Dhanashri D. Jawarkar *et al*, Int. Journal of Pharmaceutical Sciences and Medicine (IJPSM), Vol.8 Issue. 5, May- 2023, pg. 80-91

ISSN: 2519-9889
Impact Factor: 5.9

HAIR GROWTH PROMOTING ACTIVITY OF *HELIANTHUS ANNUUS* IN TESTOSTERONE INDUCED ALOPECIA IN MICE MODEL

Dhanashri D. Jawarkar[1]; J.V. Vyas[2]; V.V. Paithankar[3]; A.M. Wankhade[4]
1234 Department of Pharmacology, Vidyabharti College of Pharmacy, Amravati, M.S. India

DOI: 10.47760/ijpsm.2023.v08i05.011

Abstract:
To study the hair promoting activity of *Helianthus annuus* in testosterone induced alopecia in mice model.
Objective: The objective of this research include to find an alternative for present allopathic medication to minimize side effects and adverse drug reactions, there is no study has been carried out on Helianthus annuus so far Therefore, the present study used to evaluate hair growth promoting activity using testosterone induced alopecia.
Material and Method: Swiss albino mice of either sex weighing 25-30 gm were used. Minoxidil (2%) solution purchased from market. This is the standard drug used for Alopecia. Testosterone (1ml ampoule) were purchased from Matoshri medical, Walgaon. It is used to induced alopecia in mice.
Result: The histological study showed that hair density was maximum i.e. 2.833 ± 0.1667 in case of standard, 2.167 ± 0.1667 in case of Helianthus annuus oil 100ml/kg, 2.667 ± 0.2108 in case of Helianthus annuus oil 200 ml/kg, 2.167 ± 0.1667 in case of Negative control group while it was minimum i.e. 1.167 ± 0.3073 in testosterone treated animals.
Keywords: Hair, Testosterone, Minoxidil, *Helianthus Annuus*

1. INTRODUCTION

Hair

Hair is one of the vital parts of the body derived from ectoderm of the skin, is protective appendages on the body and considered accessory structure of the integument along with sebaceous glands, sweat glands and nails. They Are also known as epidermal derivatives as they originate from the epidermis during embryological development. [1]

Hair is a unique character found on all mammals but not on other animals. In humans it is a special and cherished Feature, especially in females, but its main functions are in protection of the skin from mechanical insults and to Facilitate homeothermy [2] eyebrows and eyelashes, for Example, stop things entering the eyes, while scalp hair Prevents sunlight, cold, and physical damage to the head And neck.[3] It also has a sensory function, increasing the Perception of the skin surface for tactile stimuli, and subserves important roles in sexual and social communication, considering the psychological impact on quality of Life seen in hair disorders, such

ISSN: 2519-9889
Impact Factor: 5.9

as hirsutism, hair [3,4] In particular, regarding this last point, a significantly higher prevalence of personality disorders in subjects with androgenetic alopecia regarding the prevalence Of such diagnoses in the general population have been Reported [5]

Mammalian skin produces hair almost all over the Body surface except for a few areas of the body, i.e., Sole of the foot, palm of the hand, buccal surface of The lip, and portions of external genitalia; in addition, Considering the distribution of human hair in different Areas of the body surface, it is possible to note that Human hair growth is reduced with tiny and virtually Colorless hair on most of the body surface, whereas hair Is longer, thicker, and heavily pigmented in other areas, Such as the scalp, eyelashes, and eyebrows. Differences Are also related to the hair's form, which can be Straight, helical, or wavy; color, depending on the Balance of different types of melanin (brown to black, Indolic eumelanin, and yellow to reddish brown, pheomelanin); length; diameter; and crosssectional [3,6,7]

Human hair is usually classified according to three conventional ethnic human subgroups, i.e., African, Asian and European. Nevertheless, a recent study showed that it is possible to classify the various hairs found worldwide into eight main coherent hair types, by the measurements of three easily accessible parameters: curve diameter; curve index; and number of wave [8]

