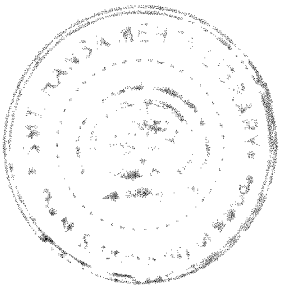
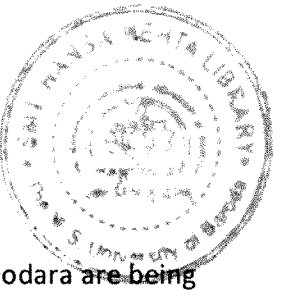


CHAPTER-7

PRELIMINARY CLINICAL STUDIES

7.0 Preliminary Clinical Studies



Out of all the four mud samples, Kerala Black, Kerala Brown, and Vadodara are being used as a mud therapy for general health rejuvenation at Shri Krishna Ayurved Center, Alleppy and Nature Cure Center, Vadodara respectively. These centers also treat patients with skin disorders, but they combine other treatments(Panchkarma ,Nasya, Massage, diet, Yogasanas etc) with mud therapy. Hence it is difficult to determine the exact role of mud therapy in treating skin disorders. Mud from Dwarka, which is commonly known as 'Gopi Chandan' is traditionally used for prickly heat and acne but its use in treatment of psoriasis and eczema has not been reported.

Hence we decided to conduct preliminary clinical studies **to study the efficacy and safety of these muds** in treatment of psoriasis, eczema and acne with nominal changes in diet (barring non-veg food) and keeping the other life styles of the patient unchanged. Unlike Nature Cure and Ayurved Centers , this treatment was taken by the patients at their home.

This study was conducted partly at Sir Sayajirao General (SSG) Hospital, Vadodara which is a government hospital of around 1000 beds and OPD (Out patient Department) of around 100 to 150 patients daily in its Dermatology Department and partly at Government Ayurved Hospital , Vadodara which has around 30 to 35 patients' OPD daily. It should be noted that all the patients referred to us for the study at SSG, and Govt. Ayurved Hospital **had disease history of minimum five years and were treatment wise categorized as failure cases according to the Dermatologists.** At both the hospitals the study was conducted under the supervision of a Dermatologist and patients were monitored and evaluated by them. In the case of psoriasis, PASI (Psoraisis Area Severity Index) score was noted, in eczema, EASI (Eczema Area Severity Index) and in acne IGA(Investigator's Global Assessment) was observed. Patients' feelings of well being were also noted down.

A protocol for the study was prepared and Informed Consent Form was filled up by the participants. A Case Report was also prepared wherein the weekly observations and results of patients were recorded. Photographs of the affected part were also taken during periodical examination.

The patients were asked to wet the soil and apply on the affected part and leave it on the skin for 30 minutes. After 30 min., mud was gently scrapped off the application site and collected in a clean dry plastic bag provided by us. These samples were analysed with respect to their chemical composition by CHNS analyser, SEM-EDS, AAS, FTIR and organic carbon chemical digestion method. The whole study continued upto one year and in all 59 patients were treated.

7.1 Protocol

7.1a Drug Profile

1. Name: Mud powder: Kerala Black, Kerala Brown, Dwarka, and Vadodara.

2. Physical Properties: It is a powder passed through 44 # sieve which on wetting with enough water forms a thick paste.

3. Ingredients: It is a natural mixture of many elements and minerals.

4. Indication: Psoriasis, Eczema, Acne.

5. Dosage and administration: Approx. 3mm. thick layer of wet mud applied topically for 25-30 min. once a day , daily at least for 15 days and after removal of mud washing with water and applying oil or cream on the mud applied part for emollient action.

6. Adverse drug reaction: dryness of skin.

7. Contraindication: open skin lesions, wet eczema, pus forming acne.

7.1b Protocol for Study

1. Study objective: For efficacy of mud on psoriasis, eczema and acne.

2. Number of subjects: Minimum 4 for each mud and each disease.

3. Subject selection criteria:

(a)Inclusion: Patients 18-60yrs., male or female suffering from psoriasis, dry eczema or acne.

(b)Exclusion:

(i)Patients having significant history of alcohol or drug abuse or smoking habits.

(ii)Patient undergoing any other therapy and taking any other systemic medication for the disease .

(iii) Patients taking any vasoactive or any otc drug.

(iv) Patients suffering from wet eczema and pus forming acne.

(c) Restrictions and prohibitions.

(i)Avoid contact with any other body parts during the time of application.

(ii) No exercise or bathing or showering while the mud is applied.

(iii) No use of creams or emollient creams to the affected area before 3 hrs. of the application.

(iv) If large body area is affected then mud shall be applied after 2 hrs. of food intake.

(v) Pulses, Brinjal, Salt, Spicy, fried , maida and non-veg food preparations are prohibited during full span of treatment .

(d) Criteria for discontinuation or withdrawal of study:

(i) If patient fails to comply with requirement of protocol.

(ii) If patient suffers from severe adverse effects .

4. Design of study: Rondomised.

5. Drug treatment: Topical application of mud.

6. Method of application and removal: Synchronised.

7. Selection of psoriatic, eczema and acne skin sites: according to severity. Avoid lesions greater than grade 3 severity.

8. Duration and method of application of mud: Mud powder is wet by enough water to make paste and kept aside for 10 minutes. It is then to be applied on the affected site with a disposable applicator and spread with the help of disposable spreader. The mud is allowed to remain on the body for 30 minutes.

9. Removal of the drug: After 30 min the mud is scrapped off by a scrapper and collected. Remaining portion of mud is to be wiped off by wet cotton.

10. Assessment of pharmacological activity: Visual assessment of erythema, plaque elevation and scaling of the lesion for psoriasis, itching and redness for eczema and no. and severity of comedones and papules in acne,is to be made and graded as severe :3, better : 2 , and good : 1.

7.1c Informed Consent Form

1. Name , Address, and Ph.No.:
2. Age:
3. Sex:
4. Body weight:
5. BMI: (body mass index):
6. Nature and purpose of trial: To study the efficacy of mud or its formulation by applying it on diseased skin.

7. Trial procedure: Natural mud will be wet by water and applied on clean affected skin by a disposable applicator. It will remain there for 30 min. and after that it will be removed with a scraper and collected for testing . The location will be wiped off by cotton. This procedure will be conducted daily for atleast 15 days or more depending upon the recovery rate.
8. Subjects' responsibility: Subject will have to avoid contact of the treated body part with any other body part or things. He/she will have to avoid pulses, brinjal, salt , spicy, fried, maida and non-veg. food preparation during the period of treatment.
9. Forseable risk and inconvenience to the subject: There is no known or reported risk of applying natural mud except for rare allergic reaction like itching and redness.
10. Reasonably expected benefits: By applying natural mud, healing of psoriatic or dry eczema affected skin lesion or acne are known to heal to normal and recurrence is reported to be negligible.
11. Duration of the treatment: Duration of the treatment may last for minimum of 15 days or longer depending upon the recovery rate .
12. Confidentiality of the subject: All the reports of the trial will be kept confidential.
13. Approximate number of subjects participating in the trial: Overall 50 subject are expected to take part in this study.

I have read the **Consent Form** completely and with my full presence of mind and am ready to participate in the trial willingly.

Sign: Place: Date:

7.1d Case Report

1.Patient No: **Date:** **Centre:**

2.Name:

3.Address

4.Sex

5.Disease:

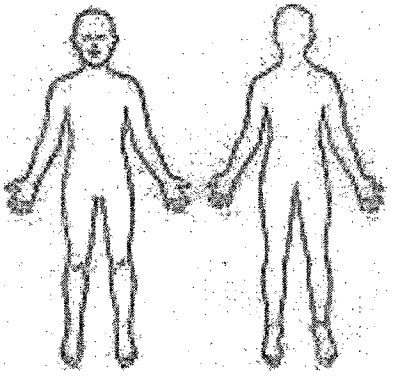
6.Prior treatment and history:

Topical				**Systemic**		
steroids	**tar**	**emollients**	**others**	**methotrexate**	**antihistamine**	**vitamins**

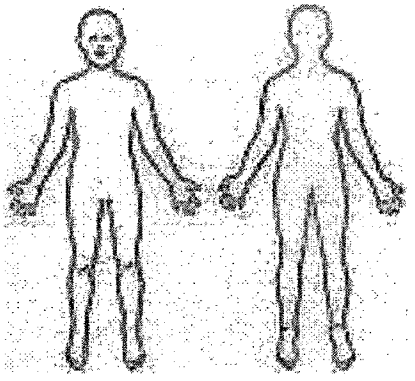
7.Application: **Daily at home /centre:**

8.Observation: Weekly

9.Area involved: (mark the body part in the figure)



10.Area treated with formulation: (mark the body part in the figure)



surface involved (per body region) (A)	Value given
<10%	1
10-29%	2
30-49%	3
50-69%	4
70-89%	5
90-100%	6

Psoriasis:

Symptoms	Base line values	After 1 week	After 2weeks	After 3weeks	After 4weeks	After 5weeks	After 6weeks
Erythema (E)	E_h=						
	E_u=						
	E_t=						
	E_i=						

Plaque elevation Or Desquamation (D)	D_h=						
	D_u=						
	D_t=						
	D_l=						
Scaling Or Infiltration (I)	I_h=						
	I_u=						
	I_t=						
	I_l=						

$$PASI = 0.1(E_h+I_h+D_h)A_h + 0.2(E_u+I_u+D_u)A_u + 0.3(E_t+I_t+D_t)A_t + 0.4(E_l+I_l+D_l)A_l$$

where h=head, u=upper extremities, t=trunk , l=lower extremities A=area of the body involved

Grading system

Degree of severity (per body region)	Value
No Symptoms	0
Slight	1
Moderate	2
Marked	3
Very marked	4

Result:

Weeks	Initial score	Final score
0		
1		
2		
3		
4		
5		
6		

Eczema:

Symptoms	Base line values	After 1 week	After 2weeks	After 3weeks	After 4weeks	After 5weeks	After 6weeks
Redness							
Itching							
Thickness							
Lichenification							

EASI = $0.1(R_h+T_h+I_h+F_h)A_h$ + $0.2(R_u+T_u+I_u+F_u)A_u$ + $0.3(R_t+T_t+I_t+F_t)$ + $0.4(R_l+T_l+I_l+F_l)A_l$

Where R=redness, T=thickness, I=itching, F=lichenification A=area of the body involved

Result:

Weeks	Initial score	Final score
0		
1		
2		
3		
4		
5		
6		

Acne:

Symptoms	Base line values	After 1 week	After 2weeks	After 3weeks	After 4weeks	After 5weeks	After 6weeks
Severity							

Result:

Weeks	Initial score	Final score
0		
1		
2		
3		
4		

IGA Scale for Acne Vulgaris Grade	Description
0	Clear skin with no inflammatory or noninflammatory lesions
1	Almost clear; rare noninflammatory lesions with no more than one small inflammatory lesion
2	Mild severity; greater than Grade 1; some noninflammatory lesions with no more than a few inflammatory lesions (papules/pustules only, no nodular lesions)
3	Moderate severity; greater than Grade 2; up to many noninflammatory lesionsand may have some inflammatory lesions, but no more than one small nodular lesion
4	Severe; greater than Grade 3; up to many noninflammatory and inflammatory lesions, but no more than a few nodular lesions

Evaluation:

Various instruments are used to evaluate the psoriatic conditions: scanning laser – Doppler velocimeter (Speight EI 1993), Xenon washout technique(Berardesca MH 1989), structural PASI (Psoriasis Area Severity Index) like instrument with visual analog scales for recording redness, thickness and scaling (Fleicher AB 1994), ultrasound imager for skin thickness as a parameter for hyperproliferation (Vaillant L 1994), High frequency ultrasound imager for assessing severity (Savolaimen I 1997), measurement of transepidermal water loss; (Berardesca MH 1989), Chromatometry for evaluating erythema, and visiometry for evaluating profilometry (Bangha E 1990), computer-image analyzer of a color segmentation method (Savolaimen I.,1997) planimetric area-detecting technique (Marks R 1989), Electrophysiologic parameters indicating damage to barrier function and epidermal mitotic activity (Cler EJ 1976), portable instrument for quantifying erythema induced by ultraviolet radiation (Diffey BI 1984), assessing scaling by densitometry of macrophotographic negatives (Marshall RJ 1986), and triple-labeling flow cytometric analysis of epidermal growth (Glade Cp , 1995, Glade Cp,1997).

In addition, a new direction in healthcare assessment of psoriatic patients has been described as the " outcomes movement", emphasizing assessment of patients' outcomes with respect to their quality of life and functional capacity, rather than techniques that rely on results of physical or laboratory tests (McKenna KE,1996).

