagen hair follicle number	127.50 (83-150)	0 (0-0)	17.00 (0-70)	4.00 (0-55)	3.00 (0-15)
Catagen hair follicle number	6.00 (1-11)	34.50 (30-50)	1.50 (1-12)	12.00 (7-20)	8.00 (5-12)
Telogen hair follicle number	8.50 (7-10)	1.00 (1-3)	7.50 (4-11)	0 (0-1)	0 (0-1)
Terminal hair follicle number	139.50 (90-157)	0 (0-1)	70.00 (37-131)	0 (0-1)	0 (0-1)
CD34 (%)	30 (0-75)	1.5 (0-25)	35 (0-75)	2 (0-25)	25 (0-75)
Bcl-2 (%)	1 (0-1)	0 (0-0)	1 (1-2)	0 (0-0)	1 (1-2)
Caspase 3 (%)	0 (0-1)	2,5 (1-5)	0 (0-1)	1 (0-1)	0 (0-1)

All results were shown as median (minimum-maximum). DTX: Docetaxel

Table 2. Statistical comparison of hair follicle phases and immuno-histochemical findings of the groups in the first stage of the study

	Control Group (n=6)	**DTX10 Group** (n=8)	**P Value**
Anagen Hair	113.33±50.25	0±0	0.001
Follicles	127.50 (83-150)	0 (0-0)	
Catagen Hair	6.67±6.09	41.75±18.80	0.002
Follicles	6.00 (1-11)	34.50 (30-50)	
Telogen Hair	8.83±3.66	1.38±0.74	0.001
Follicles	8.50 (7-10)	1.00 (1-3)	
Terminal Hair	121.00±52.28	0.25±0.46	0.001
Follicles	139.50 (90-157)	0 (0-1)	
Bcl-2Value (%)	0.83±0.75	0±0	0.009
	1.00 (0-1)	0 (0-0)	
Caspase 3 Value (%)	0.33±0.52	2.75±1.75	0.005
	0 (0-1)	2.5(1-5)	

Results were shown as both mean±standart derivation and median (minimum-maximum). DTX: Docetaxel

Table 3. Statistical comparison of hair follicle phases and immuno-histochemical findings of DTX10 group of the first stage with the solution groups of the second stage of the study.

	DTX 10 Group (n=8)	DTX10+HDDPiW-jSB I Group (n=7)	DTX10+HDDPiW-jSB II Group (n=7)	P Value
Anagen Hair	0±0	22.29±29.42	7.00±9.45	0.022
Follicles	0 (0-0)	4.00 (0-55)	3.00 (0-15)	
Catagen Hair	41.75±18.80	13.86±7.65	9.00±5.29	0.001
Follicles	34.50(30-50)	12.00 (7-20)	8.00 (5-12)	
Telogen Hair	1.38±0.74	0.57±0.79	0.29±0.49	0.016
Follicles	1.00 (1-3)	0 (0-1)	0 (0-1)	
Terminal Hair Follicles	0.25±0.46	0.29±0.49	0.29±0.49	0.984
	0 (0-1)	0 (0-1)	0 (0-1)	
Bcl-2 Value (%)	0±0	0±0	1.43±0.53	0.001
	0 (0-0)	0 (0-0)	1.00 (1-2)	
Caspase 3 Value (%)	2.75±1.75	0.71±0.49	0.57±0.79	0.009
	2.50(1-5)	1.00 (0-1)	0 (0-1)	

Results were shown as both mean±standart derivation and median (minimum-maximum). DTX: Docetaxel