Alopecia

Alopecia is a distressing dermatological disorder characterized by hair loss. Although hair Severe no critical physiological function in humans, hair loss can be psychological Devastating and adversely affect the selfconfidence of the patient [9]. The significance of this Effect can be determined from the fact that each year in the US alone, about 60 million people Spend approximately $US1.5 billion on various hair regrowth treatments. [10] With increasing Longevity adding to the ancient pre-occupation with hair, the desire to extend youthfulness is Inevitably fulfilled This ever-increasing fascination with hair-care is reflected in the in cessant Growth of the hair-care market, already a multi-billion dollar enterprise world-wide. The recent phenomenal growth of hair care industry has been attributed to the thriving Economy, earlier- aging crisis, and the commercialized awareness of organic benefits. Regulatory bodies and pharmaceutical industry all over the world are waking up to the Various prospects that indigenous medicines have to offer. Hair is really one of human biology's most intriguing structures, although it is commonly dismissed as being of superficial importance and an ornamental feature only. [11]

Causes of Alopecia

Alopecia or hair loss is a common dermatological problem that transcends demographic, Economic, racial, gender and age barriers. The etiology of hair loss is uncertain though it has Been attributed to many probable factors ranging from hormonal imbalance, chemotherapy, Thyroid imbalance, genetic predisposition, abnormal

ISSN: 2519-9889
Impact Factor: 5.9

kidney function, abnormal liver function Lupus erythematosus, thermal damage, childbirth, fungal infection, diabetes, vitiligo, stress trauma, autoimmune diseases, rheumatoid arthritis, use of excessive chemicals on hair such as bleaches, dyes, blow drying, etc.

Hair growth is a result of the proliferative activity of matrix keratiiiocytes that form the hair shaft and inner root sheath. Matrix keratinocytes are localized in the bulb where they rest on the dennal papilla, which is the condense of specialized mesenchymal cells with important inductive properties. The surgical removal of the dermal papilla and the lower dermal sheath prevents hair growth, indicating that these specialized mesenchymal cells are the key signalling centres in hair follicles. The fact that a capillary loop is located within the dennal papilla of terminal hair follicles that provides nutrition to these cells; whereas vellus hair follicle dennal papillae typically do not contain capillaries illustrates the significance of nutrition to the papilla and the overlying matrix cells.

Types of Alopecia

- **Anagen effliiviiim** is sudden shedding of anagen hair caused by intemiption of active anagen Hair-foilicle growth by external stresses (chemotherapeutic agents, ingestion of toxic Substances etc). Recovering is usually rapid and starts with the removal of stress causing factor.
- **Alopecia** (**areata** AA) is an organ-specific autoimmune disorder characterized by nonscaring hair loss that may be patchy or generalized. AA has been observed occurring in association with several autoimmune diseases including thyroid and vitiligo and nail abnonnalities. [12,13]Alopecia areata is commonly seen throughout the world. It is estimated that 0.2% to 2% of the US population alone is affected by alopecia areata and the individual time risk is estimated to be approximately 1.7%. [14,15] About 25% of patients have a genetic history of the disorder, however, various report show familial incidences range from 3-42%. Recently, alopecia areata has been linked with specific HLA class II alleles, Especially D Q B l*03 and DRB1=^1104.
- **Androgenetic alopecia** (AGA) or male/female pattern hair loss attributes to over 95% of hair Loss reported worldwide. It is the most common type of alopecia, affecting about 50% of Caucasian males and females beyond age 40 years. The severity of the disease varies from merely accentuated recession of the frontal hairline to loss of all hair except along the Temporal and occipital margins. It is a progressive disease resulting in decrease in density of Terminal hair and a resulting increase in vellus hairs. Although etiology of the disease is still unclear, cell culture work by Hibberts and co-workers proposes that increased perception of hair follicles to dihydro testosterone leads to Androgenetic alopecia especially post puberty. Sawaya and Price [16]demonstrated that the Concentration of androgen receptor protein within the dermal papilla fibroblasts and outer Root sheath are 30% greater in the balding frontal follicles as compared to the non-balding Frontal hair follicles. Increased androgen binding at these receptor sites resulted in distinct Effects on the physiology of hair follicles.

ISSN: 2519-9889
Impact Factor: 5.9

2. MATERIAL AND METHODS

Experimental Animals

Swiss Albino mice of either sex or 8-12 week age would be used for study. The animals were housed on a 12-light/dark cycle under controlled temperature (22°C ± 2°C) and humidity (50 ± 10%). Experiments are performed in accordance with the committee for the purpose of control and supervision of experimental animals CPCSEA) guidelines after approval of the experimental protocols by the institutional animal ethics committee (IAEC).

