

CHEMOTHERAPY INDUCED ALOPECIA (CIA): EFFECT OF HIBISCUS ROSA-SINENSIS AND CALOTROPIS GIGANTEA LEAVES EXTRACT OINTMENT

Dr. Namrata Singh*
Shaheed Bhagat Singh College of Pharmacy. Palampur HP

Correspondence for Author: Dr. Namrata Singh
Email ID: singh.nam8@gmail.com

ABSTRACT
Chemotherapy-induced alopecia (CIA) is a distressing side effect of chemotherapy that can cause hair loss in part or in total, and in areas all over the body. It's caused by chemotherapy interfering with the rapidly dividing cells of the body, including hair follicles. To evaluate the hair growth activity of hydro-alcohlic leaves extract ointment of Hibiscus rosa-sinensis, Calotropis gigantea and their polyherbal formulation on chemotherapy induced alopecia. In chemotherapy-induced alopecia albino rat model, alopecia induced by cyclophosphamide (i.p.). 5% ointment of hydro-alcohlic leaves extract was applied topically over the depilated skin of albino rats for 45 days. Hair growth was observed from depilated area, in the physical parameters- hair length and density, in biochemical parameters- total protein estimation, testosterone and in histopathological parameter- percentage ratio of different cyclic phases, like anagen, catagen and telogen, of hair follicles were determined.

Keywords: Hibiscus rosa-sinensis, Calotropis gigantea, Chemotherapy, Alopecia, Cyclophosphamide, Histopathology.

1. INTRODUCTION
The incidence of chemotherapy-induced alopecia (CIA) is high. In general, CIA presents as acute temporary diffuse hair loss, also known as anagen effluvium. Cytostatics stop hair production and severe damage causes hair breakage or accelerated transition from anagen to catagen phase **(Wilson B.E., et al., 2019).** CIA results from induction of follicle dystrophy or the premature induction of follicle regression (catagen) in growing (anagen) hair follicles **(Lever WF et al., 1990; Rook A et al 1991; Tierney A et al., 1990).** The three major and frequent toxicities of cytotoxic cancer chemotherapy are bone marrow suppression, gastrointestinal disturbances and alopecia **(Carelle N et al., 2002; Door V. J. et al., 1998)**. The incidence of alopecia is 65%. Alopecia negatively affects a patient's perception of physical appearance, body image, sexuality and self-esteem, and deprives patients of the privacy of having cancer **(Lindley C et al., 1999; Pickard-Holley et al., 1995).** Chemotherapy-induced alopecia (CIA) is considered one of the most negative factors in cancer patient care. The National Coalition for Cancer Survivorship cites CIA as one of the most emotionally upsetting aspects of coping with cancer. Female patients are particularly affected; a survey shows that 47% patients consider CIA the most traumatic side effect of chemotherapy, and 8% would reject chemotherapy due to fear

of CIA **(McGarvey, E.L et al., 2001; Munstadt, K et al., 1997).** Alopecia also results in reduced social interactions in school-age children and teenagers **(Baxley, K.O et al., 1984; Harrison,S et al., 1003; Wagner, L et al., 1979**). The negative psychological impact of CIA may have additional undesirable biological consequences, as depression lowers immune function and is associated with cancer progression **(Spiegel, D et al., 2003).**

Substantial efforts have been expended and consequently, multiple crude drugs like the fruits of Embelica officinalis, leaves of Bacopa monnieri, leaves of Murraya koeniigi, seeds of Trigonella foenugraecum **(Pande, M et al., 2007),** Glycyrrhiza glabra**, (Shirode, D et al., 2005)** green tea **(Kwon, O.S et al., 2007)**, grape seeds **(Takahashi T et al., 1998),** Ginkgo biloba leaf **(Kabyashi, N et al., 1993),** Lawsonia alba, Rosmarinus officinalis, Cuscuta reflexa roxb **(Roy, R.K et al., 2006),** have been developed, to manage the hair loss, but no effective treatments for CIA are available at present. This work was done with an attempt to collect some experimental evidences, use of herbs, Hibiscus rosa-sinensis and Calotropis gigantea and their polyherbal formulation that having the potential of preventing hair loss in such cases of chemotherapy-induced alopecia.

