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## International Journal of Pharmaceutical Chemistry and Analysis

Journal homepage: <https://www.ijpca.org/>

## Original Research Article

## Evaluation of betulin for hair growth promoting activity in rats

Vinay Vishnudas Sarode<sup>1\*</sup>, Sadhana P. Gautam<sup>1</sup><sup>1</sup>Dept. of Pharmacology, Institute of Pharmaceutical Education and Research, Wardha, Maharashtra, India

## ARTICLE INFO

## Article history:

Received 01-02-2024

Accepted 22-02-2024

Available online 26-03-2024

## Keywords:

Betulin

Minoxidil

Testosterone

Alopecia

ANOVA

## ABSTRACT

**Background:** Hair has historically been associated with beauty and a person's unique personality. Numerous elements, including metabolism, hormones, inheritance, and the adverse effects of immunosuppressive and anti-neoplastic medications, can adversely impact hair health and lead to hair loss, or alopecia. It is typical everywhere in the world.

**Aim:** The current study examined the effect of betulin for hair growth promoting activity in rats.

**Materials and Methods:** The study used 48 albino wistar rats, weighing  $200 \pm 30$  grams and aged between 12 and 16 months. Testosterone was given at a dose of 0.5 mg/kg to induce hair loss. During 28 days, a once-daily topical application of minoxidil solution was made. On a daily basis for 28 days, betulin was administered orally at concentrations of 10 mg/kg, 20 mg/kg, and 40 mg/kg. A combination of Betulin (10 mg/kg p.o.) and 3% Minoxidil was administered to one group, while the other was treated with Topical Betulin Solution (3 mg/ml once daily for 28 days). ANOVA analysis was used to ascertain the data's statistic.

**Results:** The results showed that effect of Betulin on Alopecia as evidenced by decreased levels of cholesterol, testosterone and increased levels of Hair length.

**Conclusions:** According to the current study, albino wistar rats with Testosterone induced Alopecia can benefit from treatment with an Betulin.

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## 1. Introduction

Due to the general interest in an individual's body form, hair, cosmetics, and attire, both men and women are under pressure from society to look a certain way.<sup>1</sup> Numerous studies on hair loss demonstrate the significant role that hair plays in social and sexual communication as well as the appearance of the human body.<sup>2</sup>

One of the essential components of the body, hair is an appendage that provides protection and is derived from the ectoderm of the skin. It plays a significant role in the human body's allure.<sup>3</sup>

## 1.1. Alopecia

Alopecia is a generic term that is generally used for hair fall and loss. Hair loss is a natural daily phenomenon, but this shedding of hair cannot be the main cause of hair loss.<sup>4</sup>

## 2. Types of Alopecia

Alopecia can be classified by different ways

A) According to their causes alopecia may be classified as follows-

1. *Non-scarring Alopecia:* It happens because of genetic issues, getting older, taking medications like contraceptives and anti-cancer chemotherapeutic drugs, applying chemical treatments topically like hair

\* Corresponding author.

E-mail address: [vinaysarode99@gmail.com](mailto:vinaysarode99@gmail.com) (V. V. Sarode).

dyes, permanent wave solutions, and straighteners, getting sick, having allergies, or getting infections in the hair follicles.<sup>5</sup>

2. *Scarring Alopecia*: It can happen as a result of trauma, which frequently destroys follicles, or burns (accidental or after surgery from cryosurgery or laser surgery).<sup>5</sup>

B) According to factor responsible alopecia may be classified as follows-

#### Androgenetic Alopecia (AGA)

AGA is a common hereditary thinning of hair induced by androgens in genetically susceptible and this condition is known in men as common baldness and as female pattern baldness in women.

AGA is major type of scalp hair loss that affects 60–70% of the population. It affects

50% of male by the age of 50 years and up to 70% of all males in the later life.

25% of women by the age of 49 years and 41% at the age of 69 years.

It is characterized by the miniaturization of the large, thick pigmented terminal hair with a diameter of  $> 0.03$  mm to small, fine, non-pigmented vellus hair with a diameter of  $\leq 0.03$  mm. This characteristic of AGA is due to premature entry of the hair follicle into the catagen phase and the delay in the transition from the telogen phase to the anagen phase, resulting in the shortening of the anagen phase.<sup>5</sup>

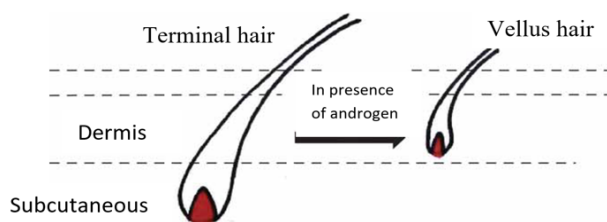


Figure 1: Characteristic of AGA.