So we adopted PASI score as the test parameter for assessing the improvement in the disease symptoms and quality of life for psoriasis (Harari M,2000). PASI 50 equates to a clinically meaningful improvement in psoriasis and represents a discerning primary endpoint (Carlin CS 2005, Louden BA 2004). .

PASI score was calculated by assessing erythema, infiltration(scaling) and desquamation (plaque elevation) of psoriatic lesions.

$$\mathbf{PASI = 0.1(E_h+I_h+D_h)A_h + 0.2(E_u+I_u+D_u)A_u + 0.3(E_t+I_t+D_t) + 0.4(E_l+I_l+D_l)A_l}$$

Where E= erythema, I=infiltration, D=desquamation, h=head, u=upper extremities, t=trunk, l=lower extremities A= area involved

Eczema

For eczema, intensity was assessed by redness , thickness, itching, and lichenification. Eczema Area Severity Index (EASI) was calculated by (www.dermetnz.org)

$$EASI = 0.1(R_h+T_h+I_h+F_h)A_h + 0.2(R_u+T_u+I_u+F_u)A_u + 0.3(R_t+T_t+I_t+F_t) + 0.4(R_l+T_l+I_l+F_l)A_l$$

Where R=redness, T=thickness, I=itching, F=lichenification

Severity for each symptom was graded from 1 to 4 and value was given for percentage of body area involved as shown in the case report.

Acne

The primary difficulty in developing a standardized ordinal scale is the pleomorphic nature of acne, as is pertinent to the mixture of lesion types, sites of involvement, the variable characteristics of the lesions (especially the inflammatory types), and the variability in the progression of acne lesions. However different methods are used for evaluating acne (Balaji A 2009). Some are based on grading severity by assigning severity index between 0-4 or 0-10 and no special equipments are required.e.g. Frank numerical grading system (Frank SB 1971). Fluorescent photography is also used for assessing severity of comedones (Lucchina LC 1994). Some scientists assess inflammatory acne by polarized light photography (Philips SB 1997). A photonumeric method: both grading and lesion counting is done using photographic standards (Allen BS 1982). Many scientists have tried to compare different systems of scoring acne but not a single system was found to be ideal (Balaji A 2009).

Use of lesion count assessments alone as an endpoint may be less than reliable because of the lack of appreciation for the variable expression of acne vulgaris with a

strictly quantitative definition (e.g., size of lesions, intensity of inflammation, and location of lesions). Combining the two approaches of ordinal global assessment scale and lesion count assessments allows for a balanced approach toward the evaluation of acne severity. We have adopted for clinical studies, investigating the effect of a therapy on acne severity, co-primary endpoints that evaluate an **IGA,(Investigator's Global Assessment)** and acne lesion counts (US Department 2005) as shown in case report form. The Case Report Forms for acne studies can allow for reporting by investigators of lesion worsening beyond Grade 4 with treatment.

Photography was also used as a test parameter for all the three diseases. Photography has been used as an evidence for acne improvement (Cook N 1979) and so all the patients examined were photographed and simultaneously evaluated by a dermatologist at Sir Sayajirao General Hospital, a public hospital at Vadodara and Government Ayurvedic Hospital at Panigate, Vadodara. The site of application of mud was photographed prior to starting the treatment and after every week.

In all 59 patients were treated and examined having either psoriasis, eczema, or acne. Out of them 23 were females and 36 males. The age group was from 18 to 60 years. All the patients treated were"failure cases" according to the dermatologists and had a history of 5 to 6 years of disease treatment by other modes of therapies.

Normal healthy persons were also applied wet mud on the ventral part of the forearm, removed when dry and taken for analysis. Those were considered as 'normal' samples.

Out of the 59 patients who were enrolled, only 52 continued the treatment for atleast 15 days and out of them 48 patients returned the samples . They were analysed for IR, SEM with EDS, CHNS analysis and Atomic absorption spectroscopy and organic carbon (chemical digestion method). IR studies were conducted at Choksi Laboratory, Makarpura, GIDC, Vadodara, SEM with EDS at Metallurgical Department, M.S.University, Vadodara, CHNS analysis at SAIF, IIT Bombay, AAS at Jewel

Metallochem Lab., Mumbai and chemical digestion method was performed at our department as per the methodology described earlier.

7.2 CHNS analysis (MAP)

The results of carbon content in treated soil samples by CHNS analyser are shown in table 37 and fig. 47.

Results:

Table no. 37 **% Carbon** by CHNS analyser of Mud Applied on Patients (MAP)

%Carbon by CHNS analyser					
	Std	Normal	Psoriasis	eczema	acne
Black	2.761	2.757	3.812	2.908	2.999
		2.846	2.858	2.845	3.234
		2.801	3.059	2.981	3.021
		2.634	3.182	3.023	3.189
Brown	0.717	0.942	1.048	0.962	1.944
		0.968	1.356	0.998	1.247
		0.854	1.345	0.872	1.256
		0.957	1.405	0.989	1.165
Dwarka	8.898	8.668	9.692	8.988	9.548
		8.608	9.768	8.996	9.487
		8.015	9.851	8.867	9.578
		8.028	9.598	9.014	9.456
Vadodara	0.289	0.359	0.585	0.359	1.622
		0.436	0.516	0.589	0.968
		0.399	0.549	0.532	0.845
		0.401	0.592	0.508	0.799

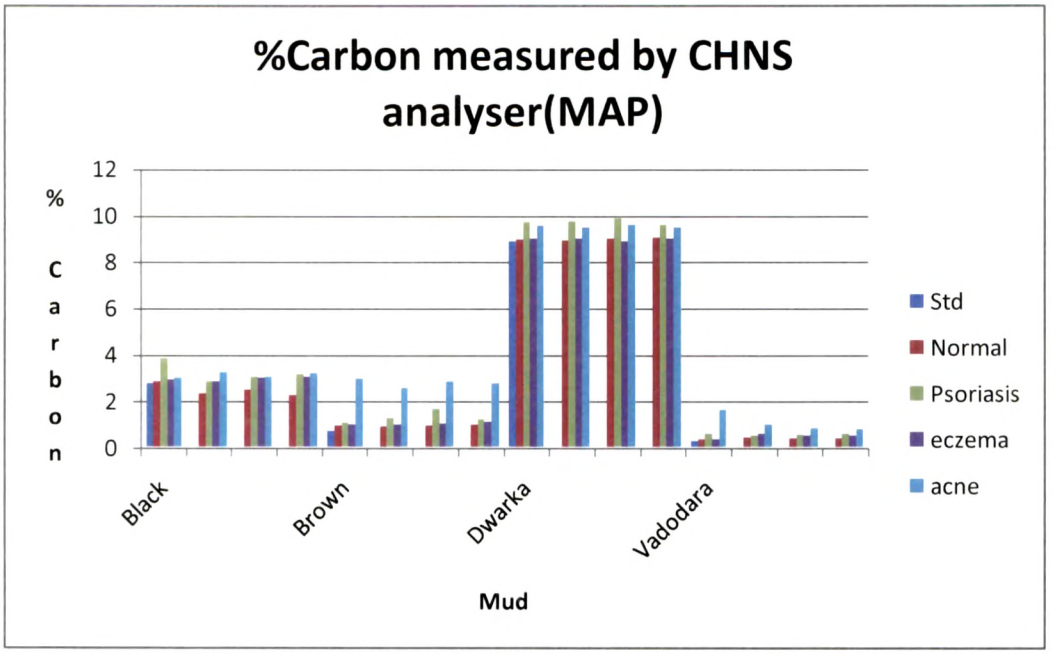


Fig. no. 47 % Carbon measured by CHNS analyser (MAP)

Discussion

It was quite evident from this data that except in two cases,(Dwarka –eczema, Vadodara – eczema) there was statistical ($P<0.05$, ANOVA) increase in Carbon concentration in the soil applied on patients compared to that of the skin of normal subjects. CHNS measures all the combustible Carbon present in the sample and since this data showed increase in carbon content compared to that of the normal subjects, it prompted us to conclude that there must be some free carbon compounds on the surface of the diseased skin which must have bonded with some soil components. It has been universally accepted that there are free fatty acids on the skin surface membranes and that these membrane lipids not only serve a fundamental role in the structure of membranes but also serve a critical role in the processes of signal transduction and cell regulation(Khan W.A. 1995). Arachidonic acid , which is present in psoriatic scales ,play an important role in inflammatory process in psoriasis and it was observed clinically, that after reduction in itching (first sign), reduction in plaque thickness was the second sign of improvement . It may be possible that free arachidonic acid present in psoriatic scales (Kouichi L 1999) may

be one of the carbon component which may be adsorbed on the soil and at the same time showed improvement in disease symptoms.

7.3 Chemical digestion method (MAP)

The percentage concentration of organic carbon in mud applied on patients, as measured by chemical digestion method is given in table no.38. and fig.no.48.

Results:

Table no: 38 % Carbon by chemical digestion method (MAP)

% carbon by chemical digestion					
	Std	Normal	Psoriasis	eczema	acne
Black	0.032	0.033	0.075	0.058	0.059
	0.024	0.049	0.085	0.094	0.069
	0.032	0.039	0.071	0.089	0.059
	0.028	0.047	0.092	0.088	0.072
Brown	0.127	0.129	0.134	0.13	0.463
	0.13	0.13	0.143	0.139	0.397
	0.123	0.126	0.142	0.141	0.402
	0.129	0.13	0.152	0.152	0.487
Dwarka	0.005	0.007	0.035	0.022	0.023
	0.004	0.024	0.049	0.087	0.062
	0.005	0.017	0.039	0.076	0.049
	0.0051	0.028	0.052	0.089	0.042
Vadodara	0.029	0.005	0.324	0.099	0.086
	0.031	0.027	0.254	0.054	0.079
	0.028	0.041	0.398	0.064	0.054
	0.032	0.039	0.401	0.101	0.0698

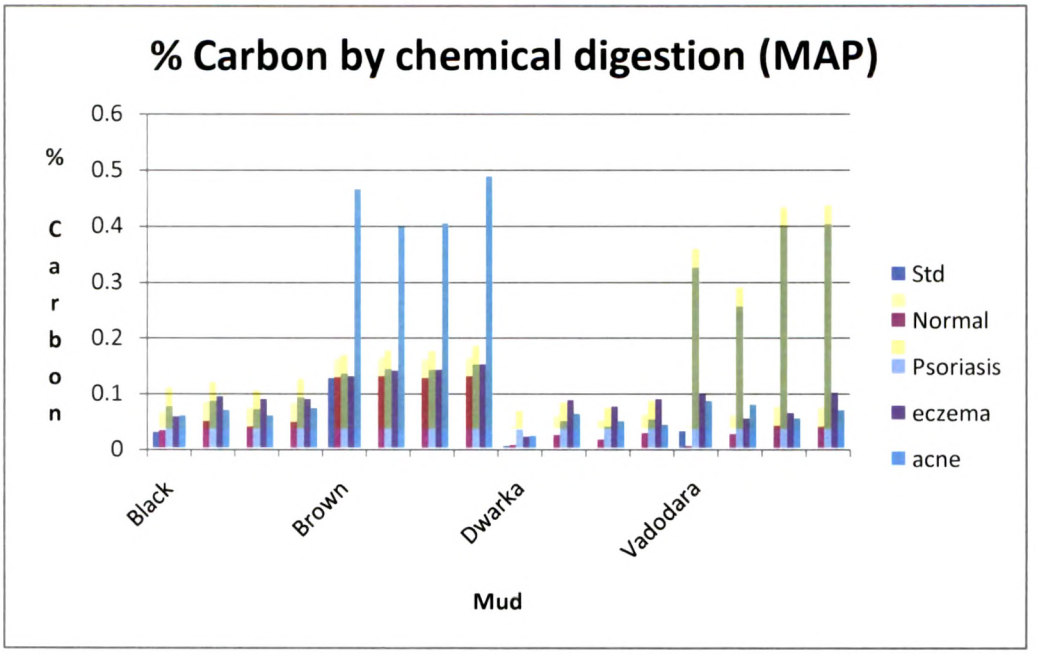


Fig.no: 48 % Carbon by chemical digestion method (MAP)

Discussion

As analogous to reports of CHNS analysis, % carbon content in soil applied on patients, showed **significant increase** in carbon content in all the four muds applied on three diseased patients, as compared to that of soil applied on normal skin ($p<0.05$, ANOVA) . This data confirmed the results shown by CHNS analyser. Moreover, it should be noted that there was **no significant** difference between carbon content of mud and that obtained after applying on **normal skin**. Hence this confirmed that carbon compounds of diseased skin were getting removed from the skin and adsorbed or absorbed on the soil. Considering the value of '$F_{critical}$' equal to 4.17, the statistical value of F was 7.16 for psoriasis,2.18 for eczema, and 4.3 for acne. Thus it could be inferred from these values that irrespective of individual mud, psoriasis was better treated than eczema and acne i.e. more amount of carbon got removed from psoriatic lesion than eczema and acne lesions.

