Formulation and clinical evaluation of topical dosage forms of Indian Penny Wort, walnut and turmeric in eczema

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Abstract: Eczema is characterized by itching, lichenification, scaling, oedema and erythema. Current management strategies include corticosteroids, which are limited due to side effects. Many herbal remedies are used traditionally but unfortunately have not been validated in controlled clinical trials. Three popular traditional treatments of eczema include Indian pennywort, Walnut and Turmeric. In this study three topical formulations (micro emulsion, gel and ointment) were prepared from extracts of Indian pennywort, Walnut and Turmeric. These formulations were monitored for stability for a period of three months. Controlled clinical trials were conducted on 360 eczema patients. Clinical parameters observed were degree of erythema, oedema, scaling, itching and lichenification. Effects of each formulation on these clinical parameters were compared with placebo formulations. Micro emulsion formulations in all cases proved to be more effective in reducing semi quantitative scores of erythema and oedema. Itching was relieved more by gel formulation. The ointment showed more efficacy towards scaling and lichenification. Comparison of the effects of placebo and the specific formulations was performed by chi-square statistics and found to be highly significant. In summary it is concluded that all the formulations could be used as promising source for treatment of eczema.

Keywords: Formulation, clinical evaluation, Indian penny wort, Walnut, Turmeric.

INTRODUCTION

Traditional herbal medicines are widely used in Asia. Evidence supporting the practice is generational, but unfortunately many traditional approaches suffer from a deficiency of stringent scientific evaluation. Rigorous quantitation of efficacy in many cases is a requirement to validate continued application. One example is the condition of eczema. Eczema is a disease characterized by inflammation of skin, accompanied by severe itching. Itching is often the first sign in eczema. In addition to debilitating physical effects, patients may feel ill-at-ease in public due to visible disfigurement. Eczema's underlying causes include environmental promoters such as pollen and food allergens, as well as clothing, jewelry and lifestyle choices such as frequent bathing or use of soap without moisturizers and detergents (Giorgia *et al*., 2005; Lippincott, 2005; Ronald, 1992). In allopathic eczema was treated by topical corticosteroids, which have a range of adverse side effects. One common side effect with long-term use of corticosteroids is dermal atrophy whereby skin becomes thin and fragile. Treated surfaces become susceptible to attack by bacteria or fungi (David, 2003). Traditional herbs commonly used among cultures of Southern Asia including Indian pennywort and walnut leaf poultices are widely used. Turmeric paste has broader folkloric dermatology indications than eczema (Nadkarni, 1976); however rigorous clinical studies remain unavailable.

The aim of the present study was to evaluate clinical efficacy of three topical formulations (micro emulsion, gel and ointment) made from Indian pennywort, walnut and turmeric on eczema patients. Clinical parameters of eczema were monitored for a period of four weeks.

MATERIAL AND METHOD

Leaves and stems of *Centella asiatica* L., fam. Mackinlayoideae, leaves of *Juglans nigra* L., fam. Juglandaceae and rhizome of *Curcuma longa* L., fam. Zingiberaceae were used as plant material. The identification of these plants was performed at the Cholistan Institute of Desert studies at The Islamia University of Bahawalpur, Pakistan.

Method

Three formulations of micro emulsion, gel and ointment were prepared from each extract of Indian penny wort, walnut and turmeric. Each formulation containing 5% of plant extract.

Formulation of micro emulsion

Micro emulsion was prepared as described by Chen *et al*. A mixture of polysorbate 20 (surfactant) and ethanol (cosurfactant) was prepared in a 2:1 ratio. 4.6g of

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surfactant mixture was added to0.5 g of oleic acid and mixing was done by magnetic stirring. Plant extract (0.5 g) was added to the surfactant mixture, which was then added to oleic acid and completely dissolved through vigorous stirring. Finally 4.4g of filtered, deionized water was added with constant stirring (1200 rpm) (Khiljee *et al*., 2010; Chen *et al*., 2006).

