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Antileishmaniasis effects of monoherbal skin ointment made from bark and leaf of *Holoptelea integrifolia* (Roxb.) Planch. in patients of cutaneous leishmaniasis

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Abstract: Prevalence of parasitic skin diseases, cutaneous leishmaniasis is very frequent in Pakistan, especially in Baluchistan where occurrence of phelbotomine sand flies (vector of protozoan parasite of genus Leishmania) is very common. This study was conducted to assess the antileishmaniasis activity of Intericutol plus ointment (2% and 3%) made with ethanolic extract of bark and leaves of *Holoptelea integrifolia* (Roxb.) Planch. The antileishmaniasis effect was observed by applying the ointments on the lesions of leishmaniasis patients. 150 patients were included in the study that had single and multiple painful wet and dry ulcerative lesions on the exposed area of the body. The patients were divided into two groups of 75 patients each, applying 2% and 3% ointment respectively for 28 days. Dose of 10gm/week was applied topically on wounds. Excellent antileishmaniasis effect was observed with significant quick healing properties in the patients receiving 3% intericutol plus ointment as compare to 2% showing 89% and 81% cure (p=0.02) respectively. It can be concluded that ointment made from medicinal plant proved to be very effective in treating cutaneous leishmaniasis.

Keywords: Leishmaniasis, ethanolic extract, medicinal plants, skin disorders, herbal drugs, sand fly.

INTRODUCTION

Cutaneous leishmaniasis (CL) is a vector borne parasitic skin diseases highly spread endemic diseases. Twenty species of phlebotomine sandflies have to be known to transmit cutaneous leishmaniasis serving as carrier/ host/vector of single celled protozoan parasite of genus Leishmania. World Health Organization has included leishmaniasis as major global diseases responsible for millions of deaths worldwide (Desjeux, 2004; Gervazoni *et al*., 2020; Volf and Peckova, 2007). Conventional treatments of this topical disorder are the use of pentavalent antimonials that are usually expensive, require long therapeutic duration, render to severe side effects and development of drug resistant strains (Alvar and Yactayo, 2006; Desjeux 1996).

Sand fly vector for cutaneous leishmaniasis is highly spread all over the Pakistan especially in Baluchistan Province (Shakila *et al.,* 2006). CL is commonly known as Kala dana by the local Balochistani population as the lesions require very long duration to heal sometimes one year. Due to improper and costly current treatment the poor and underprivileged population of Baluchistan Pakistan is facing the risk of this painful disease (Khan *et al*., 2021; Khan and Khel, 2004).

There are many challenges to treat this complicated skin diseases worldwide. As far as we know this is open wound so purpose of this study is to introduce the new highly effective herbal treatment for this skin disease (Santos, 2008; Oliveria, 2011). Recently the world health organization estimated that 80% people rely on herbal medicine for some aspect of their primary health care (Bannerman *et al.,* 1983). For the welfare and benefit of humanity, researchers investigate the alternate herbal preparation for the treatment of cutaneous leishmaniasis also (Rocha, 2005).

Holoptelea integrifolia (Roxb.) Planch (syn. *Ulmus integrifolia* Linn) belongs to family Ulmaceae held whitish or yellowish grey bark and leaves. Based on phytochemical investigations the plant contains terpenoids, alkaloids, glycosides, steroids, saponin, tannins, sterols, flavonoids, friedelin, lupeol (Ganie and Yadav, 2014; Somwong *et al.,* 2022). Our research group had also isolated two pentacyclic triterpenoid named as betulinic acid and betulin from bark of this plant (Ahmed *et al.,* 2013). From literature it is evident that *H. integrifolia* exhibits a variety of medicinal actions like antibacterial, antidiabetic, anti-inflammatory, antioxidant, antineoplastic and wound healing activities. The bark and leaves of this plant were used for leprosy, skin disease and externally for ring worm (Srinivas and Reddy, 2008; Gou *et al.,* 2013; Rizwani and Mahmud, 2012; Khalid *et al.,* 2014; Yasin *et al.,* 2019).

