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An In-vitro, In-vivo and Clinical Trials of *Somaraji Taila* in the management of *Dadru Kushta*

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ABSTRACT

Dermatophytosis has become a significant health problem affecting children, adolescents, and adults worldwide. In India 5 out of 1000 people suffer from Tinea infections. The present study evaluated the antifungal activity of *Somaraji Taila* in Tinea (Dermatophytosis) in Invitro & Invivo model. Cultures were brought from MTCC a government body and inoculated in SDA media under aseptic condition. Later it was subjected to microscopic and macroscopic examination to identify the organism. A sensitivity test was done using SDA media by well diffusion method, with 4 different concentrations of Hydro-Methanolic extraction of *Somaraji Taila* ingredients. After the incubation period, the zone of inhibition was checked and was measured in mm. As the next phase of the study, animal study was also conducted to check antifungal properties & anti-dermal toxicity on albino rat skin and conducted clinical study on 30 Diagnosed subject *Dadru Kushta* who were randomly allocated with 15 each in two groups. *Somaraji Taila* was taken for one group which was compared against widely used clinical formulation *Chakramarda Taila* taken as standard for local application in another group. The application was done for 14 days, and the data was collected from the subjects at baseline, 8th day (during treatment), 15th day (after treatment) and 21st day (follow-up). The assessment was based on the KASI method of grading. The results of the study showed that there was there was highly statistically significant difference between the effect of *Somaraji Taila* and *Chakramarda Taila* in *Dadru Kushta*.

Key words: *Ayurveda, Dadru Kushta,* Tinea Infection, Ringworm Infection, *Somaraji Taila.*

INTRODUCTION

The health of a person is reflected in their skin, which serves as a protective organ.[1] It is a frequent target organ for infections. Around 10–20% of patients in routine clinical setup experience skin illnesses, of which fungal infections account for up to 20%.[2]

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A World Health Organization (WHO) review of prevalence studies done on skin diseases reported an overall prevalence ranging from 21% to 87%.[3] In Ayurveda, references are given regarding the testing of drug and food on animals for evaluating administration to the safety before human beings. Yogya Vidhi, a separate chapter in Sushruta Samhita Sutrasthana, addresses this. Any operation that is done on humans should ideally first be tested on animals or other models that have comparable features.[4] Therefore, it is imperative to conduct an in vitro and in vivo study to assess the effectiveness of *Somaraji* Taila on dermatophytosis before administering it on the patients. *Dadru* is a variety of *Kushta* with *Rasa, Rakta, Mamsadhatu* involvement.[5] Its aetiology includes *Aharaja, Viharaja, Chikithsaapacharaja, Upasargaja* and *Krimija* factors.[6] It is identified by symptoms such as *Kandu, Deergha Pratana*, *Utsanna Mandala,*[7] *Raaga, Pidaka* with predominance of *Pitta Kapha Dosha*.[8] Tinea is a group name for a highly contagious,

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segmented mycelia fungus.[9] Tinea is analogues to *Dadru* in contemporary science.[10]

Materials and Methods

Sample Collection

The fungal sample for invitro and *In Vivo* were collected from MTCC Chandigarh in the form of Freezed Dried Samples later they were activated by adding 0.4ml of Sterile Distilled water and transferred into a slant SDA media to grow for 7days once grown then confirmed under microscope (40X).

The ingredients used of *Somaraji Taila* are *Bakuchi* seeds, *Haridra Khanda, Daruharidra Moola, Sarshapa Seeds, Kushta Moola, Karanja Seeds, Chakramarda Seeds, Aragwadha Patra*. All the used parts were authenticated by Dravyaguna Department of Alva's Ayurveda Medical College, Moodubidire. *Somaraji Taila* was prepared in RSBK Lab of AAMC, Moodubidire.

