Original Article

Evaluation of Methanolic Extract of Polyherbal Cream on DNCB-induced Atopic Dermatitis in Wistar Albino Rats

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ABSTRACT

Background: Atopic dermatitis, is a multifactorial chronic inflammatory and immunological disease affecting the skin, with the serious impact on psychological and life style of the patients. Unique polyherbal combination of *Aloe vera, Erngium foetidum, Passiflora edulis, Syzygium samarangense,* based on established profile of relevant to anti-inflammatory, immunomodulatory and anti-oxidant property and formulated as cream for effective therapy. **Materials and Methods:** Wistar albino rats were induced AD by sensitisation of DNCB-BSA on 1st day of 1st week followed by re-sensitisation of 1st day of each week for additional 5 weeks to all group except control with intervention of Pimecrolimus and low and high dose of MEPC for group-3, 4 and 5 respectively, however for group-1 and 2 no treatment was given. Parameters such as scratching behaviour, frequency and ear thickness are recorded on a weekly basis, whereas the Serum total and IgE, ear weight and histopathology were done at the end (42nd day) of experiment. **Results:** MPEC has significant and dose dependent decrease in all parameters, the standard drug has highest positive results with nearly equivalent result of high dose of MEPC in parameters such as scratching behaviour, frequency, IgE and histopathology, however as in the case of ear weight high dose of MEPC has more positive results than Pimecrolimus (standard drug), reflecting MEPC has more immunomodulatory effect in long term use as compare to Pimecrolimus. **Conclusion:** MEPC might provide good alternative therapy against the treatment of AD to improve the life style of patient without ADR.

Keywords: Atopic Dermatits, Methanolic Extract of Polyherbal Cream, Gas Chromatography-Mass Spectrometry, 2,4-dinitrochlorobenzene, Dinitrophenyl-bovine serum albumin, Acetone-olive oil solution.

INTRODUCTION

Atopic dermatitis (AD) is a chronic inflammatory skin disease with unusual and multifactorial etiology characterized by erythema, edema, thickening of the skin and pruritic, eczematous skin lesions,[1-2] prominently occurs in children than adult and often accompanied with medical history of allergic diseases, such as allergic rhinitis and asthma[3] with worldwide increase prevalence.[4] Pathologically AD initiated with Type-2 allergic mediation[5] follows the progression by type-2 helper cells, further T-helper-2 cytokines[6] such as (IL) - 4, IL-5, IL-10, and IL-13 as well as innate cytokine interferon-γ (IFN-γ) activate and aggravate the immune system to chronic inflammatory episode. The treatment primary treatment involves topical systemic administration of steroids and antihistamines[7] as it is effectively inhibiting both acute and chronic skin inflammation[8] by its potent anti-inflammatory activity, however the usages is limited by recurrence and long term notable ADR such as osteoporosis, brittle skin, muscle weaknesses and diabetes.[9]

Ayurveda work effectively due to appropriate selective combination of medicinal plants complementary to the ailment, here the unique polyherbal combination of *Aloe vera, Erngium foetidum, Passiflora edulis, Syzygium*

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samarangense, imitated based on established profile of relevant to anti-inflammatory, immunomodulatory and anti-oxidant property of the plants with complementary phytochemicals, reinforces the promising selection to combat the pathophysiology of AD at multiple levels and thereby the pharmacotherapy might be effective than currently available drugs as well as alternative therapy.

A.vera inhibit cyclooxygenase enzyme involved in arachidonic acid pathway,[10] thereby it possesses potent anti-inflammatory activity, it has potent immune modulatory activity as it activates of IL-1 and 6 and TNF-α in also lovers glutathione peroxidases and superoxide dismutase in pancreatic cells thereby it has potent anti-inflammatory activity peroxyl radicals, respectively.[11] Phytosterols (lophenol, 24-methyl-lophenol, 24-ethyl-lophenol, cycloartanol, and 24-methylene-cycloartanol) isolated from *A.vera* gel extract showed a significant decrease in the fasting blood glucose and glycated hemoglobin (HbA1C) levels in diabetic mice.[12]

