

FORMULATION AND EVALUATION OF HAIR TONIC FROM *ZIZIPHUS JUJUBA* LEAF EXTRACT

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ABSTRACT

Background: The reasons for loss of hair are exposure of the hair to alkaline medium, decreased blood flow, and scalp diseases. Therefore, it is important to develop a novel formulation that prevents the loss of hair, which promotes the hair growth. **Aims and Objectives:** The aim of the study was to formulate and evaluate a Hair tonic from *Ziziphus jujuba* leaf extract using the coconut oil as a base. This novel formulation maintains acidic pH (≤ 5), increases blood flow and prevents the scalp infections and the above three parameters promotes the hair growth. **Materials and Methods:** Acute toxicity studies were carried out to *Ziziphus jujuba* extract as per OECD test guidelines 423. The prepared formulations were assessed for Primary skin irritation test. The hair growth was observed for 30 days and evaluated for the qualitative parameters like initiation time, completion time and quantitative parameters like hair length, hair weight. **Results:** The study of the hair growth revealed that the formulation F3 had similar growth rates comparable to that of standard (2% Minoxidil). **Conclusion:** From this work, it was concluded that this novel hair tonic formulation possesses hair growth promoting activity.

Keywords: *Ziziphus jujuba*, Hair tonic, Qualitative, Initiation time, Hair length, Minoxidil.

INTRODUCTION

Hair is one of the essential parts of the body derived from the ectoderm of the skin and it is one of the protective appendages on the body. It is an important overall appeal of the human body. Although there are many medicaments available in the market, many people suffer from hair loss.¹ The reasons for loss of hair are exposure of the hair to alkaline medium, decreased blood flow, and diseases of the scalp. Therefore, it is important to develop novel formulations that prevent hair loss and promote hair growth. Because of the side effects associated with the drugs of synthetic origin, people are interested in

the usage of novel herbal hair growth formulations.²

Ziziphus jujuba is a thorny plant, belongs to the family Rhamnaceae that is widely distributed in Europe and South Eastern Asia. The leaves are used for hypoglycemic effect³, Antiulcer⁴, Sweetness inhibitors⁵, Anti-Pyretic⁶, Anti-inflammatory⁷⁻⁸, Anti-obesity⁹, Anti-allergic¹⁰, Antibacterial effect¹¹, Diuretic, Emollient, Expectorant, Sedative, Blood purifier and Anti-diarrheal¹⁸. The bark of the plant is used as Anti-helmenthic¹², Anti-fertility and in wound healing¹³. Fruits are used as a liver tonic, weight gain, Antioxidant¹⁶, Hepatoprotective¹⁸, Anti-

diabetic¹⁶, Anticancer effect¹⁷. Stones of the plant show Antifungal¹⁴ and Anxiolytic activity¹⁵.

The present study focused on scientific investigation of the hair growth potential of the leaf extract of *Ziziphus jujuba*. As previously reported *Ziziphus jujuba* leaf has Antibacterial activity¹¹ which make it beneficial against dandruff and scalp infections. The addition of stearic acid to the coconut oil maintains acidic pH (≤ 5), this acidic pH increases the blood flow, because human hair and scalp oil, sebum have a pH balance between 4 -5. This natural hair acidity prevents growth of fungi and bacteria in the hair, scalp and keeps the cuticle closed and healthy. Coconut oil is selected as a base for formulation development as it locks moisture on the scalp and protects the hair from environmental factors. It also increases blood circulation and makes it possible for hair follicles to get more oxygen and nutrients, minerals, etc.

MATERIALS AND METHODS

Leaf collection and Authentication

The leaves were collected in August-September from the Garden of Santhiram College of pharmacy, Nandyal, Kurnool district, Andhra Pradesh. The leaf specimens were authenticated by Professor P. Prasada rao, PSC and KVSC Government College, Nandyal, Kurnool district, Andhra Pradesh. After a thorough investigation, leaves were checked for any pathological disorders and contamination of other plants.

Preparation of Extract

The fresh leaves were collected from the plant of *Ziziphus jujuba* and rinsed with distilled water. Dried under shade, powdered and passed through sieve no 40. It is then defatted by maceration with n-hexane. The defatted powder was extracted with ethanol. The extractive value was found to be 13% w/w.

PREPARATION OF HAIR TONIC FORMULATIONS

Three different formulations containing 1% w/w, 2% w/w, 3% w/w of ethanolic extract of *Ziziphus jujuba* were prepared by using coconut oil as a vehicle. 1% stearic acid was incorporated into the formulation with the help of alcohol as co-solvent. The composition of formulations given in table 1.