Source of Data

For In vitro Study

The *Somaraji Taila* ingredients were tested on 4 fungal sample i.e., *Trichophyton Rubrum* (MTCC7859), *Trichophyton Mentagrophytes* (MTCC 7687), *Microsporum Gypsum* (MTCC 2819), *Microsporum Canis* (MTCC 2820) in Microbiology Department of Alva's Health Center, Moodubidire.

For In vivo Study

Before proceeding animal study, the room where animal was kept was bubble wrapped entirely to limit the spread the infection. Wister albino rats aged 14-15weeks weighing 200-250gms obtained from Alvas Animal House, Alvas Ayurveda Medical College and Hospital, Moodubidire. The rats were individually housed in propylene cage at room temperature. The animals were feed pellet diet and water.

For Clinical Study

OPD & IPD of PG Department of *Kayachikitsa*, Alva's Ayurveda Medical College, Moodubidire.

Method of Collection of Data

Selection of Subjects: Irrespective of gender, religion, occupation, marital status, socio-economic status and education status.

- **Sample size:** 30 participants
- **Grouping:** 2 arms (A and B)
- **Number:** 15 in each arm
- **Study design:** A Randomized Parallel-Group Comparative Clinical Study
- **Blinding:** Single-blind
- **Method of sampling:** The lottery method

Diagnostic Criteria

Screening of Organisms like *T. Rubrum, T, Mentagrophytes, M. Gypsum, M.Cannis, E.Floccosum* is done by KOH Mounting Method

Based on following Lakshana of *Dadru.*

- *Kandu*
- *Daha*

With or Without

- *Udgata Mandala*
- *Raga*
- *Pidaka*
- *Rookshata*

Inclusion Criteria

- Subjects who gave written consent
- Subjects who fulfilled diagnostic criteria
- Subjects with age group between 18 to 60 years

Exclusion Criteria

- Subjects who have lesion with secondary infections.
- Subjects with any other systemic disorder.

Interventions

- **Group T:** Subjects were given *Somaraji Taila* for Local application for 14 days BD dose.

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- **Group S:** Subjects were given *Chakramarda Taila* for Local application for 14 days BD dose.

Observation Period

Treatment Period: 14 Days

Days of Assessment:

0th Day - At Baseline

8th Day - During Treatment

15th Day - After Treatment

21st Day - Follow up

Assessment Criteria

- KASI[11] method of grading

Statistical Methods

- Central tendencies and dispersions were measured using Mean, Median, Standard Deviation, Standard Error and Quartiles.
- Tests of significance were done using paired 't' test and the unpaired 't' test, Anova Test, Mann Whithey Test, Wilcoxson Signed Rank Test.

OBSERVATIONS

Gender: Among 30 subjects 17 (63.3%) were males & 13 (36.7%) were females Tinea is more common in boys than girls during adolescence, because boys will have more sebaceous secretion, excessive sweating & they are more exposed to sunlight than girls.

Age: Total 30 Subjects maximum number of subjects 63.3% belongs to the age group of 21-30 years This may be due to increased sebaceous gland activity in adolescent age group which favours the growth of fungal infection & other contributing factor is lack of hygiene.

Religion: Among 30 subjects, 27 of them belong to Hindu Religion. The fact may be the area where the study was conducted was having predominance of Hindu Religion.

Socio-Economic status: Among 30 subjects, maximum number of 19 (63.3%) belong to middle class, 11 (36.6%) were from Upper-middle class. This is due to the region prominent with middle class.

Occupation: 33.6% of the subjects were students and 36.3% were housewives. This may be due to excessive house work lead to sweating and creates moist area which helps in growing fungus and fungal hyphae.

Diet: Most of the patients i.e., 70% were of mixed diet. As the study was conducted in coastal area and more over eating habits of coastal area is mixed diet.

Chronicity: Among 30 Subjects 73.3% were having Acute onset due to the spreading nature of the fungal spore and unhygienic maintenance of the body, clothes and surrounding area.