Drugs and Chemicals

Testosterone solution was prepared as suspension in the vehicle (ethanol/propyleneglycol 90:10) Minoxidil 2%soultion was prepared in vehicle (ethanol/propylene glycol, 90:10)

Plant material

Seeds extract of *Helianthus annuu*s linn were purchased from Shivay herbal and healthcare, Jaipur, Rajasthan

Phytochemical Analysis

The extract would be then subjected to different phytochemical tests for identification different phytochemical principles. The plant extract would be studied for the presence of important phytochemical which may be involved in the action of plant viz total alkaloids, flavonoids Triterpenoids content, steroids, etc.

Acute dermal toxicity study

The acute toxicity of prepared oil was performed using OECD guideline 402 in following manner

Table -1. Treatment protocol

Sr.no.	Group	No. of Animals	Treatment and Doses	Route of Administration
1.	I	6	Negative control	Oral
2.	II	6	Positive control (Testosterone)	Subcutaneous
3.	III	6	Testosterone (1mg/kg) +Minoxidil (2%)standard drug	Subcutaneous and Topical
4.	IV	6	Testosterone(1mg/kg) + *Helianthus annuus oil1(*100ml/kg)	Subcutaneous Topical and
5.	V	6	Testosterone(1mglkg) + *Helianthus annuus oil1(*200ml/kg)	Subcutaneous Topical and

ISSN: 2519-9889
Impact Factor: 5.9

Experimental procedure

Animals were divided into five groups of six animals each and were administered Testosterone subcutaneously. Animals of group III, IV and V were givenapplication of Minoxidil *Helianthus annuus oil* respectively. Approximetly100 ml/kg and 200 ml/kg of solution was topically applied on the back skin day for 20 days. After 20 days, mice from each group were selected randomly and sacrificed. Skin biopsy was undertaken from balding site of each group mice.

Histopathological examination

Skin samples were kept in phosphate-buffered formalin for paraffine sectioning. Sections Were cut and stained with hematoxyline and eosin Follicular density (number of hair Follicles per mm) and anagen/telogen ratio was calculated with the help of ocular Micrometer. Later the microscopic slides of the skin sections were photographs

Statistical analysis

Values are expressed as mean +-SEM. The data were analyzed using one analyzed using one way analysis of variance (ANOVVA) followed by Dunnets Multiple Comparision test

3. RESULT

Table -2 Observation of Phytochemical Test

The phytochemical investigation of *Helianthus annuus oil* are as follows.

Sr.No.	Natural Product	Test Performed	Inference
1	Alkaloids	Wagner's reagent	+
2.	Steroids	Libermann-Burchard test	+
3.	Tannins	Ferric chloride Test	-
4.	Terpenoids	Libermann-Burchard test	+
5.	Carbohydrates	Molisch's Test	+

ISSN: 2519-9889
Impact Factor: 5.9

6.	Saponin	Foam test	-
7.	Glycosides	Keller-killiani test	-
8.	Flavonoids	Alcoholic test	+

Acute dermal toxicity testing

Table -3 Acute toxicity testing carried out according to 402 OECD guideline

Sr.no.	Dose(ml/kg)	Observation
1.	50	No death
2.	1000	No death
3.	2000	No death

According to demal toxicity studies (OECD-402) Helianthus annuus oil showed no toxicity upto doses of 2000ml/kg nor any skin irritation of animal was observed.

Table-4 Result of skin irritation test

Formulation	Visual observation	
	Erythema	Oedema
Helianthus annuus oil 100ml/kg solution	Nil	Nil
Helianthus annuus oil 200ml/kg solution	Nil	Nil

ISSN: 2519-9889
Impact Factor: 5.9

The result of skin irritation test of Helianthus annuus oil and the solution did not produce erythema and oedema on the dorsal area or skin of Mice. The result indicated that the prepared solution was not produce any dermatological reaction and are well tolerated by Mice it means it was safe to use.