2. MATERIALS AND METHODS

2.1. Plant material

Leaves of Hibiscus rosa-sinensis Linn. and Calotropis gigantea Linn. were collected in the month of September-October from the campus of Barkatullah University Bhopal. Plants were identified and authenticated in the Department of Pharmacy, Barkatullah University, Bhopal (M.P.). Voucher specimen no. BUPH- 4024/A for Calotropis gigantea Linn. and voucher specimen no. BUPH- 4024/B for Hibiscus rosa-sinensis Linn. was deposited.

Approximately 200g of powdered crude drugs were separately extracted with hydro-alcoholic solvent (H. rosa-sinensis Linn. 50:50, C. gigantea Linn. 70:30) by double maceration process. Phytochemical tests reveals the presence of carbohydrate, alkaloid, flavonoid, steroids, protein, tannin, amino acids in H. rosa-sinensis whereas the hydro-alcoholic extract C. gigantea tested positive for carbohydrate, alkaloid, flavonoid, steroids, protein, tannin, amino-acids and tannins **(Mukherjee PK et al., 2003; WHO 1998).**

2.2 Formulation

Hydrophilic ointment USP (5% w/w) of different extracts prepared by fusion method. Test one containing H.rosa-sinensis leaf extract, test two containing C.gigantea leaf extract, and test third containing polyherbal (H.rosa-sinensis C.gigantea ratio 1:1) in hydrophilic USP base. **(Allen Loyd V et al., 2005)** .

2.3. Animals

Albino rats, weighing 120-150, from Barkatullah University Bhopal (MP) India, were used for hair growth studies, based on the OECD Guideline No. 423 of CPCSEA. The rats were placed in

cages and kept in standard environmental conditions, fed with standard and diet ad libitum and allowed free access to drinking water.

2.4. Hair growth activity (Chemotherapy Induced Alopecia model)

All albino rats were depilated with the help of wax (2.5 cm^2 area) and rest for 14 days for anagen induction. After 14 days of depilation administered cyclophosphamide (120 mg/kg, i.p.) to all albino rats in divided dose of 40mg/kg/day for 3 days. The albino rats were rest for 9 days of the cyclophosphamide administration and observed hair fall. After that all albino rats were divided into four groups. Each group contains six albino rats. On 24^{th} day of depilation, in group 1, received Ointment base, group 2 received 5%w/w ointment (hydralcoholic extract of H. rosa-sinensis), group 3 received 5%w/w Ointment (hydralcoholic extract of C.gigantea), and group 4 received total 5%w/w Ointment (hydralcoholic extract of polyherbal), for next 21 days, once daily. On 45^{th} day of depilation blood were collected from retro-orbital plexus for biochemical parameter, skin were collected (from depilated area) for histopathological parameter and hair for physical parameter **(Wang J et al., 2006).**

2.4.1. Physiochemical Parameter

Hair length: Hair was plucked randomly from the depilated area from each group on 25^{th} day of the treatment with the help of clipper and measured the hair length of each rat with the help vernier caliper and the mean of hair length was calculated **(Adhirajan, N et al., 2003).**

Hair density: A hole of 1c.$m.^2$ was made on card board. Then the card board set on the desired depilated area (where hair fall patches observed) on the back of rat after 45 days of depilation. The hair was trimed of desired depilated area and the hair was cut with the seizure. The hair was count manually **(Patni P et al., 2006).**

2.4.2. Biochemical parameter

Total Protein estimation: The total protein content of tissue homogenate measured by Lowery's method(121). 0.5 ml of tissue homogenate was mixed with 0.5 ml of 10% TCA and centrifused for 10 min. The precipitate obtained was dissolved in 1.0 ml of 0.1 N NaOh. From this 0.1 ml used for the protein estimation **(Shreejayan and Rao et al., 1997).**

Testosterone: Testosterone play major role in androgenetic alopecia.[122] The blood sample that was collected and send to Dr. Lal path labs Pvt. Ltd. Bhopal, for the estimation of blood testosterone level **(Fischer T W et al., 2005).**

2.4.3. Histopathology

Skin biopsies were taken from the depilated area and fixed in 10% formalin buffer. Tissues were embedded in paraffin wax and sectioned into uniform thickness of 10 µm and were stained with haematoxylin and eosin. From the sections, the number of hair follicles per millimeter of the skin (Sawada et al., 1987), and the percentage ratio of different cyclic phases, like anagen and

telogen, of hair follicles were determined using the microscope fitted with an ocular micrometer **(Adhirajan N et al., 2003; Sawada M et al., 1987).**

