Effect of *Lawsonia Inermis* Linn in Treatment of Alopecia

Syeda Shazma Kanwal[*], Asma Wazir[**], Nudrat Fatima[***], Ayeman Owais Ghauri[****], Maria Rahat[*], Sanober Kamal[*], Umbreen Farrukh[*****], Saniya Shuja[******], Fehmida Banaras[******]

[*]PhD Fellow, Jinnah University for Women, Karachi, Pakistan
[**]Assistant Professor, Federal Urdu University of Arts, Science and Technology, Karachi, Pakistan
[***]Professor, Faculty of pharmacy, Ziauddin University, Karachi, Pakistan
[****]Associate Professor, Department of Pharmacognosy, Jinnah University for Women, Karachi, Pakistan
[*****]Assistant Professor, Dow University of Health Sciences, Karachi, Pakistan
[******]Pharm-D, Jinnah University for Women, Karachi, Pakistan

Abstract - In this research, effect of *Lawsonia inermis* Linn flowers were evaluated in the dose of 200mg/Kg and 400mg/Kg to treat alopecia in mice models. 18 mice were selected (average wt. 25gm) for this purpose. Total 6 groups were formed, and each group contained 3 mice. First, alopecia is induced in mice by injecting Dihydrotestosterone (1mg diluted in 0.1ml of vehicle) subcutaneously in upper dorsal side daily for 12 weeks. Then mice were treated with *Lawsonia inermis* Linn flowers ethanolic extract and its extract mixture in Olive oil in the dose of 200mg/Kg and 400mg/Kg till 3 months (12 weeks). Their effects in treating alopecia were noted by measuring alopecia index (measuring baldness area). Then their effects were compared with standard drug Minoxidil 5% solution.

It was observed that after 3 months, hair fall area reduced from 3.47±0.33cm^2 (Alopecia Index 4) to 3.41 ±0.37 cm^2 (alopecia index 4) and very slight reduction of hair fall area was noted in control. Hair fall area reduced from 2.83±0.73 cm^2 (alopecia index 4) to 2.73±0.74 cm^2 (Alopecia Index 4) in Test 1 (200 mg/Kg ethanolic extract was applied topically on an alopecia induced area).

From results, it was evaluated that *Lawsonia inermis* Linn flower's ethanolic extract in the dose of 400mg/Kg was significantly effective in treating alopecia. 200mg/Kg ethanolic effect was also found to be effective. Ethanolic extract mixture inolive oil also possess hair growth promoting potential in treating testosterone induced alopecia.

Key words: *Alopecia, Alopecia index, Dihydrotestosterone, & Lawsonia inermis Linn flowers*

Introduction

Alopecia is a condition which is patchy, non-scarring, not predictable hair loss (in some types) and starting loss of hairs at different time of age due to genetic problem. In pathogenesis of sometypes of alopecia includes immune reactions (organ specific and non-specific) which is caused by T-Cells (T-lymphocytes) and may be genetic as in androgenetic alopecia. It is also caused by other factors like harmful and infectious chemicals, neurologic factors, anxiety and stress, cytokines, irregular keratinocytes and melanocytes (Madani et al., 2000)

There are various FDA approved drugs and off-Label pharmacological drugs for the treatment ofalopecia. Two FDA approved drugs are minoxidil and finasteride are used to treat alopecia whileother drugs include spironolactone, oral contraceptives, glucocorticoids, cyclosporin A, bimatoprost and diphenylcyclopropenone (Pourang A et al., 2020). These pharmacological treatments can cause many side effects like hypersensitivity reactions, hypopigmentation, atrophy, local irritation, abnormal liver function test, immune-suppression, abnormal lipids profile, nephrotoxicity, folliculitis, contact dermatitis, itching, rash, fainting, tachycardia, impotence, sexual dysfunction and decrease libido in males, sexual dysfunction may also lead todepression (Haber RS et al., 2019 and Ferreira SB et al., 2021).

These pharmacological therapies are expensive and requires long term treatment. In many cases, regrowth of hairs occurred but sometimes regrowing of hairs not seen. Recurrence of alopecia andhair loss is very common after discontinuation of therapy (Ashique S et al., 2020). Due to the above-mentioned side effects of pharmacological treatments for hair loss and recurrence of alopecia on discontinuation of therapy, create an urge to discover the phytotherapy or herbal drugs for the treatment of alopecia.

The non-pharmacological treatments or herbal drugs which can use to treat alopecia are *Avicennia marina*, *Cuscuta reflexa*, *Curcuma aeruginosa*, *Camellia sinensis*, *Serenoa repens*, *Phyllanthus niruri*, *Sophora flavescens*, *Glycyrrhiza glabra*, *Thuja orientalis*, *Ocimum sanctum*, *Allium cepa*., *Azadiracta indica*, *Ginkgo biloba*, *Simmondsia chinensis*, *Juglans regia*, *Trigonellafoecum granecum*, *Cucurbita pepo*, *Trifolium pretense*, *Saw palmetto* and Panax ginseng (Sundaram SS et al., 2019).

Alopecia can also be treated by other alternative and complementary therapies by givingpsychological and spiritual treatments like Acupuncture, Aromatherapy, Hypnosis and psychotherapy (Hosking AM et al., 2019).

