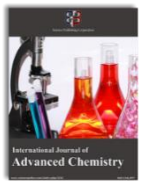
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Research paper



Evaluation of retardant property of green hair growth retardant formula

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Abstract

Background: The relevance of depilatory creams has appreciated significantly in recent time and their demand is on the increase. However most commercial depilatory creams contain chemicals with questionable health safety. These synthetic chemicals cause scaring, itching and burns on the skin.
Objectives: The study attempted to formulate green hair growth retarding cream, testing the formula for phytochemicals, hair growth retarding property and toxicity.
Methods: the cream was formulated from extracts of Tumeric (Curcuma longa), Aloe vera (Aloe barbadensis miller), Guava (Psidium guajava) and Neem (Azadirachta indica), evaluated for its phytochemical constituents and tested for its hair growth retardant property and toxicity on mice.
Results: The results show the presence of phytochemicals like tannins, phenols, flavonoids, terpeniods, alkaloids, volatile oil, anthroquinone, glycosides, saponins and a 25% hair growth retarding efficiency on tested mouse, no signs of itching, redness, swelling and topical injuries on the mouse.
Conclusion: overall, the research therefore provide convincing evidence of the safety of the formula over the conventional ones and also exhibit satisfactory hair regrowth retarding property.

Keywords: *Depilatory Cream; Extracts; Phytochemicals; Retarding Property; Toxicity.*

1. Introduction

The average number of human hair is about 135000 and each grows and falls through the various cycles of hair growth which include anagen, catagen and telogen. The life cycle of hair are affected by factors such as nutrition, medical history, hereditary, physical constitution, hormone, secretion and aging (Schlossman 2008, Schueller 2009 and Postajian 2011)
Hair growth is a natural process that needs to be control by regular shaving to prevent excess body and facial hair. Hair removal from certain location on the human body has received much attention as the growth of hair on other parts of the body. Getting rid of hair on areas of the body where it is not desired is a problem applicable to all human (Nanda et al, 2006). In Nigeria and the world over, having facial hair happen to not only men but to women too. Today most women worry about their facial hair and will do anything to get rid of it. Facial hair is quite embarrassing and may reduce self-esteem, especially when it becomes obviously unhidden even in the best of makeups (Nwalsial 2013). Thioglycolic acids whose calcium and potassium derivatives (calcium and potassium thioglycolate) are the main active ingredients in hair removal products cause skin irritation and severe skin burns. Additionally, the thioglycolates class has been shown to be rapidly absorbed through the skin in experimented animals, resulting in systemic toxicity.
Hair growth retardant is a substance that establishes and maintains a long-term and stable reduction in the frequency of hair regrowth. Although the action of hair growth retardant on hair is too slow to be studied, the rapid reaction of hair removal creams is a representative of hair growth retardant acting at an accelerated rate. The constituents of hair are primarily 88% keratin, a hard fibrous type of protein. The cytoskeleton of all epidermal cells is formed from keratin. The amino acid found in the keratin protein is cysteine. Disulphide (S-S) chemical bond firmly hold together the sulphur in cystine molecule and therefore making the cystine molecule difficult to break. The durability and resistance of hair fibre to degradation under environmental stress is a consequence of these disulphide chemical bonds. In an acidic environment, the hair fibre demonstrate great resistance to breakage, however the sulphide bond are susceptible to breakage under alkaline condition. (Depilatories 2006).
Forming the S-S bond require removal of two hydrogen atoms, each from a cysteine molecule.

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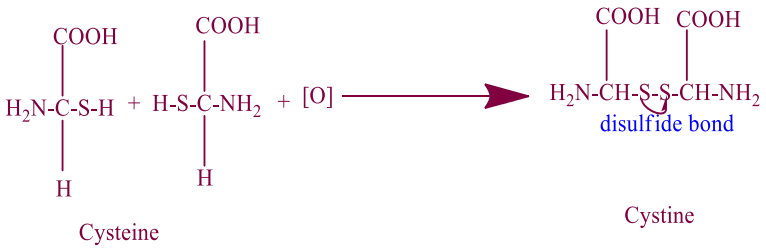


Fig. 1: S-S Bond Formation in Cysteine Molecule.

This is an oxidation process and therefore an oxidizing agent [O] is required. The reaction can be reverse by using a suitable reducing agent, resulting to loss of hair from the skin. That is, the key to chemical hair removal is breaking the S-S bond in cystine molecule using suitable reducing agent such as thioglycolic acid.