Eryngium foetidum is rich source of carotene, riboflavin, proteins, and vitamins A, B, and C content,[13] with significant quantity of volatile oil,[14] E-2-dodecenal (Eryngial) with calcium, iron in it. It has established pharmacological activity such as anti-carcinogenic, anti-diabetic, anti-inflammatory, anti-convulsant, anti-clastogenic, anti-bacterial activity and anthelmintic.[15-18]

The *P. incarnate* known to contain phytochemicals such as alkaloids, flavonoids, glycosides, cyanogenic glycosides, carbohydrates amino acids, benzopy-rones and volatile constituents.[19-23] *P. incarnate* areal parts reported to possess analgesics, anti-convulsant, sedative, anxiolytic and wormicidal activity, in addition methanolic extract of 125/kg of areal parts and 100mg/kg leaves has potent relieve against cough due to whooping cough, bronchitis, asthma and anxiolytic activity respectively.[24-28]

Syzygium samarangense (Blume), Merr popularly called wax apple has pharmaco-therapeutic value as analgesic, ant inflammatory, immune stimulant, antidiabetic, spspalytic, myolytic antipyretic and diuretic probably attributed by its phytochemicals such as flavonoids, chalcones exemplified by 2',4'-dihydroxy-6'-methoxy-3',5'-dimethylchalcone and its isomer 5-*O*-methyl-4'-desmethoxy matteucinol, it also contain Gallic and ellagic acids, and tannins such as ellagitannins as vescalagin.[29-30]

MATERIALS AND METHODS

Collection and Authentication of Plant

Leaves of all 4 medicinal plants were collected from villeges of Kerala, India during February'2020 and authenticated by Botanical survey of India (BSI) southern circle, Coimbatore, Tamilnadu. The authentication certificate number are No:BSI/SRC/5/23/2020/Tech/732,No:BSI/SRC/5/23/2020/Tech/733,No:BSI/SRC/5/23/2020/Tech/734,No:BSI/SRC/5/23/2020/Tech/735.

Extraction of Methanolic Polyherbal Leaf Extract

In a cold maceration technique 500 gm (125g of each plant) coarse powdered leaf of A*loe vera, Syzygium samarangense, Passiflora edulis, Eryngium foetidum with* 1 L of petroleum ether for 72 hr and the obtained marc was further extracted with 70% methanol (700 ml of methanol: 300ml of water) in a conical flask was kept with intermittent shaking for 72 hr the mixture was filtered by using muslin cloth and concentrated using heating mantle at 40°C, and the resultant residue was stored at cold storage with refrigerator (−20°C) till the induction of atopic dermatitis on animals.

Formulation of Polyherbal Cream

The MEPC was formulated, initially by melting beeswax and stearic acid on a water bath then liquid paraffin was added and heated the mixture to 70°C followed the addition of Polyherbal extract powder (Table 1) with continuous stirring then the mixture was allowed to cool with stirring at 40°C.[31]

Phytochemical Analysis

In our study GS-MS was employed inorder to identify and correlate the phytocomponents present in the MEPC. It was carried out at Sitra laboratory, Coimbatore, Tamil Nadu. The GC-MS separates chemical mixtures, identifies each chemical at a molecular level in a most accurete manner and considered to be an definitive analytical tool. GS-MS uses inert gas (helium) to carry the separated chemicals emerge from column opening, when it flows into the MS it identifiy the anlyte molecule using a library of spectra with corresponding compounds stored in computer.

Table 1: Composition of Methanolic Extract of Polyherbal Cream.

Sl. No.	Ingredients	Low dose 3% (g)	High dose 6% (g)
1	Steric acid	5	5
2	Lanolin	0.5	0.5
3	Triethanolamine	0.3	0.3
4	Methyl paraben	0.01	0.01
5	Glycerin	2.5	2.5
6	Water	q.s to 25gm	q.s to 25gm
7	Poly herbal extract	0.75	1.5

Animals: Male Albino Wister Rats

Wistar albino rats of 3-4 weeks old and 150-160g body weight were purchased from Biogen Laboratory, Bangalore. All rats were housed and maintained under standard conditions of temperature (22°C - 24°C), relative humidity (55 ± 10%), and 12/12 hr light/dark cycle, fed with commercial pellet diet and water *ad libitum* freely throughout the study. Protocols for the study were approved by the Institutional Animal Ethical Committee (IAEC) for Animal Care under CPCSEA guidelines by Government of India by due process (Approval No.: KMCRET/M.Pharm/4/20-21).