Figures

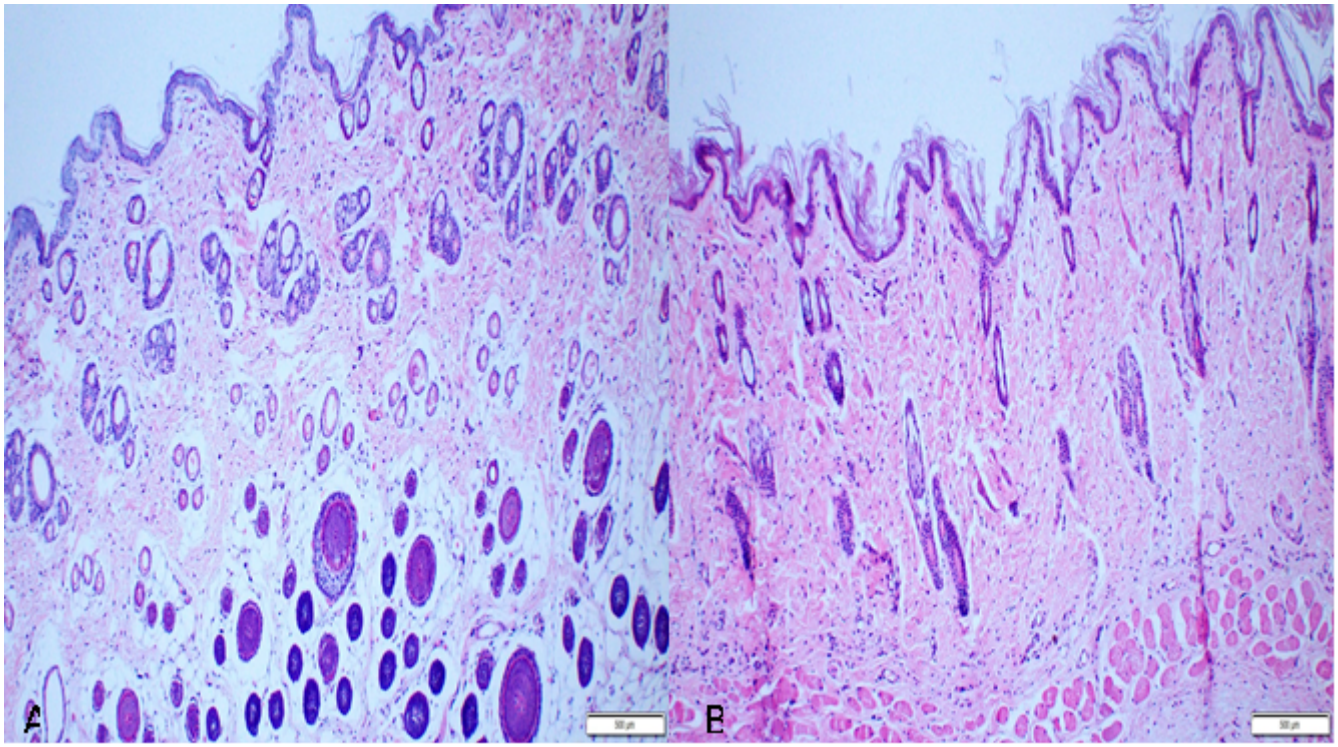

Figure 1

Hematoxylin-eosinstaining of hair follicles. **A:** Control group (X100), **B:** DTX 10 group (X100)