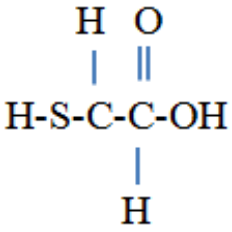


Fig. 2: Thioglycolic Acid.

Thioglycolic salt (especially K salt) is contain in most hair removal products, potassium thioglycolate react with hydroxide ions in KOH or NaOH (also a component of hair removal products) to form a dianion.



The dianions is the active reducing agent. It is the S end of the dianion that is active in the S-S bridge breaking, thus $^-SCH_2COO^-$ can be simplify as RS^- (R = CH_2COO^-).
The chemical reactions that lead to complete removal of hair follicle are represented in the figure below. These reactions occur at a low pH.

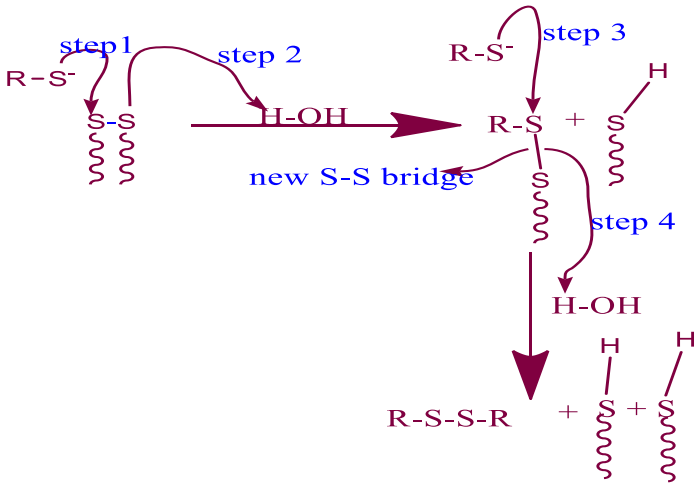


Fig. 3: Mechanism of Disulfide Bridge Breakage in Cysteine.

Step1; nucleophilic attack by S in RS on an S atom in cystine molecule breaks the S-S bridge and generate a new one between a cysteine molecule and the thioglycollate ion.
Step2; the anions formed in step1 strips a proton off water to generate a cysteine molecule.
Step3; a second thioglycollate molecule attack the first to break the newly formed
S-S bridge and remake it between the thioglycollate molecules.
Step4; the anion generated in step3 strip a proton off water to form the second cysteine molecule (chemistry in your cupboard 2013).

2. Materials and methods

2.1. Materials

The different plant materials were gotten from Muda-Lawal market in Bauchi, the chemical reagents and mice were gotten from Abubakar Tafawa Balewa University, Bauchi State, Nigeria. Deionised water was used throughout the study.

Table 1: Formula for Preparing the Hair Growth Retardant Cream

Components	Amount
Tumeric extract	5 g
Aloe-vera extract	1 g
Guava extract	1 g
Neem extract	1 g
Calcium carbonate 2 g	2 g
Cetyl alcohol 3 g	3 g
Honey	4 ml
Deionized water	2 ml
Paraffin oil	2 ml

2.2. Methods

2.2.1. Cold extraction

The leaves of the plants were washed lightly to remove dirt, dried for four weeks in the shade to avoid chemical degradation due to sunlight. The dried leaves and dried turmeric tuber was separately pulverized using electrical blender. 150 g of powdered plant were separately subjected to cold extraction in 70% ethanol at ambient temperature with occasional agitation for five days. The mixture was filtered and the filtrate concentrated by evaporation at 45^{o}C. The extracts were dried and stored in air-tight containers in the refrigerator at 4^{o}C until later use.

2.2.2. Preliminary phytochemical screening

The preliminary phytochemical tests were performed to identify different chemical groups present in the extract in each plant.

2.2.2.1. Alkaloid test (dragendorff's test)

An extract (0.1 g) from each plant was treated with few drops of dragendorff's reagent (potassium bismuth iodide solution). The appearance of an orange brown precipitate indicates the presence of alkaloid (Seema 2008).

2.2.2.2. Flavonoid test

An extract (2 ml) from each plant was treated with few drops of NaOH solution. The appearance of intense yellow coloration which turns colourless on addition of few drops of dilute sulphuric acid solution indicates flavonoids presence (Alupuli et al, 2009).