Considering the value of '$F_{critical}$ 'equal to 3.49 , the values of F (irrespective of diseases) for different methods, of determining carbon content are given in table

no. 39. It could be deduced that when organic carbon was measured by chemical digestion method, Kerala Brown had highest value of F ,in mud applied on patients (psoriasis, eczema, acne). CHNS analyser reports showed that Dwarka had the highest value of F , in mud applied on patients (psoriasis, eczema,acne). This variation in the result may be because CHNS analyser determines total combustible carbon while chemical digestion method measures organic carbon. Irrespective of the method, it could be observed that Kerala Brown had the highest F value so it could be assumed that it could adsorb highest quantity of carbon from the diseased lesions.

Table no. 39 Values of F for two methods irrespective of diseases

	Values of F for F critical =3.49	
	CHNS	Chemical digestion.
Black	3.37	12.2
Brown	5.49	168.4
Dwarka	42.7	4.64
Vadodara	8.75	55.93

7.4 Atomic Absorption Spectrometry (MAP)

Table no. 40 shows the percentage concentration of **elements** in muds applied on patients (MAP) measured by AAS .

Table no: 40 % Concentration of elements (Ca, Mg) measured by AAS (MAP)

	Std.		Normal		Psoriasis		Eczema		Acne	
	Ca	Mg	Ca	Mg	Ca	Mg	Ca	Mg	Ca	Mg
Black	0.049	0.33	0.062	0.34	0.056	0.28	0.06	0.39	0.065	0.41
			0.076	0.58	0.046	0.3	0.045	0.26	0.041	0.21
			0.087	0.37	0.052	0.22	0.051	0.19	0.045	0.12
			0.056	0.46	0.036	0.26	0.037	0.23	0.034	0.18
Brown	0.004	0.017	0.003	0.005	0.003	0.008	0.002	0.006	0.016	0.007
			0.004	0.012	0.0003	0.003	0.001	0.002	0.021	0.0026
			0.008	0.01	0.0025	0.001	0.0025	0.001	0.019	0.0039
			0.006	0.009	0.0015	0.002	0.0015	0.004	0.024	0.0013
Dwarka	0.011	0.06	0.029	0.019	0.024	0.018	0.017	0.009	0.03	0.023
			0.035	0.029	0.021	0.012	0.019	0.007	0.017	0.019
			0.026	0.031	0.015	0.01	0.023	0.003	0.014	0.014
			0.043	0.026	0.026	0.014	0.028	0.006	0.02	0.012
Vadodara	0.005	0.005	0.005	0.003	0.009	0.005	0.057	0.003	0.002	0.004
			0.003	0.009	0.094	0.0007	0.045	0.002	0.0005	0.00007
			0.006	0.006	0.085	0.0005	0.049	0.001	0.0009	0.00006
			0.002	0.005	0.1	0.0002	0.052	0.0016	0.0001	0.00004

Discussion:

Calcium and Magnesium play an important role in skin chemistry (cell regulation) of psoriasis, eczema and acne and so our work was focused on monitoring their levels in mud applied on patients.

It could be observed from the data shown in table no.40 that there was significant difference in the concentration of calcium and magnesium ($P < 0.05$ ANOVA test) in all the four muds applied on patients suffering from psoriasis, eczema and acne. Amount of magnesium and calcium was significantly decreased.

It is observed that extracellular calcium levels is less in psoriasis, eczema and acne which favors proliferation, (Menon GK 1991) while there is increase in levels of intracellular calcium. It may be possible that since penetration of components of soil into skin is a simple diffusion process, the calcium must be entering into skin and thus helping it to heal. It was observed from the F value that Brown had maximum penetration of calcium into the skin as shown in table no. 41. Indicating, superiority of Brown over 3 other muds. If we compare the order of value of 'F' with the concentration of calcium and magnesium in std. mud then it could be observed, that rule of **optimum concentration for maximum penetration** was followed.

Table no: 41 Value of 'F' for Calcium and Magnesium(AAS method MAP)

Mud	F value	
	Ca	Mg
Black	3.89	3.85
Brown	66.7	4.18
Dwarka	3.9	15.7
Vadodara	10.7	4.6

7.5 Scanning Electron Microscopy and Energy Dispersive Spectrometry (SEM-EDS) (MAP)

SEM-EDS measures all the elements present in the soil sample, but as our focus was on the carbon, Ca, and Mg content only data of these elements is given in table no.42.

Table no: 42 % concentration of C,Ca,Mg as measured by SEM-EDS of MAP

	Std			Normal			Psoriasis			Eczema			Acne		
	C	Ca	Mg	C	Ca	Mg	C	Ca	Mg	C	Ca	Mg	C	Ca	Mg
Black	3.78	0.61	1.4	2.69	0.626	1.5	11.633	0.433	1.17	7.785	0.515	1.31	5.57	0.412	1.26
				3.79	0.738	2.34	11.78	0.378	1.2	9.587	0.312	1.11	6.37	0.325	0.16
				2.89	0.666	1.54	10.49	0.165	0.23	10.345	0.245	1.03	5.72	0.409	1.09
				4.94	0.854	3.3	12.57	0.272	0.19	8.123	0.437	0.07	4.85	0.217	1.15
Brown	4.89	0.41	1.03	6.3	0.43	1.0121	19.589	0.45	0.94	15.7325	0.47	0.62	9.67	0.41	0.95
				7.5	0.39	1.129	20.356	0.04	0.11	11.256	0.07	0.53	10.32	0.39	0.77
				6.4	0.55	1.0123	17.789	0.17	0.89	25.432	0.38	0.47	9.56	0.4	0.83
				8.15	0.68	1.12	16.931	0.48	0.97	13.894	0.24	0.06	11.34	0.42	0.41
Dwarka	5.66	12.05	5.29	6.5	11.92	5.29	24.34	10.67	4.67	19.56	5.37	2.89	16.872	7.915	4.0475
				7.3	9.89	5.712	20.67	6.73	4.54	17.94	6.52	1.98	8.896	9.646	4.323
				6.46	10.907	6.302	23.76	9.59	2.61	20.36	4.69	3.12	16.754	7.945	4.0412
				9.39	12.901	7.309	14.15	10.62	3.63	15.79	8.32	4.07	11.912	6.926	4.0498
Vadodara	2.17	0.79	1.02	2.4	0.959	1.3	11.177	0.6625	0.9425	6.83	0.412	0.645	4.567	0.567	0.665
				1.5	1.967	1.2	10.234	0.5789	0.6534	9.96	0.228	0.356	5.381	0.443	0.7451
				0.2	0.756	1.01	9.456	0.6543	0.9654	8.47	0.417	0.683	6.527	0.561	0.5523
				3.1	1.945	0.93	13.009	0.4612	0.5378	10.65	0.516	0.532	7.478	0.354	0.7972

Discussion

It could be observed from data of table no. 42 that there was drastic rise in carbon content of soil ($P<0.05$) applied on psoriasis, eczema and acne patients. These results are in co-ordination with the results of CHNS analysis and chemical digestion method. So it could be confirmed that carbon compounds do come out from the diseased skin which may be helping in relieving the symptoms. It is a well known fact, as professed by our ancient literature, that mud pulls out toxins from our body and so it may be possible that carbon containing toxins must be getting removed from the lesional skin. However it is speculative and it remains to be confirmed, whether changes in carbon content in the soil , could be used as a marker of improvement of disease symptoms.

In this case also ,as analogous to AAS data, levels of Ca and Mg also were found to decrease significantly ($P<0.05$) in all the muds applied on all three diseases . Thus, it could be concluded that there is mobility of Ca and Mg ions from soil to the skin , resulting in recovery of the lesions. Data of invitro diffusion studies explained earlier also endorses this finding. Ca helps regulate cell proliferation and Mg reduces antigen presenting capacity of Langerhans cells and may thus contribute to the efficacy of mud in the treatment of inflammatory diseases (Christoph MS 2000).

7.6 Clinical observations

Patients were observed clinically for improvement in disease symptoms and as mentioned earlier they were graded and scored accordingly as shown in table no.43

Table no: 43a Disease improvement score

	Psoriasis		Eczema		acne	
	Initial score	final score	Initial score	Final score	initial	final
Black	5.6	2.6	2.4	0.2	3	1
	34.4	1.9	2	0.8	3	1
	12.2	1.2	5.6	4	4	2

	8.8	4.9	0.4	0.02	4	3
Brown	5.6	5.04	1.6	1.2	6	1
	2.4	1.2	2.4	0.8	4	1
	4.7	1.8	1	0.8	4	1
	5.3	4.2	1.6	0.28	4	1
Dwarka	14.4	4.8	0.2	0.1	4	1
	8.9	2.4	9.8	4.2	2	1
	6.2	3.8	2.4	0.8	2	1
	10.3	5.6	4.1	3.2	3	1
Vadodara	12.6	10.6	12.6	0.01	2	1
	9.6	0.5	9.6	1	1	1
	6.8	3.8	6.8	0.02	2	1
	4.6	1.2	4.6	0.7	1	1

Table no. 43b Disease Improvement in percentage

	Psoriasis	Eczema	Acne
Black	54	92	67
	95	60	67
	91	29	50
	45	95	25
Brown	10	25	83
	50	67	75
	62	20	75
	21	83	75
Dwarka	67	50	75
	74	57	50
	39	67	50
	46	22	67
Vadodara	16	100	50
	95	90	50
	45	100	50
	74	85	50

Discussion

Table no.43a shows the data of initial and final score of the disease symptoms after 15 days of treatment and table no.43b shows percent improvement in disease symptoms. Statistical test (ANOVA $p<0.05$) proved that all the four muds were effective in treating psoriasis, eczema and acne.

As mentioned previously the patients allotted to us by the dermatologists of SSG and Ayurvedic hospital for treatment, were "failure cases" with long term history of the disease. Hence, their treatment was expected to be prolonged.

Depending upon the intensity and % of area affected, there was different reduction in score but on an average, at the end of minimum 15 days , there was at least 50% reduction in PASI score which is a clinically significant end point in the assessment of psoriasis (Carlin CS 2005). In all the three diseases, overall, 80% people showed more than 50% improvement in all the three disease symptoms within 15 days of mud treatment.

It was observed during the treatment that itching was the first symptom which was improved within 2 days of application in psoriasis and eczema. Then gradually, scaling got removed and then the plaque thickness decreased in psoriasis. This phase of reduction of plaque thickness was a long process but during that time , there was no eruption of any new patch and there was complete eradication of itching. Depending upon the intensity of the disease, it took around 3 to 4 months for the skin to become normal in case of plaque psoriasis.

In case of eczema, the recovery was comparatively faster, may be because in eczema, usually skin becomes very thin, which leads to increased permeability of calcium and magnesium which play an important role in cell proliferation. Eradication of itching was the first sign of improvement and the skin lesions was healed within 10 to 15 days.

In acne , the progress was slow in some cases while fast in some. It was observed that if a person had oily skin then the recovery was fast . Normally if acne is

untreated then comedone bursts and fluid oozes out; but on application of mud, the comedones subsided and the skin became normal. Moreover, it was observed that when the mud was washed off from the face with water, there was flushing on the face lasting for 5 to 7min., which indicated increased blood circulation. However in case of young girls nearing their menstruation period time, it was commonly observed that eruption of comedones increased and application of mud gave little response at that time period. This implies that mud therapy was not able to improve symptoms which were mediated by hormonal changes in the body.

All the patients admitted that there was feeling of coolness on application of mud and this pleasant feeling helped reduce mental tension. Mental stress is one of the main cause of psoriasis, eczema and acne. It is commonly observed that young girls who are susceptible to acne experience aggravation during stress conditions like exams, in such situations application of mud improved the disease symptoms.

The score mentioned here is of 15 days of treatment but it was also observed that when the treatment was continued further, there was further improvement in the symptoms. In certain patients where the treatment had been extended for more than 15 days, the recovery increased upto 90%. Also, though no follow up study could be done for all the patients, but for 3 of psoriasis 6 of acne and 4 of eczema, feedback could be obtained after 6 months of discontinuation of our treatment (and no other treatment tried). They reported no recurrence of symptoms of the disease and experienced good mental and social well being.

Dwarka showed very good results for acne patients. As such, Dwarka is used traditionally for prickly heat and pimples. This may be attributed to the high calcium content in it (SEM-EDS report).

