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Topical application of ointment containing Ashvgandha, vetiver and stropanthus indicus linn, (PHF) used in albino wistar rats by using 2,4-Dinitrochlorobenzene for treatment of Different types of dermatitis.

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A B S T R A C T :

Aim of the study: According to survey of World health organization (WHO) world 80 % population depends upon allopathic medicines. And such allopathic medicines having large number of Adverse Events and side effects. Hence, to overcome such side effects there is need to develop new treatment having less side effect and good efficacy. Medicinal plants have rich source of antimicrobial, antifungal anti-inflammatory and moisturizing properties. Sphaeranthis indicus, Linn, Vetivera zizaniodies traditional herbal medicines dried Roots reported that used in treatment of various chronic skin disease like antifungal as well as antibacterial activity. Dried bark, roots of Ashwagandha plant reported that it anti-inflammatory activity due to presence of alkaloids and withanolides.

Material and Method: To evaluate the poly herbal formulation (PHF) in different types of dermatitis. By using 100 µL of 1% 2, 4-Dinitrochlorobenzen (DNCB)-induced atopic dermatitis in dorsal region (back side) shaved albino wistar rats. On 6th day rats were sensitized by using 200 µL of 0.5% DNCB to produce dermatitis. Two different conc. ointments were prepared and evaluated. To study the effect of PHF in different dermatitis by using ear thickness, ear weight, skin lesion

Animal: Albino wistar rats (weighing 250-350 gm.) were procured from Rajarshi Shahu College of Pharmacy Buldana. Maintained under specific temperature-controlled room (23±1 °C) with a 12 h light/dark cycle to food and water. Animal experiments were conducted in strict accordance with the recommendations of the CPCSEA Guidelines. The experimental protocol was approved by the Institutional Animal Ethics Committee.[17]

2) Preparation of Ointment:

Solids are finely powdered are passed through a sieve (# 50, # 85, #30).The powder is taken on an ointment-slab and triturated with a small amount of the base. A steel spatula with long, broad blade is used. To these additional quantities of the base are incorporated and triturated until the medicament is mixed with the base. Finally liquid ingredients are incorporated. To avoid loss from splashing, a small volume of liquid is poured into a depression in the ointment an thoroughly incorporated before more is added in the same way. [17]

Formula for herbal ointment:

Sr. No	Ingredients	Conc.%	Low conc.	High conc.
1	Cholesterol	3.2 %	1.5 gm	1.5 gm
2	Steryl alcohol	5.2 %	2.5 gm	2.5 gm
3	White wax	95 %	45 gm	45 gm
4	White petroleum,	2.1 %	01 gm	01 gm
5	Vetivera zezanniodes	2.1 %	01 gm	02 gm
6	Sphaeranthus Indicus	2.1 %	01 gm	02 gm
7	Ashwagadha	2.1 %	01 gm	02 gm

Table No.1 Formula for herbal ointment

Evaluation of ointments:

1) PH: The pH of various formulations was determined by using Digital pH meter (Digital pH meter). The0.5 g of the weighed formulation was dispersed in 50 ml of distilled water and the pH was measured.

2) Homogeneity: All the developed ointments were tested for homogeneity by visual inspection. They were tested for their appearance with no lumps.

3) Viscosity: The measurement of viscosity of prepared ointments was carried out with Brookfield Viscometer.

4) Solubility: Soluble in boiling water, miscible with alcohol and chloroform

5) Wash ability: Formulation was applied on skin and then ease extends of washing with water and then checked the hand form any kind of stickiness are appeared.