1. *Alopecia Areata*: Alopecia areata is characterized by patchy scalp baldness. Most common reasons of alopecia areata are pregnancy, hormone pills, thyroids disorder, and sexually transmitted disease like syphilis, gonorrhoea, anemia and arthritis.<sup>6</sup>
2. *Alopecia Universalis*: Alopecia universalis is a type of alopecia in which total body hair loss occurs. Patients are usually healthy, it occurs due to the thyroid disease and vitiligo and hair loss all over the body.<sup>6</sup>

#### Factors related to Hair Loss

There are various factors which affects growth of hairs.<sup>7</sup>

Table 1: Factors affecting to hair loss

Factors	Description
Major physical–emotional stress	Surgery, severe illness, diet or nutrition changes and emotional stress can cause hair loss.
Chemotherapy	Parkinson medications, anti-ulcer drugs, anticoagulants, agents for gout, anticonvulsants for epilepsy, antidepressants, beta-blocker drugs, anti-thyroid agents, anti-neoplastics, blood thinners, male hormones.
Smoking	Tobacco smoke damages the lining of blood vessels, leading to less production of nitric oxide and thus inducing hair loss.
Genetic pre-disposition	Genetic component to androgenetic hair loss exists (polygenic inheritance)
Cardiovascular	Diseases high levels of LDL in cardiac patients are converted to 5-alpha reductase enzyme, which produces DHT from testosterone, causing hair loss.
Dihydro- testosterone (DHT)	Increased level of DHT (the testosterone metabolite) shortens the hair cycle and progressively minimizes scalp follicles and this may be due to the atherosclerotic process blocking the microvasculature that nourishes the hair follicles.

### 3. Drug Profile

Name of Drug: Betulin

IUPAC Name: Lup-20(29)-ene-3 $\beta$ ,28-diol

Chemical Formula: C<sub>30</sub>H<sub>50</sub>O<sub>2</sub>

#### 3.1. Structure of betulin

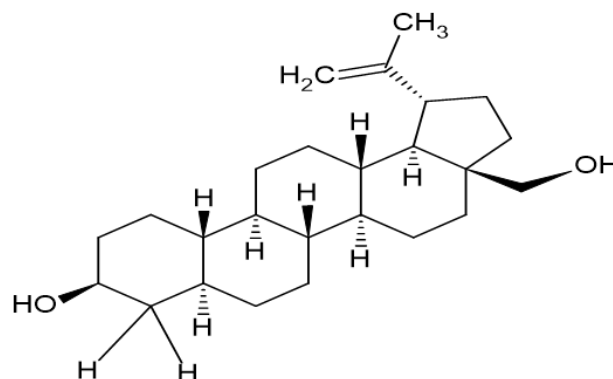


Figure 2: Structure of bitulin

**Category of Chemical Constituent:** Pentacyclic triterpene alcohol with lupane skeleton

**Source:** The major source of betulin is outer bark of Birch Trees. A lesser amount can be found in the skin and leaves of birches.<sup>8</sup>

**Mechanism of Action:** Betulin inhibit the maturation of Sterol Regulatory Element Binding Protein (SREBP) and thus decrease the biosynthesis of cholesterol and fatty acids. It decreases the lipid contents in serum, tissues and increases insulin sensitivity.<sup>9</sup>

**Pharmacological Activity:** Betulin has shown antimicrobial, anti-bacterial, antiviral, anti-HIV, anti-inflammatory, anti-diabetic, hepatoprotective, gastroprotective, anti-proliferative, anti-hyperlipidaemic, anti-malarial and anti-cancer activities.<sup>10</sup>

#### 4. Materials and Methods

##### 4.1. Procurement of animals & their diet

The Albino rats (Wistar strain) of age 12-16 months, weighing between  $200 \pm 30$ g, were procured from the animal house of Institute of Pharmaceutical Education and Research (I.P.E.R.), Wardha. The animals were housed in the polypropylene cage at a temperature of  $24^\circ \pm 2^\circ\text{C}$  with the relative humidity of 40-60% and 12 hour light / dark cycle. Animals were fed with balanced diet and water during the complete experimental period. The experimental protocol was approved by the Institutional Animal Ethics Committee of Institute of Pharmaceutical Education and Research, Wardha (Registration Number 535/PO/Re/02/CPCSEA) on dated 01.12.2018 with Protocol No. IAEC/2018-19/02

##### 5. Preparation of Testosterone (Sustanon 100)

The dose of Testosterone 0.5 mg/kg, by subcutaneous route for 21 days was selected on the basis of literature survey.<sup>11</sup>

Testosterone (Sustanon 100) 100 mg injection was diluted with 200 ml arachis oil, this was able to produce concentration 0.5mg/ml.

The dose of Testosterone (0.5 mg/kg, s.c) was calculated according to the body weight of the animal and was administered for the preparation of alopecia model.

##### 6. Preparation of 3% Minoxidil Solution

The topical 3% minoxidil solution was selected on the basis of literature survey.<sup>12</sup>

3% Minoxidil solution was prepared by using propylene glycol, alcohol and water in the ratio of 5:3:2 and was applied to the animals topically for 28 days.

