



HISTOLOGICAL FINDINGS ON FORMULATION AND STABILITY OF AA-21 IN ALOPECIA ON CHEMOTHERAPY INDUCED ALOPECIA MODEL OF RAT.

Shumaila Shaikh^{1*}, Sadaf Ibrahim², Mirza Tasawer Baig³, Aneela Atta Ur Rahman⁴

^{1*}Department of Pharmacology, Bibi Aseefa Dental College, larkana, Sindh Pakistan.

¹Department of Operative Dentistry, Bibi Aseefa Dental College, larkana, Sindh, Pakistan.

²Department of Pharmacology, Faculty of Pharmacy, Ziauddin University, Karachi, Pakistan.

³Department of Pharmacy Practice, Faculty of Pharmacy, Ziauddin University, Karachi, Pakistan.

⁴Department of Community Medicine and Public Health Sciences at LUMHS, Jamshoro, Sindh, Pakistan.

***Corresponding author:** Dr. Shumaila Shaikh

*Department of Pharmacology and Operative Dentistry, Bibi Aseefa Dental College, larkana, Sindh Pakistan. Shaikhshumaila489@gmail.com

Abstract:

Introduction: Hair is the human body's major esthetic display part, especially in cultural and social interactions. Hair serves many vital functions which includes defense from contaminants and sweat-gland dispersion substances e.g. pheromones. Alopecia is a common term for loss of hair, the hair matrix comprised (inside and outside) medulla, cortex and hair cuticle. The inner root sheath composed of the cuticle of the inner root sheath, the sheet of Huxley and the sheet of Henley (inside to out). The occurrence of hair loss is reported to increase gradually with increasing age in each of these males and females in populous-based surveys of White people aged 20-70 aged.

Methodology: This pre-clinical animal study was performed on wistar albino rats at animal house of faculty of pharmacy Ziauddin University Karachi. The duration of this study was eight to ten months. The successful induction of Alopecia was done, the animals were divided into three groups with six animals per group and all the CIA wistar rats were tested for effects of AA-21 on Alopecia as topical formulations and H&E was performed and our

Results were satisfactory.

Keywords: Animal Model, Chemotherapy, Alopecia.

Introduction:

Hair is the human body's major esthetic display part, especially in cultural and social interactions. Hair serves many vital functions which includes defense from contaminants and sweat-gland dispersion substances e.g. pheromones. Alopecia is a common term for loss of hair, (Hassan and Jwaid, 2021). Within the matrix cells of the hair bulb are melanocytes that are often responsible for hair color. The hair matrix comprised (inside and outside) medulla, cortex and hair cuticle. The inner root sheath composed of the cuticle of the inner root sheath, the sheet of Huxley and the sheet of Henley (inside to out). The sheath of the inner root is solid, and its form defines whether the hair is curled or smooth. And the outer sheath to core (Zarbo et al., 2017). The middle part (isthmus), expanding

mostly from opening of the arrector pili muscle to the entrance of the sebaceous duct. This section includes the HF's "bulge," in which the epithelial stem cells exist. Disruption to the HF bulge leads to persistent alopecia scarring, as is medically observed with conditions like discoid lupus and lichen planopilaris. The upper part (infundibulum), which continues to the follicular orifice from the entry of the sebaceous duct (Liu et al., 2003). The occurrence of hair loss is reported to increase gradually with increasing age in each of these males and females in populous-based surveys of White people aged 20-70 aged, often utilizing updated classification measures, the occurrence of hair loss ranged from 45% to 90%. In the Hamilton-Norwood classification scale, the incidence of alopecia was approximately 50% (Ellis and Harrap, 2001). Alopecia areata is not limited to the gender and can affect male and female sex equally and is independent of the age group of patients. It has been found that about 0.1% to 0.2% of the population has been suffering from Alopecia areata. It has been reported that diagnosis of AA among males is diagnosed at earlier age as compared to female sex. Some studies have also reported that pediatric populations is more prone to AA (Strazzulla *et al.*, 2018). Pull test can be used to confirm the active state of the disease, especially at the periphery of the lesion (Madani and Shapiro, 2000). Loss of hairs at beard is likely to occur more (50.5%) than the involvement of scalp (39.3%) as shown in Fig 2 (Kyriakis *et al.*, 2009). It is found that the pathogenesis of AA is incompletely understood but it is viewed as an organ-specific autoimmune disease. Androgenetic alopecia, or patterned alopecia, is the most common form of hair loss in both men and women and is characterized by a progressive loss of hair diameter, length, and pigmentation. The genetic inheritance of androgenetic alopecia is well known, although the causative genes have yet to be elucidated. (Gordon and Tosti, 2011).