***Prakrithi*:** Among 30 subjects, 80% subject having *Pitta-Kapha Prakriti* where as 20% are of *Vata-Pitta* as *Dadru Kushta* is *Kapha Pittaja Vyadhi* same *Prakriti* patient has been diagnosed.

Aggravating factors: In the present study 93.3% had excessive sweating and 3.3% was due to Dust exposure. This may be because sweating creates moist environment around the area which triggers fungal hyphae to grow further.

Skin Texture: Among 30 Subjects 80% were having Oily skin texture where as 20% were having dry skin texture, oily skin texture plays an role by nourishing the fungal cells hence sporulation of fungal spore is excess when compared to dry skin texture.

Organism: Among 30 subjects 60% subject were affected due to infection by *Trichophyton Mentagrophytes* where as 40% subjects were affected by *Trichophyton rubrum*, when we consider prevalence rate of fungal infection in Dakshin Kannada 45-87% of fungal infection is because of *Trichophyton Mentagrophytes* and *Trichophyton Rubrum* due to presence of moist climate.

Affected Area: Among 30 subjects 8 (26.6%) had affected on groins, 7 (23.3%) had affected under breast, 5 (16.6%) had affected on abdomen and hands, 2 (6.6%) had affected on hands and on scrotum, 1 (3.3%) had affected on gluteal, area of infection also plays role in spreading and severity due to moist area and friction of the area helps fungal spore to spread.

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RESULT

The whole study was carried out in Phase 0 (Laboratory Study), Phase 1 (Animal Study), Phase 2 (Clinical Study), of Research Methodology.

Phase 0: Results is, there is Anti-Fungal activity observed in any one or more than one organism with one or more extracts and formulations in well diffusion method using SDA in In Vitro study as shown in Table No. 1.

Phase 1: Results is, there is Statistically significant effect of *Somaraji Taila* which is better than control and standard or equal to standard group as shown in Table No 2

Phase 2: Result, the study was carried out on 30 subjects divided into 15 each group. Group S received *Chakramarda Taila* whereas, Group T received *Somaraji Taila.* The data was collected from subjects at baseline, 8th day (during treatment), 15th day (After Treatment), and 21st (After Follow up). The obtained data were subjected to statistical evaluation by applying Paired and unpaired T test, Anova test, Mann Whitney test and Wilcoxson signed rank test to evaluate the effectiveness of the interventions. There was statistically significant difference in assessment parameter in both group on 8th Day, 15th Day and 21st Day. When compared to the baseline shown in Table No 3.

On ***Kandu*** it shows There is Statistically Significant with p<0.05 when results compared with BT & D8, BT & D15, BT & FU shown in Table No 4. On ***Daha*** it shows There is Statistically Significant with p<0.05 when results compared with BT & D8, BT & D15, BT & FU shown in Table No 5. On ***Udgata Mandala*** it shows There is Statistically Significant with p<0.05 when results compared with BT & D8, BT & D15, BT & F6 shown in Table No 6. On ***Raga*** it shows There is Statistically Significant with p<0.05 when results compared with BT & D8, BT & D15, BT & FU shown in Table No 7. On ***Pidakas*** It shows There is Statistically Significant with p<0.05 when results compared with BT & D8, BT & D15, BT & FU shown in Table No 8. On ***Rukshata*** it shows there is Statistically significant with p<0.05 when result compared with BT & D8, BT & D15, BT & FU shown in Table No 9. On **KASI Score** it shows there is statistically significant with p<0.05 when result compared with BT & D8, BT & D15, BT & FU, shown in Table No 10.