##### 7. Preparation of Betulin

The dose of betulin 10mg/kg, 20mg/kg, 40mg/kg p.o and betulin topical was selected on the basis of literature

survey.<sup>13</sup>

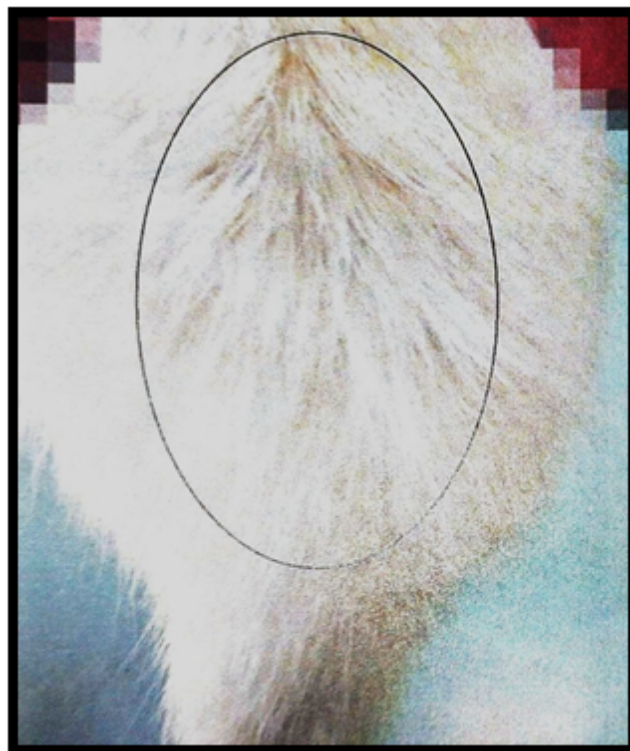
The dose of betulin (10mg/kg, 20mg/kg, 40mg/kg) was calculated according to the body weight of the animal and was administered to the rats per orally (p.o) for 28 days.

The betulin topical (3mg/ml) solution was prepared by using olive oil and applied topically for 28 days. The drug solutions for the studies were freshly prepared.

#### 8. Experimental Design

After induction period of 21 days i.e. on 22<sup>nd</sup> day and after treatment period of 28 days i.e. on 49<sup>th</sup> day the initial and final weight were taken respectively. Blood was withdrawal by retro-orbital plexus method and biochemical parameters were performed on 22<sup>nd</sup> and 49<sup>th</sup> day. 3 mm area of skin of two rats from each group were taken on 22<sup>nd</sup> day and 49<sup>th</sup> day. Samples were stored in 10% formalin solution and sent for histopathological studies.

#### 9. Induction of Alopecia



**Figure 3:** Photograph of animal showing natural hair.

#### 10. Results

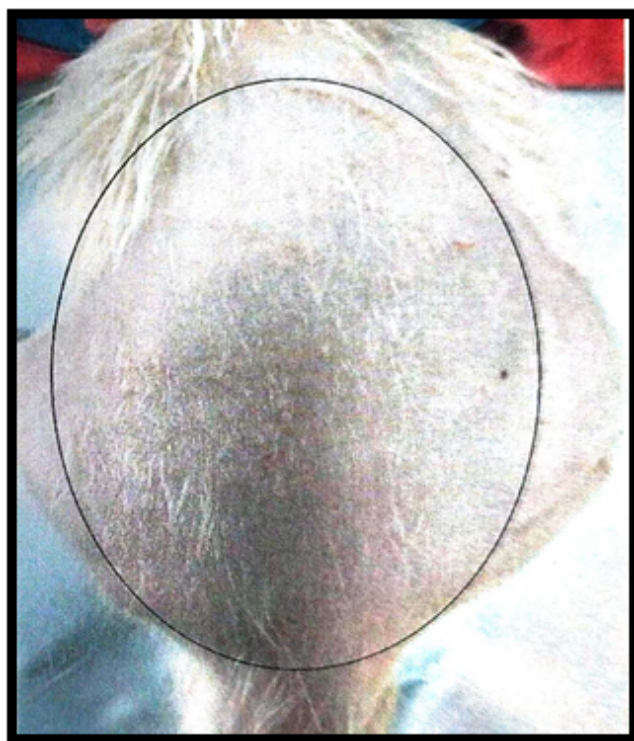
##### 10.1. Determination of physiological parameters

###### 10.1.1. Measurement of body weight

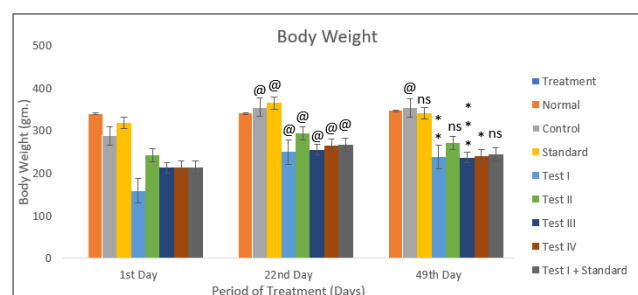
The body weight of animals of each group was measured on 1<sup>st</sup> day i.e. initial weight, on 22<sup>nd</sup> day after induction

**Table 2:** Differential groups of experimental animals

Sr. No.	Group	Treatment	Doses and Routes	No. of Animals
1	Group I (Normal)	Saline	Saline solution, p.o.	6
2	Group II (Control)	Testosterone	0.5mg/kg, s.c.	6
3	Group III (Standard)	Minoxidil	Topical	6
4	Group IV (Test I)	Betulin	10mg/kg, p.o.	6
5	Group V (Test II)	Betulin	20mg/kg, p.o.	6
6	Group VI (Test III)	Betulin	40mg/kg, p.o.	6
7	Group VII (Test IV)	Betulin	Topical (3mg/ml)	6
8	Group VIII (Test + Std.)	Betulin + Minoxidil	10mg/kg, p.o. + topical	6

**Figure 4:** Photograph of animal showing alopecia after treating with Testosterone.

of alopecia i.e. intermediate weight and on 49<sup>th</sup> day after treatment i.e. final weight.

