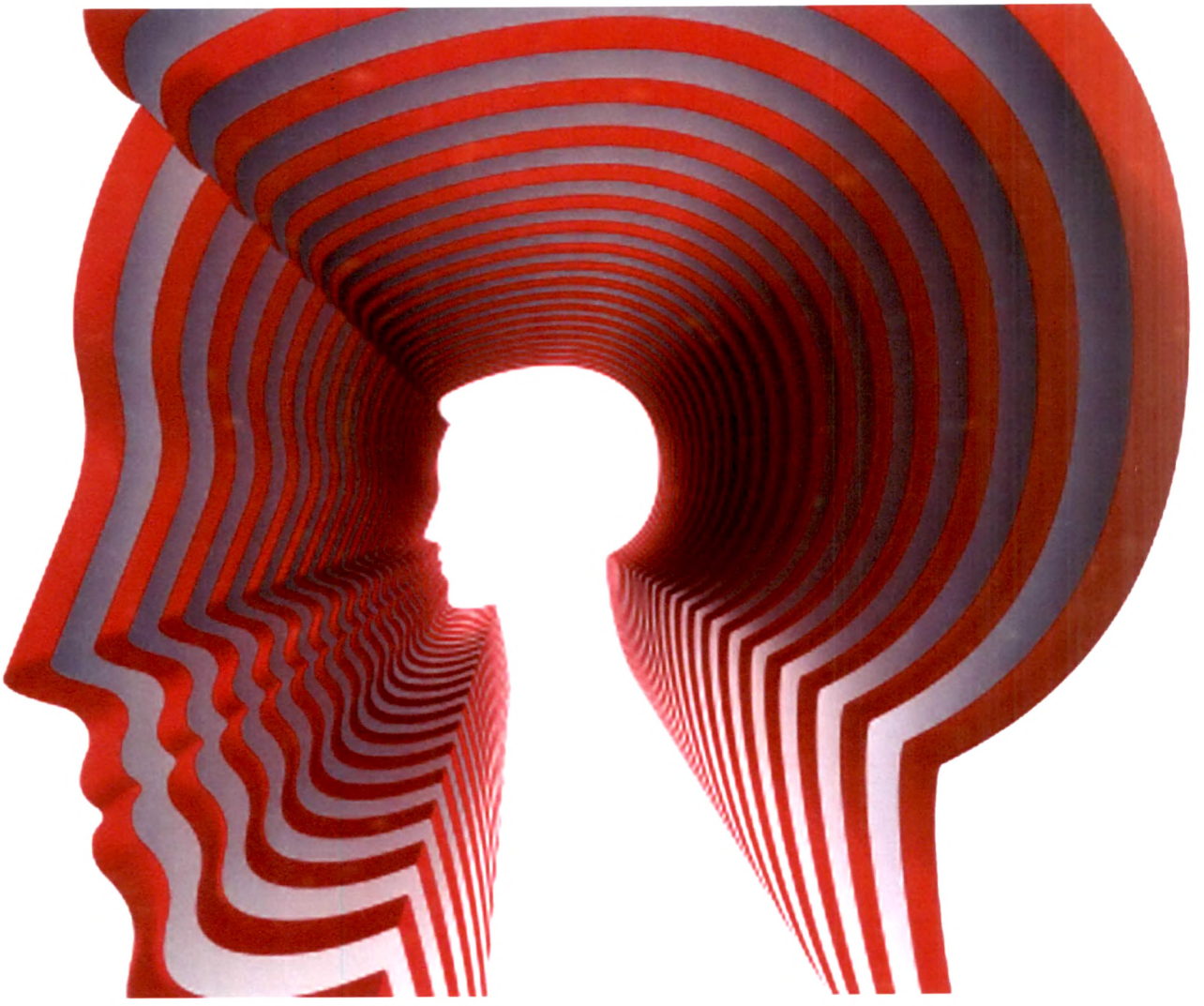
CHAPTER-9

SUMMARY CONCLUSION



9.0 Summary & Conclusions

The present study was aimed at investigating mud for skin disorders like psoriasis, eczema and acne and to prepare patient friendly formulations from it. Mud has been used therapeutically, since olden times, for various purposes like rheumatoid arthritis, constipation, skin diseases etc, but a scientific study on the therapeutic activities of Indian mud for treatment of skin disorders has not been reported so far and so we aimed to give a scientific insight into the conventional use of mud as a therapy for skin disorders. Moreover mud is used as a bath therapy, which is not very convenient for all the patients so we also aimed to prepare patient friendly formulations of mud.