During our study, it was observed that overall, Brown mud gave good and promising results in healing clinical symptoms. Black mud also was very effective, but because of its slightly acidic pH, patients complained about irritation at the first instance of application.

Psoriasis is a purely proliferative inflammatory disease caused due to many reasons, eczema results in breakdown of barrier skin, and acne is a sebum inflammatory disease. Though all 3 of them have common causative factors but over and above these factors, acne also has hormonal causative factors on which, probably mud, must not be working effectively and so compared to psoriasis, mud was less effective in treatment of acne caused due to hormonal imbalance.

When statistical t test was applied to data of each mud irrespective of diseases, for 't critical' value of 1.72, the't statistical' values was 1.95 for Black , 2.99 for Brown, 2.35 for Dwarka, and 2.86 for Vadodara. **Thus, statistical analysis and clinical observations favored Brown as best mud amongst the four for therapeutic use.**

Photographs of diseased lesions of patients were taken before starting the treatment and also after 15 days of treatment. Improvement in the symptoms were clearly noticed. In the case of psoriasis fig. no. 50, the plaque thickness was reduced and the colour of skin was also seen to be improved. The whitish tinge seen on the lesion is the scaling (Photo no. 49), this is reduced as seen in fig. no. 50 which is taken after 15 days of mud treatment. On comparing fig.no.51 and 52 improvements in disease symptoms was quite evident.

Healing of skin was quite evident from photographs no.53, 54, 55, & 56 of eczema. These results were seen after 7 days of treatment.

Photograph no. 57 shows severe nodules. The number and size of these nodules are reduced to 80% as seen in photo no.58.

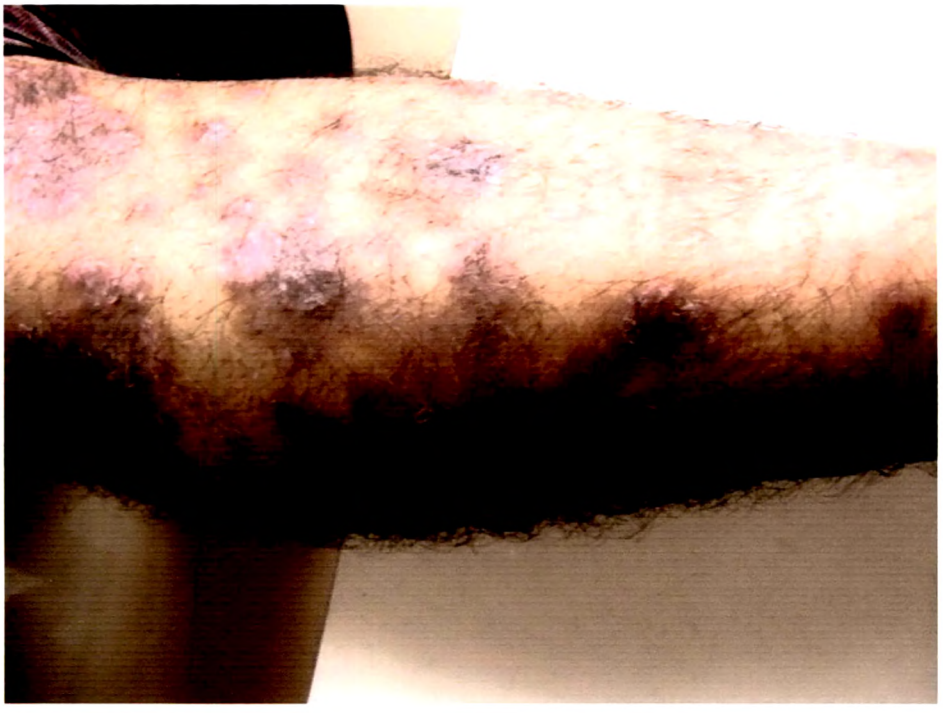


Fig. no.49 Psoriasis before treatment. The thickness of the lesion is clearly visible.

Fig. no. 50 Psoriasis after treatment (reduction in plaque thickness)

Fig.no. 51 Psoriasis before treatment

Fig.no. 52 Psoriasis after treatment. Third and last finger has recovered and plaque thickness is reduced.

Fig. no. 53 Eczema before treatment.(left leg)

Fig. no. 54 Eczema after treatment. (left leg) Index finger has completely recovered.

Fig.no . 55 Eczema before treatment (right leg)

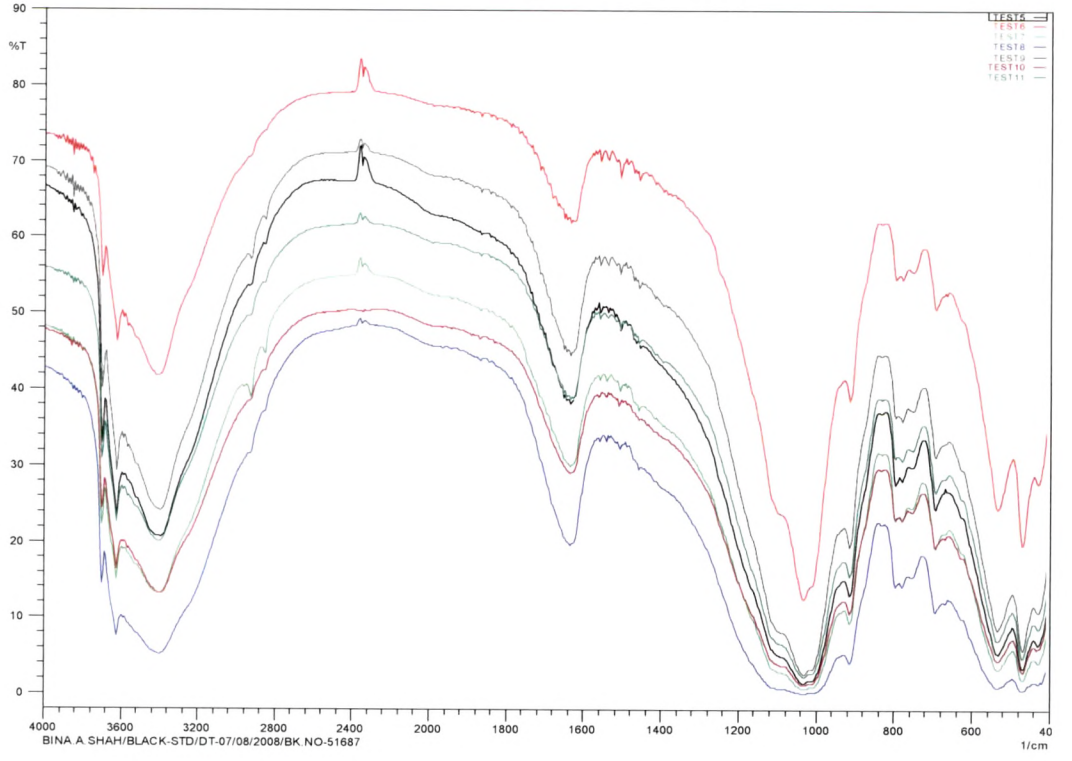
Fig.No.56 Eczema after treatment. (right leg) 95% recovery in the first finger.

Fig. no 57 Acne before treatment

Fig.no. 58 Acne after treatment. No. and size of pustules has reduced.

7.7 FTIR Spectroscopy of (MAP)

Infra red spectrographs of Black, Brown, Dwarka and Vadodara applied on patients (psoriasis, eczema and acne) are given in fig. no.59, 60, 61, 62 respectively. Different colored lines indicate different disease.



_____ Black std.

_____ Black eczema

_____ Black acne

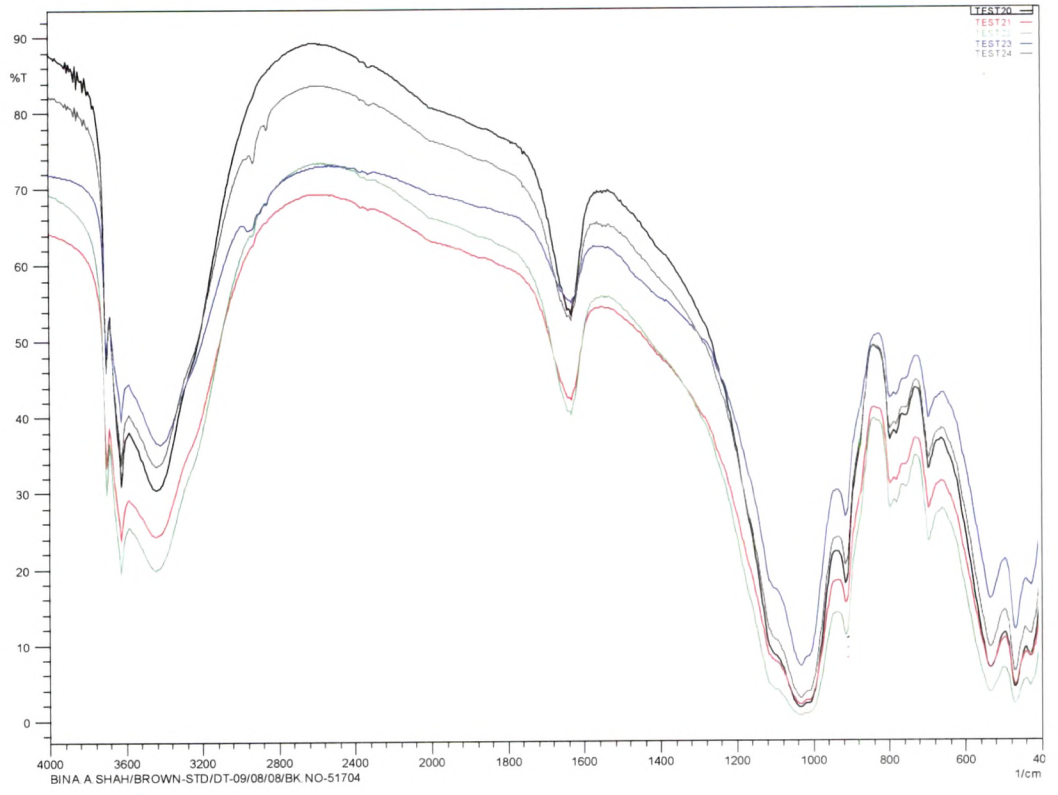
_____ Black psoriasis

_____ Black normal

_____ Black eczema

_____ Black psoriasis

Fig No: 59 Infra red spectroscopy of **Black MAP** . Different colors indicate different diseases.



Brown std.

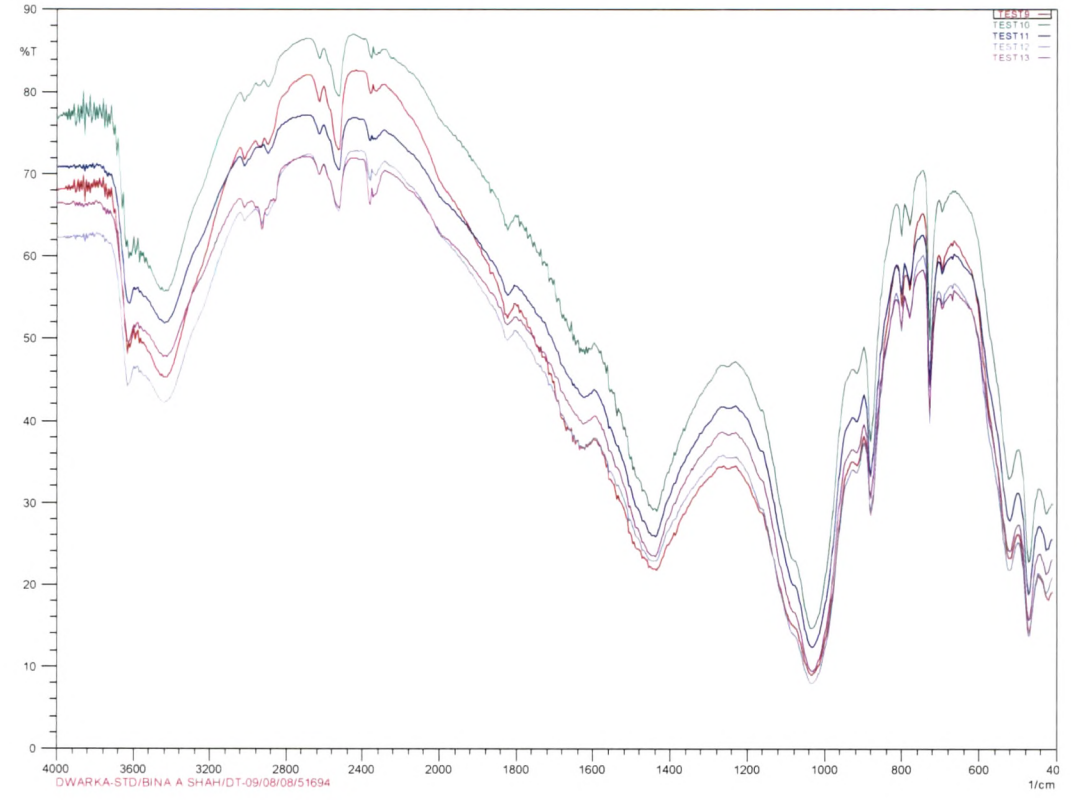
Brown normal

Brown acne

Brown eczema

Brown psoriasis

Fig.No. 60 Infrared spectroscopy of Brown MAP.Different colors indicate different disease.