2.2.2.3. Cardiac glycosis test

An extract (2 ml) of extract from each plant was treated with 0.4ml of glacial acetic acid containing few drops of $FeCl_3$, concentrated sulphuric acid will be added along the test tube wall to settle at the bottom. The appearance of reddish brown colour changing to bluish green colour at the junction of the two reagents indicates the presence of cardiac glycosides (Alupuli et al, 2009).

2.2.2.4. Saponin test

About 2 ml of extract from each plant was shaken with 1ml of lime water. The formation of semi-permanent foam (15min) indicates the presence saponins (Ashutush 2003).

2.2.2.5. Tannin test

About 1ml of extract from each plant was treated with few drops of 1% $FeCl_3$. The appearance of blue colour indicates the presence of hydrolysable tannins, while the appearance of green colour indicates the presence of condensed tannins (Ashutush 2003).

2.2.2.6. Anthraquinone test (borntrager's test)

About 2 ml of extract from each plant was boiled with 1ml of dilute HCl in a test tube. The content would be cooled and extracted with chloroform. The chloroform layer would be separated and ammonia solution added. The appearance of a rose-pink colour indicates the presence of anthraquine glycoside (Bartram 1995).

2.2.2.7. Terpenoids test

An extract (0.2 ml) from each plant was mixed with 2ml of chloroform followed by the addition of 3ml of concentrated H_2SO_4. A reddish brown colouration of the surface indicates presence of terpenoid (Trease and Evans 1989).

2.2.2.8. Phenols

Few drops of 1% $FeCl_3$ was added to 2 ml solution of extracts. A violet colour indicates the presence of phenol (Lecture Demostration manual general chemistry).

2.2.3. Hair growth retardant procedure

The procedure adopted is a modified version of Neelam, 2011 in which

- Accurate weight of calcium carbonate, cetyl alcohol, unadulterated Honey and liquid paraffin were measured and mixed thoroughly. This mixture forms the base of the cream.
- Measured deionised water was added to the base.
- Accurate weight of turmeric powder was incorporated into the above mixture

- 1 g each of neem, aloe vera and guava extract were added and mixed well into a cream like texture.

2.2.4. Hair growth retarding test using mice

A modified version of the method described by (Wakisaka et al, 2009) was adopted in which two albino mice A and B, back were shaved 3x4cm^2 with a clipper (to lower the density of the hair). The formula was applied topically the following day to the shaved portions of mouse A, daily topical application of the formula continued for 10 days. The same procedure was observed using 50% ethanol on the control mouse B. In order to observe the retarding effect of the cream on hair growth, pictures of the shaved portion was taken at an interval of 7days for 2 weeks beginning from the day of last application of the formula.

2.2.5. Evaluation of hair growth retarding efficiency

In this model, cello tape was adhered to and pulled off from the shaved portion of the back of each treated mice two weeks after last treatment. The hair density was calculated in accordance with equation A and the hair growth retarding efficiency was calculated according to equation B
Equation A:
Hair density = number of hair on cello tape/ unit area of shaved portion of mice
Equation B:
Retarding efficiency = (density of hair on control mice - density of hair on experimental mice/density of hair on control mice) x 100%

2.2.6. Toxicity test of the hair growth retardant

During the 10 days of topical application of formulated hair regrowth retardant on the mice, the mice were observed for itching, swelling, redness, and topical injuries.

3. Results and discussion

3.1. Results

Table 2: Percentage Recovery of Ethanol Extract from 150 G Powder of Plants

	Guava	Neem	Aloe Vera	Tumeric	Balsam Fruit
Amount of Extract(g)	9.6	7.8	5.4	7.2	10.2
Recovered (%w/w)	6.40	5.20	3.60	4.80	6.80
Appearance	Dark Green	Green	White	Yellow	Dark brown