To give a scientific background to our study, we determined physicochemical properties of our muds and tried to correlate them with their mechanism of action. We also tried to grade the muds as regards to their therapeutic effects on skin disorders, and to find out optimum concentration of elements in them for highest skin penetration.

We collected soils which were used therapeutically in Nature Cure Centers, from 3 different geographical locations of India. Two samples were collected from Allepy, Kerala one from Dwarka and one from Vadodara namely: Kerala Black, Kerala Brown, and Dwarka & Vadodara. They were studied for different physicochemical characteristics, all of which were either related to investigation of its activity or preparation of formulations.

Soil Colour depends on the types of soil constituents (iron oxide, calcium carbonate, humic acid). Black colour of Kerala black indicated richness in humus and reddish brown colour of Vadodara indicated presence of iron. Blackish brown colour of Kerala Brown also indicated richness in humus while yellowish white colour of Dwarka indicated presence of large amount of $CaCO_3$. Moreover, brown and black colour is indicative of large amount of clay in Kerala Black and Kerala Brown.

Particle size analysis was done by wet and dry sieve analysis. Wet sieve analysis report, suggested that Dwarka was rich in silt while Kerala Brown was rich in clay. Organic matter is adhered to clay and so particle size analysis indicated that Kerala Brown was rich in organic matter, (organic carbon) which was confirmed by chemical digestion method. In general, distribution of particle size was below 150 um., out of which, maximum was in the range of 75um to less than 25um.

From the **textural** diagram, it was observed that Kerala black and Kerala brown fell in silty clay loam region while Dwarka and Vadodara could be classified in silty loam region because clay content was less in these two soils which could be reflected in their colour also.

Presence of humus and texture of the soil influenced their **bulk density.** Kerala black (2.314 gm/cc) had least bulk density and Dwarka (2.82 gm/cc) had the highest.

Moisture content was found highest in Vadodara (6.32%) and least in Kerala Brown (3.73%). Soils rich in clay (Kerala Black and Brown) had less moisture content and so they were very **hard** when dry while Vadodara containing more silt (thus more moisture content) could be crumbled by hands.

Swelling index is a measure of incorporation of maximum amount of water by soil. Swelling index of the soils helped to judge the amount of water to be added to prepare paste, lotion and spray type formulations. Kerala Brown had the highest swelling index (120%) because it was very rich in clay.

Electrical conductivity and pH was measured which gave indication of presence of soluble salts in them. Acidic pH of Kerala Black may be attributed to high concentration of calcium ions present in it because they are held strongly by the micelle than sodium ions which can be easily hydrolyzed. High electrical conductivity of Kerala black (9.5±0.4mS) reflected more dissociable soluble salts in it.

FTIR characterization of all four soils indicated presence of many functional groups like carboxylic acid, aldehyde, pyridines, pyrolles, ketones, aliphatic carbons,

aliphatic primary amines, amides, amino acids containing NH_2 group, alcohols, silicates, iron oxides, P-O-C stretching vib and BrO_3^- ions. Absorption bands corresponding to total carbon content, total Nitrogen content, minerals, carbonate and humic acids were also observed in FTIR spectra of all four soils, the presence of which were confirmed by other tests like SEM-EDS, CHNS analysis and AAS .

Scanning electron microscopy with energy dispersive spectra (SEM-EDS) was conducted which gave an idea of concentration of elements present in them. Since carbon content of soil includes many compounds which are medicinally important, it was used as a parameter to grade the soils. Dwarka showed highest amount (5.66%) while Vadodara showed the least (2.17%). Dwarka also showed high concentration of calcium and its greyish white color indicated that the carbon present in Dwarka was in the form of $CaCO_3$ rather than organic carbon. This was also confirmed by determining carbon by chemical digestion method.