DISCUSSION

Probable Mode of Action

The *Somaraji Taila* contains a total of 8 ingredients and they are *Bakuchi, Haridra, Daruharidra, Sharshapa, Karanja, Kushta, Chakramarda, Aragwadha* all these 8 ingredients have synergetic effect with one another but individually having different actions and they are as follows

Bakuchi (*Psoralea corilifolia*) belonging to Fabaceae family having *Katu, Tikta Rasa, Laghu, Rooksha Guna, Ushna Veerya, Vata-Kapha Hara* which help in reducing *Kledata* acts as *Kandughna, Krimighna, Kushtaghna,* used part is *Beejas* and contains Psoralen, Isopsoralen, Bavachromene, Bakuchiol. Psoralen a compound has the ability to disrupts fungal cell wall and inhibits fungal DNA synthesis, Bakuchiol exhibits broad spectrum antimicrobial activities.

Haridra (*Curcuma longa*) belonging to Zingiberaceae family having *Tikta, Katu Rasa, Laghu Rooksha Guna, Ushna Veerya,* and *Tridosha Hara* helps in reducing *Daha, Kledata, Kandu* and acts as *Kandughna, Kushtaghna, Krimighna, Sophahara, Dahahara,* used part is *Moola* and contains Curcumin, Curcuminoids, Volatile Oils. Curcumin has strong antifungal properties which exerts by inducing oxidative stress within fungal sells, disrupting membrane integrity and interfering with fungal cell signaling pathway.

Daruharidra (*Berberia aristata* DC) belonging to Berberidaceae family having *Tikta, Kashaya Rasa, Laghu Rooksha Guna, Ushna Veerya* and *Kapha Hara* which help in reducing *Kleda, Daha* and acts as *Kandughna, Kushtaghna, Sophahara, Krimighna,* used part is *Moola* contains Berberin, Berbamine, Oxycanthine, Berberrubine. Berberine an alkaloid with significant antifungal effect acting by inhibition ergosterol synthesis which is vital to fungal cell wall membrane.

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Sharshapa (*Brassica nigra* L. Koch) belonging to Cruciferae family having *Katu Rasa, Teekshana Guna, Ushna Veerya, Vata-Kapha Hara* Property which help in *Kleda, Daha Hara* and acts as *Sophahara, Krimighna, Kandughna, Kushtaghna,* used part is Seeds and contains Steraric acid, Oleic Acid, Linolenic Acid, Esosenic Acid, Indole. Seeds contain allyl-isothiocynate, this compound disrupts the cell membrane integrity of fungi leading to leakage of cellular contents & leads to cell death. Glucosinolate a compound when hydrolyzed it produces various compound including isothiocyanate which have potent antifungal effect.

Kushta (*Saussurea lappa* CB. Clarke) belonging to Asteraceae family having *Tikta, Katu, Madhura Rasa, Laghu, Rooksha, Teekshna Guna, Ushna Veeyra,* having *Vata-Kapha Hara* which helps in *Kleda Hara* and acts as *Kushtaghna, Krimighna*, as it helps stopping the spreading of infection. Used part is *Moola* and contains Kusthin, Saussureal, Taraxasterol. Sesquiterpene lactone, dehydrocosten lactone, costunolide known to their strong antifungal & antimicrobial activities. Sesquiterpene exhibits antifungal property by inhibiting the synthesis of fungal cell wall & interfering with fungal enzyme activity. Castanosides help in apoptosis in fungal cells by generating reactive oxygen species (ROS) & disrupting mitochondrial function.

Karanja (*Pongamia pinnata*) belonging to Fabaceae family having *Tikta, Katu, Kashaya Rasa, Laghu Teekshna, Ushna Guna, Vata-Kapha Hara* which help in *Kleda, Daha Hara* and acts as *Kushtaghna, krminghna,* Used part is *Beeja* and contains Glabarin, Karanjin, Pongapin, Pongamol. Flavanoids, Karanjin & Pongamol having antimicrobial properties. Karanjin & pongamol interfere with fungal cell wall synthesis and disrupts membrane integrity leading to cell lysis and cell death. Flavonoids is having broad spectrum antimicrobial activity & enhance the overall antifungal efficacy by inhibiting fungal enzyme & DNA synthesis.