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Table 1: Demographics of volunteer participated in study

Age	Male	Female	Total
1-10	5	3	8
11-20	7	5	12
21-30	41	17	58
31-40	32	11	43
41-50	11	7	18
51-onwards	7	4	11

Table 2: Numbers and location of lesions in participants

Lesion site	Number	%
Face	Multiple	20%
Neck	Single	3%
Arm	Single	17%
Legs	Multiple	50%
Others	Multiple	10%

Table 3: Composition of 2% Intericutol Plus ointment

Sr. No.	Ingredients	Quantity	Role of ingredients
01	Methanolic extract of Bark of *H. integrifolia*	2 g	Active ingredient
02	Methanolic extract of Leave of *H. integrifolia*	2g	Active ingredient
03	Emulsifying wax	30 g	Emulsifying agent
04	Liquid paraffin	20 g	Emollient
05	White soft paraffin	50 g	Ointment base

Table 4: Composition of 3% Intericutol Plus ointment

Sr. No.	Ingredients	Quantity	Role of ingredients
01	Methanolic extract of Bark of *H. integrifolia*	3 g	Active ingredient
02	Methanolic extract of Leave of *H. integrifolia*	3g	Active ingredient
03	Emulsifying wax	30 g	Emulsifying agent
04	Liquid paraffin	20 g	Emollient
05	White soft paraffin	50 g	Ointment base

Table 5: Descriptive statistics of 2 % and 3% Intericutol Plus ointment against CL

Data	Number of Patients	Minimum Lesion size	Maximum Lesion size	Mean Lesion size	Std. Deviation
2% Intericutol Plus ointment					
Before Treatment	69	.10	3.95	2.7	.79
After Treatment	69	.00	2.80	.84	.79
3% Intericutol Plus ointment					
Before Treatment	71	1.00	4.01	2.6	.65
After Treatment	71	.00	1.80	.59	.52

Keeping in view the wound healing properties of bark and leaves of this plant the present study was designed to optimize this natural product for the benefit of humanity. The objective of present study was to carry out clinical evaluation in order to explore the efficacy of Intericutol plus ointment (2% and 3% concentration) prepared from bark and leaves of *Holoptelea integrifolia* (Roxb.) on cutaneous leishmaniasis lesions.

MATERIALS AND METHODS

Study design

The clinical study was conducted among 150 volunteers in Rural health Center Winder Baluchistan Pakistan, which was continued from September 2016 to February 2017 in Rular Health Center Winder, Baluchistan. This research study was approved by Hamdard University (HU/DRA/2017/137).

Sample size

Randomized convenient sampling technique was employed during this study. The sample size was n=150. Demographic information of patients is described in table 1.

Inclusion criteria

- All age people who have lesions on the body
- Free from othertopical diseases

- Willing for treatment
- And those who had not already taken any other therapy

Exclusion criteria
- Patients with treatment of antibiotics
- Pregnant ladies
- Having other types of scars and skin problems

Methodology
One hundred seventy volunteers had been taken in the study however a total of 150 patients were selected on the basis of positive leishmaniasis parasite test through Giemsa stained techniques under electron microscope. The consent form had been signed by all patients after giving all information about leishmaniasis and treatment. Consort 2010 flow chart was followed for graphically outline the design and conduct of study shown in fig. 1.

Grouping of patients
One hundred fifty patients were divided into two groups (75 patients in each) on the basis of ointment concentration that is 2% and 3%. Location and numbers of lesions are mentioned in table 2.

Composition of mono herbal skin ointment (Intericutol plus ointment)
Two different topical formulations of intericutol plus ointment (2% and 3%) were made from bark and leaves extracts (1:1 ratio) of *Holoptelea integrifolia* (Roxb.) Planch. Composition of both ointments is given in table 3 and 4 respectively.

After formulation both ointments were separately filled in labeled, air tight standard sealed jars and stored at room temperature. Fig. 2 represents the photographic picture of both ointments showing their final packages as well as ointment consistency.

Clinical application of intericutol plus ointment
Pilot study was done by performing Patch and prick tests to check irritation of the ointment on the skin prior to evaluate the efficacy of ointment (2% and 3%) on CL lesions.

In this regard formulation of 2% ointment was given to each patients of group 1, while the formulation of 3% ointment was given to each patients of group 2. Total duration of therapy was one complete month but all patients of both groups were provided 10g ointment in standard sealed jar per week and asked to visit the hospital for follow up. Patients were advised to apply their respective ointments (2% and 3%) twice a day covering entire lesion superficially without covering and rubbing it for a one month. Follow-up from patient each week had been done for query about any irritation and side effects of ointment. Improvement in healing of lesion noticed despite any pain or allergy.