CHNS analysis of soils once again confirmed the presence of highest amount of carbon in Dwarka (8.898%). Sulphur was present only in Kerala Black. Typical sulphurous smell of Black was confirmed by the presence of sulphur detected by CHNS analysis.

Carbon was also determined by **chemical digestion method** because this method determined organic carbon (easily oxidisable) which is medicinally important rather than total carbon (combustible) as measured by CHNS analyzer and total carbon as measured by SEM-EDS. This method showed that Kerala Brown had highest organic carbon (0.127±0.027 %) amongst the four. This finding was also supported by its blackish brown colour and highest clay content which indicated highest organic carbon content.

Organic carbon content was also found of **particulate fractions** which helped us to select the fraction with high carbon content for formulations. It was observed that organic carbon content increased with decreasing particle size. These results were in correlations with the fact that color is reflected by the clay content which in turn is

reflected by organic carbon content. It was observed that there was 26.52% rise in carbon content in fraction passed thorugh 500# sieve (less than 25um size) than in fraction retained in 44# sieve (greater than 355um size).

Atomic absorption spectrometry (AAS) results showed that heavy metals like cadmium, copper, zinc, cobalt, & lead were present in trace quantities (<0.5ppm). Magnesium (0.22%), calcium (0.035%), phosphorus (0.022%), potassium (0.023%) were found highest in Kerala Black.

Element concentration in the soils was also found by **Inductively Coupled Argon Plasma Atomic Emission Spectrometry (ICP-AES).** Only five dermatologically important (phosphorus, magnesium, sodium, potassium, calcium) elements were determined. Kerala Black showed highest amount of calcium and magnesium. An AAS and ICP-AES method determines elements soluble in water, while SEM-EDS and CHNS analyzer determines total concentration of elements. The former two methods were useful to draw conclusion regarding penetration of elements while the latter two methods helped in adsorption (into soil from skin) studies.

Content of **Humic acid**, a pharmacologically important soil constituent, was determined and Kerala Black was found to have highest amount (0.182%). This result was In accordance with the black colour which signifies abundance of humic acid.

Microbial examination of all four soils revealed that there was no growth in plates after 48 hrs. of incubation but significant CFUs were observed after 7 days of incubation indicating presence of spore forming bacteria. However, the soils also exhibited **antimicrobial activity**. Vadodara expressed maximum zone of inhibition against *E.coli* (14.0±0.1 mm), *S. aureus* (15.02±0.2mm) and *Candida albicans* (9.5±0.3mm) which was comparable to that of ampicillin and fluconazole. Antimicrobial activity may be attributed due to high salt concentration and presence of antibiotics in soils.

In vitro diffusion studies on human skin showed that amount of diffusion of **elements from** soil into the skin depended on **optimum** concentration of that

element in the soil. i.e. more concentration of element did not imply higher penetration. It was observed that if a soil sample contained soluble concentration of P = 0.26 - 0.28 ug/100mg soil , Mg = 10-12 ug/100mg soil, Na = 38-40 ug/100mg soil, K = 2-3 ug/100mg soil, Ca = 5-7 ug/100mg soil, then it would ensure about 90% skin penetration of that element. It was observed that Kerala Brown had maximum skin penetration of calcium (45%) even though its concentration was less than Kerala Black and Dwarka. Magnesium which is also a dermatological important element, penetrated 83% (which is maximum) from Kerala Brown while its concentration in soil was (10.37ug/ml), less than that in Kerala Black (45.61ug/ml). Phosphorus which is responsible for itching showed 84%penetration from Kerala Black and 73% from Kerala Brown.

There was approximately 1.9% diffusion of **humic acid from** Kerala Brown through excised human skin **in vitro.** Humic acid plays a vital role in skin chemistry for regulating cell proliferation and differentiation. So one of the actions of mud in improving disease symptoms must be through its humic acid content.