Lawsonia inermis is a small dicotyledonous shrub and about 2.6 meters in height. It has multiple and smooth branches, branchlets are covered with spines. Leaves are elliptical, smooth, complete(entire), acuminate, opposite arrangement, dorsal side has depressed veins, sub –sessile and 1.5 to 5cm x 0.5 to 2 cm lanceolate. Its petiole is small, apex acute and base is tapered. Its branches are green when young then became red on aging. Its bark is greyish brown. Young shrub is unbranched but developed spined tip with age. It grows two times in a year. It is native to South West Asia and North Africa. It is cultivated broadly in hot tropical countries. It is used for ornamental and dying purposes (Shekhar TC et al., 2016 & Chaudhary G et al., 2010). Different phytoconstituents were detected in different parts of *Lawsonia inermis* Linn, like in seed Arachidonic acid, Linoleic acid, Palmitic acid and Stearic acid were found, in bark Isoplumbagin, Triterpenoids-Hennadiol, Aliphatics or 3-methyl-nonacosan-1-ol & Napthoquinone were detected, in leaves β-Sitosterol, Apigenin, gallic acid, 2-hydroxy-1,4 Napthoquinone (Lawsone) were reported, in roots 24 β-ethylcholest-4-en-3β-ol were reported and in flowers 0.02% Essential oil with 90% Ionones and β-Ionones, Carbohydrate, Amino acids& Proteins, Phytosterols, Glycosides, Flavonoids, Starch, Alkaloids and Tannins were found an in whole plant Laxanthone I, II & III and n-triacontanol were reported (Chaudhary G et al., 2010& Fathima SN et al., 2018). *Lawsonia inermis* L. has pharmacological properties like hepatoprotective, antioxidant, antifertility, wound healing, antiparasitic, anticoagulant, antifungal, antidiabetic, analgesic, antiviral, anti-inflammatory and enzyme inhibitory properties(Ahmed S et al., 2000, Dasgupta T et al., 2003, Munshi SR et al., 1977 and Okpekon T et al., 2004).

Materials and methods

Collection of Flowers and authentication

Lawsonia inermis flowers were collected from horticulture garden of Karachi vicinity in the spring season from April to June 2021. The flowers were identified by Prof. Dr. Iqbal Azhar, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi, Pakistan and issued herbarium voucher number as **LIF-05-21**.

Microscopic Evaluation

Microscopic evaluation of Lawsonia inermis Linn flowers were done by using reagents like 50% glycerin through electronic microscope Olympus by setting magnification 10X. Powder microscopy photographs were taken by digital camera model M863(B) and smart phone OPPO model A57.

Preparation of extracts of Lawsonia inermis Linn. flowers

The *Lawsonia inermis* flowers were washed with tap water then with distilled water. Then cleanthe flower and shadow dried them at room temperature for two to three days.

Ethanolic extract

Dried *Lawsonia inermis* Linn flowers under the shade for 7 days. Then reduced the size of flowers by grinding them into a dry grinder. Take 1 Kg of dried powder of flower and extractedin ethanol for 7 days and shaking time to time. After one week filtered the extract through Whatman filter paper. Then filtrate was evaporated in a rotary evaporator. After evaporation, dried the extract in a fume hood. The dried extract then collected in a glass container.

Mixture with olive oil

Measured the amount of *Lawsonia inermis* ethanol's extract and mixed with specified amount ofOlive oil to prepared doses of 200 and 400 mg/kg for 24 hours and keep the oil in glass container.

Phytochemical studies

Phytochemical studies were performed to detect Alkaloids, Glycosides, Carbohydrate, Flavonoids, Tannins, Proteins, Saponins, Phytosterols & Phenols as per the standard phytochemical analysis method (Arora R et al., 2020, Mounika A et al., 2020 & Shaikh JR et al.,2020).

Experimental animals

In this study, male and female Swiss Albino mice were taken. These mice were purchased fromDow University of Health Sciences, Karachi, Pakistan. The mice were grouped separately in plastic boxes in animal house of Faculty of Pharmacy, Jinnah University for Women, Karachi. The mice were kept at 20 ± 2ºC temperature and recurring cycle of 12 hours of dark and light periods. The animals of average 25gm weight were distributed in 6 groups, 3 mice per group inseparate cage for each group. During the whole study period, the mice could move freely for water and food. The mice were adjusted for laboratory condition for at least 7 days before starting the experiment.

Ethical approval

All procedures of experiment were reviewed and approved by Institutional Animal Ethics Committee and issued ethical approval letter with number as JUW/IERB/PHARM- ARA013/2022

Methodology

Toxicity studies

Dermal irritation test

The test was done to evaluate the irritational sensitivity of *Lawsonia inermis* Linn extract inwhite Rabbits via topically application. As per the OECD guidelines 6 white male Rabbits having weight around 2.7 kg to 3.2kg weretaken for the dermal irritation test.

On first day of study, at Day 0, right side of each Rabbits were taken as Control. Rabbits were shaved from lateral sides and trunk region (around 10% surface area of body) via electric shaver. The left shave side (6 cm^2) were taken for toxicity test of the Lawsonia inermis Linn flower's ethanolic extract. Then abrasions were made on left shaved side.