Histopathological examination

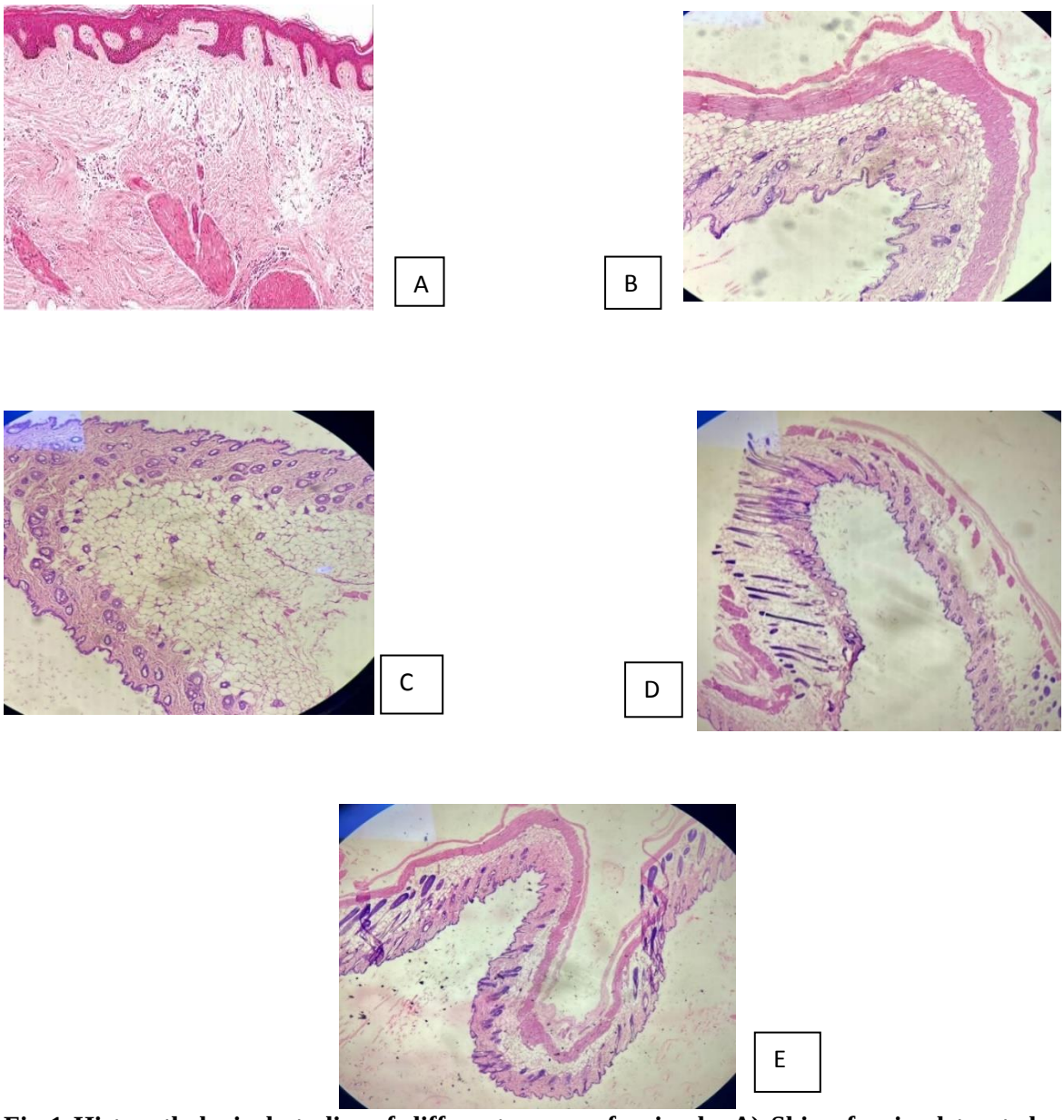

Fig-1 Histopathological studies of different group of animals. A) Skin of animal treated with vehicle B) Skin of animal treated with testosterone C) Skin of animal treated with testosterone and minoxidil D) Skin of animal treated with testosterone and *Helianthus annuus oil* E) Skin of animal treated with testosterone and *Helianthus annuus oil.*

ISSN: 2519-9889
Impact Factor: 5.9

Table-5 Hair follicular density and A/T ratio in section of skin of different group animals.