Preliminary clinical studies were conducted in patients of psoriasis, eczema and acne. Patients were asked to apply mud samples on the diseased lesion and then carefully remove it after 30 minutes. The removed samples were analysed for carbon content, element concentration and structural bonding changes by different methods.

CHNS analysis of MAP (mud applied on patients) and **chemical digestion method** indicated significant ($p<0.05$) increase in carbon content. On applying ANOVA, the value of F for Kerala Brown was 168.4 (highest) for F critical =3.49, indicating that Kerala Brown far exceeded other muds in adsorbing carbon containing compounds from lesional skin. This was in accordance with its highest clay content which is a powerful adsorbing component of soil.

SEM-EDS reports of MAP (mud applied on patients) revealed significant ($p<0.05$) rise in carbon content. These results were in coordination with those of CHNS and

chemical digestion method analysis. Moreover this report showed significant decrease in calcium and magnesium content of soil applied on patients. Improvement in disease symptoms and decrease in calcium and magnesium content in soil, both confirmed the fact that these elements play an important role in pathogenesis of psoriasis, eczema and acne.

CHNS analysis and SEM-EDS, both give total concentration of elements while chemical digestion method and AAS study give water soluble concentration of those elements. **AAS study** results indicated significant decrease ($p<0.05$) in calcium and magnesium content in soil applied to lesional skin. In vitro diffusion studies was conducted on **healthy skin** while AAS study was conducted on soil applied to **lesions** (psoriasis, eczema or acne), but both showed mobility of calcium and magnesium ions into the skin. Moreover, improvement in disease symptoms was observed on application of mud and so it could be conclusively said that one of the action of mud in improving disease , may be due to its calcium and magnesium content.

FTIR studies on mud applied on patients **(MAP)** showed the presence of many new absorption bands in the region of amines, amino acids, carboxylic acids. Moreover, psoriatic lesions contains free arachidonic acid and changes in amino acid (2600-3100cm-1), carboxylic acid (1620-1680cm-1) regions and CHNS and SEM-EDS reports of increased carbon content led us to conclude that chemical moiety relating to arachidonic acid had adsorbed from the lesions, thus decreasing concentration of free arachidonic acid from the skin, resulting in less availability of arachidonic acid in inflammatory and cell regulation and protein kinase C cascade which leads to abnormal cell proliferation and differentiation. However, further studies are recommended to isolate the adsorbed compounds, and if that would be successful then a day may not be far when it (isolated arachidonic acid) may be used as a marker for the improvement of the disease.

400-700cm-1 range corresponds to exchangeable Ca+ and Mg+ ions and new peaks and change in intensity was noted in this region for all three diseases. This data was once again in correlation with that of AAS and SEM-EDS data.

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 analyzed by CHNS, SEM-EDS and chemical digestion method.

Evaluation of psoriasis was done by PASI score, of eczema by EASI score and acne by Investigator's Global Assessment scale when **evaluated clinically** at Sir Sayajirao General (SSG) Hospital and Government Ayurvedic Hospital, Panigate Vadodara. It was observed that eradication of itching was the first sign of symptom in all the three diseases. After a few days of treatment, the inflammatory symptom was reduced and in cases where the treatment was continued for a long time i.e. 3 to 4 months, depending upon the intensity of the disease, the skin resumed normal texture in psoriasis and eczema. Statistical evaluation ($P<0.05$) indicated that overall, Kerala Brown was the best mud for all the three diseases.

Patient friendly, economical and environment friendly formulations like paste, lotion, mud compress, spray and powder were prepared from mud and evaluated.

Paste was prepared by adding, methyl & propyl paraben, glycerin and water to mud. 8-10% glycerin was kept as optimum concentration for optimum drying time (30min). 0.1% concentration of methyl and propyl paraben each was found optimum to prevent microbial growth.