Induction of dermatitis:

The back skin hairs of rats were removed by electric shaver. 30 rats were divided into 5 groups (n= 6 per group).To induce AD-like immunologic and skin lesions, DNCB 100 µl of 1% DNCB on 1stday dissolved in acetone and olive oil (4:1) and the mixture was applied on back of rats. All groups are treated with DNCB except Normal control group. For sensitization of Atopic dermatitis in Rats 200 µl of 0.5% DNCB on 6th day of same rats acetone mixture (4:1) used as vehicle was applied on back of rats. All experimental protocols for animal care were performed according to the rules and regulations of the Animal Ethics Committee [13]

5) Experimental protocol:

After 6 days following treatment was started and continued for 16 days.

Sr. No	Group	Treatment
1	Normal control group	No any kind of treatment given to this group
2	Toxic control group	2,4, Dinitrochlorobenzene (1 %) only
3	Standard group	2,4, Dinitrochlorobenzene(1%)+Standard marketed Cream (Dexamethasone)
4	Test group 1	2,4, Dinitrochlorobenzene (1%) + PHF (low conc.)
5	Test group 2	2,4, Dinitrochlorobenzene (1%) + PHF (High conc.)

Table No. 2 Treatment of dermatitis.

6) Evaluation of ear thickness: Ear thickness was measured with Vernier Caliper.Ear thickness were measured before sensitization phase (day 06) and at end of study (day 16)

Sr. No	**Group**	**Ear thickness (mm)**	
		6th Day	**16th Day**
1	Normal control	1.060 ±0.0400	1.060 ±0.0678
2	Toxic control	5.000 ±0.3162	5.060 ±0.0748
3	Standard	4.000 ±0.3162	1.940 ±0.0599
4	Test 1	5.000 ±0.3162	3.040 ±0.0748
5	Test 2	5.000 ±0.3162	2.440 ±0.2315

Table No. 3 Evaluation of ear thickness

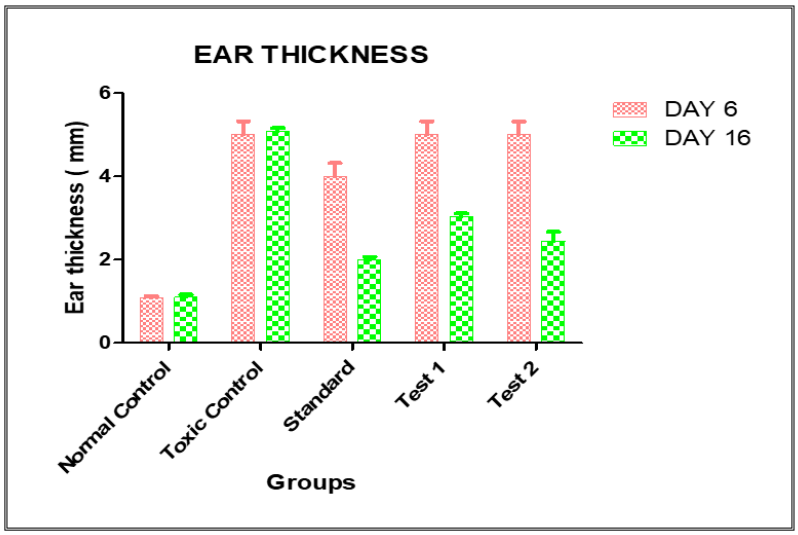

Fig. No.1 Evaluation of ear thickness

7) Evaluation of ear weight: Anesthesia was given to rats using chloroform as anesthetic. In aseptic condition the rat's ear get cut and weight of ear was measured with digital weighing balance at end of study (day 16).

Sr. No	Groups	Ear weight (gm)
1	Normal control	2.18 ± 0.0833
2	Toxic control	4.00 ± 0.1390
3	Standard	2.35 ± 0.0563
4	Test 1	3.50 ± 0.0775
5	Test 2	2.67 ± 0.0494