Formulation of gel

Carbopol gel was prepared as mentioned by Proniuk *et al*. 1% carbopol 934P solution was prepared in water with continuous stirring at 1200rpm. Triethanolamine was added drop wise until a gel was formed. Plant extracts (0.5g) were added and Indian pennywort, walnut and turmeric had pH of 5.5, 6.2 and 5.6 respectively (Khiljee *et al*., 2012; Proniuk *et al*., 2001).

Formulation of ointment

Simple B.P. ointment was prepared. At 60^{o}C 4.75g of hard paraffin and wool fat was melted, into which 4.75g of cetostearyl alcohol was added. The mixture was cooled to room temperature by stirring and then white soft paraffin 80.75g and 5g of the respective extracts were added (Khiljee *et al*., 2010; Chen *et al*., Marriott, 2006).

Characterization

All formulations were characterized by performing pH, conductivity and viscosity tests and monitored for stability for a period of 3 months as shown in table. 1. *In vitro* release was evaluated using Franz cells (Khiljee *et al*., 2010).

Electrical conductivity (σ) of formulated samples was measured using a conductivity meter WTW Cond 197i (Weilhein, Germany) (Djordjevic *et al*., 2005). The pH value of prepared formulations kept at different conditions was determined by a digital pH-Meter. Centrifugal tests were performed for formulations immediately after preparation. The centrifugal tests were performed at 25^{o}C at 5000 rpm for 10 minutes by placing a 5g sample. Viscosity of the formulations was determined using a Brookfield RVDV III ultra, Programmable rheometer (Brookfield Engineering Laboratories, Middleboro, MA) using the ULA (Ultra Low Adaptor). The software used for the calculations was Rheocalc V2.6. All experiments were carried out in triplicate for each sample and results presented as average ± S.D (Ueda *et al*., 2009).

Stability tests

Stability tests were performed at different storage temperature conditions for all formulations made from three plants named Indian pennywort, walnut and turmeric. The tests were performed on samples kept at 0^{o}C±1, 8^{o}C±0.1^{o}C (in refrigerator), 25^{0}C±0.1^{o}C (in incubator) and 40^{o}C±0.1^{o}C (in incubator). All formulations were analyzed for the change in appearance, pH, conductivity and rheology (Shivhare *et al*., 2009; Shinde *et al*., 2005; Reddy *et al*., 2006; Saeedi *et al*., 2003).

Clinical evaluation

The study was approved by institutional ethic committee. The study was a randomized clinical trial (RCT), double blind (patient, caregiver, and investigator) and placebo (control). Controls were prepared without plant extract for the three formulations for each of the three plants. The study course was 4 weeks with 2 visits. Mild to moderate eczema patients were included in this study and formulations were applied to lesions on hands, legs, feet, neck and face. Each subject underwent a preliminary 24 hr patch test and no signs of erythema and any allergy were observed with any formulation or control. Written informed consent was obtained from each subject if age above 18 years or from caregiver or parent if under age 18 before commencement of the study. A total of 450 male and female patients from all socioeconomic classes were included as were children. All patients who were taking other medication or with co-existing skin diseases, pregnant or breast feeding women; or patients with hepatic or kidney disease or known drug hypersensitivities were excluded from study (Saeedi *et al*., 2003). After exclusion, 360 participated and completed the study (Mohanta, 2007). The data were collected in the period from June 2009 - Jan 2011. Three plants were used in this study. From each plant three topical formulations (micro emulsion, gel and ointment) were prepared. Each of the nine formulations was tested on 30 patients. Controls of micro emulsion, gel and ointment were each given to 30 patients (total 90). Signs and symptoms of 270 eczema patients were noted with these formulations. All formulations results were compared with control formulations to evaluate any effect on the sign and symptoms.

All formulations were applied two times a day for 4 week period. Formulations effect on clinical parameters of eczema like scaling, itching, oedema, erythema and lichenification were compared with control formulations. Patient enrolled were mostly mild to moderate cases. Patients signs were assessed using a semi quantitative 0-3 score (Saeedi *et al*., 2003) (none to severe) as assessed by physician.