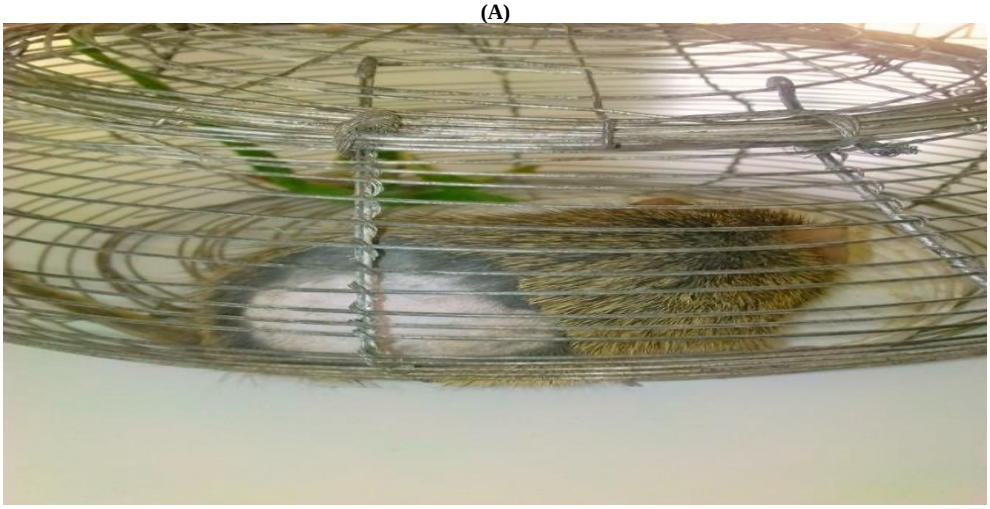
Table 3: Phytochemical Screening of Extracts

Phytochemicals	Guava	Aloe V.	Neem	Tumeric
Alkaloids	+	+	-	+
Saponins	+	-	-	-
Glycosides	-	-	+	+
Anthroquinone	-	+	+	+
Tanins	+	-	+	+
Flavonoid	+	+	+	+
Terpenoid	+	-	+	+
Phenol	+	+	-	+

(+): Present, (-): Absent.

Hair Growth Retardant Test
The effect of the formulated hair retardant on body surface of mice A and B is shown in plate I-III. Hair regrowth began 14-21 days after the last dose of the retardant cream (plates II-III)
20th March

(A)



(B)

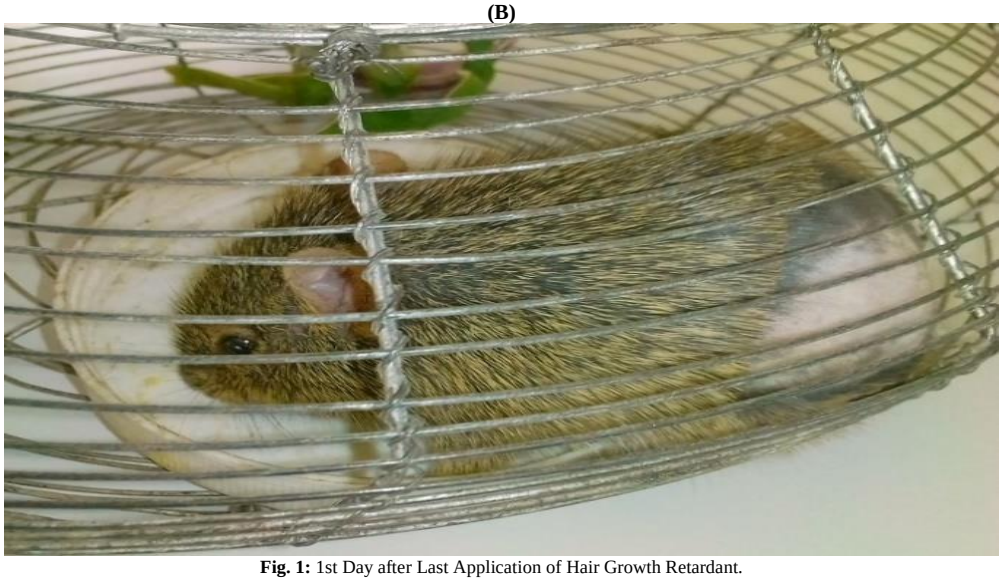
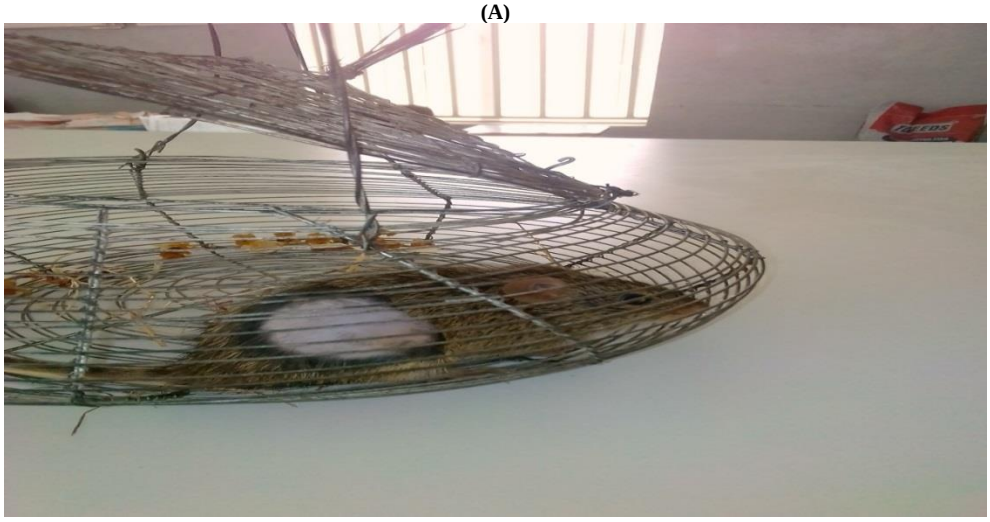