Dwarka std

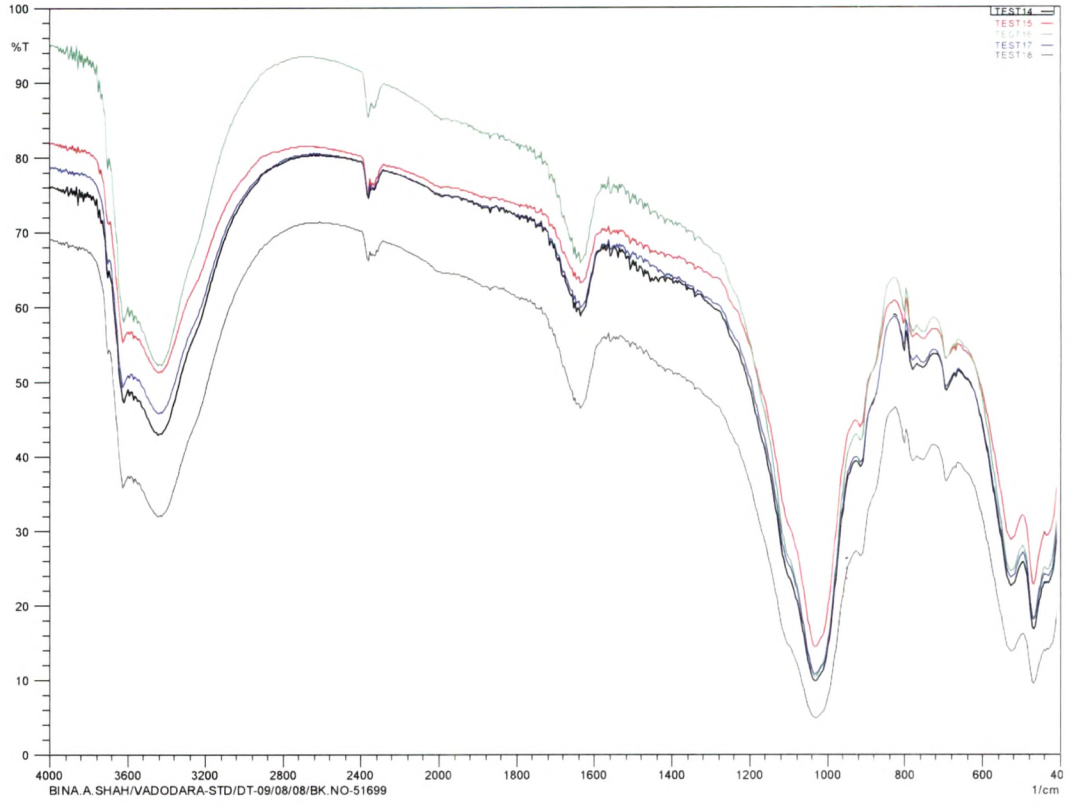
Dwarka normal

Dwarka acne

Dwarka eczema

Dwarka psoriasis

Fig.No.61 Infrared spectroscopy of Dwarka MAP. Different colors indicate different disease.



_____ Vadodara std.

_____ Vadodara normal

_____ Vadodara acne

_____ Vadodara eczema

_____ Vadodara psoriasis

Fig. no.62 Infrared spectroscopy of Vadodara MAP. Different colors indicate different disease.

Results and discussion

The bands (cm-1) of intensities and peaks of Black , Brown, Dwarka and Vadodara for **psoriasis** is given in Table no. 44. The comparison is done amongst peak and intensity of mud applied on **normal healthy** persons with that of peak and intensity on mud applied on **patients** (MAP).

Table No: 44 Infrared spectroscopy data of four muds for **psoriasis**

Colored cells: Peak cell indicate common peaks of Std. mud and MAP and intensity of MAP i.e. colored cells indicate change in intensity of transmission with respect to Std.mud.

Colored fonts : Peak and intensity of Std. mud which is not shown by MANP(mud applied on normal persons) and MAP i.e.that peak is lost in MAP

Black fonts : Peak and intensity of MAP i.e. new peak generation

IR data	**Psoriasis**																
Brown MAP		Brown Normal		Black MAP				Black Normal		Dwarka MAP		Dwarka Normal		Vadodara MAP		Vadodara Normal	
Peak	Intensity	Peak	Intensi	Peak	Inten	Peak	Inten	Peak	Intensi	Peak	Intensity	Peak	Intensit	Peak	Intensity	Peak	Intensity
										420.5	17.936			420.5	14.731		
		422.42	8.33														
										424.35	21.046	424.35	28.549				
428.21	11.166			428.21	4.064	428.21	8.541										
430.14	8.166			430.14	6.624	430.14	6.624							430.14	22.93		
								432.07	27.521								
																434	29.522
														466.79	9.544	466.79	22.931
468.72	6.261	468.72	4.564			468.72	4.699	468.72	19.622					468.72	16.667		
				470.65	1.809					470.65	15.505	470.65	22.604				
										518.87	22.999						
										520.8	23.897	520.28	32.745				
														526.58	13.821	526.58	28.952
				532.37	3.191	532.37	7.021	532.37	24.309								
534.3	9.404	534.3	6.797														
				671.25	21.08	671.25	28.24										
694.4	34.287	694.4	27.78	694.4	19	694.4	26.37	694.4	50.388	694.4	53.436	694.4	65.215	694.4	36.513	694.4	53.103
										727.19	40.764	727.19	49.809				
																750.33	55.757

								752.26	55.392								
754.19	39.902			754.19	24.43	754.19	32.05							754.19	39.503		
756.12	40.904	756.12	33.59														
														777.34	39.215	777.34	55.922
779.27	38.644	779.27	31.43	779.27	22.84	779.27	30.58	779.27	54.404	779.27	52.354	779.27	63.702				
796.63	38.144	796.63	30.92	796.63	22.7			796.63	54.383								
						798.56	30.95										
										800.49	51.269	800.49	62.327	800.49	41.562		
														802.41	54.044	802.41	57.845
				831.35	31.47	831.35	38.69	831.35	61.771								
				833.28	37.01	833.28	37.01										
										852.56	44.938	852.56	55.422				
										879.57	28.267						
										881.5	30.323	881.5	37.236				
912.36	20.267	912.36	15.38	912.36	9.291	912.36	15.43	912.36	38.579					912.36	26.503		
										914.29	34.397			914.29	38.5	914.29	44.071
										916.22	35.895	916.22	45.575				
		1008.8	2.544	1008.8	1.919	1008.8	1.919										
				1012.7	0.841												
1032	2.749	1032	1.972	1032	0.631	1032	2.217	1032	12.428	1031.95	9.305	1032	14.51	1032	4.856	1032	14.457
										1249.91	38.186	1249.9	46.403				
										1271.13	34.185						
												1336.7	41.173				

										1340.57	33.926						
										1359.86	28.433						
												1361.8	38.676				
										1384.94	25.925						
												1386.9	35.727				
						1394.6	43.56							1394.6	50.936		
										1435.09	21.528	1435.1	28.825				
										1437.02	23.277						
												1446.7	28.849				
				1456.3	37.22												
				1506.5	39.61	1506.5	48.03							1506.5	54.489		
				1516.1	40.97												
				1541.2	40.97												
				1558.5	41.16												
						1568.2	49.8										
		1570.1	54.25	1570.1	41.43												
												1606.8	48.553				
										1608.69	37.014						
										1618.33	39.542						
				1628	30.12	1628	38.82	1628	62								
1631.8	52.532	1631.8	42.15														
				1633.8	38.18	1633.8	38.18							1633.8	46.447	1633.8	63.257
				1635.7	29.73												

												1737.9	59.891				
										1766.85	51.158						
														1770.7	60.7		
										1822.79	52.44	1822.8	63.029				
				1824.7	49.52					1824.72	51.561						
														1861.4	62.562		
						1867.2	57.4							1867.2	62.206		
				1869.1	50.15												
														1990.6	64.506		
				1917.3	51.5												
				1942.4	51.76												
						1990.6	59.13										
				1992.5	52.14												
						2189.3	61.24										
						2241.4	61.46										
												2243.3	83.993				
								2279.9	79.056								
				2281.9	54.88												
														2310.8	67.534		
														2312.7	76.655	2312.7	77.518
2318.5	81.004																
		2324.3	67.31														
														2328.2	66.868		

										2330.09	67.384						
				2349.4	55.94	2349.4	62.07										
														2343.6	66.862		
										2353.23	79.69						
														2359	66.209	2359	75.458
		2364.8	67.63							2360.95	66.022						
						2397.6	61.6										
				2393.7	54.87												
												2395.7	86.31				
				2399.5	67.41	2399.5	67.41	2399.5	79.016								
				2409.2	54.83												
																2413	80.375
				2430.4	54.78												
						2432.3	61.51										
				2447.8	54.73												
						2451.6	61.48										
														2467	70.701		
						2469	61.43										
				2470.9	67.43	2470.9	67.43										
				2482.5													
				2497.9													
						2501.8	61.36										
				2505.6	67.49	2505.6	67.49										

				2515.3													
		2521.1	69.28														
						2523	61.31			2522.98	65.826	2523	79.337				
				2536.5													
														2538.4	71.132		
						2542.3	61.25										
				2553.8													
		2559.6	69.37														
		2575.1	69.39	2575.1													
						2578.9	61.12										
		2594.3	69.42														
						2598.2	61.01										
		2613.6	69.37											2613.6	71.311		
				2617.5	67.31	2617.5	67.31										
										2625.21	69.817	2625.2	83.931				
														2632.9	71.29		
						2636.8	60.69										
		2640.6	69.3														
														2650.3	71.308		
														2681.1	71.191		
														2723.6	70.914		
										2735.15	81.377						
																2779.5	81.267

														2800.7	70.08		
														2831.6	78.878		
2854.7	77.814			2854.7		2854.7	53.81	2854.7	73.914								
										2856.67	66.531						
		2858.6	65.62														
										2895.25	73.463	2895.3	80.427				
																2908.8	80.115
				2926.1						2926.11	63.09						
2928	73.436																
						2931.9	49.52										
										2933.83	73.286						
								2937.7	70.384								
										2955.04	65.811						
				2960.8													
2966.6	73.959																
												2991.7	79.564				
												3018.7	78.706				
										3016.77	65.858						
				3387.1	20.71	3387.1	20.71										
				3394.8	20.61	3394.8	20.61										
						3396.8											
				3400.6													
						3402.5											

								3404.5	41.64								
				3418	20.77	3418	20.77	3418	41.662								
3419.9	33.702									3419.9	45.245	3419.9	55.687	3419.9	32.072		
										3421.83	47.686					3421.8	51.53
										3429.55	45.161						
												3435.3	55.579				
										3437.26	47.69						
		3439.2	24.42													3439.2	51.489
3441.1	33.527													3441.1	32.015		
						3524.1											
								3526	46.431								
										3545.72	50.673						
														3564.6	47.677		
														3566.5	36.492		
										3587.72	51.286						
3601.2	39.398																
														3618.6	35.854		
				3620.5				3620.5	46.024							3620.5	55.541
						3620.5				3620.51	49.454						
3622.4	33.727	3622.4	23.75														
														3626.3	47.157		
3697.7	45.582	3697.7	33.56	3697.7		3697.7		3697/66	54.525								
														3699.6	64.024		

										3747.81	65.412						
				3904.1													
				3969.6													

Table No: 45 Infrared spectroscopy data of four muds for **Eczema**

Colored cells: Peak cell indicate common peaks of Std. mud and MAP and intensity of MAP i.e. colored cells indicate change in intensity of transmission with respect to Std.mud.