Table No. 4 Evaluation of ear weight

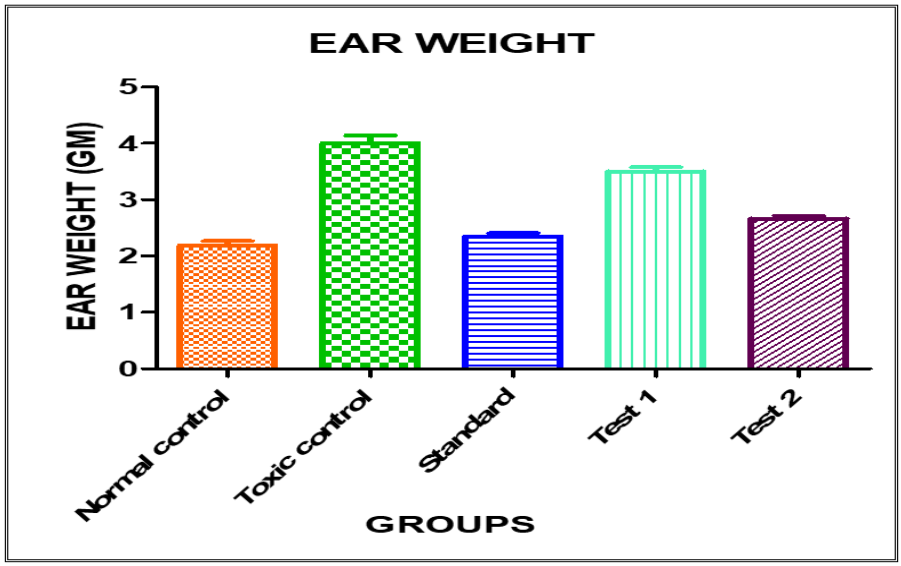

Fig. No. 2 No. 4 Evaluation of ear weight

8) Evaluation of lesions: Skin status was photographed every 2–3 weeks when hair was removed. Development of 1) dryness and scaling, and 2) erosion, excoriation, and hemorrhage on the skin was scored as 0 (none), 1 (mild), 2 (moderate) and 3 (severe). Scoring was performed by two volunteers who were unaware of the treatment status. Scores were accumulated and taken as an average for each group.

Sr. No	Group	Skin lesion score	
		6th Day	16th Day
1	Normal control	0.000 ±0.0000	0.000 ±0.0000

2	Toxic control	3.800 ±0.2000	3.900 ±1.224
3	Standard	3.900 ±0.2000	2.000 ±0.5477
4	Test 1	3.500 ±0.2000	3.200 ±0.2000
5	Test 2	3.600 ±0.2000	2.400 ±0.4000