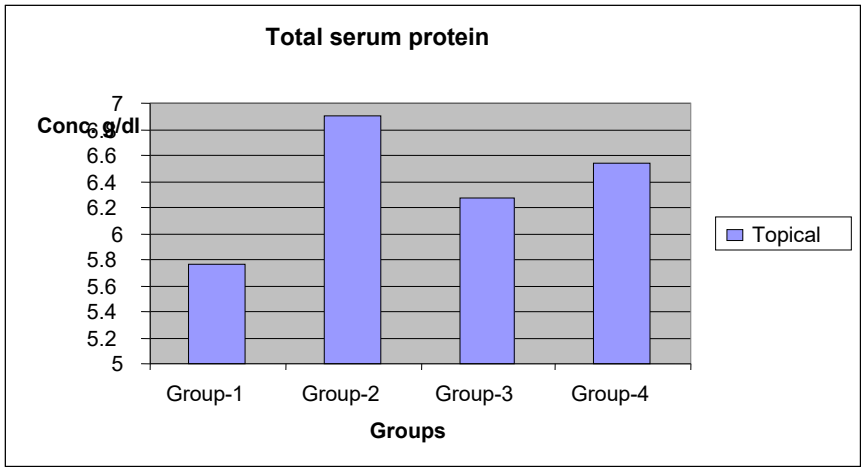
3. RESULTS

In Chemotherapy induced alopecia model the physical parameter like hair length and density of all groups shown in Table 1, biochemical parameter like Total Protein estimation and testosterone of all groups shown in Graph 1 & 2, and Histopathological parameter shown in table 2.

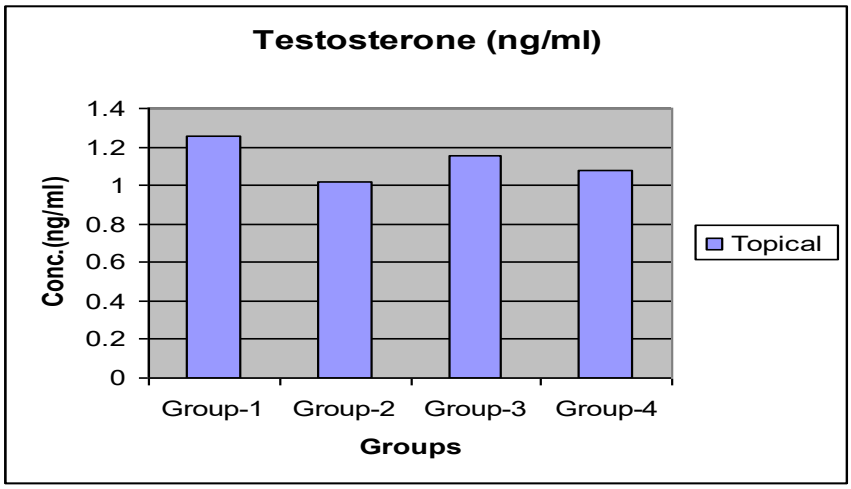
Table 1. Effect of different formulation on Hair Length and Hair Density of albino rats in CIA alopecia

S.N.	Groups	Drug	Formulation	Hair Length Mm(mean±s.d	Hair Density (mean±s.d.)
1.	Group 1	Vehicle	Ointment Base	2.03±0.082	1242.5±38.69
2.	Group 2	Hydroalcoholic Extract of H.rosa-sinensis	Ointment (5% w/w)	4.46±0.314*	2073.3±53.17*
3.	Group 3	Hydroalcoholic Extract of C.gigantea	Ointment (5% w/w)	2.73±0.0162	1480±40.49
4.	Group 4	Polyherbal hydroalcoholic Extract of (H.R.+C.G.)	Ointment (5% w/w)	3.86±0.318	1810±41.95

Group-1: Control, Group-2: H.rosa-sinensis, Group-3: C.gigantea, Group-4: Polyherbal
*Significance (P value < 0.01) as campared of control group to to all 3 test group.