Sr.no	Group no.	Treatment	Hair follicular density (no./mm)	A/T ratio
1	I	Negative control	2.167± 0.1667	1.54:1
2	II	Positive control (Testosterone)	1.167±0.3073	0.5:1
3	III	Testosterone +Standard (2%Minoxidil Topical)	2.833±0.1667	1.60:1
4	IV	Testosterone + Helianthus annuus oil 3ml/kg	2.167±0.1667**	1.12:1**
5	V	Testosterone + Helianthus annuus oil 6ml/kg	2.667±0.2108***	1.23:1**

Value expressed as a mean ± SEM (n=6) one way ANOVA followed by Dunnett`s Multiple Comparision test

**P< 0.05 compare to positive control for follicular density.

****P<0.0001 compare to positive control for A/T ratio

ISSN: 2519-9889
Impact Factor: 5.9

- **Assessment of follicular density**

The histological study showed that hair density was maximum i.e. 2.833 ± 0.1667 in case of standard, 2.167 ± 0.1667 in case of Helianthus annuus oil 100ml/kg, 2.667 ± 0.2108 in case of Helianthus annuus oil 200 ml/kg, 2.167 ± 0.1667 in case of Negative control group while it was minimum i.e. 1.167 ± 0.3073 in testosterone treated animals.

- **Assessment of Anagen/ Telogen ratio**

Anagen telogen ratio was significantly affected by Helianthus annuus oil which was 1.12:1 and 1.23:1 as against 0.5:1 for testosterone – treated animal and 1.60:1 for minoxidil-treated animal. The predominance of hair follicle in anagenic growth phase indicates the reversal of androgen induced hair loss in Helianthus annuus oil-treated animals. As it is evident from the above data, the activity of Helianthus annuus is comparable with minoxidil.

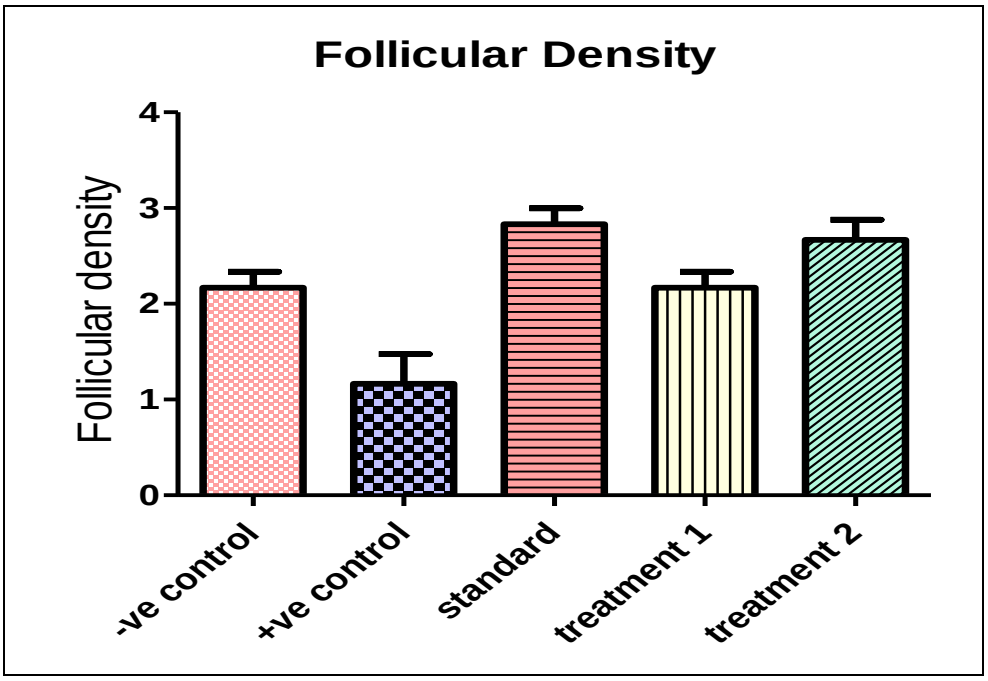

Fig -2 Hair follicular density in section of skin of different groups of animal.