On Day 1 of study, the *Lawsonia inermis* Linn extract in the dose (0.5ml/Site) were applied to both non-abraded intact Skin and Skin with abrasion (Abraded till Stratum Corneum) on left shaved side. Abrasions were made carefully. So, that it would not cause bleeding. The skin then covered with patches of gauge and fixed with tape then the Rabbits were kept backin their cages.

After 1 day (24 hours period), the gauge patches were detached, and dermal reaction was notedby viewing the signs of oedema and erythema in each six rabbits between 1 hour to 24 hours period. If skin irritation was reported, then it will record as per the "System of Draize Score" (Djerrou Z et al., 2013).

Testing for treatment of alopecia

Induction of alopecia

Dihydrotestosterone (1 mg in 0.1 ml of vehicle) will subcutaneously administer at upper dorsalside of mice daily for 12 weeks except the weekends to create the models for alopecia (Matias JR.,1989).

Dosing in groups of mice

To test the plant extract of *Lawsonia inermis* Linn Flower's, ethanol extract and Olive oil will beapplied topically at doses of 200 and 400 mg/kg in mice of test groups for 12 weeks. Standard drug will be applied to positive control group and only vehicle will apply topically in negative control group for 3 months. 6 groups of mice will be formed, and 3 mice will be kept in each group.

(1) Control (alopecia induced animals, without any treatment).
(2) Standard (alopecia induced animals, treatment with standard drug).
(3) Test group 1 (alopecia induced animals, treatment with 200 mg/kg of ethanol extract).
(4) Test group 2 (alopecia induced animals, treatment with 400 mg/kg of ethanol extract).
(5) Test group 3(alopecia induced animals, treatment with 200 mg/kg plant extract + Olive oil).
(6) Test group 4 (alopecia induced animals, treatment with 400 mg/kg plant extract + Olive oil).

Measurement for reduction of hair fall

In the period of treatment, mice will be screen weekly for reduction of hair fall based on alopeciaindex, measured as 0, 1, 2, 3 and 4 described no hair fall, less hair fall, alopecia affected area of 1cm^2, alopecia affected area of 2 cm^2, alopecia affected area of 3 cm^2 and alopecia effected area of 4 cm^2 respectively (see Table 1) (Matias JR.,1989).

Statistical analysis

The experimental Data was analyzed Statistically by One Way ANOVA by using IBM SPSS Statistics Software, Version 21. The data presented in the form of Mean ± S.E.M. Significancewas taken as $p < 0.05$.

Results

Powder microscopy of lawsonia inermis flowers

Lawsonia inermis dried powder of flower was brown in color. During powder microscopy of *Lawsonia inermis* flowers, different structures or features were observed in microscope (as shown in fig 1),

a) Epidermal Cells
b) Coloring matter
c) Glandular trichome
d) Trichome
e) Epidermal cells containing coloring matter
f) Inulin
g) Oil cells
h) Pollen grains
i) Tannin cells
j) Perisperm
k) Cuticular cells
l) Vessels
m) Lignified fibers
n) Glandular cells in Testa
o) Sclereids

Phytochemical screening of lawsonia inermis flower's

Ethanolic extract

Phytochemical screening of ethanolic extract of *Lawsonia inermis* flowers showed the presenceof different phytoconstituents as mentioned in the following table 2 and fig 1. Different colors and ppt showed the presence of alkaloids, glycosides, flavonoids, phytosterol and tannins whilesaponins and fixed oil were absent in their respective tests.

Toxicity study

Dermal irritation test

After performing dermal irritation test on 6 white male rabbits as per OECD guidelines. It wasobserved that after one day (24 hours period), no signs of edema & erythema were observed inbetween 1 hour to 24 hours period. Hence, *Lawsonia inermis* Linn. Flowers extract is safe to apply topically as a drug to treat alopecia.

Evaluation of effect of Lawsonia inermis flowers in the treatment of alopecia

Hair fall area in all groups were measured in cm^2 by subcutaneous administration of testosteroneinjection. Hair fall area was measured as 3.47±0.33 cm^2, 2.83±0.73 cm^2 ,2.5±0.76 cm^2, 3.33±0.67 cm^2 ,3.58±0.30 cm^2 and 3.00±0.58 cm^2 in control, Test 1, Test 2, Test 3, Test 4 and standard group of mice respectively as shown in Table 3.

Topical application of test doses of l. Inermis flower extract orthe treatment of alopecia in different groups of mice

The doses were given to 6 groups of mice as per the method given in methodology section. Afterthe 3 months period of treatment, reduction of hair fall area was measured weekly and alopecia index were noted for each group of mice as 0, 1, 2, 3, 4 and 5 means no hair fall, less hair fall, alopecia affected area of 1 cm^2, alopecia affected area of 2 cm^2, alopecia affected area of 3 $cm^2$and alopecia effected area of 4 cm^2 respectively.

Hair fall area after 3 months of treatment

It was observed that after 3 months, hair fall area reduced from 3.47±0.33cm^2 (Alopecia Index 4) to 3.41±0.37cm^2 (alopecia index 4) and very slight reduction of hair fall area was noted in control.