Patients with moderate severity were chosen to facilitate evaluation. Those having reduction in scores of erythema, oedema, scaling, itching and lichenification were tabulated as improved and those, which did not show any improvement were tabulated as not improved.

Data were analyzed with statistical analysis software SPSS, version 10.0. The proportion of patients' signs and symptoms recovery was compared with placebos using chi square analysis. The p- values were used to analyze the change in improvement measurements over time.

Table 1: Physicochemical evaluation of dosage forms

Formulations parameters		pH	Conductivity	Viscosity (cps) Mean ± SD
Indian penny wort formulations	Micro emulsion	5.8	0.1	23.74±0.003
	Gel	5.5	0.2	28.43±0.002
	Ointment	5.3	0	120.21±0.003
Walnut formulations	Micro emulsion	5.7	0.2	15.81±0.001
	Gel	5.9	0.1	24.39±0.002
	Ointment	5.6	0	112.25±0.004
Turmeric formulations	Micro emulsion	5.8	0.1	16.52±0.002
	Gel	5.6	0	22.03±0.001
	Ointment	5.5	0	154.21±0.005

Table 2: Chi- Square test of all formulations verses control

Chi Square Tests for Indian Penny formulations	
Parameters	P Value
Micro emulsion Vs Control	.000
Gel Vs Control	.000
Ointment Vs Control	
Chi Square Tests for Walnut formulations	
Parameters	P Value
Micro emulsion Vs Control	.000
Gel Vs Control	.000
Ointment Vs Control	
Chi Square Tests for Turmeric formulations	
Parameters	P Value
Micro emulsion Vs Control	.000
Gel Vs Control	.000
Ointment Vs Control	

Probability or p-value was set at $p<0.05$, or a confidence interval of 95%. Hence the level of significance is 5% (also called an alpha level), so any result with a p-value of less than 0.05 is significant and $p>0.05$ is statistically not significant. The acceptable level of statistical significance for all tests was $p<0.05$ (Usmanghani *et al*., 2007).