**Figure 5:** Results of body weight

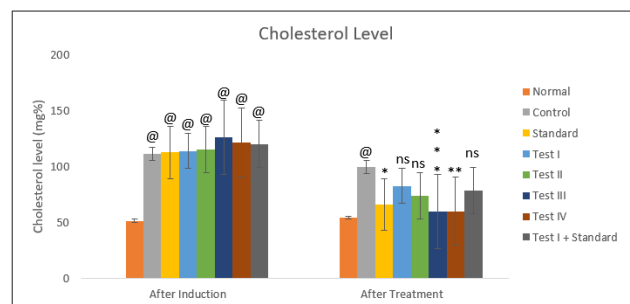
Observation shows that after induction of alopecia with testosterone on 22<sup>nd</sup> day there was significant increase in body weight of animals of all groups as compared to 1<sup>st</sup> day of study but there was no significant change in weight of normal group animals was decreased after the treatment with standard drug, various doses of betulin and combination of betulin with standard drug on 49<sup>th</sup> day as compared to 22<sup>nd</sup> day.

## 11. Estimation of Biochemical Parameters

### 11.1. Cholesterol level

The measurement of cholesterol makes it possible to identify hypercholesterolemia, which is linked to hypertriglyceridemia. Elevated cholesterol levels are linked to an increased risk of vascular accidents and the development of atherosclerosis. The ratio should be taken into account when assessing one's risk of cardiovascular disease.

For determination of cholesterol level the collected blood sample was sent to Amey Pathology Laboratory, Socialist Square, Wardha.

**Figure 6:** Results of cholesterol level

Observation shows that after induction of alopecia with testosterone on 22<sup>nd</sup> day there was increase in level of cholesterol in blood of animal.

After treatment with standard drug and various doses of Betulin, cholesterol level decreases. Significant decrease observed in animals treated with Betulin 40 mg/kg p.o., Betulin topical and standard drug Minoxidil topical solution where as other group animals treated with betulin 10 mg/kg

**Table 3:** Observation of body weight

Group No.	Treatment	Body Weight		
		1 <sup>st</sup> Day	22 <sup>nd</sup> Day	49 <sup>th</sup> Day
I	Normal (Saline Solution)	339.66 ± 19.68	340 ± 17.49	346.16 ± 17.34
II	Control (Testosterone)	287.5 ± 8.71	354.83 ± 11.18@	353.5 ± 9.78@
III	Standard (Minoxidil)	317.5 ± 4.77	364.5 ± 6.10@	341.33 ± 6.23 <sup>ns</sup>
IV	Test I (Betulin 10mg/kg,p.o)	158.66 ± 10.08	249.5 ± 5.85@	237.83 ± 3.63**
V	Test II (Betulin (20mg/kg, p.o)	241.66 ± 6.24	293.33 ± 3.87@	271 ± 4.10 <sup>ns</sup>
VI	Test III (Betulin (40mg/kg, p.o)	212.66 ± 8.28	255.16 ± 3.70@	236.83 ± 16.80***
VII	Test IV (Betulin (Topical)	213.66 ± 12.86	265.33 ± 13.25@	240 ± 13.70*
VIII	Test I + Std. (Betulin + Minoxidil)	214 ± 11.41	267.16 ± 18.12@	245.16 ± 16.26 <sup>ns</sup>

Values expressed as mean ± S. E. M., (n=6,6 and 6) Two way analysis of variance (ANOVA) followed by Bonferroni's test @P<0.0001 compared to normal and \*P<0.05, \*\*P<0.01, \*\*\*P<0.001 compared to control.

**Table 4:** Observation for cholesterol level

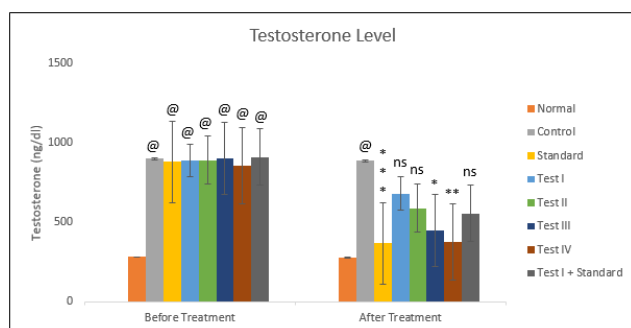
Group No.	Treatment	Cholesterol Level	
		22 <sup>nd</sup> Day	49 <sup>th</sup> Day
I	Normal (Saline Solution)	51.5 ± 2.71	54.16 ± 3.36
II	Control (Testosterone)	111.33 ± 3.72@	99.66 ± 2.71@
III	Standard (Minoxidil)	112.833 ± 6.81@	66.16 ± 3.71*
IV	Test I (Betulin 10mg/kg,p.o)	114 ± 5.77@	82.83 ± 3.55 <sup>ns</sup>
V	Test II (Betulin (20mg/kg, p.o)	115 ± 7.46@	73.5 ± 5.07 <sup>ns</sup>
VI	Test III (Betulin (40mg/kg, p.o)	126.33 ± 6.82@	59.5 ± 4.63***
VII	Test IV (Betulin (Topical)	121.83 ± 10.18@	60.16 ± 3.32**
VIII	Test I + Std. (Betulin + Minoxidil)	120.33 ± 6.58@	78.5 ± 3.59 <sup>ns</sup>

Values expressed as mean ± S. E. M., (n=6,6 and 6) Two way analysis of variance (ANOVA) followed by Bonferroni's test @P<0.0001 compared to normal and \*P<0.05, \*\*P<0.01, \*\*\*P<0.001 compared to control.

p.o., betulin 20 mg/kg and combination of test and standard drug does not show significant decrease in cholesterol level in blood of animals. These observations show that Betulin 40 mg/kg p.o. is best effective dose.