Paste was filled in aluminium collapsible tube and observed every month, upto six months. There was no change in **colour, odour or appearance**. There was 1.23% w/w (highest amongst four) **loss in water** in Vadodara, which may be due to least clay content in it. There was no sign of **microbial growth** when incubated on McConkey's agar, nutrient agar and sheep blood agar for 48 hrs. There was no significant change in **pH** of all the four pastes even after six months indicating its chemical stability. **Spreadability** was in the order of Dwarka > Black > Brown >

Vadodara ranging from 3.9 to 4.9 cm. There was no change in texture even after storage for six months.

Lotions were prepared by adding, HPMC (2% w/w), methyl paraben and propyl paraben 0.1% w/w of soil and water sufficient enough to produce lotion like consistency to mud. HPMC (Hydroxy propyl methyl cellulose) was added to impart viscosity to the formulation. The zeta potential of lotions ranged from -14.1mV to -26.6mV. Kerala Brown showed zeta potential of -26.6 mV which indicated maximum dispersion stability. This was reflected in **redispersibility** test wherein with 4 inversions the lotion was completely redispersed. Humic acid which is an intrinsic constituent of soil is a polymer and so it, along with HPMC, must be responsible for good redispersion. Lotions were also examined for any microbial growth till the end of six months. There was no sign of growth indicating appropriate concentration of preservatives. There was no change in **pH of** lotions also, after six months, reflecting chemical stability. **Sedimentation volume** of all the four lotions ranged from 0.9 to 0.95 after 24 hrs. which indicated negligible settling rate.

Mud compresses (patch) were prepared by placing mud paste between two pieces of clothes which was placed on surgical adhesive tape. Different shapes and sizes were prepared and stored in closed box. After six months of storage, **no cracks** were found in the patch.

Powder passed through 500# sieve (<25um) was used as such without adding any ingredient. **Angle of repose** of powders ranged from 14 to 19 which indicated poor flow characteristic, which may be due to very fine size and so it was directed to be applied with puff.

Spray formulation was prepared with a special intention to enable the sprayed mud to reach body parts which are not reachable by hands eg. Back. Muds of particle size of <25um was used. Methyl and propyl paraben was used as preservative. **Sedimentation volume** ranged from 0.87 to 0.92. Kerala Brown showed highest sedimentation volume, as expected, from its zeta potential data and clay content.

Spray suspensions were **redispersed** with 4 to 5 rigorous pendular shakings. Since our formulation contains very high solid content, it did not show a sharp spray **pattern** of fine mist but the pattern consisted of droplets which adhered to the skin but did not trickle down. With six to seven sprays, a uniform layer of mud was formed on 7 x 5 sq.cm area. The only drawback using this formulation was that if after using once, it is not cleaned properly by removing the nozzle, it would get clogged, thus preventing subsequent ejection of spray suspension.

Comparing all the physicochemical properties, characteristics of formulations and the results of preliminary clinical trials, it was concluded that Kerala Brown was the best mud from amongst the four and so formulations made from it were taken up for clinical studies.

Clinical trials were conducted at Dahod General Hospital under supervision of a dermatologist. **Clinical observations** showed improvement greater than 50% in psoriasis with **paste.** Reduction in **itching** was the first sign of improvement and gradually the **plaque thickness** was reduced. Eczema patients showed better response than those with psoriasis and acne.

On applying **lotions**, due to more water content, the **feeling of coolness** was the first pleasing experience expressed by patients of acne, specially when applied on face. This coolness helped relieve mental stress (one of the cause for acne, psoriasis, eczema), resulting in improvement in disease symptoms. Due to more water content, the **diffusion** of water soluble constituents from the soil into the skin would be facilitated, so recovery was greater with lotions (74%) than paste.

It was difficult to find patients with lesions which matched the shape and size of **mud compresses (patch)** prepared. In eczema, patients, the skin surrounding the lesion becomes very thin, so applying the adhesive tape was a bit uncomfortable. When applied on forehead for acne patients, the results were very good due to combination of two effects: **adsorption** due to very high content of paste and

increased **diffusion** due to occlusion effect. The **cooling effect** lasted for longer time than lotions and so gave a pleasant feeling which helped reduce stress.