RESULTS

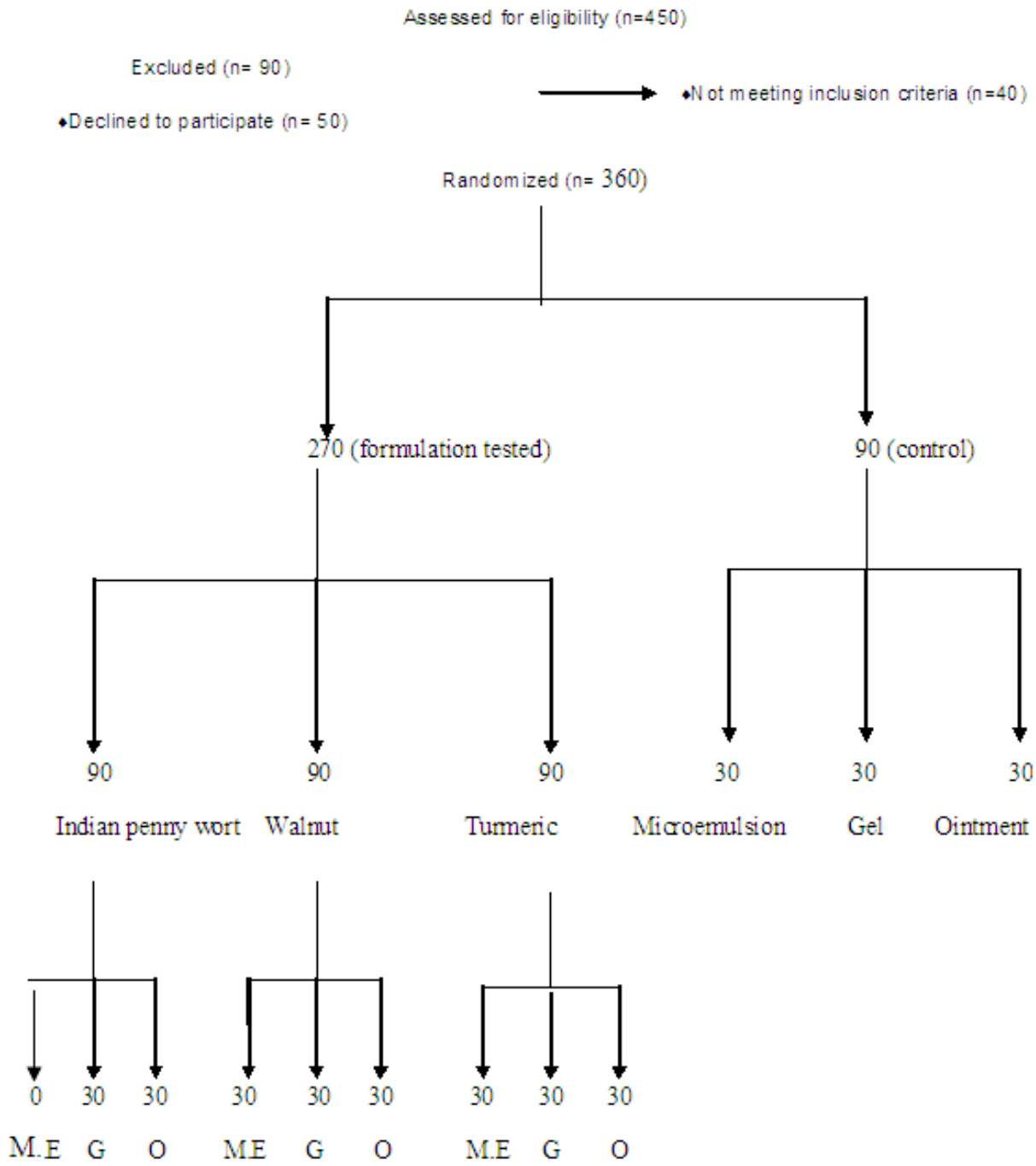
Stability of formulations kept at different storage conditions i.e. 0°C, 8°C, 25°C and 40°C was determined for a period of 90 days at predetermined time intervals. The three topical formulations were prepared from extracts of Indian pennywort, walnut and turmeric. No previous data were available on the dosage made from these plant extracts. All the formulations were characterized by performing conductivity, pH and rheology tests (table 1). No change in their physical characteristics including color, liquefaction and phase separation; and other parameters like pH, viscosity and conductivity was observed over a period of three months. This was consistent with a previous study in which topical dosage forms prepared from an extract of *Eupatorium odoratum* L (Panda & Ghosh., 2010).

Normal human skin has a pH ranging from 4.5-6 (Jennifer *et al*., 2003). Our formulations were in good compliance with the skin pH. In the topical dosage forms, product stability is one of the most important quality criteria (Mostefa *et al*., 2006). Our formulations were stored under conditions which limited potential oxidation (Panda & Ghosh, 2010).

A comparative study of the nine formulations plus placebo (without plant extract) was conducted. Three hundred-sixty patients were enrolled and among them 270 were tested for anti-eczema effects by application of topical formulations of Indian pennywort, walnut and turmeric. Ninety patients were given placebo formulations. The clinical study was completed within two year time period. Parameters taken in sign and symptoms were erythema, oedema, scaling, lichenification and itching. These parameters were chosen since they represent the most common expressions of disease. Herbal formulations were evaluated on the basis of improvement in the sign and symptoms. All formulations were applied two times a day for a period of 4 weeks.