### 11.2. Testosterone Level

Elevated testosterone level indicates hyper-androgenism. The collected blood samples by the retro orbital plexus route were sent to Amey Pathology Laboratory, Socialist Square, Wardha. The serum testosterone level was stimulated by Electro chemiluminescence.

**Figure 7:** Results of testosterone level

Observation shows that testosterone level increased after induction of alopecia with testosterone on 22<sup>nd</sup> day but after treatment testosterone level decreased. Treatment with standard drug significantly lowers the testosterone level. Betulin 40mg/kg p.o. and Betulin when applied topically also significantly decreased testosterone level.

## 12. Evaluation of Biological Parameters

### 12.1. Skin irritation test

The Betulin (3mg/ml) in Olive Oil was assessed for primary skin irritation test. All group animals were used for the study. Each animal rat was caged individually and food and water given during the test period for 24 hours, prior to the test. The dorsal skin 1cm<sup>2</sup> area of animal was cleaned with spirit. Betulin (3mg/ml) solution was topically applied to animals and observed for erythema and edema for 48 hours after application.

Observation found that no erythema and edema occurred in any animal within 48 hours after topical application of Betulin (3mg/ml) solution. It indicated that it is safe for topical use.

### 12.2. Measurement of hair length

Quantitative model for the evaluation of hair growth measurement was performed. Hair was plucked randomly

**Table 5:** Observation for testosterone level

Group No.	Treatment	Testosterone Level	
		22 <sup>nd</sup> Day	49 <sup>th</sup> Day
I	Normal (Saline Solution)	280.94 ± 6.40	276.59 ± 6.58
II	Control (Testosterone)	900.3 ± 13.71@	888.03 ± 10.74@
III	Standard (Minoxidil)	881.3 ± 34.05@	368.04 ± 6.66***
IV	Test I (Betulin 10mg/kg,p.o)	890.11 ± 27.20@	682.12 ± 12.02 <sup>ns</sup>
V	Test II (Betulin (20mg/kg, p.o)	892.76 ± 33.21@	589.21 ± 14.75 <sup>ns</sup>
VI	Test III (Betulin (40mg/kg, p.o)	901.78 ± 26.90@	445.96 ± 12.42*
VII	Test IV (Betulin (Topical)	857.26 ± 36.32@	377.48 ± 12.26**
VIII	Test V (Betulin + Minoxidil)	910.76 ± 17.62@	555.56 ± 14.45 <sup>ns</sup>

Values expressed as mean ± S. E. M., (n=6,6 and 6) Two way analysis of variance (ANOVA) followed by Bonferroni's test @P<0.0001 compared to normal and \*P<0.05, \*\*P<0.01, \*\*\*P<0.001 compared to control.

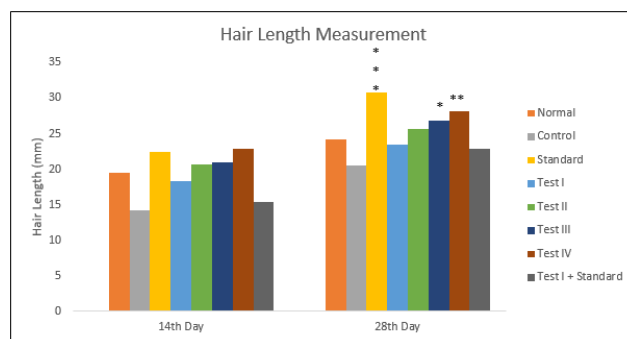
using sterile forceps from the depilated area on 30<sup>th</sup> day of treatment and the hair length was measured with the help of scale and the results were calculated as mean length ± SEM of 25 hairs.

**Table 6:** Observation for hair length measurement

Group No.	Treatment	Hair Length (mm) (Mean±SEM)	
		14 <sup>th</sup> Day	28 <sup>th</sup> Day
I	Normal (Saline Solution)	19.51±0.57	24.12 ± 0.47
II	Control (Testosterone)	14.19±0.28	20.52 ± 0.41
II	Standard (Minoxidil)	22.43±0.56	30.82 ± 0.48***
IV	Test I (Betulin 10mg/kg,p.o)	18.36±0.42	23.41 ± 0.47
V	Test II (Betulin (20mg/kg, p.o)	20.62±0.39	25.61 ± 0.63
VI	Test III (Betulin (40mg/kg, p.o)	20.89±0.47	26.89 ± 0.60*
VII	Test IV (Betulin (Topical)	22.88±0.55	28.09 ± 0.46**
VIII	Test V (Betulin + Minoxidil)	15.33±0.48	22.89±0.55

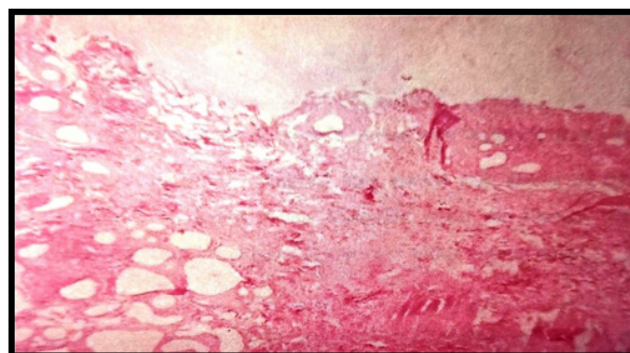
Values expressed as mean ± S. E. M., (n = 25 Hairs) @P<0.0001 compared to normal and \*P<0.05, \*\*P<0.01, \*\*\*P<0.001 compared to control.