Powder was very well accepted by acne patients because it kept the skin dry by adsorbing skin secretions. Here, mechanism of action by adsorption was more prominent than diffusion. Around 70% improvement was observed in acne , by application of powder.

Spray was not used for clinical trial because of inability of patients to clean spray device and nozzle properly.

Thus it was observed that application of our developed formulations **only on the affected part** rather than full body, which is done in traditional mud method, showed therapeutic effectiveness against psoriasis, eczema and acne.

Analysis of correlation between mud properties and therapeutic activity

Mud is such a complex, natural, god gifted material and a mixture of so many compounds that unlike modern medicines, it does not have a single mode of action. There are multifaceted actions of mud on our body. It exerts its action due to **(1) its constituents (which we measured) (2) its adsorption property,(3) its cooling property (4) its Occlusion property (5) Activity due to other reported constituents.**

Hence, we analyzed the data gathered from our investigations to correlate with various proposed mechanisms as discussed below.

(1) Constituents

We have seen in chapter 1 that soil comprises of variety of chemical compounds and many scientists have isolated therapeutically active compounds from mud. The fact is that even though these compounds are present in very small concentrations, they penetrate through and into the skin and elicit pharmacological action. This has been confirmed by Beer AM (2002) and Tateo FT (2007).

The involvement of elements in the enzyme systems of cell i.e. protein kinase and calcium level and psoriasis, role of magnesium in cAMP cascade and psoriasis, etc has been very well discussed in chapter 6.1 of the text (invitro diffusion of elements through human skin) . Our study indicated that elements like Ca, Mg, K, & Na, penetrated through stratum corneum and were concentrated in the skin. Humic acid, an important component of mud having many pharmacological actions was also accumulated in the skin .

There is evidence that minerals depleted mud does not elicit pharmacological functions and so one can conclude that beneficial effects of mud are due to its constituents (Laurie Barclay 2002). Probably this may be the reason why mud once used is discarded in Nature Cure Centers and Ayurved centers.

Due to presence of high salt concentrations (our findings) and other antibiotics (reported) it acts as an antibacterial agent in case of psoriasis, eczema and acne. The antimicrobial activity of muds was comparable to that of ampicillin and fluconazole.

(2) Adsorption:

Our body cannot detoxify all the toxins produced and so they should be removed by one way or the other or they will accumulate and manifest as a disease (principle of Naturopathy). It has been mentioned in Indian medicinal literature that mud removes toxins from the body. Effective adsorption of organic toxins from intestines and Pseudomonas aeruginosa toxin (Said, S.A. 1980, Johns T 1991, Mahaney WC 2000, Dominy, N.J 2004) by mud has been reported.

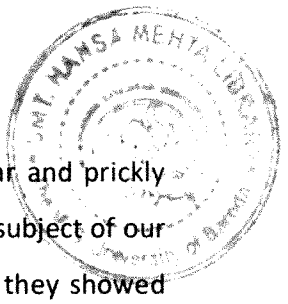
One of the objectives of our study was to find a scientific approach to the use of these muds in treatment of psoriasis, eczema and acne. And so mud was first characterized by IR study, SEM-EDS, CHNS analyser, AAS, chemical digestion method and then applied to the diseased part of the body for 30 min and then carefully recovered from it for testing. On analyzing the samples once again by the above mentioned methods it could be observed that muds do extract (adsorb) substances

from the lesional skin. It is for the first time that this type of study has been conducted and it is recommended that more studies be done to exactly define the substances adsorbed by the mud, from the diseased skin.

(3) Cooling Property:

Addition of water to mud is an exothermic reaction (Morrill LG 1982) and so when water is added to mud, it reduces the temperature of the mixture. When mud is applied to the skin, in the initial few minutes, it causes cooling effect which in turn causes vasoconstriction. . This coolness helps to reduce the mental stress of the patient and gives a good psychological feeling of well being. However as a natural body response and heat transfer, the temperature of the mud gradually rises, and at the end of 30 to 40 minutes the cooling effect is lost as the mud attains equilibrium with body temperature.i.e. 37°C**Thus it indirectly helps in treating patients, as stress is a trigger factor in psoriasis, eczema and acne** (Al-ahmar HF 1976).