The follow up was taken from each patient weekly to check healing of lesion. The size of each lesion was measured on every weekly visit. Wound is being considered healed and repaired when epithelial layer of cell was covered the ulcerative lesion with reduced size of lesion and pus in center become got dried. An evaluation response into the treatment was assessed in accordance with the criteria of visual observation and by measuring the lesion size with scale.

Moreover follow up had been taken after two month and after one year about any unconditional and unwanted effect of topical ointment but no any allergy and adverse effect noted then after.

STATISTICA ANALYSIS

Statistical analysis was performed to compare the results of 2% and 3% ointment using IBM SPSS Statistics (version 20). t-test was used to compare the mean lesion size before and after treatment. P=0.05 was considered significant.

RESULTS

A total of 150 patients were assigned to receive topical Intericutol plus ointment. Group 1 received 2% while group 3 received 3% concentration. Follow up data were available for every week. Table 5 contains the descriptive analysis of effect of 2% and 3% Intericutol Plus ointment against coetaneous leishmaniasis (CL) comprising of mean maximum and minimum lesion size with standard deviation. Results showed that after one month follow-up healing of lesion was found better in patients receiving 3% concentration of ointment as compare to 2% concentration.

Fig. 3 demonstrates the percentage of cure, partially cure *and* fail to cure patients. Fig.4a and 4b give the pictorial view of effect of 2% and 3% ointment on the patient respectively.

Effect of 2% intericutol plus ointment
The 2% concentration proved good topical antileishmaniasis therapy for patients. It took more than four weeks to heal the wound completely without leaving any scar or allergy after terminating treatment (fig.4a).

Effect of 3% intericutol plus ointment
Patients who were treated with 3% ointment exposed a very excellent effect with perfect healing of lesion of leishmaniasis and no scar was left behind. The 3% concentration of ointment proved curable therapeutic remedy for leishmaniasis, showing very miraculous effect within one month of time period. This topical remedy proves very beneficial, painless and easy applicable for patients of leishmaniasis.