Chakramarda (*Cassia tora* Linn.) belonging to Caesalpiniaceae family having *Katu Rasa, Laghu Rooksha Guna, Ushna Veerya, Vata Kapha Hara* and acts as *Krimighna, Kauhstghna,* which help in reducing spread of infection. Used part is *Beeja* and Contains Oleic Acid, Lenolic Acid, Palmitic Acid, Sitosterol's. Seeds are rich ion flavonoids, anthraquinones and phenolics compound which contributes to its antifungal activity. Anthraquinones exhibits antifungal effect by interfering with the mitochondrial electron transport chain by fungi leading to energy depletion and leads to cell death. Flavonoids enhances membrane permeability, causing leakage of cellular content and inhibit fungal spore germination.

Aragwadha (*Cassia fistula*) belonging to Caesalpiniaceae family having *Madhura Rasa, Mrudhu, Guru Snigdha Guna, Sheeta Veerya, Kapha-Vata Hara* helps in Kandu, Daha *Hara* and acts as *Kushtaghna, Krimighna, Kandughna.* Used part is *Patras* which contains Anthraquinone, Lenoceric Acid, ß-Sitosterol's. Anthraquinone, flavonoids, and tannins which has antimicrobial properties. Anthraquinones these help in disrupt mitochondrial function and energy production in fungal cells. Tannins precipitate proteins and inhibit fungal enzymes, hence preventing fungal growth and reproduction. Flavonoids act by inhibiting fungal cell wall synthesis & inducing oxidative stress within fungal cell wall.

These all herbs work in synergetic effect to balance the impaired *Doshas*, purify the blood and direct combating fungal infection, their unique bioactive compound disrupt fungal cell wall and membrane inhibiting essential enzymes, generating oxidative stress and interfere with fungal DNA and energy synthesis. This synergetic effect enhances the efficacy of *Somaraji Taila* making it a potent natural *Taila* preparation for *Dadru Kushta.*

CONCLUSION

Dadru Kushta being a *Pitta Kapha Pradhana Vyadhi* and show symptoms like *Kandu, Udgata Mandala, Raga, Pidaka, Daha, Rookshata*. It mainly occurs due to *Krimija* and *Upasargaja Nidanas*, so one should maintain hygiene to prevent the spread. Both *Somaraji Taila* and *Chakramarda Taila* were effective in treating *Dadru Kushta* but *Somaraji Taila* gave better relief than *Chakramarda Taila* due to presence of 8 anti-fungal ingredients when compare to *Chakramarda Taila* has

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only one ingredient. In comparison between two group there was highly statistically significant with p<0.05. Since all the *Lakshanas* statistical shown highly statistically significant conclusion was drawn that, There is a Statistically significant difference in the effect of *Somaraji Taila* and *Chakramarda Taila* with *Somaraji Taila* having better effect than *Chakramarda Taila* in the management of *Dadru Kushta.*

Table 1: In-Vitro Zone of Inhibition.

Organisms	Standard Drug	Trial Drug(µg/l)			
		25	100	250	500
Trichophyton Rubrum	34 mm	0	13 mm	18 mm	21 mm
Trichophyton Mentagrophytes	17 mm	0	11 mm	16 mm	18 mm
Microsporum Gypsum	16 mm	0	0	0	14 mm
Microsporum Canis	10 mm	0	0	13 mm	14 mm

Table 2: Showing Scoring details of In-Vivo Study

Group	Day 0	Day 8	Day 15	Day 21
Group 1	0	0	0	0
	0	0	0	0
	0	0	0	0
Group 2	3	3	3	3
	3	3	3	3
	3	3	3	3
Group 3	3	2	1	1
	2	2	2	1
	3	2	1	0
Group 4	3	3	2	0
	3	2	2	0
	3	2	1	0
Group 5	3	2	2	1
	3	2	2	1
	3	3	2	1