Traditionally, mud therapy involves either direct application of mud on the full body or indirectly by packing in a cloth and placing on the body part. This utilizes large amount of mud and reduces patient's mobility. Hence, one of our aims was to prepare patient friendly formulation. In foreign countries mud based creams, soaps, shampoos, and packs are marketed while in India there is only one company (Himalaya Herbals) who manufactures mud paste. So we developed formulations like Pastes, lotions, patch (compress), spray, and dry powder which have shown quite promising results in the treatment of skin disorders. Uptil now there, has been no reference about using mud as dry powder for external application as a medicinal agent but in our findings, it was shown that dry powder(particle size approx.25um) could be used for healing psoriasis eczema and acne. This formulation was specially very useful in case of acne where it can be used similarly as a face powder. As it can be kept for 24 hours, it is cosmetically appealing as well as medicinally active.



In India, hot & humid climate persists for nearly 8 months of the year and prickly heat is a common complaint in summer. Though prickly heat was not a subject of our study but we gave Dwarka soil to such patients on their demand and they showed 90% recovery within two days.

(4) Occlusion Property:

The absorption promoting effect of occlusion has been attributed to the suppression of insensible water loss and subsequent imbibitions of water by the skin. In 1921 Stephen Rothman (1921) found that insensible water loss is decreased, on the average, by 60% if patches, 50 % if ointments, by 25% if pastes, and not at all if dusting powders, are applied to the skin. It reduces TEWL (total epidermal water loss) and thus improves symptoms of psoriasis and eczema (Comacchi C 2004). Hence this may be one of the mechanisms of action of mud and its formulations in treatment of skin disorders.

Conclusions

Mud therapy has been a very old therapy and its mention is also obtained in Ayurveda. Though its mechanism of action has been a subject of contemplation, a lot of efforts have been made to make an insight to it. It has still been quite confusing and complex due to large number of constituents and varied physico chemical properties of different muds.

The present investigation was undertaken to give a scientific background to the traditional use of mud as a therapy for skin diseases. It was observed that some simple examinations like color, & texture could **partially** help decide its physicochemical chemical properties and give idea about its constituents and therapeutic activity .i.e. blackish brown (Kerala Brown) coloured soil would be more effective therapeutically for skin diseases because of high organic carbon content, but to have maximum advantage of its therapeutic efficacy its constituents have to be optimized. Since mud is a natural element and its constituents differ according to

topographical areas which are influenced by climate, rainfall, vegetation and the depth from which it is collected, it is very difficult to obtain an ideally composed soil, in that case colour and texture of soil becomes the deciding factor to get a general idea regarding its therapeutic activity.

Our literature survey guided us to correlate the activity of the constituents, we monitored in our study and also the multifaceted and manifold mechanism of actions of mud on skin disorders.

We have succeeded in comparing various physicochemical properties of soils and correlating them with their therapeutic activity against psoriasis, eczema and acne and finding out the criteria for judging the best soil amongst the four (organic carbon, humic acid, elements and their penetration).

Moreover, to overcome the limitations of its traditional (mud therapy) use, due to time consuming body mud baths and restriction of patients' mobility, we have been successful in preparing patient friendly formulations which have been effective against psoriasis, eczema and acne.

Amongst the many existing evaluation parameters for improvement of the skin diseases, we have partially been successful in correlating the penetration (diffusion) or adsorption of certain components with the therapeutic efficacy of the muds. We could reasonably conclude that diffusion of substances like Ca, Mg, humic acid, Na, K etc. from the mud into the skin whereas adsorption of carbon containing compounds were definitely involved in showing the therapeutic potential of muds in the treatment of various skin disorders like posriasis, eczema and acne. However more in depth studies using sophisticated technologies are required to exactly identify the compounds removed from the lesional skin of psoriasis, eczema and acne to enable us to quantify the marker compound (s) that can be used for assessing the **disease improvement index.**

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