Fig. 1: 1st Day after Last Application of Hair Growth Retardant.

27th March

(A)



(B)

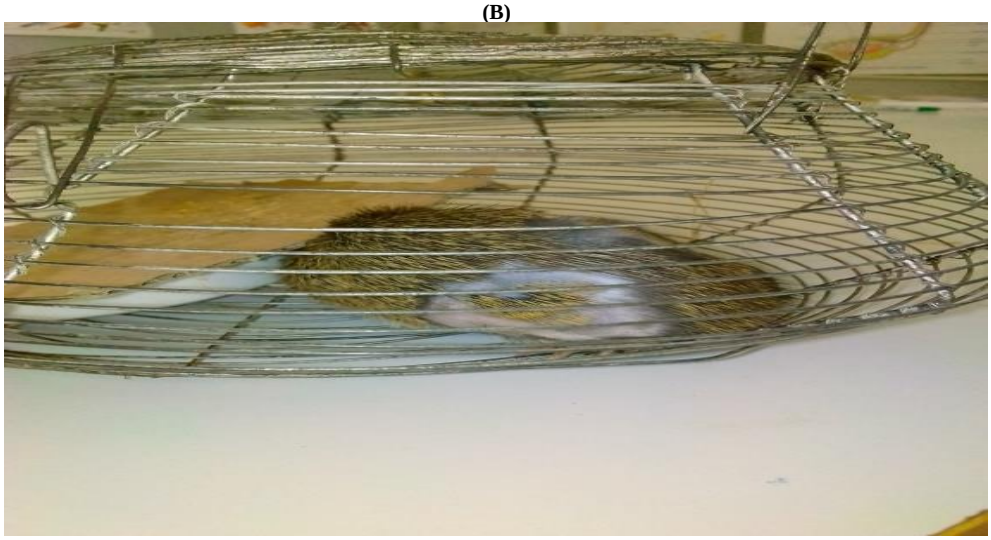
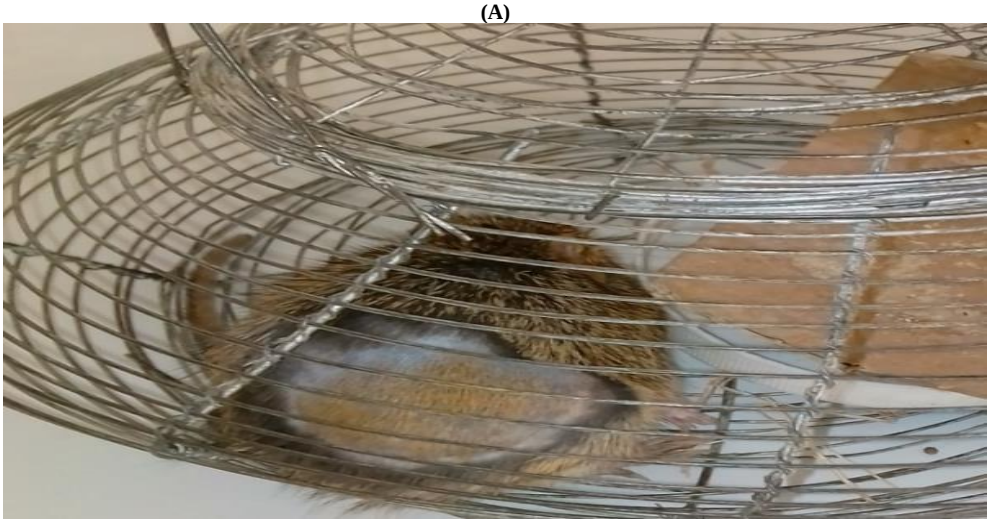


Fig. 2: 7 Days after Last Application of Hair Growth Retardant.