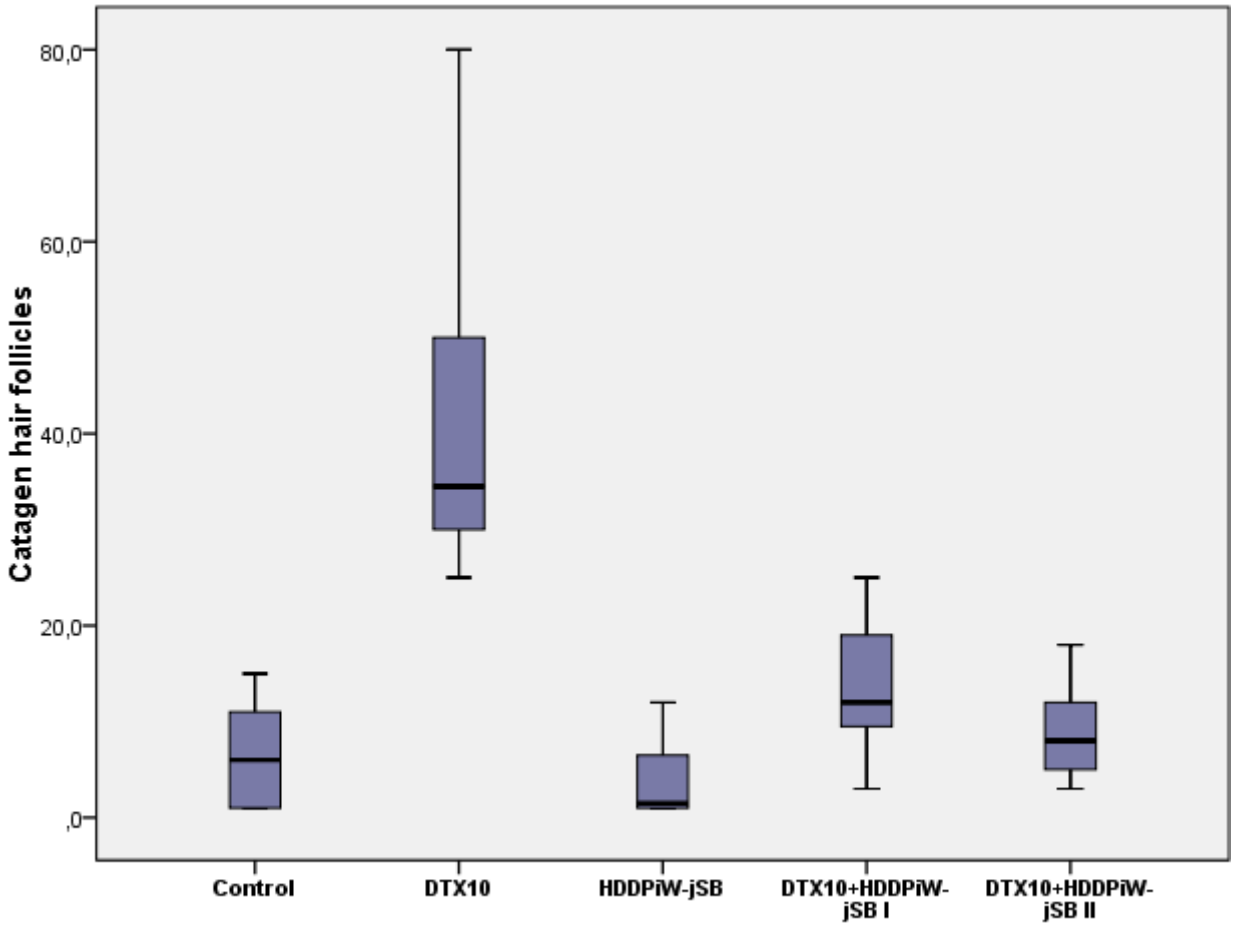

Figure 2

Box-plot graphics of catagen hair follicle numbers in the study groups.

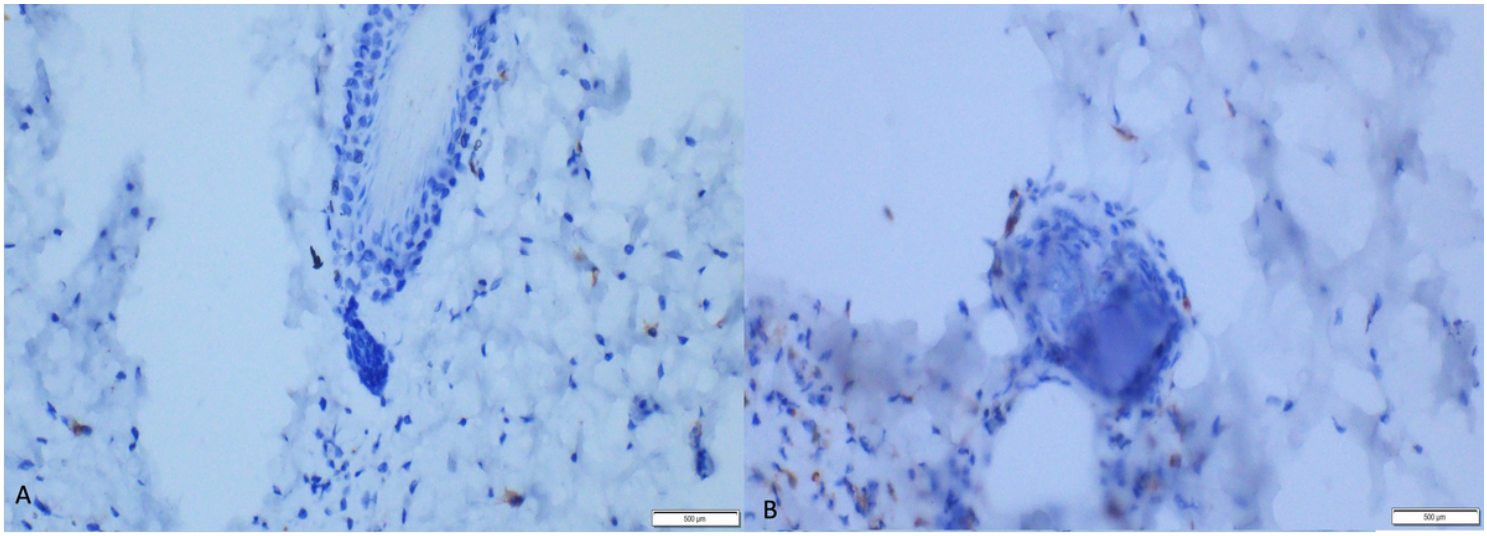

Figure 3

Bcl-2 staining of follicular epithelium. A: DTX 10 group (X400), B: DTX10 + HDDPiW-jSB II group (X400)