After treatment, when hair length was measured it found that length of hair increases. Faster hair growth observed in animals treated with standard drug. Animals treated with topical Betulin also show hair growth with 28.09mm length as compared with other doses of betulin.

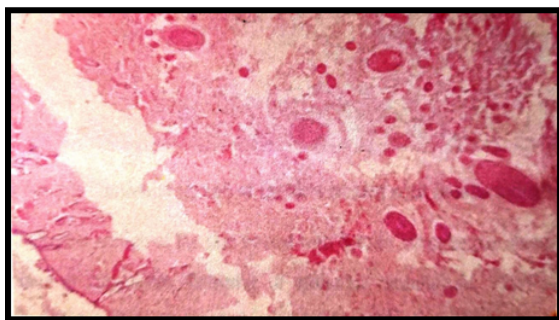
**Figure 8:** Results of hair length measurement

### 12.3. Histological Study

After the treatment with standard drug and various doses of Betulin, on 28<sup>th</sup> day of treatment, two rats from each group were selected for cutting the section of the skin. Skin biopsies were obtained from the depilated portion of the rats and preserved in 10% formalin solution. The samples were sent to Amey Pathology Laboratory, Socialist Square, Wardha for the further studies.

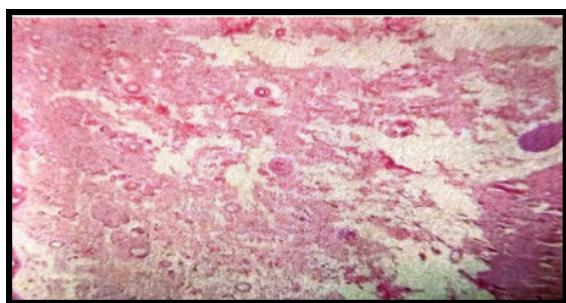
**Figure 9:** Photomicrograph of normal skin of rat

Histopathological section of the normal rat skin shows normal epidermis or dermis layer



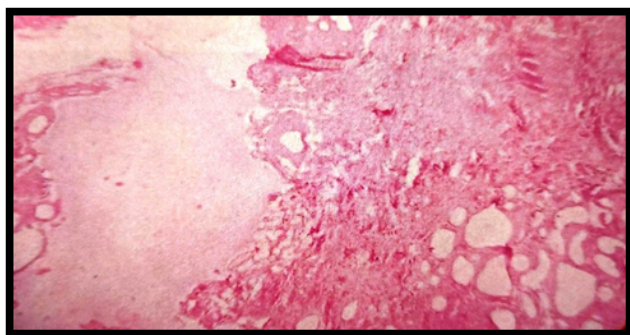
**Figure 10:** Photomicrograph of alopecia induced skin of rat

Histopathological section of alopecia induced skin shows heavy infiltration of lymphocytes, plasma cells, macrophages in deep dermis. Epidermis shows diffuse atrophy and ballooning degeneration. Histological features thus suggested the appearance of Chronic Dermatitis.



**Figure 11:** Photomicrograph of rat skin treated with standard drug (Minoxidil)

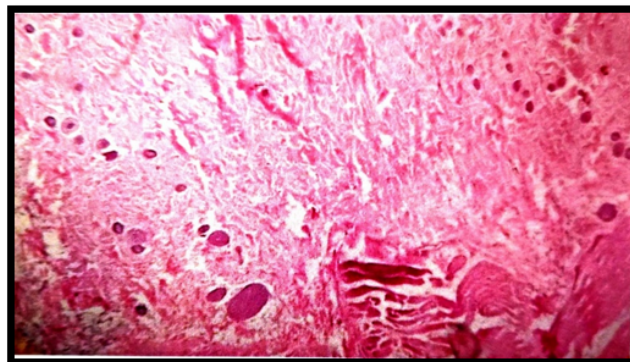
Histopathological section of the standard drug treated skin shows no remarkable changes in epidermis or dermis means Minoxidil repair the changes occur after induction of alopecia. Thus histological features are within normal limits.



**Figure 12:** Photomicrograph of rat skin treated with betulin 10mg/kg p.o.

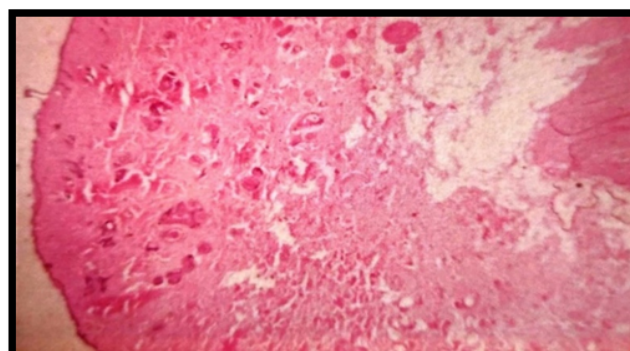
Histological section of the skin treated with Betulin 10mg/kg p.o. shows diffuse degeneration of keratinocytes,

flattening of epidermis, acantholysis. There is heavy infiltration of lymphocytes in deep dermis. This histological feature indicates drug/chemical induced dermatitis.