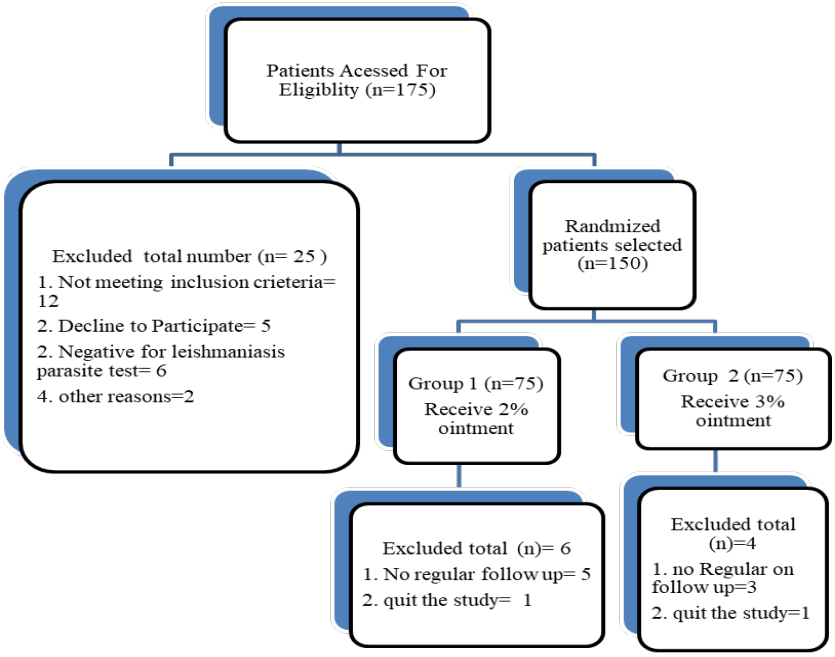

Fig. 1: Consort flow chart

Fig. 2: Monoherbal Intericutol Plus ointment 2% and 3%

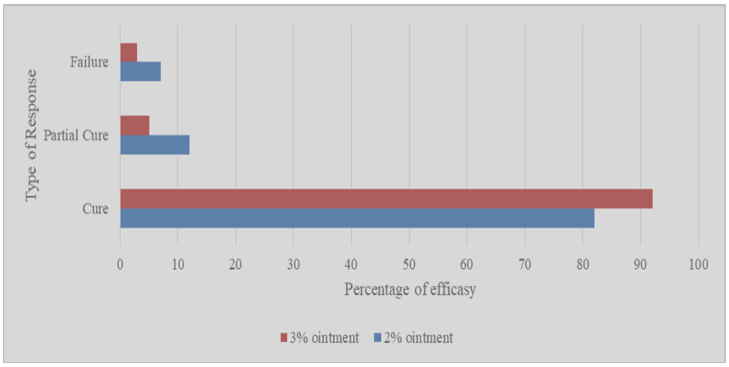

Fig. 3: Effectiveness of 2% and 3% ointment on CL patients
Note: %age of effectiveness of 2% and 3% Intericutol Plus ointment was calculated after statistical evaluation of average lesion size before and after treatment

Fig. 4a: Healing effect on 2% intericutol plus ointment

Fig. 4b: Effect of 3% Intericutol Plus ointment

Fig.4b showed wet ulcerative cutaneous lesion, which after 15 days of treatment with 3% ointment showing skin layer covered center pus. In third picture after approx four week showing healed lesion with complete superficial skin layer.

DISCUSSION

Epidemiological studies showed that Patients of Baluchistan had been severely in attack of cutaneous leishmaniasis is scattered in Baluchistan like normal fever (Khan and Khel 2004; Khan *et al.,* 2021). Cutaneous leishmaniasis is a very irritating skin disease presenting dry/wet itchy harsh lesions which can take almost a year to heal spontaneously. These lesion may be single are in multiple numbers which make your routine life disturb. These lesions may easily prone to other bacterial infection. Currently no effective treatment option is available instead of pentavalent antimonial drugs that are too toxic and costly injection. A unique feature of the disease is the skin ulcerative lesion which is filled with pus/ matrix caused by bite of sand fly. Lack of the knowledge in the complexity of disease caused by organism has hampered the development of the drug targeting organisms.

Medicinal plants have curative properties due to the presence of various complex phytochemicals constituents (Mishra *et al.,* 2008). *Holoptelea integrifolia* was selected as active ingredient for our Intericutol Plus ointment (2% and 3% concentrations). Regarding this plant different researches have been carried out by our research team on Pharmacognostic and phytochemical parameters (Rizwani and Mahmud, 2012; Ahmed *et al.,* 2013; Guo *et al,* 2013; Khalid *et al.,* 2014). In the current study we had formulated an ointment from the extracts of bark and leaves of this plant with different concentrations 2% and 3% for topical use to treat cutaneous leishmaniasis.

This study was carried out at rural health center Winder Baluchistan. The monoherbal skin ointment that was applied to treat CL. Topical ointment had been given for one month in 40g of dose divided in 10gm /week and follow up was taken each week up to one month until the recovery of lesion seen. The herbal topical ointment proved a very excellent therapy for lesion of cutaneous leishmaniasis. Intericutol plus ointment in 2% and 3% concentration showed desirable effects (table 5, fig.4a and 4b). The ointment formulation was found to be effective and dermatologically non-toxic in both groups.

CONCLUSION

In this study topical ointment intericutol plus was formulated with bark and leaves extract of medicinal plant *H. integrifolia* Roxb. Planch in 2% and 3% concentration. Topical application of this formulation showed remarkable wound healing effect in cutaneous

leishmaniasis patients of Baluchistan having multiple external lesions and explains the success claimed in the folk use of plant in the treatment of common skin condition. It can be concluded that formulating Intericutol plus ointment from medicinal plant is effective approach in the treatment of cutaneous leishmaniasis lesions and should be further explored in future to harness the potentials of this medicinal plant in the treatment of topical parasitic disease.

REFERENCES

Ahmed M, Rizwani GH, Mohammad FV, Mehmood I and Ahmed V (2013). A terpenoid antioxidant agents found in *Holoplelea integrifolia* (Roxb.) plant. *Int. J. Pharm. Chem. Biol. Sci.,* **3**(1): 63-67

Alvar J and Yactayo S (2006). Leishmaniasis and poverty. *Trends Parasitol.,* **22**(12): 532-557.