Table No. 5 Evaluation of ear weight

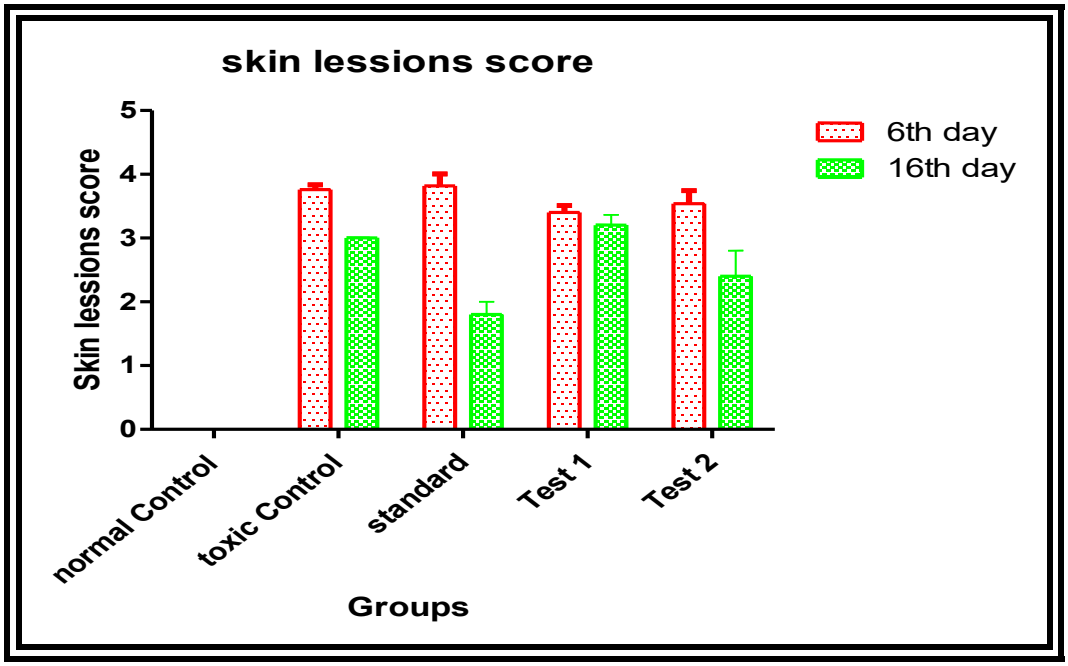

Fig. No. No. 3 Evaluation of ear weight

9) Histopathological evaluation:

The ears of rat were stained with eosin (0.5 %) ,toluene (100 mg /L) and xylene (0.5 %) normal control group animals, showed no change in architecture and no abnormalities in architecture of cells. Where in toxic control group showed damaged architecture of cells epidermal hyperplasia. Whereas in control group the group of animals showed damaged epidermal hyperplasia, and immune cells in the dermis. However Histopathological examination of animals treated with standard cream dexamethasone showed very much decrease in abnormalities of epidermal hyperplasia. Whereas animals of test 1 and test 2 groups treated with low and high conc. PHF showed dose/ conc. Dependent change in architecture of cell respectively. In the test group experimental animals were treated with herbal ointment showed decrease in abnormalities of epidermal hyperplasia and. The effect of herbal ointment is slightly less effective than that of standard treatment.