**Figure 13:** Photomicrograph of rat skin treated with Betulin 20mg/kg p.o.

Histopathological section of skin treatment with Betulin 20mg/kg p.o. shows spongiosis, diffuse atrophy of squamous epithelium and ballooning. It also shows degeneration of basal cells of epidermis. Section shows dermal adnexae are normal. These histological features are indicated that all changes are drug/chemical induced changes.

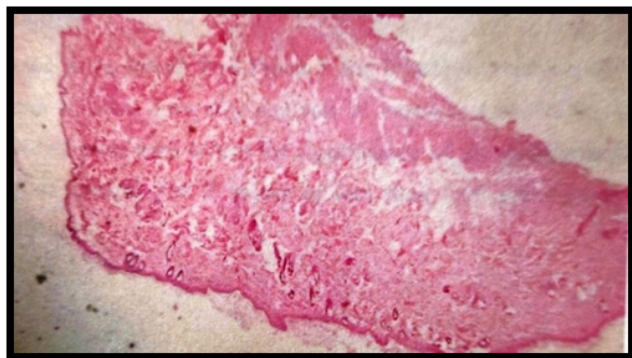


**Figure 14:** Photomicrograph of rat skin treated with Betulin 40mg/kg p.o.

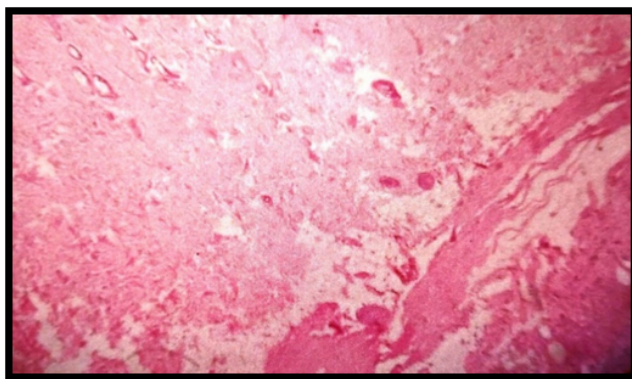
Histopathological section of the skin treated with Betulin 40mg/kg p.o. shows no remarkable changes in epidermis or dermis which may be due to restoration of the changes observed during induction of alopecia.

Histopathological section of the skin treated with Betulin Topical shows no remarkable changes in epidermis or dermis. Betulin Topical repair the changes occur after induction of alopecia. Thus histological features are within normal limits.

Histopathological section of skin treated with test drug i.e. Betulin 10mg/kg p.o. and Standard drug (Minoxidil) shows epithelioid granulomas, Langhans' Giant cell, necrosis in deep dermis. Epidermis is histologically



**Figure 15:** Photomicrograph of rat skin treated with Betulin topical solution



**Figure 16:** Photomicrograph of rat skin treated with combination of test and standard drug

unremarkable. Histological feature indicate appearance of Tuberculosis.

#### 12.4. Statistical Analysis

Data were analysed using Graph Pad Prism 5 for Windows (version 5.00). Results expressed as Mean  $\pm$  SEM. Two-way analysis of variance (ANOVA), Bonferroni's and Tukey's post-test were used to test the significance of the difference between the variables in various groups. The P values of less than 0.05 were considered to be statistically significant.

### 13. Discussion

Hair has been sign of beauty and a contribution to an individual personality. Many factors such as metabolism, hormones, heredity and side effects of anti-neoplastic and immune suppressant drugs are negatively affects the healthy growth of hair and results in hair loss that generally called alopecia. It is common throughout the world.<sup>7</sup>

Androgens like testosterone are mediators of terminal hair growth all through the body. An elevated testosterone level is not the cause of hair loss. Dihydrotestosterone (DHT) (metabolised form of testosterone) is more potent

androgen, is responsible for hair loss.<sup>14</sup>

Hair loss results when certain hair follicles on scalp are highly sensitive to DHT. As a result of this sensitivity, hair follicles shrink and eventually disappear. Based on these findings, DHT is held responsible for miniaturization of hair follicles in androgenic alopecia.<sup>15</sup>

There are number of ways by which drug may stimulate hair growth like it may increase the linear growth rate of hair, increase the diameter of the hair fibre, alter the hair cycle, either shortening telogen or prolonging anagen or act through a combination of both effects.

Minoxidil (Topical) is the FDA approved drug used for the treatment of alopecia.

Minoxidil is a vasodilator and potassium channel opener known to prolong the anagen phase and converts vellus hair to terminal hair. Minoxidil solutions causes scalp irritation, headaches, dizzy spells, itchiness, redness, contact dermatitis.

