Research Article

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EVALUATION OF DIAGNOSTIC PARAMETERS TO DETECT INDUCTION OF ACNE, ACUTE DERMAL IRRITATION AND CORROSION POTENTIAL OF A POLYHERBAL FORMULATION

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ABSTRACT

The current protocol was designed to evaluate and detect the new parameters of acne induction (Histological lesion), Acute Dermal Irritation and corrosion potential of a polyherbal nano emulsion (obtained from research and development of Venus Medicine Research Centre, Baddi, H.P, India) according to Organisation for Economic Co-operation and Development (OECD) guidelines. Materials and Methods: Polyherbal drug, a new micro emulsion for topical use applied on the dorsal area of the trunk, has been developed as an anti-acne herbal nano-emulsion. Polyherbal drug's active ingredients include herbal extracts of *Melaleuca alternifolia* oil, *Rosmarinus officinalis* oil, *Mentha arvensis* oil along with *Citrus limon* are tested for their antibacterial, antimicrobial, anti-inflammatory and antiseptic effects. Serialise testing approach is recommended for developing scientifically sound data on the corrosivity/irritation of a new substance. Young healthy albino rabbits were used for the initial corrosivity/irritation study. If corrosive response was not found in the initial test, the irritant or negative response was confirmed using three additional animals each with one patch for an exposure period of four hours. Each animal served as its own control. After application of polyherbal emulsion the degree of irritation/corrosion and histological lesions were read and scored. Results: No severe erythema, oedema or any histological lesions were observed after polyherbal nanoemulsion application. The results revealed no irritation potential of polyherbal nanoemulsion. Conclusion: polyherbal nanoemulsion is safe formulation for topical use.

Keywords: Acne, Polyherbal, OECD, Irritation, Corrosion

INTRODUCTION

Acne vulgaris is a chronic inflammatory condition of skin that characterized by blackheads, whiteheads, and spots with inflammation[1, 2]. The evaluation of histological and dermal lesions and their complications are important to assess the complexity of acne[3]. Polyherbal emulsions are novel topical anti acne approaches that may protect acne in acne vulgaris[4, 5]. In present study the emulsion contains most of the new chemicals/agents from plant origin. So, the formulations could be irritative or corrosive for skin. Thus, the formulation must be checked for any irritation by applying on skin of the animal for a specified period of time. The purpose of the present study was to evaluate the dermal effects of polyherbal emulsion in vivo.

MATERIAL AND METHODS

Selection of the animal species

Healthy young adult albino rabbits with healthy intact skin were selected and housed individually at 20 ºC ± 3ºC, RH 50-60%. Artificial lighting was used to sequence being 12 hours light and 12 hours dark. For feeding, conventional laboratory diets was given with an unrestricted supply of drinking water[7]. The experiment was carried out after approval Institutional animal ethics committee (IAEC/CS/11/2011) of Venus Remedies Pvt. Ltd.

Preparation of animals

Approximately 24 hours before starts the experiment, animals were placed and fur were removed by closely clipping the dorsal area of the trunk of the animals with care to avoid any abrading of the skin.

Application of the test substance

The test substance weighing 0.5ml was applied topically once a day to the test site area (approximately 6cm sq.) of the skin and covered with the gauze patch, which was held in place with non-irritating tape.

Initial Test (in vivo dermal irritation/corrosion test using one animal)

It was performed initially using one animal, since the test substance was not expected to produce any corrosion. A single patch was applied to a single animal for four hours. Serious skin reactions (if any) were observed timely at 15min, 30min, 45min, 60min, 2 h, 3 h & 4h and the responses were graded[8].

Confirmatory Test (in vivo dermal irritation/corrosion test using three animals)

When corrosive effect was not observed in the initial test, the irritant or negative response was confirmed using three additional animals each with one patch for an exposure period of

four hours. Serious skin reaction (if any) was observed and the responses were graded.

Clinical observations and grading of skin reactions

All animals were examined for the signs of erythema and edema, and the responses were scored at 60min, 2 h, 3 h, 4h, 8h, 12 h, 24hr, 48h, and 72h and so on till fourteen days after patch removal. For the initial test in one animal, the test site was also examined immediately after the patch has been removed. Dermal reactions were graded and recorded according to the table 2 below. If there is any damage to the skin which cannot be identified as irritation or corrosion at 72 h, observation may be needed until day 14th day to determine the reversibility of the effects. In addition to the observations of irritation, all local toxic effects, such as defatting of the skin, and any systemic adverse effects (e.g. effects on clinical signs of toxicity and body weight) were fully described and recorded [9].