Induction of Atopic Dermatitis

The rat's epidermal barrier was modified after removal of dorsal body hair (6 cm × 6 cm) by an electric razor after exposure to soft hair-removal cream, 24 hr. followed. All animals are randomly divided into 5 groups of 6 animals each, all groups except control were sensitized at day-1 with DNP-BSA precipitated in 7.8 mg of aluminium hydroxide gel ($Al(OH)_3$ in 1mL saline by *i.m.* injection with consequent *Bordetella pertussis* vaccine as an adjuvant, 0.5 mL of containing 10–15 × 10^9 heat-killed bacilli/mL by *s.c.* On days 7, 14, 21, 28, 35, and 42, animals were re-sensitized with a topical application of 60µL of 1.5% w/v DNCB prepared in A-OO solution (4:1) to both sides of right ear love of the rats including control group as depicted on (Table 2) with standard and test drug treatment respectively but not to negative control group for 42 days.

($Al(OH)_3$; in 1 mL of saline solution simultaneously with an adjuvant of 0.5 mL of *Bordetella pertussis* vaccine containing 10–15 × 10^9 heat-killed bacilli/mL was injected by *s.c* route. On days 14, 16, 18, 20, 22, and 36, animals were re-sensitized with a topical application of 60 µl of 1.5% w/v DNCB prepared in A-OO solution (4:1) to both sides of the right ear lobe of the rats. Control group was only injected with adjuvants and topically applied with A-OO solution.

Table 2: Experimental Design.

Groups	Sample size	Group specification
Group-1 (Control)	6	Control group will receive normal saline (*i.p.*)
Group-2 (Negative Control)	6	(DNP-BSA) + $Al(OH)_3$ in 1mL saline by *i.m.* with *Bordetella pertussis* vaccine adjuvant, 0.5 mL by *s.c.*
Group-3 (Positive Control)	6	(DNP-BSA) + $Al(OH)_3$ in 1mL saline by *i.m.* with *Bordetella pertussis* vaccine adjuvant, 0.5 mL by *s.c.*+ Standard (Pimecrolimus 1% w/v - route)
Group-4 (Test – Low Dose)	6	(DNP-BSA) + $Al(OH)_3$ in 1mL saline by *i.m.* with *Bordetella pertussis* Vaccine adjuvant, 0.5 mL by *s.c.* + MEPC (Low Dose - 500 mg)
Group-5 (Test – High Dose)	6	(DNP-BSA) + $Al(OH)_3$ in 1mL saline by *i.m.* with *Bordetella pertussis* vaccine adjuvant, 0.5 mL by *s.c.* + MEPC (High Dose - 1000 mg)

Parameters

Evaluation of Total IgE

The total IgE level in serum was determined by ELISA kit on blood sample collected at the end of 42nd day DNCB induced AD rats as per the instruction given by the manufacturer.

Evaluation of Cutaneous Ear-inflammatory Reaction

Application of DNCB challenge cause acute inflammatory reaction on the epidermis and dermis layer of the ear due to accumulation of infiltration inflammatory cells such as eosinophils and macrophages, can be measured by ear thickness and ear weight parameters. Ear thickness measured by Vernier apparatus after 1hr exposure as the inflammatory reaction attain the peak after DNCB challenge and the progress of ear thickness is measured on 7,14,21,28,35 and 42nd day. At the end of 42nd day, rats were killed and ears were exercised from the base and individual ear weighs of all groups were measured and tabulated.

Evaluation of Scratching Behavior

The scratching episode is the part of inflammatory reaction and the progress of the dermatitis condition, the scratching moment denotes the series of one (or) more scratching moments by hind paw directed toward the DNCB challenged site and the episode end by either liking of hind paw (or) placed it back on the floor. The scratching behavior is measured by total number of scratching observed in 10 min instantly after 7,14,21,28,35 and 42nd day of DNCB application.