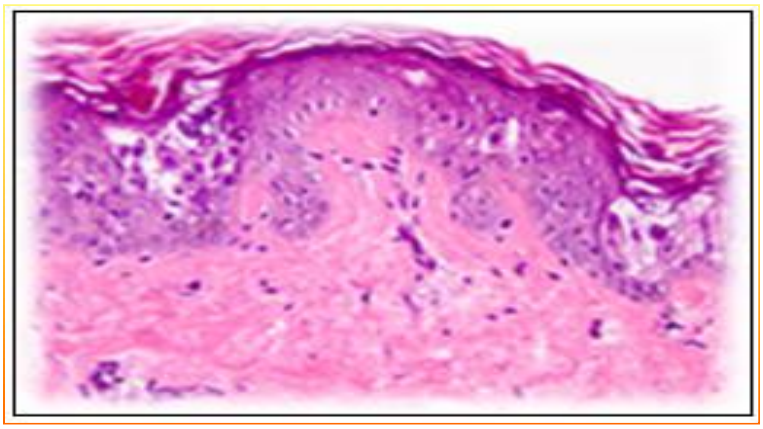

Fig. No. 4 Normal Control

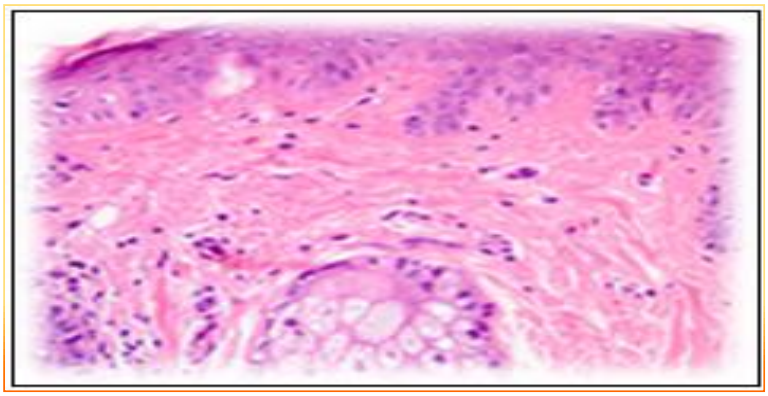

Fig. No. 5 Standard

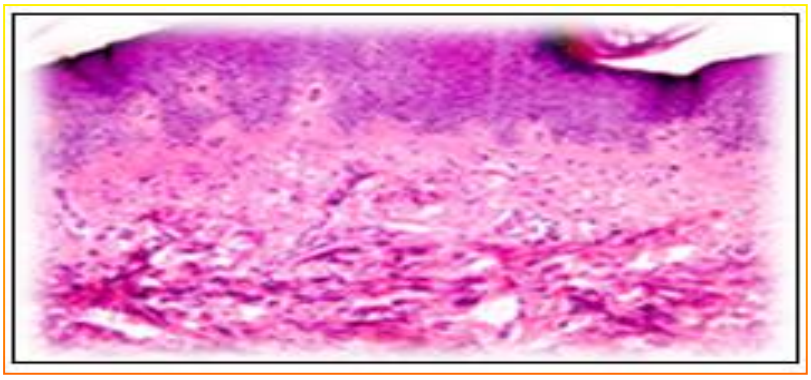

Fig. No. 6 Toxic control

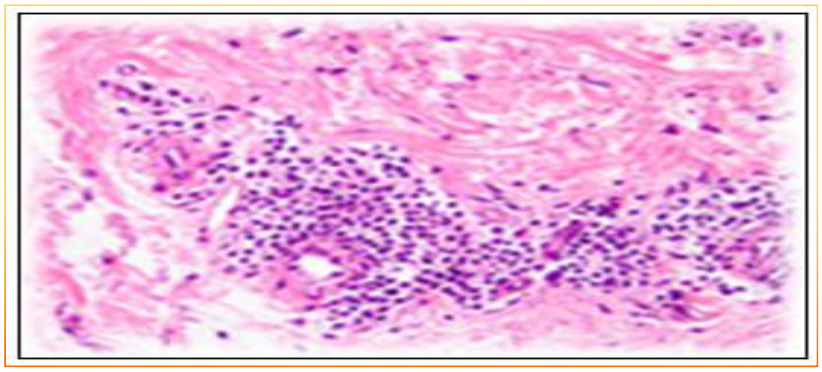

Fig. No. 7 Test 1 (Low Conc.)

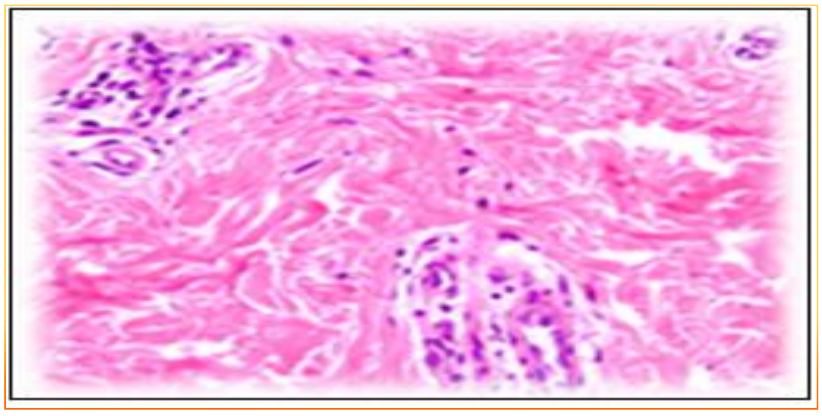

Fig. No. 8 Test 2 (High Conc.)

Conclusion:

From the present findings it can be concluded that the Poly Herbal formulation having high conc. shows significant reduction in skin lesion, dryness of skin, ear weight. However histological analysis observed that decreased thickening of the dermis and dermal infiltration by inflammatory cells of albino Wister rats as compared with standard marketed formulations.

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