Table 1: Grading of skin reaction

Erythema and Eschar formation (maximum possible:4)	
No erythema	0
Very slight erythema (barely perceptible)	1
Well defined erythema	2
Moderate to severe erythema	3
Severe erythema (beef redness) to eschar formation preventing grading of erythema	4
Oedema formation (maximum possible : 4)	
No oedema	0
Very slight oedema (barely perceptible)	1
Slight oedema (edges of area well defined by definite raising)	2
Moderate oedema (raised approximately 1mm)	3
Severe oedema (raised more than 1mm and extending beyond area of exposure)	4

Histological lesion count

Skin tissue samples from the patch of drug application site were collected in 10% buffered formalin saline for histological studies. The formalin fixed tissues were washed overnight in running tap water, dehydrated in ascending grades of alcohol, and cleared in benzene. The 4-5 micron thick tissue sections were cut from the paraffin embedded tissues and was stained with haematoxylin and eosin stain (H&E) for routine histopathology (Luna 1968)[10]. Morphological changes (lesion count) were assessed by using routine light microscopy. Skin tissue slide were examined for inflammation, sebaceous gland and hyperkeratization as a lesion count grading. The grading was scored as per grading index for lesion count reference.

RESULTS

The evaluation of safety parameters was based on estimating the irritancy and oedema formation on 4 point scale accounting for very slight erythema (barely perceptible), well defined, moderate and severe (Skin redness) erythema responses. Polyherbal emulsion was well tolerated by all the animals and did not show any serious corrosive or irritant effect during the crucial four hour of initial test.

Table 2: Skin reaction initial test status in vehicle treated and Polyherbal drug treated group

INITIAL TEST (using one animal)						
ERYTHEMA/OEDEMA SCORING						
Groups	15 min	30 min	1 hour	2 hour	3 hour	4 hour
Group-I (Vehicle applied group)	2/0	1/0	0/0	0/0	0/0	0/0
Group-II (VRP-0110 applied group)	2/0	1/0	1/0	0/0	0/0	0/0

Slight erythema was observed during first 30 minutes of application which vanished by end of one hour of application. No serious/ adverse effect, pathophysiological abnormality or reversibility of signs of erythema and oedema was found till the 14th day of completion of experiment. All animals showed normal body weight.

Table 3: Skin reaction confirmatory test status in vehicle treated and Polyherbal drug treated group

CONFIRMATORY TEST (using three animal)																			
ERYTHEMA/OEDEMA SCORING																			
Groups	1 hour	2 hour	3 hour	4 hour	12 hour	24 hour	48 hour	72 hour	4th day	5th day	6th day	7th day	8th day	9th day	10th day	11th day	12th day	13th day	14th day
Group-I (Vehicle applied group)	1/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0
Group-II(VRP-0110 applied group)	1/0	1/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0

Histological lesion count

Lesions count were performed and graded after 14 days application. The microscopic examination of skin tissue sections of vehicle treated (Figure 1) and Polyherbal treated (Figure 2) shows no infiltration of lymphocytes, neutrophils and monocytic cells and sebaceous glands were also found intact. Lesions revealed infiltration of mixed population of inflammatory cells **(Red arrow)**.

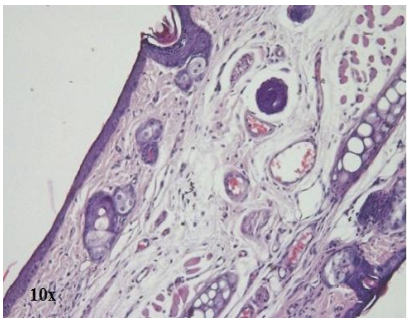

Figure 1: Vehicle Treated

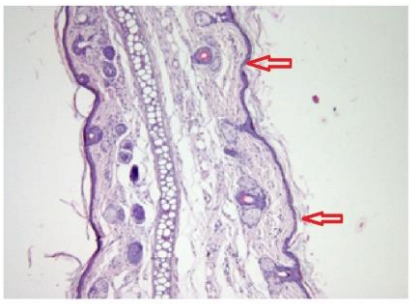

Figure 2: Polyherbal Treated

Table 4: Histology Lesion Count grading system in vehicle treated and Polyherbal drug treated group

Groups	**Observatory events**			**Overall lesion count**
	Inflammation	Sebaceous gland	Hyper-keratinization	
Vehicle Treated	--	--	--	--
Observation Treated	+	--	--	+

Reference grading index for lesion count

++++ very high
+++ high
++ normal
+ low
-- not present

DISCUSSION

Introducing new substances into the clinical trials for developing scientifically sound data on the corrosivity/irritation requires the recommended stepwise testing approaches in animals [11, 12]. Literature review suggests that active ingredients of plant origin may possess toxicological potential[13]. Dermal irritation is sign of reversible damage to the skin following the application of any test substance[14]. In current work the Acute Dermal Irritation study of polyherbal emulsion in rabbits was conducted. No signs of skin corrosivity/irritation were observed with polyherbal emulsion application. The herbal constituents of emulsion i.e. *Melaleuca alternifolia* oil, *Rosmarinus officinalis* oil, *Mentha arvensis* oil *along with Citrus limon* are reported to possess antibacterial, antimicrobial, anti-inflammatory and antiseptic potential [15, 16, 17]. So the formulation in present study was found to be non corrosive/irritant.

CONCLUSION

Based on the diagnostic and analysis of all the parameters studied, it is concluded that polyherbal nanoemulsion was tolerated in experimental rabbits and there were no skin lesions in animals up to 72hr time interval. The overall observations indicated that Polyherbal nano emulsion is safe formulation for topical use.

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