Clinical evaluation of micro emulsion of Indian penny wort, Walnut and turmeric

The effects of Indian pennywort micro emulsion observed in 30 patients are outlined in fig. 2. Out of 30 patients 23 showed improvement in the erythema, while 7 patients



Abbreviations: M.E = Microemulsion, G = Gel, O = Ointment

Fig. 1: Flow diagram of patient recruitment

showed no improvement. Oedema was improved in 22 patients. Scaling and itching were improved in 19 patients. Lichenification improved in 17 patients. Indian pennywort micro emulsion comparison with control micro emulsion has P-Value of 0.000 as shown in table 2.

A parallel study using walnut micro emulsion showed improvement in 24 out of the 30 subjects with erythema. Oedema showed improvement in 23 patients and scaling showed improvements in 19 individuals. Itching was minimized in 20 patients. Lastly lichenification got recovered in 18 patients. P value of micro emulsions made from Walnut versus control formulation is 0.000 as shown in table 2.

Results for the turmeric micro emulsion in 30 eczema patients were similar to the two sister studies outlined above. Twenty-three patients showed improvement in the

erythema, oedema was improved in 19 patients and scaling was improved in 22 patients. Itching was minimized in 21 patients and lastly lichenification recovered in 20 patients. P value of micro emulsions made from Turmeric versus control formulation is 0.000 as shown in table 2.

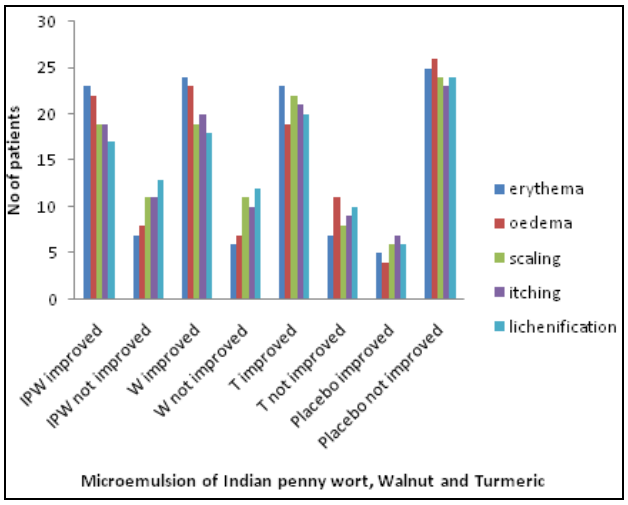


Fig. 2: Effect of Micro emulsion of Indian pennywort, walnut and turmeric on clinical parameters of patients

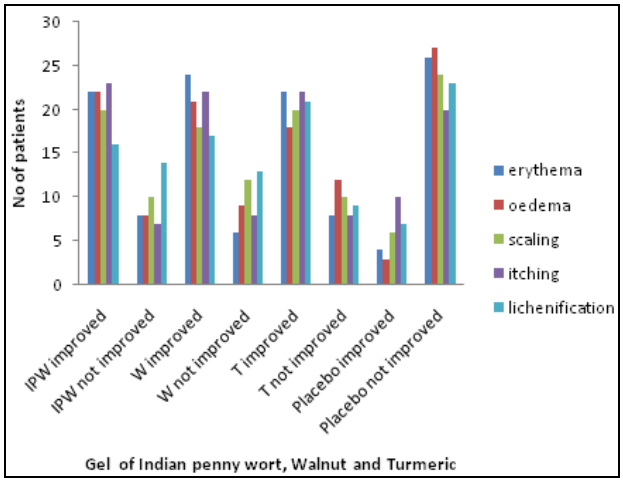


Fig. 3: Effect of gel of Indian pennywort, walnut and turmeric on clinical parameters of patients

Clinical evaluation of gel of Indian penny wort, Walnut and turmeric

Effect of Indian pennywort gel was observed on 30 patients. Parameters observed were erythema, oedema, itching, scaling and lichenification as shown in fig. 3. It was noted that out of 30 patients 22 showed improvement in the erythema (redness and inflammation), while 7 patient showed no improvement. Oedema showed improvement in 22 patients and in 8 patients no improvement was found. Scaling showed improved in 20 patients while 10 patients showed no improvement. Itching was minimized in 23 patients while 7 showed no improvement. Lastly lichenification got recovered in 16 patients and 14 showed no improvement. P value of gel made from Indian penny wort versus control formulation is 0.000 as shown in table 2.