3rd April

(A)



(B)

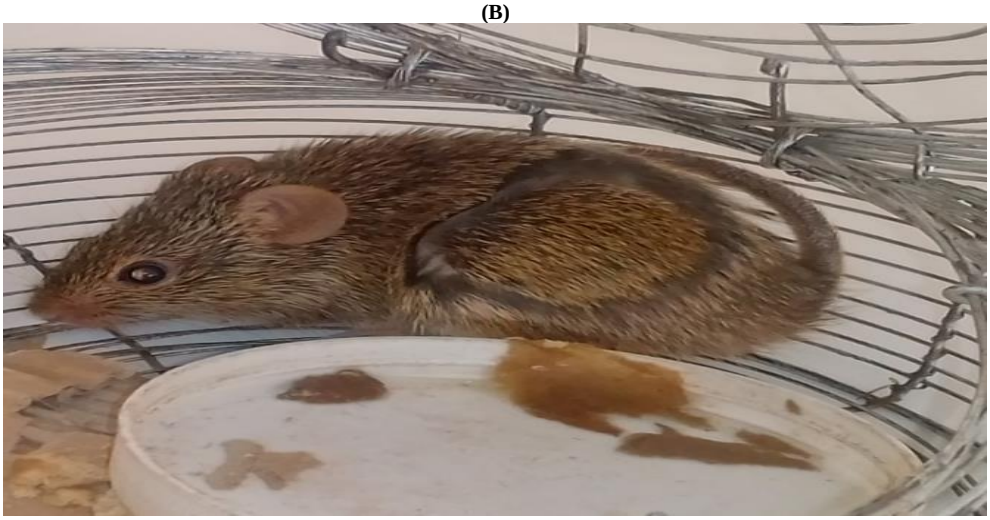


Fig. 3: 14 Days after Last Application of Hair Growth Retardant.

Hair Growth retarding efficiency
Table 4 show that the formula has significant potential of retarding hair regrowth after shaving.

Table 4: Hair Growth Retarding Efficiency

Mice	Hair density	Retarding efficiency (%)
A	2.1	25
B	2.8	

3.2. Discussion

3.2.1. Percentage recovery

The percentage recovery of plant extracts shows that Ethanol extract exhibit 5.36% recovery. This constitute a very small portion of the plants indicating that the concentrations of these bioactive components in plants are very low.

3.2.2. Phytochemical tests

The result from the tests shows phytochemicals such as in table 3. The presence of these bioactive compounds in the extracts makes them medicinal especially as antimicrobial, anti-inflammatory and antioxidative agents. According to Igbinosa et al., 2009, tannins interfere with bacteria cell protein synthesis and are vital in treating ulcerated and inflamed tissues and also intestinal disorder. Alkaloids have been reported also to ease pains and saponin to manage inflammation (Igbinosa et al, 2009 and Hussain et al, 2009). Studies have also shown that flavonoids have greater potential benefit to human health. A study reveals a significant inverse relationship between flavonoid intake and myocardial infection (Jouad 2001).

3.2.3. Hair growth retardant test

The Hair growth Retardant showed evidence of slowing down hair growth. Significant regrowth of hair on mouse A started two weeks after the last application of the formulated hair retardant (plate III) while regrowth of hair started one week in the control, mouse B (plate II) after the last application (plate I). This result is in agreement with that of Neelam (2011). Hair growth inhibition is a reduction process involving the breaking of the S-S bond in the cystine molecule of the hair follicle. Some herbaceous plants extracts contain components with hydroxyl -OH, carbonyl –CO and carboxylic –COOH functional groups which act as reducing agent in an alkaline environment.

Curcumin contained in the Tumeric used in the formula contain four reduction sites that could facilitate the reduction process and consequently slow down hair growth.

3.2.4. Toxicity test

There were no signs of itching, swelling, redness and topical injuries (plate I-III).

3.2.5. Evaluation of hair growth retarding efficiency

It has been noticed that Mouse A treated with the formula showed a 25% significant reduction in the density of hair in the tested area of the mouse (table 4). This implies that the formula has hair growth retarding property

4. Conclusion

Overall, the result of the research provides convincing evidence of the hair growth retarding property of the herbal formula. The research proffers an alternative way forward on how safe natural materials can replace the synthetic ingredients in depilatory creams.

Acknowledgements

I express my sincere thanks to God almighty

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