The scratching behavior is measured as scratching frequency and further scores are given based on macroscopical evaluation of skin lesion, in order to assess the disease progress and the effect of standard and test drug intervention. The skin dermatitis severity score was calculated by summing up the scores for erythema Haemorrhage edema excoriation/erosin and scaling dryness on the following scale:0 none, 1 mild 2 moderate and 3 severe.[32-35]

Histopathology

At the end of 42nd day, the dorsal skin tissue of rats was fixed in formalin embedded in paraffin deparaffinised

with xylene and 5µm thick section of skin and were cut and mounted on the slides. The mounted tissues were stained with either hematoxylin-eosin for the identification of inflammatory cells. Evaluation of section was conducted under a light microscope with10x, 40x magnificent for the qualitative analysis.

RESULTS

Phytochemical Analysis

The quantitative phytoconstituent analysis MEPC by GS-MS and further by comparing the chemical library showed the presence of specific phytochemicals among that majority of the phytochemicals such aschrysyn, beta-carolene, squalene, eicosane, delta-tocopenol were found and attributed to anti-inflammatory and immuno-modulatory activity as presented in Figure 1 and Table 3.

Evaluation of Total IgE

The expression of IgE by B-cell mediated immunity is one of the prominent feature of AD was imitated by DNCB induction on rats, the total IgE count was increased almost 5 times as control. the test drug MEPC has dose dependent and moderate potency in reducing the expression of IgE on rats, the percentage reduction of Low dose and high dose of MEPC was 28.1 and 41.9 respectively, whereas the standard group has significant percentage reduction of about 73.7 as observed on Figure 2.

Evaluation of Cutaneous Ear-inflammatory Reaction

The sensitization of rats by DNCB-BSA, followed by DNCB-AOO challenge by one time on each week interval showed a marked inflammatory response in negative control and moreover it is an incremental response with progression of weekly challenge, except the first week drastic spike.

Ear thickness was increased to almost 2 fold in the first week challenge followed by mild gradual increase from 2nd to the 6th week as compare to the control. However

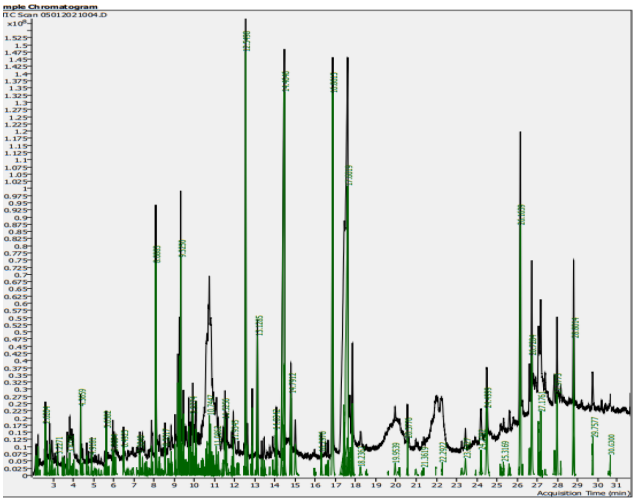

Figure 1: GS-MS Chromatogram for Phytochemical Constituents.

Table 3: Major Phytochemical Constituent of MEPC - Identified by GC-MS.

Sl. No.	Major component	Clinically Proven Properties
1	2(5H)-Furanone	Antitumor effect,Antioxidant
2	2-Pyrrolidinecarboxylic acid, 1,2-dimethyl-5-oxo-,methyl ester.	Used in treatment of any condition in which dry skin and pruritus icting is a feature.
3	Phytol	Antinociceptive, Antioxidant, Antiinflammatory, Antiallergic activity
4	Maltol	Antiatopic properties
5	Norbornane, 2 isobutyl	Antimicrobial
6	Chrysin	Antianxiety, Antinflammatory, HIV/AID, Preventing Cancer
7	Beta-Carolene	Antioxidant, Immunomodulator
8	Squalene	Antioxidant, Antitumor and Immunostimulant
9	Eicosane	Antioxidant, Antimicrobial, Healing properties
10	Delta-Tocophenol	Antioxidant