TESTS FOR OILS AND EXTRACT²⁰

- Acid value
- Saponification value
- Test for pH

Experimental animals

Five week old rats were kept in polypropylene cages and maintained on a standard laboratory diet, water ad libitum. They were housed in an air-conditioned room with 12:12 h light and dark cycle for 7 days prior to the experiment.

Acute toxicity study²²⁻²³

Acute toxicity studies were carried out for the ethanolic extract of *Ziziphus jujuba*. Approval from the Institutional ethical committee was taken before starting the experiment. The Acute toxicity study was carried out as per OECD guidelines. Animals were starved for 12hrs and divided into 5 groups (n= 6). A divided doses 0.5g/1g/ 2g/ 3g/4g/ 5g were given orally based upon the body weight of each animal. Animals were observed for every 2 hours up to 24 hours.

Treatment

The Wistar albino rats were divided into 5 groups of six rats each. A 4-cm² area of the hair from the dorsal portion of all the rats was removed with a hair removing cream (Veet hair remover cream), wiped with cotton and surgical spirit. 1ml of Control (coconut oil), Standard (2% Minoxidil), Formulations (F1, F2, and F3) was applied to denuded area of the respective groups once a day. This treatment was continued for 30 days and the hair growth pattern was recorded.

EVALUATION²⁴⁻³⁶

QUALITATIVE HAIR GROWTH STUDY

Primary Skin Irritation test

The prepared formulations were assessed for primary skin irritation test. Six healthy rats were selected for the study. Each rat was caged individually. Food and water were given during the test period, 24 hours prior to the test. The hair from the back of each rat of 1cm² was shaved on either side of the spine to expose sufficiently large test areas, which could accommodate three test sites. The sites were cleaned with surgical spirit. 1 milliliter of each formulation F1, F2, F3, were applied over the respective test sites and observed for erythema and edema for 48hrs after application.

Hair growth initiation time and completion time

During 30 days of study period each animal of every tested group was observed for hair growth initiation and completion time by using a magnifying lens that enabled observation of minute changes in the pattern of hair growth. The

point at which a small prickle of hair length is observed was noted as the initiation time and the time taken to cover the denuded skin with new hair completely is taken as hair growth completion time. The recorded growth initiation time and completion time are shown in figure 1.

QUANTITATIVE HAIR GROWTH STUDY

Hair length determination

The hair growth at 7th, 14th, 21st and 30th day after beginning of topical application was observed visually and recorded. Hairs were plucked randomly from the test area of all rats on 7th, 14th, 21st and 30th day after initiation of the treatment. The length of 10 hairs from each rat was measured and the average length was determined. The results are expressed as the mean length \pm S.D of 10 hairs.

Hair weight determination

At the end of the experimental period, the rats were sacrificed by cervical dislocation. A 1 cm² area of dorsal skin with hair was cut from all the rats of each group and weighed (W_1 g). The hair was removed from each place and the skin without hair was weighed (W_2 g). The weight of the hair was obtained by subtracting W_2 from W_1 .

Statistical analysis

Each experiment was run in triplicate, and mean values were calculated. A student's t-test was computed for the statistical significance of the results.

RESULTS AND DISCUSSION

The results revealed that, the ethanolic extract of *Ziziphus jujuba* has no mortality up to 5000 mg/Kg body weight of experimental animals. The present formulation achieved the three parameters, i.e., creating the antibacterial properties, maintaining an acidic pH (≤ 5) which increases blood circulation to the scalp. Physical evaluation tests like Acid value, Saponification value, and pH were determined for Coconut oil and Formulations (F-1,

F-2, and F-3). Acid values and Saponification values for oils were found. The acid values of the formulations were found to be increased proportionately to the concentration of the extract in the formulation, thus imparted more acidic environment in the formulation. Saponification values found to be similar to coconut oil and all the formulations. The results are shown in the table 2.

Primary skin irritation test was done in 48 hours and there was neither erythema nor oedema observed in rats. Qualitative tests like Initiation time and Completion time was noted. The initiation time in an F3 group was observed on the 3rd day and it was on the 8th day for Minoxidil. Time taken for complete hair growth was 18 days in F3 group and 20 days in Minoxidil treated group and the results are shown in table 3.

Quantitative tests like Hair length and hair weight were evaluated. Hair length on 30th day was found to be more in F3 i.e., 1.82 ± 0.03 compared to that of the standard and control and the results are shown in table 4 and figure 2. Hair weight was found to be more in F3 group i.e., 1.21 ± 0.01 gm. The results of hair weight are shown in table 5 and figure 3.

The F3 formulation was found to be very promising and results are comparable to that of minoxidil and as the prepared formulation consists of natural ingredients it will be having no side effects compared to that of standard formulation. All the other formulations also showed considerable hair growth promoting activity comparable to that of standard drug, Minoxidil (2%).