Hair fall area reduced from 2.83±0.73 cm^2 (alopecia index 4) to 2.73±0.74 cm^2

(Alopecia Index 4) in Test 1 (200 mg/Kg ethanolic extract was applied topically on an alopecia induced area).

Hair fall area reduced from 2.5±0.76 cm^2 (alopecia index 3) to 2.14±0.86 cm^2 (alopecia index 3) in Test 2 (400mg/Kg ethanolic extract was applied topically on an alopecia induced area).

Total reduction of hair fall area was 0.11cm^2 during treatment period of 3 months. Hair fall area reduced from 3.33±0.67cm^2 (Alopecia Index 4) to 3.18±0.66cm^2 (Alopecia Index 4) in Test group 3 (200mg/Kg extract in Olive oil was applied topically on an alopecia induced area,). Total reduction of hair fall area was 0.15cm^2 during treatment period of 3 months.

Hair fall area reduced from 3.58±0.30cm^2 (Alopecia Index 4) to 3.17±0.18cm^2 (Alopecia Index 4) in Test 4 in which 400mg/Kg extract in Olive oil was applied topically on an alopecia induced area. Total reduction of hair fall area was 0.41cm^2 during treatment period of 3 months.

In standard group, 5% minoxidil solution was applied topically and hair fall area reduced from 3±0.58cm^2 (Alopecia Index 4) to 2.65±0.39cm^2 (Alopecia Index 4) in Standard Group. Total reduction of hair fall area was 0.35cm^2 during treatment period of 3 months. The results showed that the effect of 400mg/Kg ethanolic extract were found to be more effective in treating alopecia.

Discussion

In this research, the effect of *L. inermis* Linn. flowers were evaluated in the treatment of hair fall or alopecia. Alopecia is not a life-threatening disease or neither caused any kind of physical pain, but it is need to be treated because it exerts psychological effects in patients whichcan hinder in their social interactions, working efficiency and confidence.

Symptoms and characteristics of alopecia depend on the type of alopecia. Genetic and immunological factors are considered the main causative factors of the disease. There are reportsabout the co-existence of alopecia with different diseases like pernicious anemia, vitiligo, thyroiditis, hormonal problems, stress or depression. Baldness may also be caused as the result ofside effects of the drugs such as antidepressants, antidiabetics and many other medicines which are used in cardiovascular disorders. Baldness may also cause by routine hair styles in which pulling of the hairs occur as in tight pony tail and braids (Johns HR et al., 2021 & Ho CH et al., 2021).

There are various FDA approved drugs and off-Label pharmacological drugs for the treatment ofalopecia. Two FDA approved drugs are minoxidil and finasteride are used to treat alopecia whileother drugs include spironolactone, oral contraceptives, glucocorticoids, cyclosporin A, bimatoprost and diphenylcyclopropenone. These pharmacological treatments can cause many side effects like hypersensitivity reactions, hypopigmentation, atrophy, local irritation, abnormal liver function test, immune-suppression, abnormal lipids profile, nephrotoxicity, folliculitis, contact dermatitis, itching, rash, fainting, tachycardia, impotence, sexual dysfunction and decrease libido in males, sexual dysfunction may also lead to depression (Pourang A et al., 2020,Haber RS et al., 2019 & Ferreira SB et al., 2021).

These pharmacological therapies are expensive and requires long term treatment. In many cases, regrowth of hairs occurred but sometimes regrowing of hairs not seen. Recurrence of alopecia andhair loss is very common after discontinuation of therapy. Due to the above-mentioned side effects of pharmacological treatments for hair loss and recurrence of alopecia on discontinuation of therapy, create an urge to discover the phytotherapy or herbal drugs for the treatment of alopecia.

The non-pharmacological treatments or herbal drugs which can use to treat alopecia are *Avicennia marina, Cuscuta reflexa*, *Curcuma aeruginosa, Camellia sinensis*, *Serenoa repens*, *Phyllanthus niruri*, *Sophora flavescens*, *Glycyrrhiza glabra*, *Thuja orientalis*, *Ocimum sanctum*, *Allium cepa*., *Azadiracta indica*, *Ginkgo biloba*, *Simmondsia chinensis*, *Juglans regia*, *Trigonellafoecum granecum*, *Cucurbita pepo, Trifolium pretense*, *Saw palmetto* and *Panax ginseng* (Sundaram SS et al., 2019).
Many of the herbal therapies are used on the basis of personal experiences and lack of scientificdata for their effectives in hair fall. It is essential to work and find the scientifically proven phytotherapy for the treatment of alopecia with less adverse effects (Hosking AM et al., 2019).

Lawsonia inermis leaves are found to be effectively used in hair dye, prevention of premature hair graying, dandruff, and some type fungal infections. Henna also reduces the hair fall by control pHof scalp. L inermis contained constituents such as flavonoids, carbohydrates and lawsone. In haircosmeceuticals, powder of dried henna leaves used as a base for the nutritional benefits as well as for its effect on hair growth and the texture (Zamani P et al. 2021, Guo EL et al., 2017 & Pal RS et al., 2018).