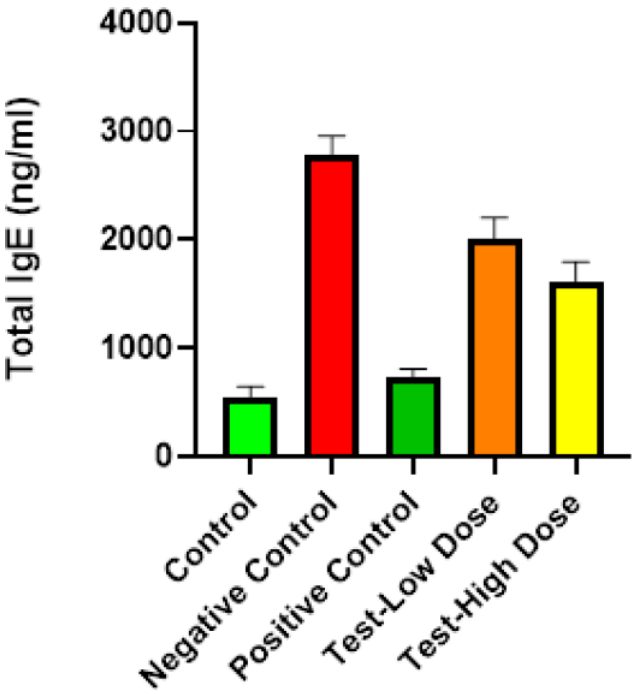

Figure 2: Total IgE evaluation on DNCB induced Atopic Dermatitis in rats.

n=6, One-way ANOVA followed by Dunnett's test.
ns= non-significant, Compared with normal control; ns,* $p<0.05$, ** $p<0.01$, *** $p<0.001$.
Compared with normal control; ns,* $p<0.05$, ** $p<0.01$, *** $p<0.001$.

there is a dose dependent substantial reduction of ear thickness reflecting potent anti-inflammatory activity was observed with MEPC and moreover the high dose of MEPC is almost same as that of standard treatment group (Pimecrolimus), depicted by Figure 3. The reduction of ear thickness was about 25.8%, 35.4% and 44.5% for low, high dose of MEPC and standard drug respectively.

Ear weight due to the inflammatory fluid accumulation has increased the net weight of the rat has increased to 4.2 fold as compare to control for the DNCB control at the end of 6th week, but due to the MEPC potent anti-inflammatory activity with the percentage reduction of 41.3, 47.4 for low and high dose and the activity is much higher even as compare to Pimecrolimus (Standard group) of 27.2 as demonstrated by Figure 4.

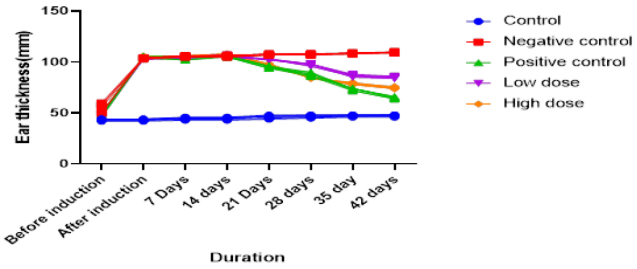

Figure 3: Ear Thickness evaluation on DNCB induced Atopic Dermatitis in rats.

n=6, One-way ANOVA followed by Dunnett's test.
ns= non-significant, Compared with normal control; ns,* $p<0.05$, ** $p<0.01$, *** $p<0.001$.
Compared with normal control; ns,* $p<0.05$, ** $p<0.01$, *** $p<0.001$.

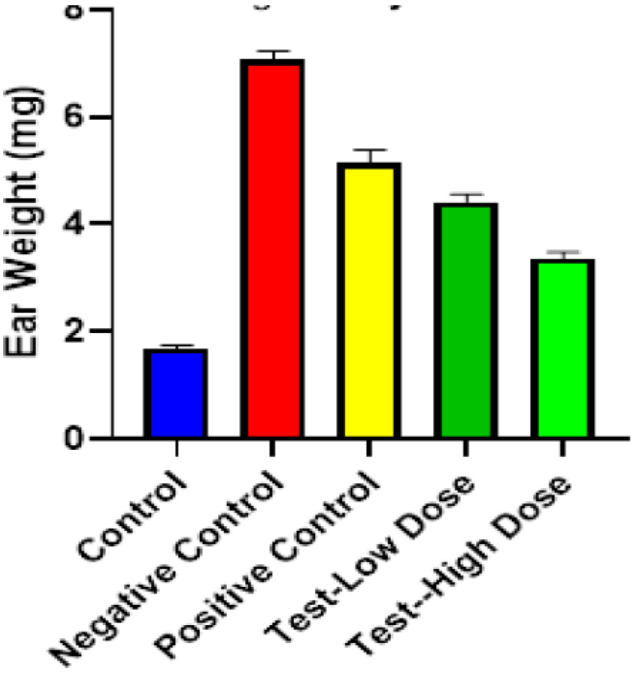

Figure 4: Ear Weight evaluation on DNCB induced Atopic Dermatitis in rats.