ISSN: 2519-9889
Impact Factor: 5.9

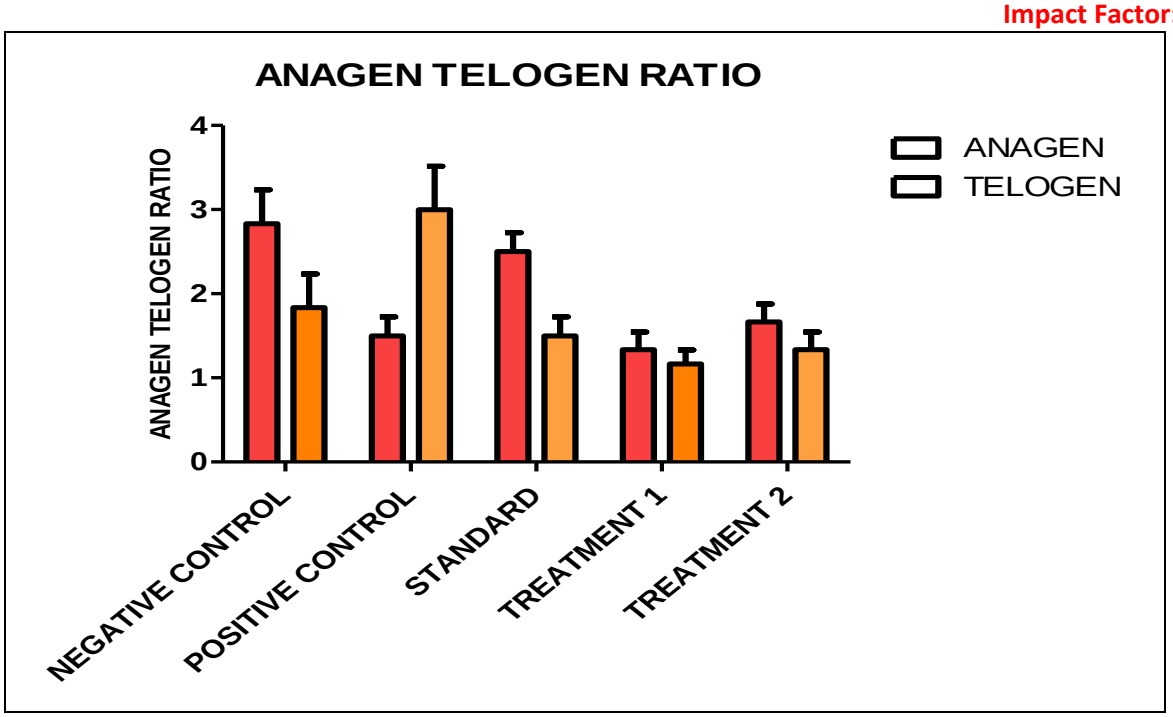

Fig-3 Anagen/Telogen ratio in section of different groups of animals.

4. DISCUSSION

The present study aimed to investigate the potential hair growth promoting activity of Helianthus annuus (sunflower) in a testosterone-induced alopecia mice model. Androgenetic alopecia, also known as male pattern baldness, is a common form of hair loss characterized by the progressive miniaturization of hair follicles due to the influence of dihydrotestosterone (DHT), a metabolite of testosterone. The testosterone-induced alopecia mice model is widely used to study the mechanisms underlying hair loss and evaluate potential therapeutic interventions. The administration of testosterone to the experimental mice induced hair loss, leading to visible alopecic areas on the dorsal skin. The severity of alopecia was quantified by measuring the size and number of bald patches. Additionally, hair follicle analysis was performed to assess the effect of Helianthus annuus treatment on hair follicle size, density, and anagen-telogen ratio.

findings of the study demonstrated that treatment with Helianthus annuus extract significantly reduced the size and number of bald patches compared to the control group. This suggests the potential hair growth promoting activity of Helianthus annuus in the testosterone-induced alopecia model. The improvement in alopecia was further supported by the increase in hair follicle size, density, and anagen-telogen ratio in the treated group compared to the control group.

The observed hair growth promoting effects of Helianthus annuus could be attributed to its phytochemical composition, which includes various bioactive compounds such as flavonoids, polyphenols, and vitamins. These

ISSN: 2519-9889
Impact Factor: 5.9

constituents possess antioxidant, anti-inflammatory, and growth-stimulating properties, which are known to play a crucial role in promoting hair growth combating hair loss.

The antioxidant activity of Helianthus annuus extract may help protect hair follicles from oxidative stress, a key factor contributing to hair loss. Additionally, the anti-inflammatory properties of the extract may reduce inflammation within the hair follicles, leading to improved follicle health and hair growth.

Moreover, the presence of certain bioactive compounds in Helianthus annuus extract may interact with androgen receptors or inhibit the enzyme 5-alpha reductase, which is responsible for the conversion of testosterone to DHT. By reducing the levels of DHT, Helianthus annuus extract could help prevent the miniaturization of hair follicles and promote hair growth.