Cicatricial alopecia includes a group of conditions characterized by inflammation and subsequent destruction of the hair follicle, resulting in irreversible hair loss. Cicatricial alopecia can be primary or secondary. Diseases that primarily affect the hair follicle cause primary cicatricial alopecia, which is classified as lymphocytic or neutrophilic, based on the type of inflammatory cell that predominates (Gordon and Tosti, 2011). Alopecia considered as most severe skin side effects that damages the skin. Several drugs influence the growth of hair and a few others induce loss of hair, the symptoms of the illness of which are important for hair growth as a direct consequence of drug action on different cells. These cells are keratinocytes, hair matrix cells, peri-follicular blood vessels, in addition those of hair bulbs connective tissues. Keratinocytes considered a first affected cite for environmental or xenobiotic damaging effects. This can be attributable to the normal feature that up to 90% from total HFs are in a rapid growth process and the high blood flow rate around hair bulbs contributes to a strong bioavailability of many drugs at these locations (Al-Joda and Zalzal, 2019; Hassan and Jwaid, 2021). Alopecia-causing triggers include hormonal disorder, impaired hepatic and liver function, lupus erythematosus, heat and chemical injury, conception, and fungal infection, hereditary predisposition, chemotherapy, fatigue, diabetics, vicious disorders, injuries, autoimmune diseases and rheumatoid arthritis (Ramos and Miot, 2015). Younger age at onset is also regarded as a less favorable prognostic indicator (Strazzulla *et al.*, 2018).

Methodology:

This pre-clinical animal study was performed on wistar albino rats (120 to 140gms) at animal house of faculty of pharmacy Ziauddin University Karachi. The animals were maintained on normal conditions of humidity, temperature and light/dark cycle. They were fed on standard rodent pellet diet and they had free access to water. The duration of this study was eight to ten months.

The alopecia was induced in the animals as they were clipped at day one of procedure and another clipping on the day six for the induction stimulation of anagen hair growth. The anagen hair follicles are more sensitive for the effect of the cyclophosphamide (CYP). The animals were kept under supervision for the complete effects of the chemotherapy for six days. After six days of intra peritoneal injection of chemotherapy the animals were analyzed for induction of Alopecia (Hassan

and Jwaid, 2021). On successful induction of Alopecia, all the CIA wistar rats were tested for effects of AA-21 on Alopecia as topical formulations. At end of treatment the animals were authenticated and samples will be taken for analysis.

Ethical Approval: Institutional animal ethical approval was taken.

Induction and Treatment of Alopecia: The animals in this study were divided into three groups; each group with six animals.

- Group 1 (Control Group), n=6: This group were given Normal Saline i.p
- Group 2 (CYP Group) n=06: This group were given topical Administration of AA-21 Formulation.
- Group 3 n=06: topical Administration of AA-21 + Minoxidil spray

Inclusion Criteria: Wistar albino male rats weighing 120-140 gm

Exclusion Criteria: Wistar albino rats below or above the weight range

Histological examination: Approximated regions of mice dorsal skin tissue were sampled and put in 10% neutral buffered formalin, all dorsal skin tissues were re-fixed again for 24 hours. Three to four mm sections were prepared after paraffin embedding. For general histological architecture, descriptive parts were stained with hematoxylin and eosin (H&E). Under a light microscope, the histological profiles of individual cross-cut dorsal skin tissues were observed.

Results:

In Figure A it is clearly shown that in control group only few natural follicles were present which was done through by the H&E staining and the negative control yields about 11.17 ± 1.161 follicles per mm².