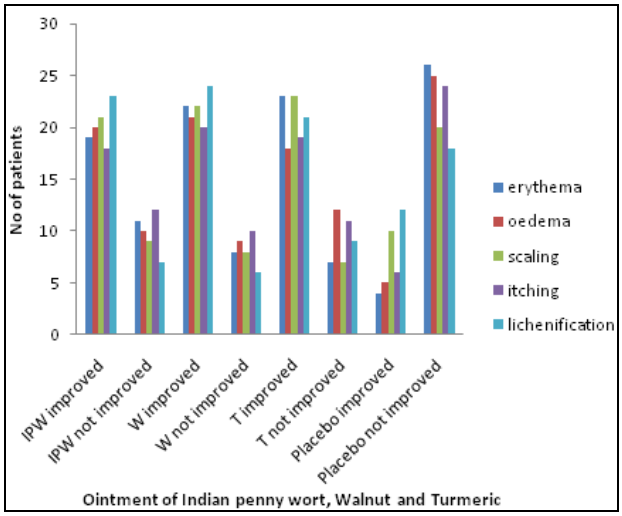


Fig. 4: Effect of Ointment of Indian pennywort, walnut and turmeric on clinical parameters of patients

Efficacy of walnut gel was observed on 30 eczema patients. Parameters observed were erythema, oedema, itching, scaling and lichenification. It was noted that out of 30 patients 24 showed improvement in the erythema (redness and inflammation), while 6 patient showed no improvement. Oedema showed improvement in 21 patients and in 9 patients no improvement was found. Scaling showed improved in 18 patients while 12 patients showed no improvement. Itching was minimized in 22 patients while 8 showed no improvement. Lastly lichenification got recovered in 17 patients and 13 showed no improvement. P value of gel made from Walnut versus control gel is 0.000 as shown in table 2.

Efficacy of turmeric gel was observed on 30 eczema patients. Parameters observed were erythema, oedema, itching, scaling and lichenification. It was noted that out of 30 patients 22 showed improvement in the erythema (redness and inflammation), while 8 patient showed no improvement. Oedema showed improvement in 18 patients and in 12 patients no improvement was found. Scaling showed improved in 20 patients while 10 patients showed no improvement. Itching was minimized in 22 patients while 8 showed no improvement. Lastly lichenification got recovered in 21 patients and 9 showed no improvement. P value of gel made from Turmeric versus control gel is 0.000 as shown in table 2.

Clinical evaluation of ointment of Indian penny wort, walnut and turmeric

Effect of Indian pennywort ointment was observed on 30 patients. Parameters observed were erythema, oedema, itching, scaling and lichenification as shown in fig. 4. It was noted that out of 30 patients 19 showed improvement

in the erythema (redness and inflammation), while 11 patient showed no improvement. Oedema showed improvement in 20 patients and in 10 patients no improvement was found. Scaling showed improved in 21 patients while 9 patients showed no improvement. Itching was minimized in 18 patients while 12 showed no improvement. Lastly lichenification got recovered in 23 patients and 7 showed no improvement. P value of Indian pennywort ointment versus control ointment is 0.000 as shown in table 2.

Efficacy of walnut ointment was observed on 30 eczema patients. Parameters observed were erythema, oedema, itching, scaling and lichenification. It was noted that out of 30 patients 22 showed improvement in the erythema (redness and inflammation), while 8 patient showed no improvement. Oedema showed improvement in 21 patients and in 9 patients no improvement was found. Scaling showed improved in 22 patients while 8 patients showed no improvement. Itching was minimized in 20 patients while 10 showed no improvement. Lastly lichenification got recovered in 24 patients and 6 showed no improvement. On chi square comparison of walnut ointment with control ointment (without walnut extract) it was seen that P value is 0.000 as shown in table 2.