While the findings of this study are promising, it is important to acknowledge some limitations. The testosterone-induced alopecia mice model, although widely used, does not fully replicate the complex pathogenesis of human androgenetic alopecia.

Therefore, further investigations employing other experimental models, such as human scalp skin organ culture or human hair follicle culture, are necessary to validate the hair growth promoting effects of Helianthus annuus.

Future studies should focus on elucidating the underlying mechanisms through which Helianthus annuus promotes hair growth. This could involve investigating its effects on hair follicle stem cells, dermal papilla cells, and the expression of genes related to hair growth regulation. Moreover, conducting dose-response studies and evaluating the long-term effects of Helianthus annuus treatment would provide further insights into its therapeutic potential for androgenetic alopecia.

5. CONCLUSION

The result provide evidence that the helianthus annuus oil shows excellent hair growth activity along with antiandrogenic activity in testosterone induced alopecia in mice model. Hair growth activity may be attributed to the presence of saponin, steroids an terpenoids in the plant , resulting into increased follicular density and anagen/ telogen ratio. Hence it can be concluded that the findings of the present study suggest that Helianthus annuus extract possesses hair growth promoting activity in a testosterone-induced alopecia mice model. These results highlight the potential of Helianthus annuus as a natural remedy for hair loss and support its further exploration as a potential therapeutic option for androgenetic alopecia. Additional research is necessary to fully understand the underlying mechanisms and evaluate its efficacy and safety profiles.

ISSN: 2519-9889
Impact Factor: 5.9

REFERENCES

[1]. Kuldeep Singh, Alopecia: introduction and overview of herbal treatment Journal of Chemical and Pharmaceutical Research, 2016, 8(8):59-642
[2]. Harrison JL, Davis KD. Cold-evoked pain varies with Skin type and cooling rate: a psychophysical study in Humans. Pain 1999; 83: 123–135.
[3]. Randall VA, Botchkareva NV. The biology of hair Growth. In: Ahluwalia GS, ed. Cosmetic Application of Laser and Light-Based System. Norwich, NY: William Andrew Inc., 2009: 3–35.
[4]. Randall VA. Is alopecia areata an autoimmune disease? Lancet 2001; 358: 1922–1924.
[5]. Maffei C, Fossati A, Rinaldi F, et al. Personality disorders and psychopathologic symptoms in patients with Androgenetic alopecia. Arch Dermatol 1994; 130: 868–872
[6]. Olabiyi AO, Akpantah AO, Oyerinde OF, et al. The Distribution of hair on the phalanges of a sample Population of Nigerian Yorubas in relation to sex, age and job type. Niger J Physiol Sci 2008; 23: 101–104.
[7]. Ito S, Wakamatsu K. Human hair melanins: what we Have learned and have not learned from mouse coat Color pigmentation. Pigment Cell Melanoma Res 2011;24: 63–74.
[8]. De la Mettrie R, Saint-Leger D, Loussouarn G, et al.Shape variability and classification of human hair: a Worldwide approach. Hum Biol 2007; 79: 265–281
[9]. Hadshiew fM, Foitzik K, Arck PC, Paus R. Bui'den of hair loss: stress and the underestimated Psychosocial impact of telogeii effluvium and aiidi-ogenetic alopecia. J Invest Dermatol. Sep 2004; I23(3);455-7
[10]. Davies K. Human genetics: Hair apparent. Nature. 5 February 1998; 391: 537-538. 001:10.1038/35264
[11]. Paus P, Cotsarelis G. The biology of hair follicles. N. Engl. J. Med. 1999; 341(7); 491
[12]. Muller SA, Winckelmann RK: Alopecia areata. Arch Dennatol. 1963; 88:290-297,
[13]. Hordinsky M. Clinical presentations of alopecia areata. Dermatol Ther. 2001; 14:291-296.
[14]. Safavi K. Prevalence of alopecia areata in the First National Health and Nutrition Examination Survey (Letter). Arch Dennatol, 1992;128(5):702
[15]. Safevi KH, Muller SA, Suman VJ, et al. Incidence of alopecia areata in Olmsted County, Minnesota, 1975 through 1989, Mayo Clin Proc. 1995;70(7):628-33
[16]. Sawaya ME, Price VH. Different Levels of 5a-reductase type 1 and 2, aromatase, and androgen Receptor in hair folicles of men and women with androgenetic alopecia, J Invest Dermatol. 1997; 109:296-9.