The severity score given based on the skin lesion after induction of AD by DNCB, has increased nearly incremental from 1st hr application to 24 hr and it is stabilized on the 2nd week and then mild decremental in score was observed for DNCB control as compare to the control group. The test drug MEPC has shown a mild dose dependent reduction of the severity score as 5.9% and 17.6% respectively as compare to standard group (41.2%) as demonstrated by Figure 5.

The macroscopical examination of the skin lessions showed development of severe dermatitis on negative control group as well as good reversal effect on the test drug on both low and high dose with dose dependend effect was observed and the positive results of the test group were even superior as compare to the standard drug treatment (Figure 6).

Scratching Behavior

The scratching frequency, provoked by DNCB-AA challenge, drastically increased and attains the peak on 3rd week by almost 29-fold increase as compare to control group, followed by gradual rise up to the 6th week of DNCB-AOO challenge. As in the case of

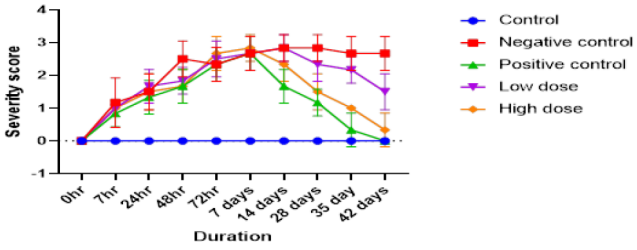

Figure 5: Severity Score evaluation on DNCB induced Atopic Dermatitis in rats.

Data are presented as mean ±SEM, *n*=6. Control, Negative control (DNCB), Positive Control (Pimecrolimus 1%), Low Dose, High Dose after AD-induction. *n*=6, One way ANOVA followed by Dunnett's test ns= non-significant. Compared with normal control; ns, * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

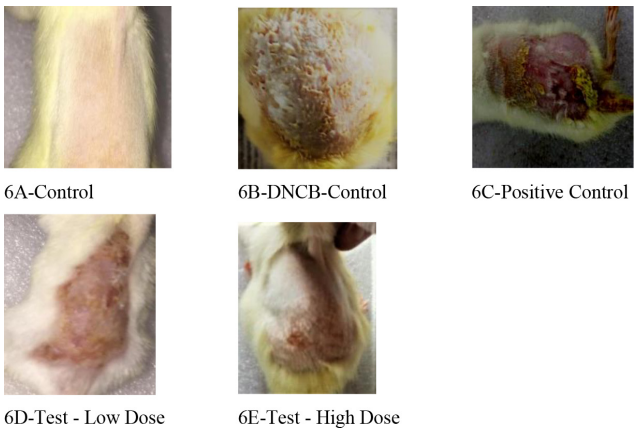

Figure 6: Macroscopic Analysis on DNCB induced Atopic Dermatitis in rats.

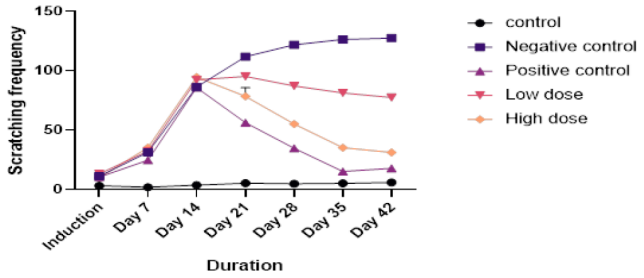

Figure 7: Scratching Frequency evaluation on DNCB induced Atopic Dermatitis in rats.

Data are presented as mean ±SEM, n=6. Control,Negative control(DNCB),Positve Control(Pimecrolimus 1%), Low Dose,High Dose after AD-induction. n=6, One way ANOVA followed by Dunnett's test ns= non-significant. Compared with normal control; ns,* p<0.05, ** p<0.01, *** p<0.001.