Graph 1. Effect of different formulation on Total serum protein of albino rats in CIA model



Graph 2. Effect of different formulation on Testosterone of albino rats in CIA model

Table 2. Effect of different formulation on No. of hair follicles in different stages of albino rats in CIA model

S.No	Drug	Formulation	Hair Follicle Population %		
			Anagen	Catagen	Telogen
Group-1	Vehicle	Ointment Base	50.67	1.83	47.5
Group-2	Hydroalcoholic Extract of H.rosa-sinensis	Ointment (5% w/w)	64.66	1.66	33.66
Group-3	Hydroalcoholic Extract of C.gigantea	Ointment (5% w/w)	51.83	1.66	46.5
Group-4	Polyherbal hydroalcoholi Extract of (H.R.+C.G.)	Ointment (5% w/w)	59	1.5	39.5

Group-1: Control, Group-2: H.rosa-sinensis, Group-3: C.gigantea, Group-4: Polyherbal
*Significance (P value < 0.01) as campared of control group to to all 3 test group.

Albino Rat Photographs (Chemotherapy induced alopecia model)

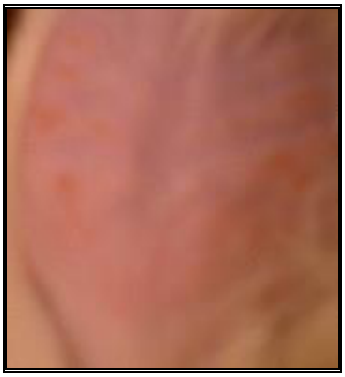


Photo. 1 After Depilation

Photo. 2 After 14 days

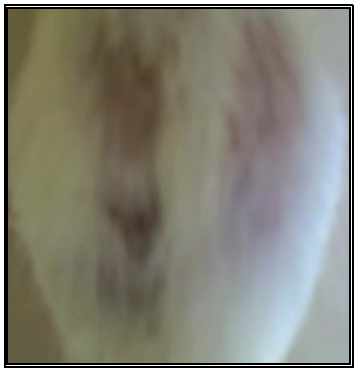


Photo. 3 After 23 days (Cpd.

Treated)

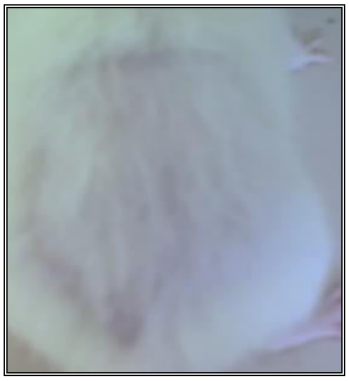


Photo. 4 Control

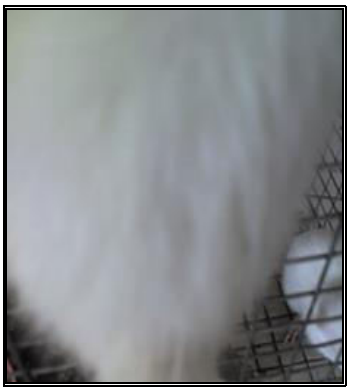


Photo. 5 H.rosa-sinensis

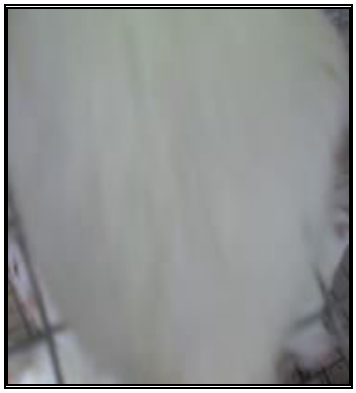


Photo. 6 C.gigantea

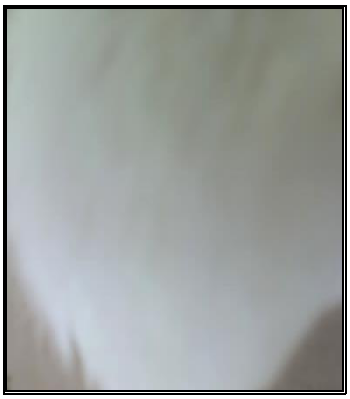


Photo. 7 Polyherbal

Histopathology Photographs (Chemotherapy induced alopecia model)