Colored fonts : Peak and intensity of Std. mud which is not shown by MANP(mud applied on normal persons) and MAP i.e.that peak is lost in MAP

Black fonts : Peak and intensity of MAP i.e. new peak generation

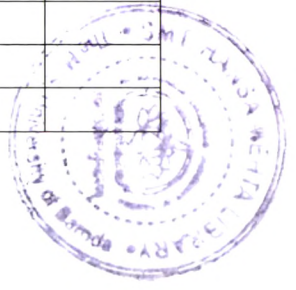
IR data Eczema																	
Brown MAP		Br. Normal		Black MAP-I		Black MAP-II		Black Normal		Dwarka MAP		Dwarka Normal		Vadodara MAP		Va. Normal	
Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity
				418.57	1.564												
420.5	5.217					420.5	6.201			420.5	17.94						
		422.42	8.33														
										424.35	24.01	424.35	28.549				
				428.21	1.392												
430.1	4.227			430.14	6.624									430.14	24.85		

IR data Eczema																	
Brown MAP		Br. Normal		Black MAP-I		Black MAP-II		Black Normal		Dwarka MAP		Dwarka Normal		Vadodara MAP		Va. Normal	
Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity
				418.57	1.564												
420.5	5.217					420.5	6.201			430.5	17.94						
		422.42	8.33														
										424.35	24.01	424.35	28.549				
				428.21	1.392												
430.1	4.227			430.14	6.624									430.14	24.85		
								432.07	27.521								
																434	29.522
														466.79	14.32	466.79	22.931
468.7	4.126	468.72	4.564	468.72	0.456	468.72	2.938	468.72	19.622					468.72	16.67		
470.7	1.953									470.65	18.71	470.65	22.604				
										518.87	27.73						
												520.28	32.745	524.66	24.7		
														526.58	22.61	526.58	28.952
532.4	3.556			532.37	0.874	532.37	4.361	532.37	24.309								
534.3	6.677	534.3	6.797														
						626.89	17.87										
				669.32	11.89	669.32	20.46										
694.4	23.45	694.4	27.78	694.4	10.67	694.4	19.25	694.4	50.388	694.4	57.78	694.4	65.215	694.4	52.99	694.4	53.103
										727.19	44.07	727.19	49.809				

IR data **Eczema**

Brown MAP		Br. Normal		Black MAP-I		Black MAP-II		Black Normal		Dwarka MAP		Dwarka Normal		Vadodara MAP		Va. Normal	
Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity
				418.57	1.564												
420.5	5.217					420.5	6.201			420.5	17.94						
		422.42	8.33														
										424.35	24.01	424.35	28.549				
				428.21	1.392												
430.1	4.227			430.14	6.624									430.14	24.85		
																750.33	55.757
														752.26	56.65		
								752.26	55.392								
754.2	39.9			754.19	15.39	754.19	23.99							754.19	51.82		
756.1	30.7	756.12	33.587														
														777.34	56.72	777.34	55.922
779.3	28.45	779.27	31.427	779.27	14.14	779.27	22.71	779.27	54.404	779.27	56.49	779.27	63.702				
796.6	27.77	796.63	30.921	796.63	14.13	796.63	22.93	796.63	54.383								
										800.49	55.25	800.49	62.327				
														802.41	59.36	802.41	57.845
				831.35	22.24	831.35	29.47	831.35	61.771								
				833.28	37.01												
										852.56	48.62	852.56	55.422				
										879.57	28.27						

IR data	**Eczema**															
Brown MAP		Br. Normal		Black MAP-I		Black MAP-II		Black Normal		Dwarka MAP		Dwarka Normal		Vadodara MAP		Va. Normal	
Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity
				418.57	1.564												
420.5	5.217					420.5	6.201			420.5	17.94						
		422.42	8.33														
										424.35	24.01	424.35	28.549				
				428.21	1.392												
430.1	4.227			430.14	6.624									430.14	24.85		
										881.5	33.01	881.5	37.236				
912.4	11.07	912.36	15.38	912.36	4.127	912.36	10.47	912.36	38.579					912.36	42.12		
										914.29	34.4			914.29	38.5	914.29	44.071
										916.22	39.75	916.22	45.575				
		1008.8	2.544														
				1008.8	1.919												
1013	0.862			1012.7	0.127												
1032	1.5	1031.95	1.972	1032	1.558	1032	1.154	1032	12.428	1032	12.22	1031.95	14.51	1032	10.59	1031.95	14.457
1034	0.608			1033.9	0.082												
				1089.8	0.695												
										1249.9	41.32	1249.91	46.403				
										1271.1	44.19						
												1336.71	41.173				
										1350.9	28.43						



IR data **Eczema**

Brown MAP		Br. Normal		Black MAP-I		Black MAP-II		Black Normal		Dwarka MAP		Dwarka Normal		Vadodara MAP		Va. Normal	
Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity
				418.57	1.564												
420.5	5.217					420.5	6.201			420.5	17.94						
		422.42	8.33														
										424.35	24.01	424.35	28.549				
				428.21	1.392												
430.1	4.227			430.14	6.624									430.14	24.85		
												1361.79	38.676				
										1384.9	25.94						
												1386.86	35.727				
										1435.1	21.53	1435.09	28.825				
										1437	25.76						
												1446.66	28.849				
				1456.3	29.5												
				1506.5	47.91												
				1508.4	32.09												
				1516.1	33.02												
				1541.2	33.14												
				1558.5	33.19												
1570	55.48	1570.11	54.247														
												1606.76	48.553				

IR data	**Eczema**																
Brown MAP		Br. Normal		Black MAP-I		Black MAP-II		Black Normal		Dwarka MAP		Dwarka Normal		Vadodara MAP		Va. Normal	
Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity
				418.57	1.564												
420.5	5.217					420.5	6.201			420.5	17.94						
		422.42	8.33														
										424.35	24.01	424.35	28.549				
				428.21	1.392												
430.1	4.227			430.14	6.624									430.14	24.85		
										1608.7	17.01						
										1616.4	42.8						
								1628	62								
														1628	66.62		
						1629.9	29.1										
1632	40.34	1631.83	42.151														
				1633.8	38.18									1633.8	58.8	1633.76	63.257
1636	40.78			1635.7	19.47												
												1737.92	59.891				
										1766.9	51.16						
										1822.8	55.22	1822.79	63.029				
				1869.1	43.59	1869.1	46.6										
				1917.3	44.99												
				1942.4	45.2												

IR data	**Eczema**																
Brown MAP		Br. Normal		Black MAP-I		Black MAP-II		Black Normal		Dwarka MAP		Dwarka Normal		Vadodara MAP		Va. Normal	
Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity
				418.57	1.564												
420.5	5.217					420.5	6.201			420.5	17.94						
		422.42	8.33														
										424.35	24.01	424.35	28.549				
				428.21	1.392												
430.1	4.227			430.14	6.624									430.14	24.85		
				1992.5	45.45												
												2243.29	83.993				
				2258.5	48.19												
				2278	48.26	2278	50.44										
								2279.9	79.056								
				2293.4	48.32												
						2312.7	50.19			2312.7	74.57			2312.7	76.66	2312.73	77.518
2319	86.02																
2322	71.23																
		2324.3	67.314														
						2345.5	50.04										
				2347.5	48.59												
										2353.2	79.69						
														2359	85.45	2359.02	75.458

IR data **Eczema**

Brown MAP		Br. Normal		Black MAP-I		Black MAP-II		Black Normal		Dwarka MAP		Dwarka Normal		Vadodara MAP		Va. Normal	
Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity
				418.57	1.564												
420.5	5.217					420.5	6.201			420.5	17.94						
		422.42	8.33														
										424.35	24.01	424.35	28.549				
				428.21	1.392												
430.1	4.227			430.14	6.624									430.14	24.85		
										2361	73.9						
2363	71.7	2364.81	67.632														
						2374.5	50.11										
				2384.1	48.21												
												2395.67	86.31				
				2399.5	48.12	2399.5	50.33	2399.5	79.016								
																2413.03	80.375
2415	72.61																
						2416.2	50.22										
				2418.8	48.03												
2434	72.76																
				2436.2	47.95	2436.2	50.22										
2452	72.91			2451.6	67.42	2451.6	50.15										
				2453.5													

IR data	**Eczema**																
Brown MAP		Br. Normal		Black MAP-I		Black MAP-II		Black Normal		Dwarka MAP		Dwarka Normal		Vadodara MAP		Va. Normal	
Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity
				418.57	1.564												
420.5	5.217					420.5	6.201			420.5	17.94						
		422.42	8.33														
										424.35	24.01	424.35	28.549				
				428.21	1.392												
430.1	4.227			430.14	6.624									430.14	24.85		
2469	73.04																
				2470.9		2470.9	50.1										
2486	73.17																
						2488.3	50.05										
				2490.2													
2506	73.29					2505.6	50										
				2507.5													
		2521.05	69.283														
2523	73.38									2523	70.42	2522.98	79.337				
						2524.9	49.97										
				2526.8													
2542	73.42																
				2544.2													
		2559.62	69.374														

IR data	**Eczema**																
Brown MAP		Br. Normal		Black MAP-I		Black MAP-II		Black Normal		Dwarka MAP		Dwarka Normal		Vadodara MAP		Va. Normal	
Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity
				418.57	1.564												
420.5	5.217					420.5	6.201			420.5	17.94						
		422.42	8.33														
										424.35	24.01	424.35	28.549				
				428.21	1.392												
430.1	4.227			430.14	6.624									430.14	24.85		
2562	73.47																
		2575.05	69.388														
						2578.9	67.49										
2581	73.49																
				2582.8		2582.8	49.81										
		2594.34	69.419														
2598	73.47																
						2600.1	49.71										
		2613.63	69.368														
2617	73.39					2617.5	67.31										
										2625.2	74.72	2625.21	83.931				
2637	73.28																
		2640.64	69.299														
2656	73.11																

IR data **Eczema**

Brown MAP		Br. Normal		Black MAP-I		Black MAP-II		Black Normal		Dwarka MAP		Dwarka Normal		Vadodara MAP		Va. Normal	
Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity
				418.57	1.564												
420.5	5.217					420.5	6.201			420.5	17.94						
		422.42	8.33														
										424.35	24.01	424.35	28.549				
				428.21	1.392												
430.1	4.227			430.14	6.624									430.14	24.85		
										2735.2	81.48						
																2779.52	81.267
										2798.8	76.22						
														2831.6	78.88		
														2833.5	92.52		
				2854.7		2854.7	42.39	2854.7	73.914								
2857	68.01																
		2858.6	65.62														
										2895.3	72.38	2895.25	80.427				
																2908.75	80.115
2932	63.99			2931.9	53.58	2931.9	53.58										
				2933.8		2933.8				2933	73.29						
								2937.7	70.384								
												2991.69	79.564				

IR data **Eczema**

Brown MAP		Br. Normal		Black MAP-I		Black MAP-II		Black Normal		Dwarka MAP		Dwarka Normal		Vadodara MAP		Va. Normal	
Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity
				418.57	1.564												
420.5	5.217					420.5	6.201			420.5	17.94						
		422.42	8.33														
										424.35	24.01	424.35	28.549				
				428.21	1.392												
430.1	4.227			430.14	6.624									430.14	24.85		
										3016.8	70.93						
												3018.7	78.706				
				3385.2		3385.2											
				3387.1	20.71	3387.1	20.71										
				3394.8													
								3404.5	41.64								
				3418	20.77	3418	20.77	3418	41.662								
3420	30.65									3419.9	45.25	3419.9	55.687	3419.9	52.45		
										3421.8	51.86					3421.83	51.53
3426																	
										3429.6	45.16						
												3435.34	55.579				
										3437.3	51.79						
		3439.19	24.423													3439.19	51.489

IR data **Eczema**

Brown MAP		Br. Normal		Black MAP-I		Black MAP-II		Black Normal		Dwarka MAP		Dwarka Normal		Vadodara MAP		Va. Normal	
Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity
				418.57	1.564												
420.5	5.217					420.5	6.201			420.5	17.94						
		422.42	8.33														
										424.35	24.01	424.35	28.549				
				428.21	1.392												
430.1	4.227			430.14	6.624									430.14	24.85		
3441														3441.1	43		
														3445	52.36		
				3526				3526	46.431								
														3564.6	58.23		
3601	37.12																
										3618.6	54.1						
														3618.6	47.2		
				3620.5		3620.5		3620.5	46.024							3620.51	55.541
3622		3622.44	23.745														
														3626.3	57.77		
										3628.2	53.81						
3698		3697.66	33.556	3697.7		3697.7		3697/66	54.525								
														3699.6	84.02		
				3801.8													

IR data	**Eczema**																
Brown MAP		Br. Normal		Black MAP-I		Black MAP-II		Black Normal		Dwarka MAP		Dwarka Normal		Vadodara MAP		Va. Normal	
Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity
				418.57	1.564												
420.5	5.217					420.5	6.201			420.5	17.94						
		422.42	8.33														
										424.35	24.01	424.35	28.549				
				428.21	1.392												
430.1	4.227			430.14	6.624									430.14	24.85		
				3821.1													
				3838.5		3838.5											
				3853.9		3853.9											
				3902.1													
						3904.1											
						3961.9											
										3975.4	70.67						

Table No: 46 Infrared spectroscopy data of four muds for **Acne**

Colored cells: Peak cell indicate common peaks of Std. mud and MAP and intensity of MAP i.e. colored cells indicate change in intensity of transmission with respect to Std.mud.