MEPC treated test group there was a moderate to high and dose dependent decreased scratching frequency was observed in Figure 7 (as compare to standard group treatment, the percentage reduction was about 66.6, 102.7 and 113.4 for low, high and standard group respectively.

Histopathology

Skin section of DNCB induced control group shows the accumulation of inflammatory infiltrates composed of neutrophils lymphocytes and mast cells, surrounded stroma with fibro-collagenous and focal congested vessels with thickened epidermal layer. The entire episode almost imitates the AD pathology of human being and also imply the MEPC might possess antioxidant, anti-inflammatory and immune-modulatory on treating the AD as the high dose of MEPC significantly reduce the infiltrates and lessen the epidermal thickening which is almost similar to that of standard group treated rats by Pimecrolimus as observed in Figure 8.

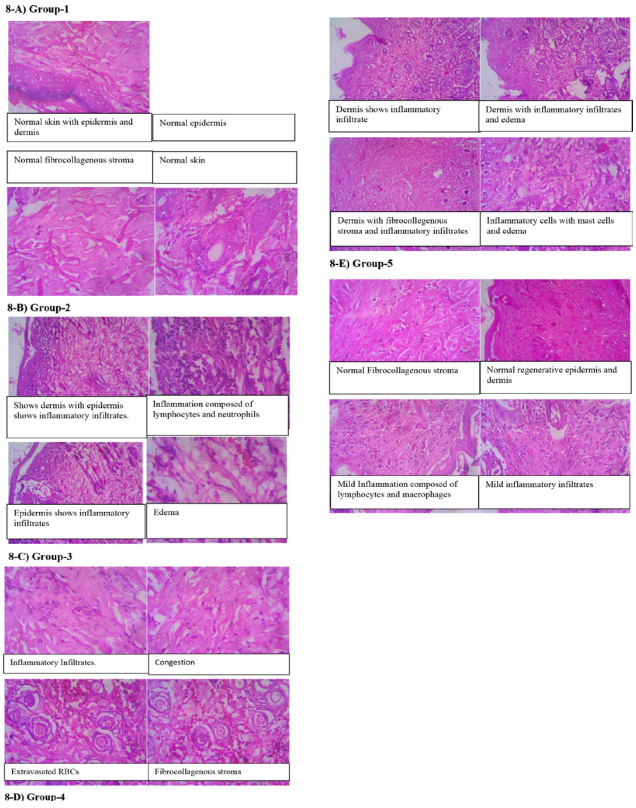

Figure 8: Histopathology evaluation on DNCB induced Atopic Dermatitis in rats.

The histopathology of skin was performed after killing the animal at the end of 42nd day, skin covered soft tissue bit measuring 3.8 x 2.4 x 0.4 cms (PE): two bits-one block. The microscopical appearance of Group-1 (control) showed normal dermis and epidermis with normal fibro-collagenous stoma, in contrast Group-2 showed infiltrates composed of neutrophils and lymphocyte with formation of edema demonstrating the pathology of AD, whereas in Group-3 treated by Pimecrolimus (standard) showed extravascular RBC with limited infiltrate and congestion as compare to Group-3 reflecting the limited chronic inflammatory reaction, whereas in the case of Group-4 treated by MEPC low dose does not showed improvement in both innate and acquired immunity as illustrated by the infiltration of macrophage and lymphocytes with edema. However, in Group-5 treated by high dose of MEPC showed mild infiltrates of lymphocyte and macrophage with mild edema which is almost similar response as observed with standard treatment (Group-3).

DISCUSSION

Atopic dermatitis, otherwise called atopic eczema type of chronic allergic skin disease, mainly affecting children starting with common allergic symptom and most of the time it is co-existing with another atopic disorder include allergic rhinitis, asthma (or) food allergy. AD is a multifactorial chronic inflammatory and immunological skin disease about 31 million people have eczema and 17.8 million people in the world with atopic dermatitis westernized countries. Approximately one in three children with AD has a moderate to severe form. For adults, the prevalence is as high as 10.2, with the serious impact on psychological and life style impact affecting the livelihood of the patients.