Efficacy of turmeric ointment was observed on 30 eczema patients. Parameters observed were erythema, oedema, itching, scaling and lichenification. It was noted that out of 30 patients 23 showed improvement in the erythema (redness and inflammation), while 7 patient showed no improvement. Signs of oedema showed improvement in 18 patients and in 12 patients no improvement was found. Scaling got improved in 23 patients while 7 patients showed no improvement. Itching was minimized in 19 patients while 11 showed no improvement. Lastly lichenification got recovered in 21 patients and 9 showed no improvement. P value of Turmeric ointment versus control ointment is 0.000 as shown in table 2.

DISCUSSION

From results it was noted that the all-micro emulsion formulations showed more efficacy towards healing of erythema and oedema that might be due to its improved penetration at the cellular level. However it did not have a significant effect on scaling and lichenification due to its non-adherent characteristics. Furthermore comparison of micro emulsion made from Indian pennywort, Walnut and Turmeric showed statistically significant from control micro emulsion.

Gel of all formulation gave good effect towards itching it showed that gel dosage form is an effective remedy for itching. In one study different compositions of gel made from *Glycerrhiza glabra* were standardized and 2% was found to work well in the *in vivo* efficacy of eczema. Clinical parameters observed were erythema, oedema, scaling and itching among all parameters it was seen that it did well (Saeedi *et al*., 2003). By applying chi square test it was found that all gel formulations effect on signs and symptoms of eczematous patients had statistically significant difference with control gel effect on patients.

From the above results it was seen that although all formulations made from herbal plant extracts showed marked effect in healing of the major clinical manifestations of eczema. In all cases the ointment showed more efficacy towards scaling and lichenification. This may be due to an emollient effect of the ointment base, which provides superior protection of the dry, damaged eczema epidermal lesions. Furthermore ointment formulations were statistically significant from control ointment. This study was consistent with previous studies employing traditional Chinese medicines were used for treatment of eczema (Armstrong & Ernst., 1999).Another study examined the clinical efficacy of herbal plants, henna (Lawsonia *innermis)* and black cumin (Nigella *sativa)*.In this study these plants were mixed in olive oil and applied to eczema patients. Clinical parameters included itching, burning sensation, oozing, erythema, oedema that included papules, vesicles, papulo-vesicles, scaling, crusting, lichenification, excoriation and hyperpigmentation (Nawab *et al*., 2008).

However, work on Indian penny wort, walnut and turmeric with special reference to topical formulations in the treatment of eczema was not found in literature and all formulations in this study work well both *in vitro* and *in vivo.*

CONCLUSION

In the present study clinical evaluation of micro emulsion, gel and ointment of Indian penny wort, walnut and turmeric were shown to impact the clinical manifestations of eczema quite effectively. Although all formulations demonstrated significant improvements, each formulation promoted differential effects on each aspect of disease. The micro emulsion performed better effect on erythema and oedema, while the gel form performed best to alleviate itching. The ointment performed best for scaling and lichenification. Statistically all formulations were significant different from placebo formulations. We conclude that herbal remedies used traditionally are indeed a promising source of therapy, but they require controlled clinical testing in order to validate their individual efficacy.

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REFERENCES

Armstrong NC and Ernst E (1999). The treatment of eczema with Chinese herbs: A systematic review of randomized clinical trials. *Br. J. Clin Pharmacol.,* **48**(2): 262-264.

Cardinali G1, Ceccarelli S, Kovacs D, Aspite N, Lotti LV, Torrisi MR and Picardo M. (2005). Keratinocyte growth factor promotes melanosome transfer to keratinocytes. *J. Invest. Dermatol.,* **125**: 1190-1199.

Chen H, Chang X, Du D, Li J, Xu H and Yang X (2006). Microemulsion-based hydrogel formulation of ibuprofen for topical delivery. *Int. J. Pharm*., **315**(1-2): 52-58.