Colored fonts : Peak and intensity of Std. mud which is not shown by MANP(mud applied on normal persons) and MAP i.e.that peak is lost in MAP

Black font : Peak and intensity of MAP i.e. new peak generation

IR data Acne															
Brown MAP		Brown Normal		Black MAP		Black Normal		Dwarka MAP		Dwarka Normal		Vadodara MAP		Va. Normal	
Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity	Peak	Intensity
								420.5	17.936						
		422.42	8.33												

								424.35	18.821	424.35	28.549				
426.48	17.704														
				428.21	10.851										
430.14	8.166			430.14	6.624							430.14	24.02		
						432.07	27.521								
														434	29.522
												466.79	18.098	466.79	22.931
468.72	11.779	468.72	4.564	468.72	5.906	468.72	19.622					468.72	16.667		
								470.65	13.546	470.65	22.604				
								518.87	22.999						
								520.8	21.546	520.28	32.745				
												526.58	23.89	526.58	28.952
				532.37	8.636	532.37	24.309								
534.3	15.94	534.3	6.797												
				671.25	33.03										
												692.47	49.406		
694.4	39.775	694.4	27.78	694.4	31.185	694.4	50.388	694.4	54.022	694.4	65.215	694.4	48.755	694.4	53.103
								727.19	39.457	727.19	49.809				
														750.33	55.757
						752.26	55.392					752.26	52.584		
754.19	39.902			754.19	37.365							754.19	51.821		
756.12	44.857	756.12	33.587												
												777.34	51.538	777.34	55.922

779.27	42.644	779.27	31.427	779.27	36.031	779.27	54.404	779.27	52.438	779.27	63.702	779.27	52.842		
796.63	42.306	796.63	30.921	796.63	27.587	796.63	54.383								
				798.56	36.432										
								800.49	50.714	800.49	62.327				
												802.41	55.058	802.41	57.845
				831.35	44.475	831.35	61.771								
				833.28	37.005										
								852.56	43.614	852.56	55.422				
								879.57	28.267						
								881.5	28.642	881.5	37.236				
912.36	26.777	912.36	15.38	912.36	19.506	912.36	38.579					912.36	39.21		
								914.29	34.397			914.29	38.5	914.29	44.071
								916.22	33.48	916.22	45.575				
		1008.8	2.544	1008.8	1.919										
				1010.73	3.384										
1031.95	7.106	1031.95	1.972	1031.95	2.717	1031.95	12.428	1031.95	7.826	1031.95	14.51	1031.95	10.857	1031.95	14.457
								1249.91	35.345	1249.91	46.403				
								1271.13	34.185						
										1336.71	41.173				
								1340.57	31.62						
								1359.86	24.433						
										1361.79	38.676				
								1384.94	25.925						

								1386.86	27.814	1386.86	35.727				
								1435.09	21.528	1435.09	28.825				
								1437.02	22.681						
								1446.66	22.664	1446.66	28.849				
				1456.3	45.373										
				1506.46	47.908							1506.46	66.506		
		1570.11	54.247												
										1606.76	48.553				
								1608.69	37.014						
								1616.4	36.771						
								1622.19	36.546						
				1626.05	44.966										
						1627.97	62								
				1633.76	38.176							1633.76	60.076	1633.76	63.257
				1867.16	65.965										
1631.83	54.923	1631.83	42.151												
										1737.92	59.891				
								1766.85	51.158						
								1822.79	49.656	1822.79	63.029				
												1867.16	73.064		
												1990.6	74.975		
										2243.29	83.993				
						2279.94	79.056								

								2312.73	70.531			2312.73	76.838	2312.73	77.518
2318.51	86.023														
2324.3	72.087	2324.3	67.314												
												2328.16	76.009		
								2330.09	69.771						
								2353.23	79.69						
								2359.02	69.067			2359.02	75.152	2359.02	75.458
2364.81	72.335	2364.81	67.632												
										2395.67	86.31				
				2399.53	71.212	2399.53	79.016								
2413.03	72.874													2413.03	80.375
2430.39	72.926														
				2451.61	67.42										
2467.04	73.004														
				2470.9	67.43										
2499.83	73.103														
				2505.62	67.493										
2521.05	73.124	2521.05	69.283												
								2522.98	65.356	2522.98	79.337				
2559.62	73.1	2559.62	69.374												
		2575.05	69.388												
				2578.91	67.486										
		2594.34	69.419												

2613.63	72.897	2613.63	69.368												
				2617.49	67.313										
								2625.21	69.812	2625.21	83.931				
		2640.64	69.299												
								2710.08	72.141						
												2750.58	80.218		
								2735.15	71.826						
														2779.52	81.267
												2831.6	78.878		
												2843.17	79.085		
				2852.81	62.31										
				2854.74	59.028	2854.74	73.914								
2856.67	68.214														
		2858.6	65.62					2858.6	66.512						
												2874.03	78.538		
								2895.25	64.844	2895.25	80.427				
														2908.75	80.115
												2910.68	77.817		
								2926.11	63.894						
				2928.04	57.109										
				2931.9	53.575										
								2933.83	73.286						
						2937.68	70.384								

2955.04	64.643														
								2991.69	64.914	2991.69	79.564				
								3016.77	64.085						
										3018.7	78.706				
				3387.11	20.707										
				3394.83	20.609										
				3396.76	24.085										
						3404.47	41.64								
				3417.98	20.767	3417.98	41.662								
3419.9	36.546							3419.9	42.145	3419.9	55.687	3419.9	43.088		
												3421.83	46.06	3421.83	51.53
								3429.55	45.161						
Brown				Black				Dwarka				Vadodara			
										3435.34	55.579				
		3439.19	24.423					3439.19						3439.19	51.489
3441.12	30.361											3441.12	42.998		
						3525.99	46.431								
												3564.57	47.677		
												3566.5	50.476		
												3599.29	50.788		
3601.22	44.072														
								3618.56				3618.58	47.201		
				3620.51	29.304	3620.51	46.024					3620.51	49.528	3620.51	55.541

3622.44	39.616	3622.44	23.745												
												3626.29	47.157		
								3628.22							
3697.66	48.292	3697.66	33.556	3697.66	40.114	3697/66	54.525					3697.66	66.023		
												3699.59	64.024		
3884.76	71.452														

Discussion

On comparing the infrared absorption data of soil applied to patients with that of soil applied to normal persons and with the standard soil (wet and dried, to nullify the peaks due to hydration of soil) as given in table nos 44, 45, 46 for psoriasis, eczema and acne respectively, we can observe several new peaks in many band regions and also change in intensities of their peaks. Several peaks which existed in std. mud were lost. This indicated that some **adsorption on soil** and **absorption into the skin,** of compounds, had taken place on application of mud to the skin.

Changes in the band positions and intensities between the mud applied to patients and that applied on normal persons explains that these changes were not due to hydration of soil because in both the cases soil was wetted and applied (Katerina MD 2004). Water of hydration (towards 3450cm-1) can be distinguished from the OH of structural hydroxyls by the presence of a HOH band towards 1630 cm-1 (Mineralogical analysis).

Soil adsorption

A study of structural changes and bonding mechanisms on a molecular scale could better be made with infrared spectroscopy (Farmer VC 1971). Scanning electron microscopy (SEM) can also be used beneficially in the study of association of organic and mineral matter in soils.

Mud is a strong adsorption agent. Many compounds get adsorbed on clay part of mud, by various adsorption mechanisms like **hydrogen bonding, Ion exchange, physical adsorption (vander waals forces), Cordination (non-ionic adsorption) and chemisorptions.**

Adsorption is an exothermic process, hence, it should decrease with increasing temperature. Wet soil's temperature is less, than the temperature of water, used, to wet it (because of adsorption of water). When this mud is applied to the patient's body , initially the patient feels cool at the site of application, but it was observed that, at the end of 30 minutes (approximately) the temperature of mud comes to the

level of body temperature(due to heat transfer or due to chemisorptions in humic acid systems) and the feeling of coolness diminishes. **This could probably explain the practice of removing the applied mud after it has reached body temperature (approx. 30 min) because as temperature will increase the adsorption (of skin compounds onto soil) process will decrease. It is also due to this reason that dried scraped off mud (MAP), should not be used again because the adsorption sites are saturated with compounds (toxins) and there are negligible chances of desorption of those compounds from those sites.**

Hydrogen bonding

Organic substance that are native to soil are also adsorbed to clays by hydrogen bonding: for example, **humic acid** is held on clay surface by hydrogen bonding to a water molecule in the primary hydration shell of the adsorbed cation on the exchange complex (Theng BKG 1976a. &1976b) .Humic acid bands are seen at 2310-2350cm-1 region. New peaks i.e. 2312 and 2318cm-1 have been observed in this region in IR graph of all three diseases. So it can be assumed that some changes in humic acid molecule (by hydrogen bonding) must be taking place in MAP.

Vander Waals forces

Vander Waals forces are important for adsorption of large molecules whose configuration conforms spatially to the adsorbing surface (Browman MG 1975). **Amines** are adsorbed on clay in the cationic form and amino acids are adsorbed as cations and / or as dipolar ions. (Grenland DJ 1965a, Cowa CT 1958, Greenland DJ 1965b).

1090-1025, 3100-2600, 1550-1485, 3550-3330 cm-1 are all amino acids and amines absorption bands. In these regions, new peaks or changes in intensities were observed in all the three diseases as shown below.

Increased intensity in absorption bands of aliphatic primary amines (range 1040-1030 cm-1) was seen in IR spectra of soil applied on psoriasis patients. Many

derivatives of eicosanoids (one of the causative agents of psoriasis) are found in psoriatic scales (Ikai 1999). **There is a possibility that these may be adsorbed on soil by weak van der Waals forces which may be reflected in increased intensity in this range.**

Psoriasis: 1031(change in intentsity) ,2613, 2632, 2636, 2650, 2681, 2723, 2800, 2856, 2926, 2928, 2933, 2955, 2960, 2966, 3396, 3400, 3402, 3419, 3421, 3429, 3437 cm-1 were all new peaks observed in amines and amino acids region , in psoriasis in one or the other muds.

Eczema: 1032, 1033, 1089, 1506, 1508, 1516, 1541, 2600, 2617, 2625, 2636, 2656, 2798, 2833, 2854, 2856, 2895, 2931, 2933, 3016, 3418, 3419, 3421, 3437, 3445, 3526 cm-1 were all new peaks observed in eczema in amines and amino acid absorption band region in one or the other muds.

Acne: 1031, 1340, 1386, 1506, 2613, 2625, 2710, 2735, 2750, 2843, 2852, 2856, 2858, 2874,2910, 2926, 2928, 2955, 2991, 3016, 3387, 3394, 3396, 3417, 3419, 3421, 3439, 3441, cm-1 were all new peaks observed in amines and amino-acid region in acne (MAP) in either of the four muds.

It could be inferred from the above data that emergence of many new peaks **in amines and amino-acid absorption band region** indicated presence of some **new** compounds (adsorbed by Vander-Waals forces), which were obtained from the mud applied on psoriasis, eczema and acne patients.

Ion exchange

Anion exchange reactions are not as well defined as cation exchange reactions in soils. Anions are adsorbed either electrostatically or with a degree of chemical bonding by a wide range of soil materials (Mott CB 1970). Polyvalent exchangeable cations and oxides and /or hydroxides of Al and Fe (major amorphous minerals) may act as bridges between clay and anions (Grenland DJ 1965a). Several new peaks have been observed in the region of 400-500cm-1. Iron oxide region, and 650-800 cm-1 Cl & Br region in MAP and not from soil applied on control subjects. This can lead

us to conclude that many anions like Cl, Br, I must be getting adsorbed into the soil electrostatically or chemically binding or being exchanged with Fe++ of epidermal scales resulting into new peaks in that region. Role of Bromine in healing of psoriasis is very well established by scientists studying the effects of Dead Sea water bathing for treatment of psoriasis (Sima H 1997). The edges of kaolinite become positively charged at low pH and form peripheral complexes with anionic compounds (Grenland DJ 1965a.).