Bannerman RB, Buton J and Chieh CW (1983). Traditional medicine and health care coverage. Africa: World Health Organization, pp.9-13.

Desjeux P (1996). Leishmaniasis, public health aspects and control. *Clin. Dermatol*., **14**(5): 417-423.

Desjeux P (2004). Leishmaniasis: Current situation and new perspective. *Comp. Immunol. Microbiol. Infect. Dis.,* **27**(5): 305-318.

Ganie SA and Yadav SS (2014). Holoptelea integrifolia (Roxb.) Planch: A review of its ethnobotany, pharmacology and phytochemistry. *Biomed. Res. Int.,* 2014: 401213.

Gervazoni LFO, Barcellos GB, Paes TF and Almeida-Amaral EE (2020). Use of natural products in leishmaniasis chemotherapy: An overview. *Front. Chem.*, **8**: Article 57989.

Guo H, Wang DS, Rizwani GH, Ahmed M, Ahmed M, Hassan A, Xu RH, Mansoor N, Tiwari AK and Chen ZS (2013). Antineoplastic activity of *Holoptelea integrifolia* (Roxb.) Planch bark extracts (*in vitro*). *Pak. J. Pharm. Sci.*, **26**(6): 1151-1156.

Khalid S, Rizwani GH, Yasin H, Perveen R, Abrar H, Shareef H, Fatima K and Ahmed M (2014). Medicinal importance of *Holoptedea integrifolia* (Roxb.) Planch. Its biological and pharmacological activities. *Nat. Prod. Chem.,* **2**: 124.

Khan A, Sajid R, Gul S, Hussain A, Zehri MA, Naz S, Simsek S, Waseem S, Afzal MS, Naqvi SKH, Qasim M and Ahmed H (2021). Epidemiological and pathological characteristics of cutaneous leishmaniasis from Baluchistan Province of Pakistan. *Parasitol.*, **148**(5): 591-597.

Khan J and Khel KS (2004). Kaal Daana (Cutaneous Leishmaniasis) in South-West Pakistan: A preliminary study. *Turkiye Parazitol. Derg,* **28**(1): 5-11

Mishra BB, Kale RR, Singh RK and Tiwari VK (2008). Alkaloids: Future prospective to combat leishmaniasis. *Fitoterapia,* **80**(2): 81-90.

OliveriaFL (2011). Systemic review of adverse effects of cutaneous leishmaniasis in the new world. *Acta Trop*., **118**(2): 87-96.

Rizwani GH, Mahmud S, Shareef H, Perveen R and Ahmed M (2012). Analgesic activity of various extracts of *Holoptelea integrifolia* (Roxb.) Planch leaves. *Pak. J. Pharm. Sci.,* **25**(3): 629-632.

Rocha LG (2005). A review of natural products with antileishmanial activity. *Phytomedicine*, **12**(6-7): 514-535.

SantosOD (2008). Leishmaniasis treatment challenge that remains a review. *Parasitol. Res.,* **103**(1): 1-10.

Shakila A, Bilqees FM, Salim A and Moinuddin M (2006). Geographical distribution of cutaneous leishmaniasis and sand flies in Pakistan. *Turkiye Parazitol. Derg*, **30**(1): 1-6.

Somwong K, Lertpatipanpong P, Nimlamool W, Panya A, Tragoolpua Y, Yongsawas R, Gritsanapan W, Pandith H and Baek SJ (2022). Effect of *Holoptelea integrifolia* (Roxb.) Planch. n-Hexane extract and its bioactive compounds on wound healing and anti-inflammatory activity. *Molecules,* **27**(23): 8540.

Srinivas B and Reddy R (2008). Evaluation of antimicrobial, antioxidant and wound healing potential of *Holoptelea integrifolia*. *J. Ethnopharmacol.,* **115**(2): 249-256.

Volf P andPeckova J (2007). Sand flies and *Leishmania*: Specific versus permissive vectors. *Trends Parasitol*., **23**(3): 91-92.

Yasin H, Mahmud S, Rizwani GH, Perveen R, Abrar H and Fatima K (2019). Effects of aqueous leaves extract of *Holoptelea integrifolia* (Roxb) Planch on liver and kidney histopathology of albino rats. *Pak. J. Pharm. Sci.,* **32**(2): 569-573.