In figure B you can see the follicles are clearly visible and the growth covers the area and reduce the alopecia which shows our formation produced satisfying results and the AA0-21 formulation yielded 109.50 ± 1.871 follicles per mm².

In figure C the combination of formulation and Minoxidil was used and combination of these drugs yields better results than the formulation of AA-21 given alone and the combination AA-21 + Minoxidil yielded 198.33 ± 2.16 follicles per mm².

Figure A: Negative control group AA -21 Vehicle using H&E stain under microscope at 10X power

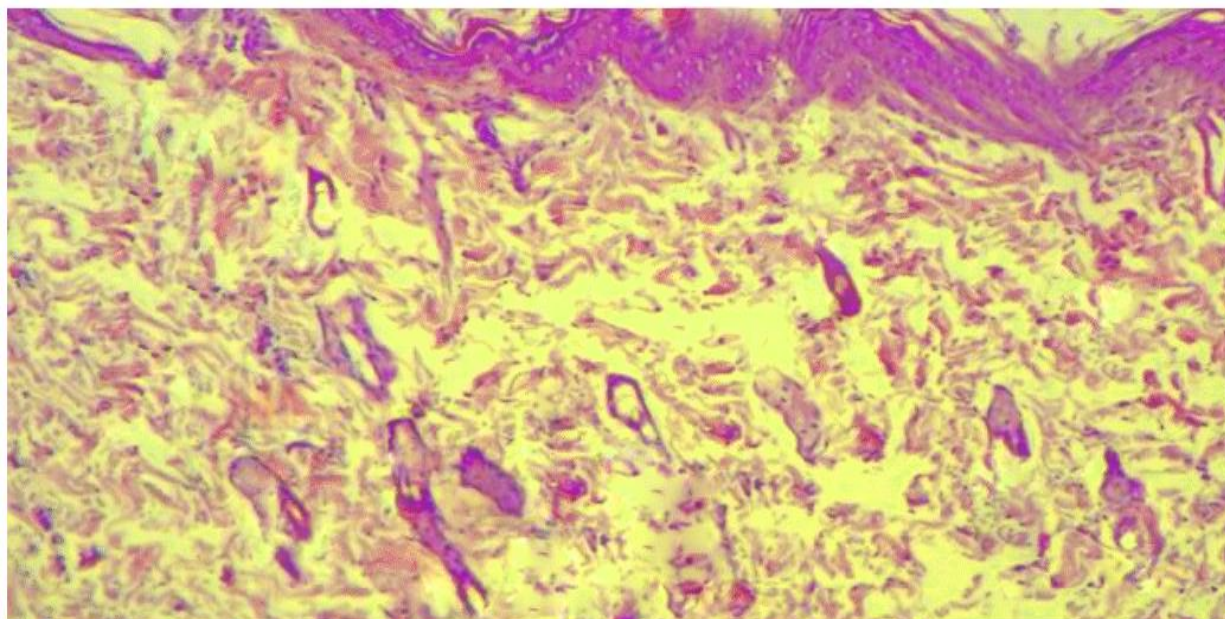


Figure B. AA-21 Formualtion Model Group using H&E stain under microscope at 10X power.