Table 3: Showing the effect of Group S & Group T on KASI Scoring

Group	Pairing	Q Value	P<0.05	Group	Pairing	Q Value	P<0.05
Group S	BT-D8	5.47	Yes	**Group T**	BT-D8	12.02	Yes
	BT – D15	5.47	Yes		BT – D15	16.2	Yes
	BT – AFU	10.59	Yes		BT – AFU	13.4	Yes
	D8-D15	11.1	Yes		D8-D15	5.47	Yes
	D8-AFU	5.47	No		D8-AFU	11.1	Yes
	D15-AFU	5.47	No		D15-AFU	8.37	Yes

Table 4: Showing the effect of Group S & Group T on *Kandu*

Kandu	Median Score		Mann Whitney Test		Remarks
	Group S	Group T	T Value	P Value	
BT - D8	0.0	1.0	165.5	P=0.006	SS
BT - D15	1.0	2.0	170.5	P=0.01	SS
BT - AFU	2.0	3.0	177.5	P=0.02	SS
D8 - D15	1.0	1.0	222.5	P=0.6	NS
D8 - AFU	2.0	2.0	224.5	P=0.7	NS
D15 - AFU	1.0	1.0	241	P=0.7	NS

Note: SS - Statistically significant, NS - Not Significant

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Table 5: Showing the effect of Group S & Group T on *Daha*

Daha	Median Score		Mann Whitney Test		Remarks
	Group S	Group T	T Value	P Value	
BT – D8	1.0	1.0	218.5	P=0.5	NS
BT–D15	1.0	2.0	198	P=0.1	SS
BT-AFU	2.0	3.0	193.5	P=0.1	SS
D8-D15	1.0	1.0	206	P=0.2	NS
D8-AFU	1.0	2.0	205.5	P=0.2	SS
D15-AFU	1.0	1.0	225.5	P=0.7	NS

Note: SS - Statistically significant, NS - Not Significant

Table 6: Showing the effect of Group S & Group T on *Udgata Mandala*

Udgata Mandala	Median Score		Mann Whitney Test		Remarks
	Group S	Group T	T Value	P Value	
BT – D8	1.0	2.0	251	P=0.4	SS
BT–D 15	1.0	2.0	162.5	P=0.04	SS
BT-AFU	1.0	2.0	150.5	P=0.01	SS
D8-D15	0.0	1.0	177	P=0.02	SS
D8-AFU	1.0	2.0	164	P=0.005	SS
D15-AFU	1.0	1.0	243.5	P=0.6	NS

Table 7: Showing the effect of Group S & Group T on *Raga*

Raga	Median Score		Mann Whitney Test		Remarks
	Group S	Group T	T Value	P Value	
BT – D8	0.0	1.0	210	P=0.3	SS
BT–D 15	1.0	2.0	176.5	P=0.02	SS
BT-AFU	2.0	3.0	180.5	P=0.03	SS
D8-D15	1.0	1.0	190	P=0.08	NS
D8-AFU	2.0	2.0	201	P=0.1	NS
D15-AFU	1.0	2.0	156.5	P=0.02	SS

Note: SS - Statistically significant, NS - Not Significant

Table 8: Showing the effect of Group S & Group T on *Pidaka*

Pidaka	Median Score		Mann Whitney Test		Remarks
	Group S	Group T	T Value	P Value	
BT – D8	0.0	1.0	186.5	P=0.05	SS
BT–D 15	0.0	1.0	209.6	P=0.03	SS
BT-AFU	0.0	1.0	187.3	P=0.05	SS
D8-D15	0.0	0.0	230.5	P=0.9	NS
D8-AFU	0.0	0.0	222	P=0.6	SS
D15-AFU	0.0	0.0	234	P=0.9	NS