Pathogenesis of AD seems to be very complex, initially mediated by innate immunity and later part adaptive immunity sensitization mediated by IgE aggravate the immunological response. The coordinated hyper immune response mediated by various cytokines such as IL-4, IL-5 and IL-13, followed by the role of cytotoxic T-cell leads to complex chronic inflammatory illustration, appears as eczematous skin condition. Currently a number a pharmacotherapy involves topical (or) and systemic administration of steroids, combination antihistamines, calcineurin inhibitors, monoclonal antibodies. Even today Skin corticosteroids by topical administration are the most commonly preferred first line therapy, with minor adverse drug reaction such as skin rot, purpura, striae, telangiectasias, dyspigmentation, and facial acneiform changes, with recurrence. The most common long term systemic side effects include, hypothalamic–pituitary–adrenal axis suppression, immune suppression, ulceration covering and advancement retardation.

Therefore, there is burning need for improving the pharmacotherapy with minimal ADR would be the ideal choice, might be promising by phytochemical based plant preparation. As the pathology of AD is complex. The polyherbal cream include equal proportion of *Erygium foetium, Syzygium samarangense, Aloe vera* and *Passiflora edulis* methonolic leaf extract of standard cream formulation, was chosen. The phytochemical analysis confirms the presence of 2-Pyrrolidinecarboxylic acid, 1,2-dimethyl-5-oxo-methyl ester, phyrol, maltol, chrysinm, beta-carolene, squlee, eicosane and deltatocophenol etc., known to possess predominant anti-inflammatory, immunomodulatory and anti-oxidant property to effectively combat the pathology of AD. Among the AD models, DECB induced model seems to be the most pomising model as it imitates the human pathology.

CONCLUSION

MEPC was found to be effective in suppressing the symptoms by acting on both early and late phase of AD. It has significant and dose dependent manner decrease effect in all parameters. The standard drug has highest positive results was found in parameters such as scratching behaviour, frequency, macroscopy of ear, IgE and histopathology and almost similar degree of results were found with high dose of MEPC, however as in the case of ear weight high dose of MEPC has more positive results than Pimecrolimus (standard drug), reflecting MEPC might has potent immunomodulatory activity in long term use as compare to Pimecrolimus. Therefore, MEPC might provide good alternative therapy against the treatment of AD to improve the life style of patient without ADR. However, the isolation and characterisation of phytochemical may facilitate the understanding of molecular mechanism and prospects in exploration of new drug discovery for the effective treatment of AD.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

AD: Atopic Dermatits; **A-OO:** Acetone-olive oil solution; **CPCSEA:** For Animal Care and were in accordance with Committee for the Purpose of Control and Supervision of Experiments on Animals guidelines; **DNCB:** 2,4-dinitrochlorobenzene; **DNP-BSA:** Dinitrophenyl-bovine serum albumin; **GC-MS:** Gas Chromatography-Mass Spectrometry instrument separates chemical mixtures; **IAEC:** Institutional Animal Ethical Committee; **MEPC:** Methanolic Extract of Polyherbal Cream.

Ethical Statement

The entire research work on animals were done by strict adherence with CPCSEA norms.

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SUMMARY

The main objective of our research work to evaluate unique polyherbal combination of *Aloe vera, Erngium foetidum, Passiflora edulis, Syzygium samarangense,* based on established profile of relevant to anti-inflammatory, immunomodulatory and anti-oxidant property and formulated as cream for the treatment of Atopic Dermatis. Based on the literature review DNCB induced atopic dermatitis was found to be more relevant pathologically as well as to make effective screening of test drugs for effective therapy. The study was done Wistar albino rats Atopic dermatitis was induced by sensitisation of DNCB-BSA on 1st day of 1st week followed by re-sensitisation of 1st day of each week for additional 5 weeks to all group except control with intervention of Pimecrolimus and low and high dose of MEPC for group-3, 4 and 5 respectively, however for group-1 and 2 no treatment was given. Parameters such as scratching behaviour, frequency and ear thickness are recorded on a weekly basis, whereas the Serum total and IgE, ear weight and histopathology were done at the end (42nd day) of experiment. The study demonstrated there was significant and dose dependent decrease in all pathological parameters for MEPC as compare to standard group (treated by Pimecrolimus), reflecting MEPC has significant anti-inflammatory and immunomodulatory compare to Pimecrolimus. Therefore, MEPC might provide good alternative therapy against the treatment of AD to improve the life style of patient without ADR.

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