Adsorption depends on **concentration, length of alkyl chain, amount of phosphate (950-1100 cm-1) in solution, amount and type of soluble salts, and pH**. Phosphate anions are adsorbed preferentially and more strongly than simple organic anions (Appelt G 1975b). Excess of phosphorus in epidermal skin has been found responsible for itching in patients of skin disorders (accompanied by itching) and removable of it by anion exchange may play a role in improving the disease symptoms.

700-400 cm-1 absorbance zone corresponds to vibrations of exchangeable cations ,Ca+ and Mg+(due to hydration) balancing the charges in interfoliaceous spaces of clays and substitution of exchangeable cations (Mineralogical analysis). **There were new peaks** found in this region in mud applied on psoriasis, acne and eczema. **This possibly indicated exchange of these ions through hydration of soil with skin cations (amines, amides N+).** Penetration of these ions into skin **was affirmed by our in-vitro studies on skin permeation of elements (** section 6 of the text.).

The dermal loss of iron is not a sweat-gland function but is due to epidermal desquamation (Adams WS 1950). **This was reflected in increase in peak intensities in 400-500cm-1 region of iron oxides.** Desquamation is a characteristic parameter for measuring psoriasis intensity and decrease in desquamation by removal of iron oxides (soil adsorption) would mean improvement in disease symptom.

Psoriasis: 420, 424, 428, 430, 466, 468, 470, 671, 754, 756, 798 1008, 1012 cm-1 were all new peaks in Cl,Br, I, HPO_4 and iron oxide absorption band region.

Eczema: 418, 420, 424, 428, 430, 466, 468, 470, 524, 532, 626, 669, 754, 756,777, 779, 796, 1008, 1012, 1033, 1089 cm-1 were new peaks in Cl,Br, I,HPO_4 and iron oxide absorption band region seen in mud applied on eczema patients.

Acne: 424, 426, 428, 466, 468, 520, 526, 692, 694, 727, 752, 756, 779, 796, 798, 1008,1010 cm-1 were new peaks in Cl,Br, I ,HPO_4 and iron oxide absorption band region seen in mud applied on acne patients.

Co ordination bonding

Ca, Mg, and the transition metals Cr, Mn, Fe, Co, Ni, Cd and Zn are capable of participation in coordination type bonding. Coordinated complexes of clays with polar organic compounds such as ketones, (Parfitt RL 1968, Sund KA 1956) pyridine (Farmer VC 1966.), amino acids (Yariv S 1970), aliphatic amines(Farmer VC 1965) and alcohols have been reported (Dowdy RH 1967b). **Presence of absorption bands of pyridines (1030-1045 cm-1), ketones (1745-1755 cm-1), amino acids (2600-3100 cm-1) and alcohols (3580-3670 cm-1) in soils are confirmed and so there is possibility of adsorption of Mn and Fe from skin by co ordination bonding.** As mentioned above there have been many new peaks in pyridines, ketones, amino acids alcohols {3620, 3626 cm-1 new peaks (alcohols) in psoriasis, eczema and acne} absorption band region so there must have been some changes in structure of constituents in soil applied on such patients.

Amides are capable of forming coordination complexes with metal cations on the clay complex through the oxygen of the amide (Mortland MM 1975). **Many new peaks were seen in the region 1940-1630cm-1 (assigned to NO+ coordination compounds) and so there is a possibility that amide adsorption had taken place. The peaks shown in Black and Vadodara soils (applied on psoriasis) between 2310-2135cm-1(R-C=N=N)+ , corresponding to diazonium salts , indicated that there may be bonding with metal cations of soil with nitrogenous compounds of the psoriatic scales..**

Chemisorption

McGlamery and Slife (1972) and Hayes et al (1968) reported, that adsorption in humic acid systems often results in an increase in temperature, indicating possible chemisorptions. Chemisorption at room temperature is usually a slower process than physical adsorption. Adsorption band of humic acid is 2310-2350 cm-1. New absorption peaks and variation in its intensity, in this region may confirm adsorption taking place in humic acid system.

Psoriasis: 2310, 2312, 2328, 2330, 2343, 2349 cm-1 were new peaks observed in humic acid absorption band region.

Eczema: 2312, 2318, 2322, 2345, 2347 cm-1 were new peaks observed in MAP (eczema) , in humic acid absorption band region.

Acne: 2312, 2324, 2328, 2330, cm-1 were new peaks observed in MAP (acne) in humic acid absorption band region.

Nonhumic substances contribute little to adsorption of organic compounds (Dunigan EP 1967) while the humic materials are highly adsorptive, as already indicated, providing larger surfaces and higher charge densities per unit weight than clays. Humic substances occur in intimate contact with other soil constituents and do not exist free (Dubach P 1963). The adsorption mechanism of soil organic matter may differ from that of clays or other amorphous soil materials (Burns IG 1973b).

Adsorption of aliphatic and aromatic compounds

Aliphatic and aromatic compounds with more than five carbon units can be adsorbed by montmorillonite (Black is rich in montmorillonite) from aqueous solutions by displacing other molecules associated with exchangeable cations (Bower CA 1963). **Water molecules in the primary hydration shell of cations adsorbed on clay can be displaced by alcohols** (Mortland MM 1970.). **This was indicated by increased intensity in adsorption bands in 3670-3580 cm-1 range , the range of OH stretching vibrations of alcohols**. Excessive activity of sebaceous glands is considered as one of

the cause of acne and sebum is composed of Squalene, wax esters, sterol esters, free fattyacids, sterols, glycerides and saturated hydrocarbons (Donald TD 1974).

Acne: 2413,2430, 2467, 2499, 2521, 2559, cm-1 (broad band OH stretching vibrations), 1437, 1446 (carboxylic acid region). , were all new peaks observed in mud applied on acne patients. These functional groups being the part of constitution of sebum there is possibility that mud must be adsorbing sebum surface lipids and thus improving acne symptoms.

2300-2600 cm-1 is also the region of P-OH stretching vibrations and there are several new peaks in this region in all the three diseases , Phosphorus is a causative factor for itching (acting through Vit.D channel). Decrease in Itching is the first improvement symptom observed in all the three diseases. New peaks in this region may probably explain this improvement.

Since aliphatic hydrocarbons lack polarity, they are poor competitors with water for adsorption sites on the exchange complex. When adsorbed, the adsorption is by van der Waals forces predominantly on the external surfaces of clay minerals (Wheatley GA 1968).

Humic acid mainly contains ketone and aldehyde groups. The adsorption mechanism for ketones is hydrogen bonding ,i.e they bond by the OH group of the adsorbent and the carbonyl group of the ketone, or via a water bridge (Bykov VT 1974). Like ketones , aldehydes are also adsorbed on clay minerals and IR analyses of aldehyde-clay complexes indicate that carbonyl groups of the aldehyde are hydrogen-bonded to hydroxyls of the silicate layer (Larson GO 1964). Initially, adsorption sites in a soil system can be considered to be occupied by water; and adsorption of an organic molecule usually involves desorption of Type II (loosely bounded) water from the colloid surface (Morill LG 1982). The absorption band of humic acid is 2310-2350 cm-1 and changes in the form of intensity or emergence of one or two new peaks are observed in this region. so there is a possibility that humic acid must be easily desorbed from the soil and diffused into the skin. Presence of humic acid in skin in in-vitro diffusion study confirms this phenomenon.

The carboxyl group of the organic acid interacts either directly with the interlayer cation or by forming a hydrogen bond with the water molecules coordinated to the exchangeable cation on the clay complex. In addition to coordination and hydrogen bonding, organic acids can be adsorbed by forming salts with the exchangeable cations (Dieguez-carbonell 1975). **The absorbance intensities of band 912.36 cm-1 which is in the range of 800-920cm-1(C-O stretching vib and OH vib of carboxylic acid,) increased and at the same time absorbance at 694.4 cm-1 also increased, which indicated that adsorption of free acid present in psoriatic skin** (Voorhees 1983, Khan WA 1995) **might be taking place by OH bonding with exchangeable cations like Mg^{++},Al +++(**Mineralogical analysis by Infra-red spectrometry) **(694 cm-1 is attributed to Mg/Al and $R_1CH=CHR_2$). 1630-1650 cm-1 range is due to C=O stretching vib of carboxylic acid and intensities of all four soils increased in this range , which prompted us to conclude that free arachidonic acid present on psoriatic skin or other free eicosanoids may be adsorbed on the soil, (thus removed from diseased lesion) , as a result of which thinning of plaques must be occuring. Free Arachidonic acid cascade is one of the major cause of triggering fast cell proliferation which occurs in psoriasis (** Khan WA, 1995). Eicosanoids play an important role in skin inflammatory diseases like psoriasis, eczema and acne also.

Total carbon was associated with the main polysaccharide envelopes at 1030 and 3300 cm-1, lignin like compounds (1513,1450, 1371,1265, and 835 cm-1) and aliphatic structures at 2920 and 2850 cm-1 (fats, waxes and lipids) and nearly in MAP of all the diseases, absorption at these frequencies was increased. This was also confirmed by increase in carbon content in MAP when analysed by CHNS, SEM-EDS and chemical digestion method.

The results of these preliminary studies indicate that a day may not be far off when adsorbed free acid content and carbon content in the MAP(mud applied on patients) might be used as a marker for improvement of disease symptoms of psoriasis, eczema and acne.

Amines can protonate in soil and can replace inorganic cations from the clay complex by ion exchange. Uncharged amino acids and peptides can be adsorbed physically

(Greenland DJ 1965b). When amines form double-layer complexes, there is a weak hydrogen bonding between the NH_2 group of the amine and oxygen on the silicate surface (Laby RH 1970). **Absorbance at 1031.95 cm-1 (silicate bonding and N bonding range) in MAP was stronger than that in mud applied to normal skin. Hence we could draw the conclusion that amines or compounds containing nitrogen (eicosanoids) must be getting adsorbed on the soil from diseased skin.** On correlating replacement of amines by inorganic cations, with invitro diffusion studies of elements(Ca and Mg) in to human skin, it could be observed that metal ions like Ca+, & Mg+ penetrate into the skin while amines of lesional skin must be coming out and getting adsorbed onto the soil.

Desorption

Desorption of organic compounds from soil organic matter is slower than that for clays (Harris CI 1964, Mcgalmery MD 1966). In some cases, organic matter adsorption is partially irreversible. Desorption of polymers like humic compounds in soil is slow because of the improbability of a simultaneous removal of all anchor-segments from the surface of the adsorbent (Kipling JJ 1965.). **So it can be assumed that no desorption has taken place in the soil after it is removed from the patients' skin until the time IR studies were carried out indicating no loss of adsorbed compounds.**

In case of acne, there were not many new peaks as compared to psoriasis and eczema but there was decrease in absorbance of various peaks (indicating absorption of elements into skin) compared to mud sample applied to normal persons , as well as std. mud sample. This report was in coordination with the fact that some of the causative factors of acne are different than that of psoriasis and eczema and that good clinical results with Dwarka mud (Clinical observations) for acne indicated that **absorption of compounds or elements from soil into skin was important rather than the adsorption by soil from skin, in healing acne.**

Moreover, all muds show many new peaks in 1200cm-1 to 3400cm-1 regions which corresponds to peaks of adsorption of organic compounds which have been reported to be responsible for inflammation {aliphatic carbons, nitrogenous compounds i.e

arachidonic acid and its precursors eicosanoids (Lkai K 1993) }. Hence, the anti-inflammatory activity of mud in relieving the symptoms of the skin diseases may be attributed to their property for adsorption of compounds that trigger inflammation.

Conclusion

Thus, applying mud to the skin as a source of elements, helped in healing psoriatic, , eczematous and acne skin lesions as observed in preliminary clinical studies.

Though statistically there was no difference in the effects of the four muds , clinically it could be observed that Black and Brown mud gave better effects on psoriasis and eczema compared to Vadodara and Dwarka. This could be attributed to the higher humic acid content of Black and Brown soils compared to Dwarka and Vadodara soils.

Various biological reactions of ions, may be attributed to transmembrane electrolytic variations, depending on diaphoretic transport. Ions might cross the skin in both directions: influx and efflux. In our study, we observed that ions penetrate the skin in microgram quantity and Beer AM (2002) have proved that this concentration is enough to elicit pharmacological action. Our clinical observations also confirm this by improvement in disease symptoms.

It can be concluded from the results of IR studies of MAP, that adsorption of compounds did take place from the skin of patients suffering from skin disorders like psoriasis, eczema, or acne. Peaks in psoriasis , eczema and acne points to role of free arachidonic acid and peaks in acne specially show change in intensities of alcohols , humic acid & P-H bonding (itching). Correlating this data to results of preclinical studies directs us to conclude that both penetration (diffusion) and adsorption play a very important role as a healing factor for inflammatory skin disorders like psoriasis, eczema and acne.

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