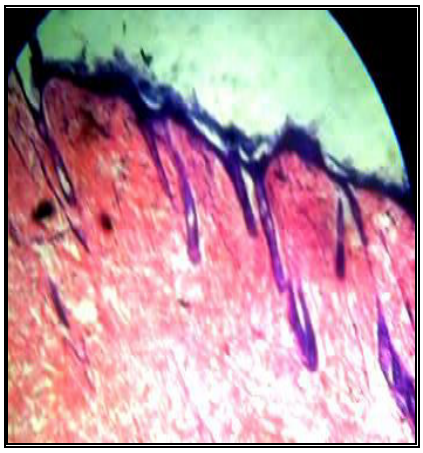


Photo. 8 Control

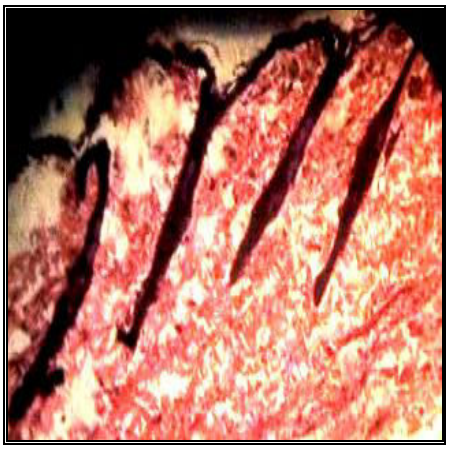


Photo. 9 H.rosa-sinensis

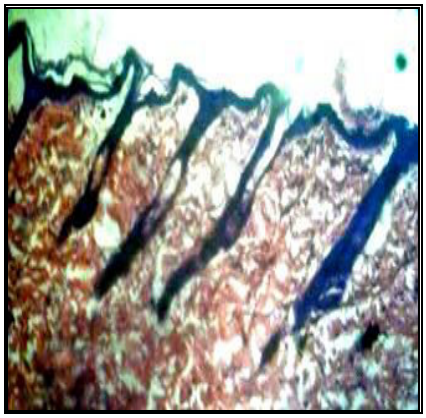


Photo. 10 C.gigantea

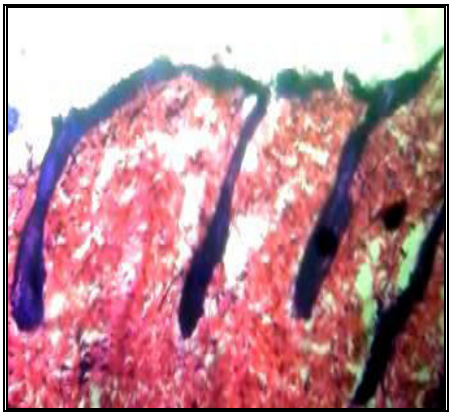


Photo. 11 Polyherbal

4. DISCUSSION AND CONCLUSION

In Chemotherapy induced alopecia model the hair length of all groups shown in Table 1. In this Table the group-2 have significant hair length as compared to control. The hair density of all groups shown in Table 2. In this Table the group-2 have significant hair density as compared to control. In this table the group-2 have significant no. of hair follicle in anagen phase as compared to control.

The total serum protein of all groups shown in graph 1. In this graph the group-2 have significant total serum protein as compared to control. The testosterone level of all groups shown in graph 2. In this graph group-2 have significant less testosterone level as compared to control.

Thus, the drugs under investigation helped in maintaining more % of hair follicles in anagen phase, subsequently promoting hair growth. Increased total serum protein and reduced level of testosterone also support hair growth due to chemotherapy.

Recently, the number of men and women who suffered from hair loss (alopecia) due to Chemotherapy. Thus it is very important to develop new therapeutic materials to stop hair loss and to enhance hair growth. Herbal medicine is one interesting area, which is getting more popular.

The hydro-alcohlic leaves extract ointment of Hibiscus rosa-sinensis, Calotropis gigantea and their polyherbal formulation showed significant effect on chemotherapy induced alopecia. Further study will be carrying out to find out chemical active constituents of these plants which promoted hair growth. Presence of active constituents like flavonoids, tannins, may be responsible for Chemotherapy induced alopecia.

5. ACKNOWLEDGEMENT

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