Lawsonia inermis seeds can also treat alopecia. It was suggested that, arachidonic acid (fatty acid) found in Lawsonia inermis seeds can inhibits 5α-reductase enzyme and encourages growthof hairs via increasing proliferation of hair follicles. It was also suggested that arachidonic acid can encourages mitosis, elongation of hairs, matrix keratinocytes proliferation, angiogenesis (formation of new blood vessels) & in dermal papilla, it enhances growth factor expression (Semwal RB et al., 2014).

But there was no any literature available on the effect of *Lawsonia inermis* flowers in the treatment of alopecia. In this study, evaluate the effect of Lawsonia inermis flowers ethanolic extract & its mixture with Olive oil at the doses of 200mg/ Kg & 400mg/ Kg in hair loss. For theinvestigation of the treatment of hair fall, alopecia was induced in 6 groups of mice by subcutaneously testosterone injection (1mg in 0.1ml) at upper dorsal side for 3 months. The test doses were administered topically for three months, results were noted by measuring the hair fallarea. The results were compared with control and standard group of mice. 5% minoxidil solution was used topically as standard drug.

Lawsonia inermis flowers contains essential oils (α-Ionone & β-Ionone), resins, nitrogenous substance. It was found that ionone (sesquiterpenes) present in flowers of Lawsonia inermis may promote the growth of hairs and stops hair falling by gene expression and protein synthesis. It was also found that ionone converts hair follicles from inactive telogen phase to active anagenphase (Rajwar S et al., 2013 & Sultana S et al., 2013).

The presence of flavonoids in the extract of Lawsonia inermis flowers may play important role inthe treatment of different types of alopecia which improved the condition of hair follicles by enhancement of blood circulation in the scalp (Singh S et al., 2023).

Amino acids, vitamins, minerals and terpene derivatives are the important phytoconstituents in the reduction and prevention of hair fall. The phytochemicals in the extract of Lawsonia inermis flowers may synergistically work with Olive oil for the treatment of alopecia. Beside the antioxidant and reduction of hair loss effect ofOlive oil, it also enhances the absorption of phytoconstituents of Lawsonia inermis flowers whichapplied topically in the region of hair fall area.

The various mechanisms may have role in the treatment of alopecia such as increase the cell division of hair matrix by increase the blood circulation, release of hormones by stimulation ofnervous system, involvement of amino acids, vitamins, minerals and other nutrients on hair growth cycle by gene expression (Liu J et al., 2009 & Bassino E et al., 2020).

Conclusion

Hair growth promoting effect of *Lawsonia inermis* Linn. flowers ethanolic extract and the mixture of extract with olive oil were evaluated in mice after inducing alopecia. It was observed that 400mg/kg of ethanolic extract of *Lawsonia inermis* flowers was effective in treating alopecia. The mixture of *Lawsonia inermis* extract with Olive oil was also produced reduction of hair fall. It was found safe and effective for topical application in the investigation of the natural treatment for hair fall. It will be supportive and helpful in future for other researchers in cosmeticformulation for hair fall and different types of alopecia.

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Conflict of interest

There is no any conflict of interest in doing this research work.

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Tables

Table 1: Measurement of Hair fall Reduction by Measuring Alopecia Index

Reduction of Hair fall Alopecia Index	**Reduction of Hair fall Alopecia Index**
No hair fall	0
Less hair fall	1
Alopecia affected area of 1 cm^2	2
Alopecia affected area of 2 cm^2	3
Alopecia affected area of 3 cm^2	4
Alopecia affected area of 4cm^2	5

Table 2: Phytochemical analysis of Lawsonia inermis Linn flower's extract

Phytochemicals	Observation
Alkaloids	Present
Glycosides	Present
Carbohydrates	Present
Flavonoids	Present
Tannins	Present
Proteins	Present
Saponins	Absent
Fixed Oil	Absent
Sterol/ Phytosterol	Present

Table 3: Reduction of hair fall area after treatment during 3 months.

Groups	Dose (Topical)	Hair fall (cm^2) After Testosterone Injection 1mg/0.1 ml	Reduction of Hair fall after treatment (cm^2) Mean ± S.E.M											
			1st Month				2nd Month				3rd Month			
		Mean ± S.E.M	1 Week	2 Week	3 Week	4 Week	1 Week	2 Week	3 Week	4 Week	1 Week	2 Week	3 Week	4 Week
Control	Only Vehicle	3.47±0.39	3.47 ± 0.39	3.47 ± 0.39	3.47 ±0.39	3.47 ±0.39	3.47±0.39	3.47±0.39	3.43±0.37	3.43±0.37	3.42 ±0.37	3.42 ±0.37	3.42 ±0.36	3.41± 0.37
Test 1	200mg /Kg Ethanolic Extract	2.83±0.73	2.83± 0.73*	2.83± 0.73*	2.83 ±0.73*	2.83 ±0.73*	2.83±0.73*	2.83± 0.73*	2.78± 0.71*	2.77± 0.71*	2.77 ±0.73*	2.76 ±0.73*	2.75 ±0.73*	2.73 ± 0.74*
Test 2	400mg /Kg Ethanolic Extract	2.5±0.76	2.50 ±0.76**	2.50 ±0.76**	2.50± 0.76**	2.50± 0.76**	2.50± 0.76**	2.50± 0.76**	2.48± 0.76**	2.46± 0.76**	2.44 ±0.76**	2.43± 0.76**	2.41± 0.76**	2.39±0.76**
Test 3	200mg /Kg Olive Oil Extract	3.33±0.67	3.33 ±0.67	3.33 ±0.67	3.33 ±0.67	3.33 ±0.67	3.33± 0.66	3.31± 0.66	3.29± 0.66	3.27± 0.66	3.24 ±0.66	3.22 ±0.66	3.20 ±0.66	3.18 ± 0.66
Test 4	400mg /Kg Olive Oil Extract	3.58±0.30	3.58 ±0.30	3.58 ±0.30	3.58±0.30	3.58 ±0.30	3.58±0.30	3.56±0.30	3.54±0.30	3.51±0.30	3.49 ±0.31	3.47 ±0.31	3.45 ±0.31	3.42± 0.31
Standard	Minoxidil (Minoxin 5% Solution)	3.00±0.58	3.00± 0.58	3.00± 0.58	3.00 ±0.58	3.00 ±0.58	2.99±0.58	2.97±0.58	2.96± 0.58	2.94± 0.58	2.91 ±0.57	2.89 ±0.57	2.87 ±0.57	2.85 ±0.58