Note: SS - Statistically significant, NS - Not Significant

Table 9: Showing the effect of Group S & Group T on *Rukshata*

Rukshata	Median Score		Mann Whitney Test		Remarks
	Group S	Group T	T Value	P Value	
BT – D8	3.0	7.0	199	P=0.1	NS
BT–D15	6.0	13.0	176.5	P=0.02	SS
BT-AFU	8.0	21.0	167.5	P<0.05	SS
D8-D15	3.0	6.0	165.5	P<0.05	SS
D8-AFU	4.0	13.0	159.0	P<0.05	SS
D15-AFU	2.0	6.0	155.5	P=0.02	SS

Note: SS - Statistically significant, NS - Not Significant

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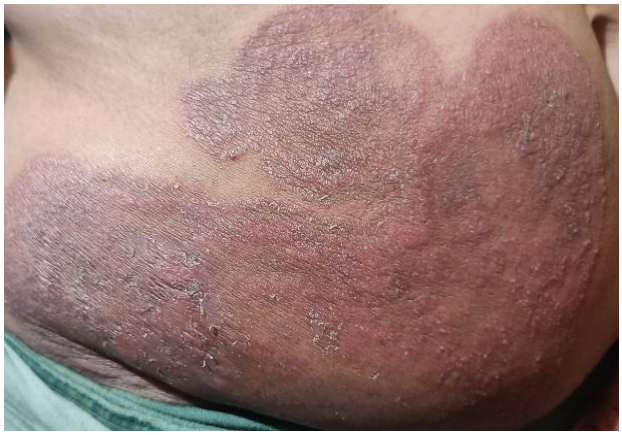
Table 10: Showing the effect of Group S & Group T on KASI Scale

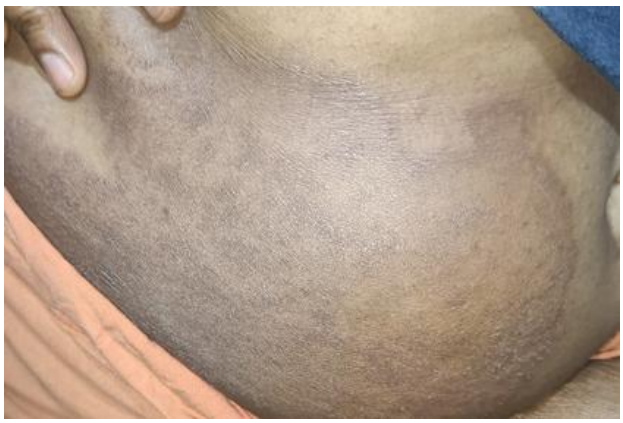
KASI Score	Median Score		Mann Whitney Test		Remarks
	Group S	Group T	T Value	P Value	
BT – D8	5.0	4.0	257	P=0.3	NS
BT– D15	12.0	8.0	284	P=0.03	SS
BT-AFU	16.0	11.0	274	P=0.08	NS
D8-D15	5.0	3.0	303	P=0.004	SS
D8-AFU	8.0	5.0	279	P=0.05	SS
D15-AFU	4.0	3.0	254	P=0.3	SS

Note: SS - Statistically significant, NS - Not Significant

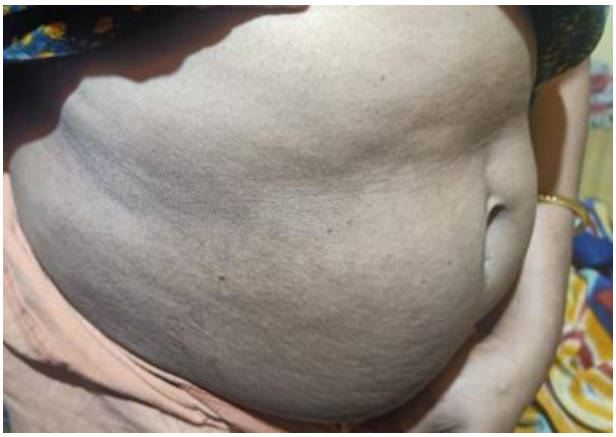
Figure 1: Showing Reduction of lesion.



0th Day



8th Day



21st Day

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