Various researches shows that betulin (Triterpene) exhibit a wide range of activities like antimicrobial activity, anit-inflammatory, antiviral, hepatoprotective and anticancer. Triterpenes now a day's mostly used for cosmetic applications. Highly pure betulin widely used in the pharmaceutical and cosmetic industries. Betulin and its semisynthetic derivatives have high potential in medicine.<sup>14</sup>

Betulin occurs in a number of plants, especially in many species of birch, where it found in large amount in the outer bark.<sup>8</sup> Triterpenoid like Lupeol, Betulinic acid present in different plants like Birch bark, Careya Arborea, Alangium salvifolium and Alstonia scholaris are used in the hair conditioners and shampoos. They help to reduce hair loss, improve keratin absorption, reduce inflammation and inhibit bacterial growth.<sup>16</sup>

Literature survey shows that Betulin is a triterpenoid also used in the hair conditioners and shampoos since 19<sup>th</sup> century. Some studies shows that increase in the cholesterol level causes the hair loss and betulin was found to lower the cholesterol level hence it may inhibit the hair loss. By considering the above fact we investigated hair growth promoting activity of betulin on testosterone induced alopecia.

In present study testosterone (0.5 mg/kg) subcutaneously administered for 21 days to rats for induction of alopecia. The fall of hair and appearance of baldness (Figure 4) confirmed the induction of alopecia. The alopecia developed by testosterone may be due to the high sensitive of hair follicles toward DHT. As a result of this sensitivity, hair follicles shrink and eventually disappear. It was further confirmed by determination of various parameters.

The body weight of animals significantly increase after induction of alopecia with testosterone on 22<sup>nd</sup> day (Table 3 and Figure 5). Whereas body weight of animals of all groups except normal group animals was decreased after the treatment with standard drug, various doses of



betulin and combination of betulin with standard drug on 49<sup>th</sup> day. Some studies have suggested that chronic administration of testosterone, increase body weight and body fat in experimental animals. Testosterone might disturb the mechanism that protect against adiposity and hyperphagia and represent the risk factor for excessive body and obesity.<sup>17</sup>

Observation of biochemical parameters shows that cholesterol and testosterone level increased after induction of alopecia (Table 4 and 5) (Figures 6 and 7). Testosterone level increased because it is used as an inducer and cholesterol level increased may be due to testosterone which may increase in microsomal enzyme activity, resulting in increased cholesterol production by the liver.

After treatment with standard drug and various doses of betulin, cholesterol level decreased. Betulin, may decreased the biosynthesis of cholesterol and fatty acid by specially inhibiting the maturation of sterol regulatory element-binding proteins (SREBP).<sup>9</sup>

Minoxidil also decreased the cholesterol and testosterone level but its mechanism of action have still not been comprehensively understood but one of the in-vitro study shows that Minoxidil changes the activity of 5- $\alpha$ -reductase type 2 inhibitor. This anti-androgenic effect of minoxidil, shows by significant down regulation of 5 $\alpha$ -R2 gene expression in HaCat cells, may be one of its mechanism of action in alopecia.<sup>18</sup>

For testing the safety of betulin, skin irritancy test was performed on animals. Observation found that no erythema and edema occurred in any animal within 48hours after topical application of Betulin (3mg/ml) solution. It indicated that it is safer for topical use.

For checking the hair growth promoting effect of standard drug Minoxidil and betulin, hair length was measured two times i.e. after 14<sup>th</sup> and 28<sup>th</sup> day of treatment. Faster hair growth observed in animals treated with standard drug with hair length 30.82mm. Animals treated with topical topical betulin also show hair growth with 28.09mm as compared with other doses of betulin (Table 6 and Figure 8). The results of betulin indicates that topical application of betulin shows better and fast hair growth than use of oral betulin. Topical betulin shows better effect may be due to more absorption in the skin as it used along with olive oil. Olive oil contains hydrophilic phenols which are rich in antioxidants effect and thus it has been used as a skin product and hair cosmetic.

For the checking of induction of alopecia, the histopathology study was conducted. Histopathological section of alopecia induced skin shows heavy infiltration of lymphocytes, plasma cells, macrophages in deep dermis. Epidermis shows diffuse atrophy and ballooning degeneration, which results in appearance of Chronic Dermatitis (Figure 10). These features indicated the induction of alopecia.

For the checking hair growth effect of betulin, the histopathology study was conducted. Histopathological section of the skin treated with Betulin 40 mg/kg p.o. and skin treated with Betulin Topical shows no remarkable changes in epidermis or dermis which means that betulin restore the changes which observed in alopecia induced skin, may be due to its antioxidant potency.

The histopathology of skin treated with test drug i.e. Betulin 10 mg/kg p.o. and standard drug (Minoxidil) shows epitheloid granulomas, langhan's giant cells, necrosis in deep dermis. Histologically epidermis is unremarkable. All these histological features indicate appearance of Tuberculosis. The cause of such effect is unknown.

#### 14. Conclusion

The present study showed that Betulin, a triterpenoid, effective against testosterone induced alopecia. It decreases the level of testosterone and ultimately level of DHT which mainly responsible for the hair loss. Various doses of betulin work against alopecia but topical application show better effect. Topical betulin shows better effect may be due to more absorption in the skin as it used along with olive oil which antioxidants. A histopathological observation also proves these results of betulin.

#### 15. Source of Funding

None.

#### 16. Conflict of Interest

None.

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### Author biography

**Vinay Vishnudas Sarode**, Student

**Sadhana P. Gautam**, Assistant Professor

**Cite this article:** Sarode VV, Gautam SP. Evaluation of betulin for hair growth promoting activity in rats. *Int J Pharm Chem Anal* 2024;11(1):62-71.