* Data presented as mean ± S.E.M; Significant (Significant result at $p < 0.05$). [Control=Only Vehicle; Test 1=200mg/Kg Ethanolic Extract; Test 2=400mg/Kg Ethanolic Extract; Test 3=200mg/Kg Ethanolic Extract mixture with Olive Oil; Test 4=400mg/Ethanolic Extract mixturewith Olive Oil; Standard= 5% Minoxidil Solution

Table 4: Measurement of hair fall reduction before & after treatment by measuringalopecia index

Groups	Dose (topical)	Before treatment Hair fall area	Alopecia Index	After treatment Hair fall area	Alopecia Index
Control	Only Vehicle	3.47±0.39	4	3.41±0.37	4
Test 1	**200eth extract**	**2.83±0.73***	**4**	**2.73±0.74***	**4**
Test 2	**400eth extract**	**2.50±0.76****	**3**	**2.14±0.86****	**3**
Test 3	**200eth extract With Olive oil**	3.33±0.67	4	3.18±0.66	4
Test 4	**400eth extract with Olive oil**	3.58±0.30	4	3.17±0.18	4
Standardd	Minoxin 5% Solution (Minox idil)	3.00±0.58	4	2.85± 0.58	4

*Data presented as mean ± S.E.M; Significant (Significant result at $p < 0.05$), * = Significant value, ** = more significant value. [Control=Only Vehicle; Test 1=200mg/Kg Ethanolic Extract; Test 2=400mg/Kg Ethanolic Extract; Test 3=200mg/Kg Ethanolic Extract mixture withOlive Oil; Test 4=400mg/Ethanolic Extract mixture with Olive Oil; Standard= 5% Minoxidil Solution].

1.0– 1.5 cm^2 (Alopecia affected area) = 2 (Alopecia Index); 1.6 – 2.0 cm^2 (Alopecia affected area) = 3 (Alopecia Index); 2.6 – 3.0 cm^2 (Alopecia affected area) = 4 (Alopecia Index); 3.6 – 4.0 cm^2 (Alopecia affected area) = 5 (Alopecia Index).