David J Atherton (2003). Topical corticosteroids in atopic dermatitis. *Br. Medical Journal*, **327**(7421): 942-943.

Djordjevic L, Primorac M and Stupar M (2005). *In vitro* release of diclofenac diethylamine from caprylocaproyl macrogolglycerides based micro emulsions. *Int. J. Pharm.,* **296**: 73-79.

Jennifer LM, Karen LC, Ibulaimu K, Philip FS and David JS (2003). Evaluation of the effect of pH on *in vitro* growth of *Malassezia pachydermatis. Can. J. Vet. Res,* **67**: 56-59.

Khiljee S, Rehman NU, Sarfraz MK, Montazeri H, Khiljee T and L–benberg R (2010). *In vitro* release of Indian pennywort, Walnut and Turmeric from topical preparations using two different types of membranes. *Dissolut. Technol.,* **17**(4): 27-32.

Marriott JF, Wilson KA, Langley CA and Belcher D (2006). Pharmaceutical compounding and dispensing. Pharmaceutical Press, London, p.162.

Mohanta GP, Jamal M and Umadevi S (2007). Formulation and evaluation of a poly herbal wound healing cream. *Indian Drugs,* **44**(4): 281.

Mostefa NM, Sadok AH, Sabri N and Hadji A (2006). Determination of optimal cream formulation from long-term stability investigation using a surface response modeling. *Int. J. of Cosm. Sci*., **28**(3): 211-218.

Nadkarni AK (1976). Dr KM Nadkarni's Indian materia medica. *Bombay Popular Prakashan,* **1**: 662-666.

Nawab MD, Mannan A and Siddiqui M (2008). Evaluation of the clinical efficacy of unani formulation on eczema. Indian. J. Tradit. Knowl, **7**(2): 341-344.

Panda P and Ghosh A (2010). Formulation and evaluation of topical dosage form of *Eupatorium odoratum* L and their wound healing activity. Int. J. Pharm. Bio.Sci, **1**(2): 1-13.

Proniuk S, Dixon SE and Blanchard J (2001). Investigation of the utility of an *in vitro* release test for optimizing semisolid dosage forms. *Pharm. Dev. Technol*., **6**(3): 469-476.

Reddy MS, Mutalik S and Rao GB (2006). Preparation and evaluation of Minoxidil gels for topical application in Alopicia. Indian. J. Pharm. Sci, **68**(4): 432-436.

Ronald M (1992). Eczema, Philadelphia: Springer-Verlag, pp.37-200.

Saeedi M, Morteza M and Ghoreishi MR (2003). The treatment of atopic dermatitis with licorice gel. J. Dermatolog. Treat, **14**: 153-157.

Shinde AJ, Bhise SB, Jarag RJ and Jadhav NR (2005). Preparation of cream containing *Tridax procumbens, Curcuma longa* and *Azadirachta indica* and its evaluation for wound healing property. *Indian. Pharm,* **4**: 107-110.

Shivhare UD, Jain KB, Mathur VB, Bhusari KP and Roy AA (2009). Formulation development and evaluation of diclofenac sodium gel using water-soluble polyacrylamide polymer. Dig. J. Nanomater. Biostruct, **4**(2): 285-290.

Springhouse. Professional Guide to Diseases (2005). 8th edition, Williams & Wilkins, Lippincott, p.516, 551.

Ueda CT, Shah VP, Derdzinski K, Ewing G, Flynn G, Maibach H, Marques M, Rytting H, Shaw S, Thakker K and Yacobi A (2009). Topical and transdermal drug products. *Pharm. Forum.,* **35**(3): 750-759.

Usmanghani K, Iqbal A, Hannan A and Akhtar ST (2007). Comparative studies of some herbal formulations Hepotin tablet and Vironil syrup for the treatment of Hepatitis C. Hamdard University Publication, Karachi, Pakistan, pp.1-18.