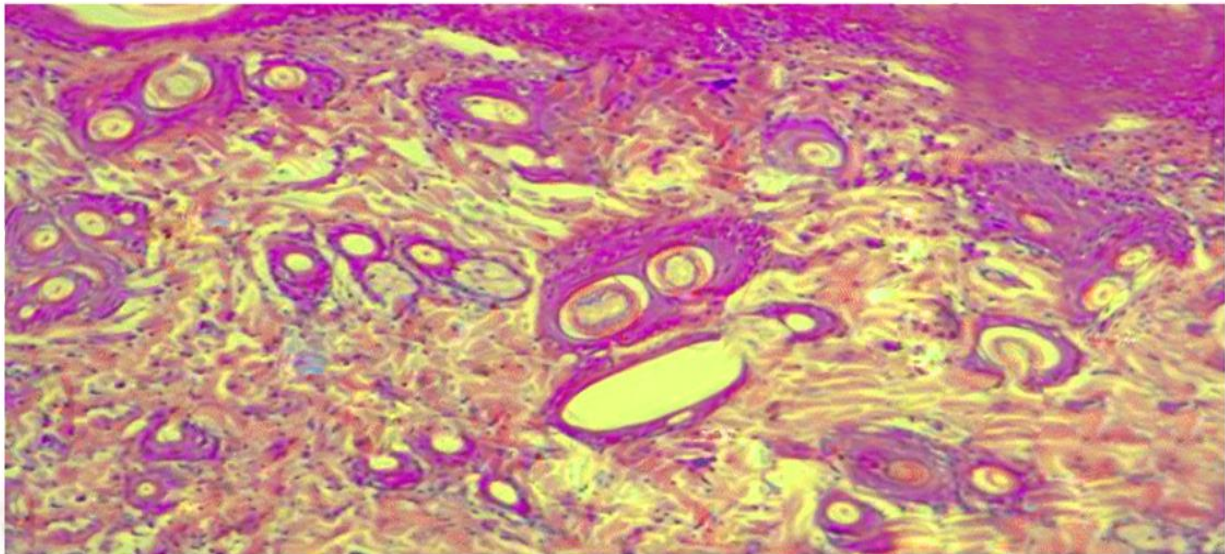
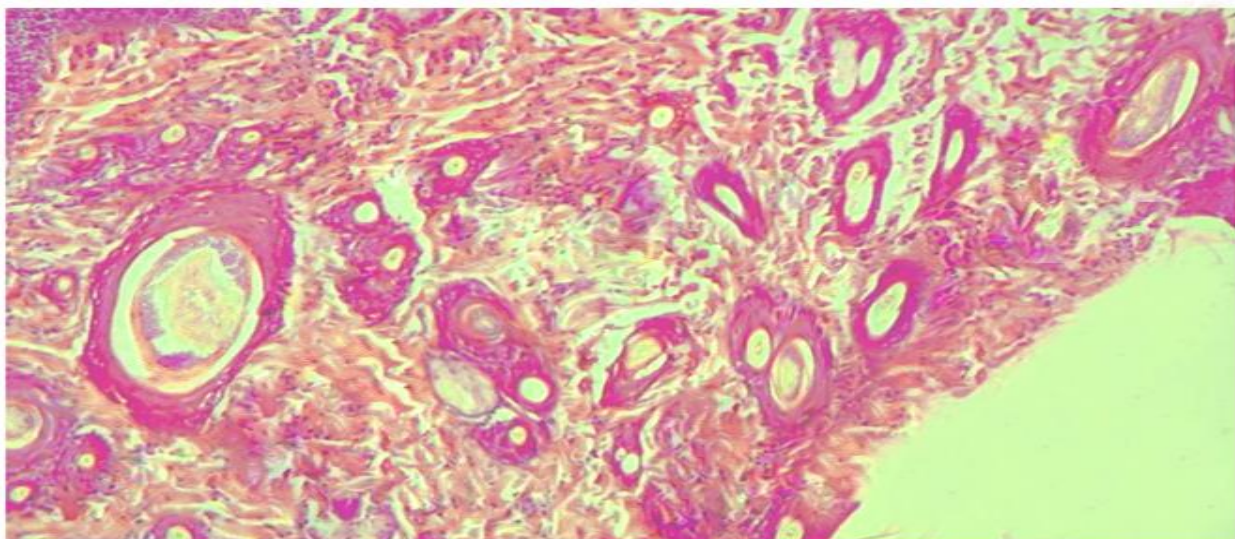


Figure c: AA -21 + minoxidil group using H&E stain under microscope at 10X power.



Discussions:

The results of current study revealed a decline in HFs count in comparison negative control group with positive control group. These results were consistent with the previous study of T. Ohtani *et al.* (2006) that revealed CYP influence on HFs.

As comparing groups that treated with AA-21 with negative control group showed significant increase in means follicles count. The number of HFs increased more significant when administered AA-21 formulation. This increase had been attributed to immune modulation and increased proliferation that reported by study of H. Tilg *et al.* (1993) on PTX pharmacological effect.

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