Figures

Fig 1: Lawsonia inermis Linn flowers

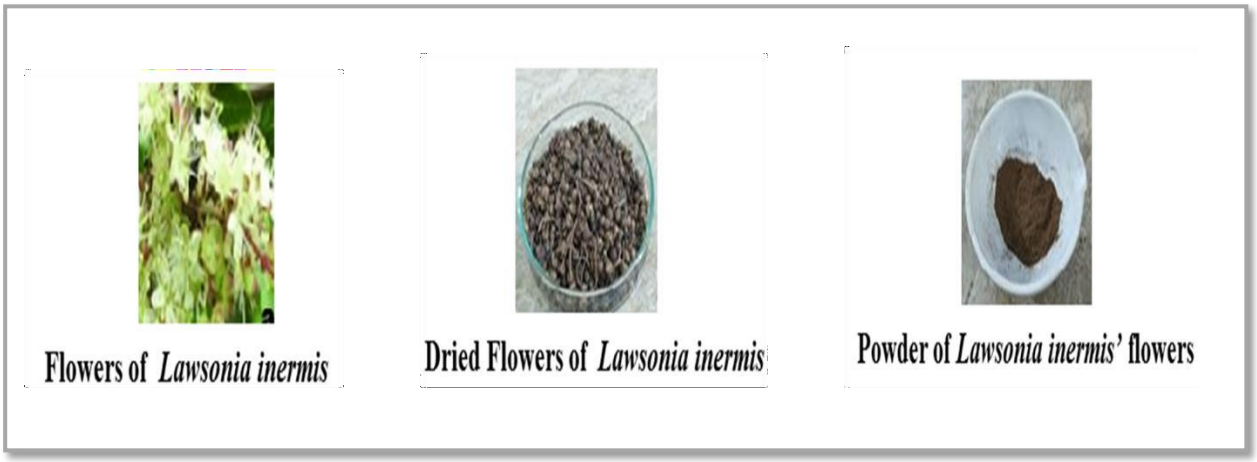


Fig 2: Powder microscopy of Lawsonia inermisLinn flowers

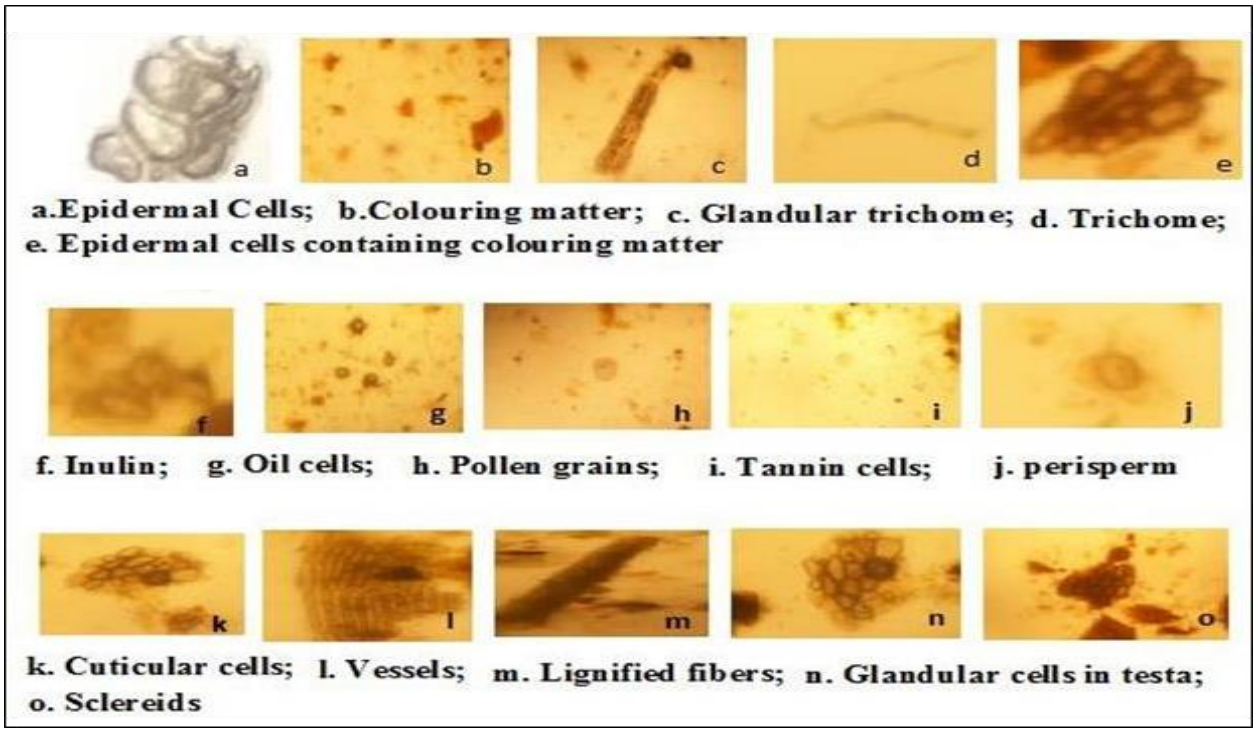


Fig 3: Graph showing reduction of hair fall area during 3 months

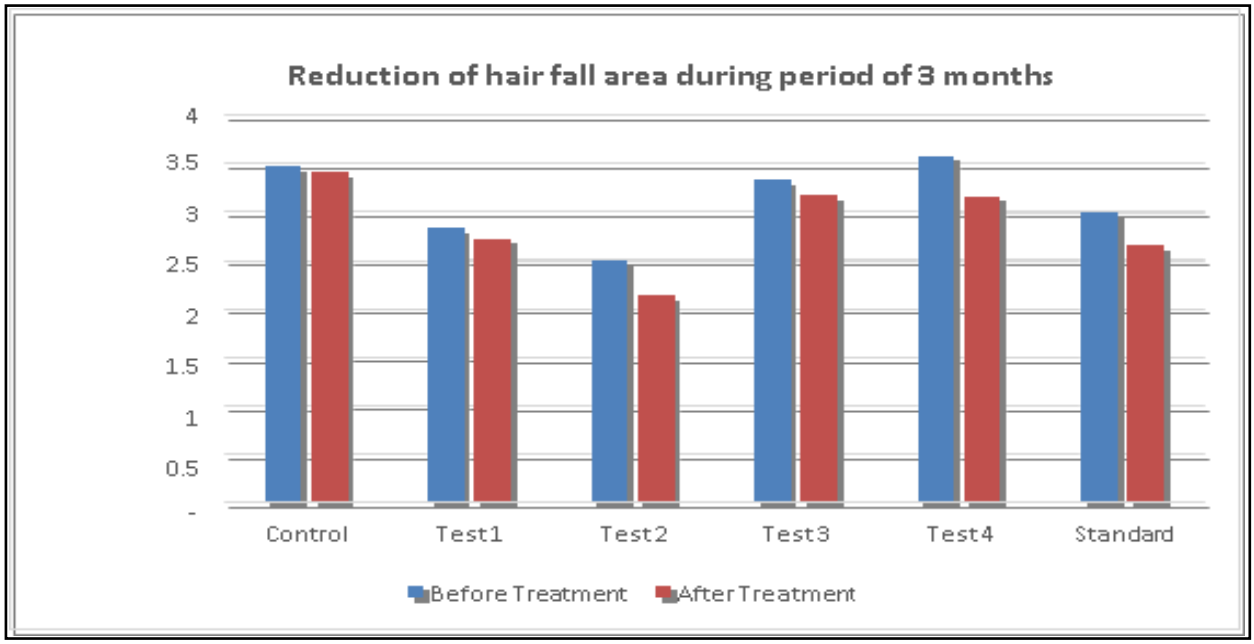
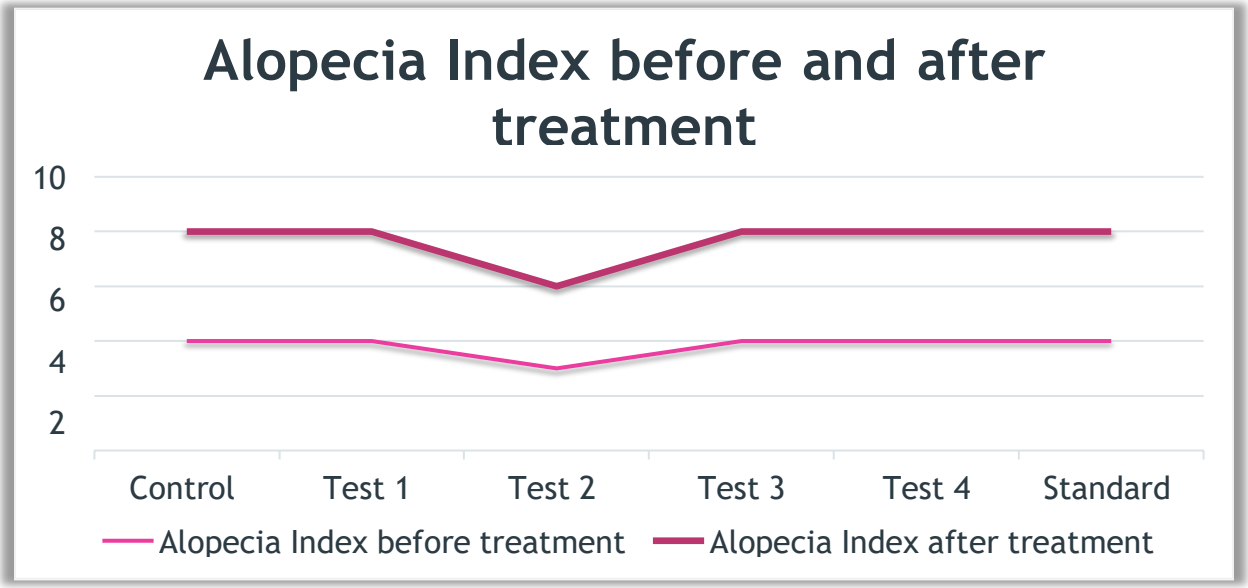


Fig 4: Graph showing alopecia index before & after treatment



Authors

First Author - Full Name: Syeda Shazma Kanwal, Qualification: Pharm-D, M-Phil (Pharmacognosy) & PhD (Pharmacognosy) Fellow, Institute: Jinnah University for Women, Karachi, Pakistan.
Second Author - Full Name: Asma Wazir, Qualification: BPharm, MPharm (Pharmacognosy) & PhD (Pharmacognosy), Institute: Federal Urdu University of Arts, Science and Technology, Karachi, Pakistan.
Third Author - Full Name: Nudrat Fatima, Qualification: BPharm, MPhil (Pharmaceutics) & PhD (Pharmacognosy), Institute: Ziauddin University, Karachi, Pakistan.
Fourth Author - Full Name: Ayeman Owais Ghauri, Qualification: B.E.M.S, M-Phil & PhD Eastern Medicine, Institute: Jinnah University for Women, Karachi, Pakistan.
Fifth Author - Full Name: Maria Rahat, Qualification: Pharm-D, MPhil (Pharmacognosy) & PhD (Pharmacognosy) Fellow, Institute: Jinnah University for Women, Karachi, Pakistan.
Sixth Author - Full Name: Sanober Kamal, Qualification: BPharm, MPhil (Pharmacognosy) & PhD (Pharmacognosy) Fellow, Institute: Jinnah University for Women, Karachi, Pakistan
Seventh Author - Full Name: Umbreen Farrukh, Qualification: PhD (Pharmacognosy), Institute: Dow university of health sciences, Karachi, Pakistan
Eight Author - Full Name: Saniya Shuja, Qualification: Pharm-D & MPhil (Pharmacognosy) Fellow, Institute: Jinnah University for Women, Karachi, Pakistan
Ninth Author - Full Name: Fehmida Banaras, Qualification: Pharm-D & M-Phil (Pharmaceutical Chemistry) Fellow, Institute: Jinnah University for Women, Karachi, Pakistan,

Correspondence Author – **Full Name**: Syeda Shazma Kanwal, **Qualification:** Pharm-D, M-Phil (Pharmacognosy) and PhD (Pharmacognosy) Fellow, **Institute:** Jinnah University for Women, Karachi, Pakistan