CONCLUSION

This study demonstrated that the Hair tonic prepared with *Ziziphus jujuba* leaf extract has promising hair growth activity. Further work needs to be carried out in order to establish the mechanism of action and the exact chemical component responsible for the hair growth potential of *Ziziphus jujuba*.

Table 1: Formulation of hair oils

Batch No.	Coconut oil (ml)	Stearic acid (g)	Ziziphus jujuba ethanolic leaf extract (g)	Ethanol	pH
F1	100	1	1	5ml	≤ 5
F2	100	1	3	5ml	≤ 5
F3	100	1	5	5ml	≤ 5

Table 2: Physical evaluation tests

Physical parameters	Coconut oil	F1	F2	F3
pH	2	5	5	5
Acid value	1.4	7.243	10.378	12.903
Saponification value	235	242	239	243

Table 3: Initiation and Completion time

S. No	Group	Drug	Initiation time (days)	Completion time (days)
1	Group 1	Control	10	25
2	Group 2	Standard	8	20
3	Group 3	F1	9	23
4	Group 4	F2	7	22
5	Group 5	F3	3	18

Table 4: Length of hair at different time intervals after beginning the treatment

Average hair length (mm)						
S. No	Group	Drug	Day7	Day14	Day21	Day30
1	Group1	Control	0.1±0.01	0.5±0.04	0.99±0.06	1.28±0.13
2	Group 2	Standard	0.2±0.015	0.9±0.07	1.31±0.12	1.79±0.01**
3	Group 3	F1	0.5±0.011	1±0.02	1.12±0.01	1.40±0.12
4	Group 4	F2	0.3±0.013	0.7±0.012	1.20±0.05	1.55±0.04*
5	Group 5	F3	0.8±0.03	1.10±0.17	1.36±0.01	1.82±0.03***

Statistical analysis: The statistical calculations were performed using the software graph pad Instat, values are mean ± S.D, where n=6 and p value is < 0.0001

Table 5: Weight of hair (gm)

S. No.	Group	Weight of hair (gm)
1	Control	0.77±0.04
2	Standard	1.19±0.05**
3	F1	0.83±0.03
4	F2	0.98±0.06*
5	F3	1.21±0.01***

Statistical analysis: The statistical calculations were performed using the software graph pad Instat, values are mean ± S.D, where n=6 and p value is < 0.0001.

**(A)**



(B)



(C)

Fig. 1: (A) Initially Shaved skin of rats (B) Hair growth initiation time (C) Hair growth completion time

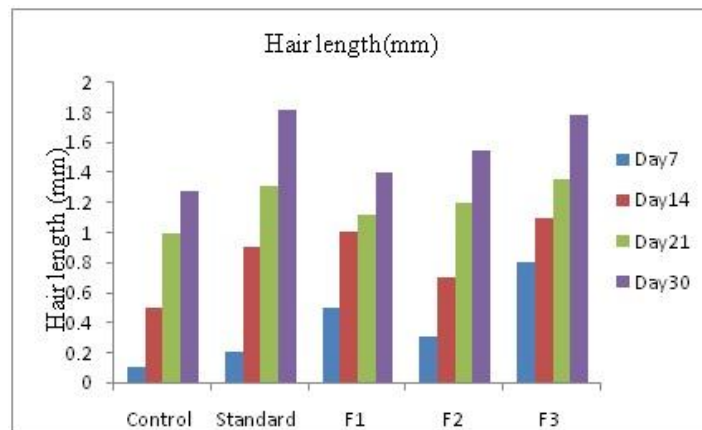


Fig. 2: Comparison of hair length (mm) between control, standard and formulations (F1, F2, F3)

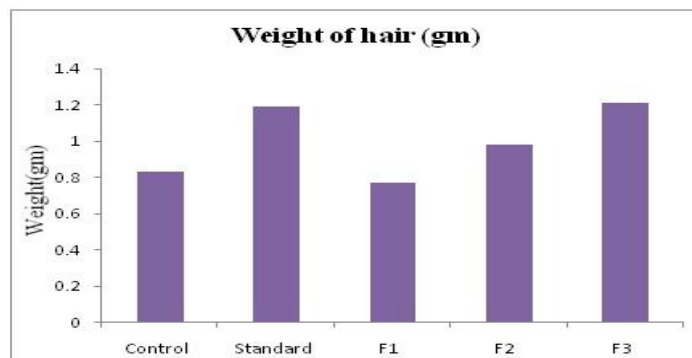


Fig. 3: Comparison of hair weight (gm) between control, standard and formulations (F1, F2, F3)

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