Original Article

Contact Dermatitis like Skin Lesion: A Dermal Toxicity of Formaldehyde Treated by Phytomedicine Extracted from *Woodfordia fructicosa and Gardenia gummifera*

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ABSTRACT

Introduction: *Woodfordia fructicosa* and *Gardenia gummifera* are traditionally claimed to be useful in treatment of number of skin diseases. However, there are no established scientific reports for their potential in contact dermatitis. The aim of this study was to investigate the phytomedicine formulated from extract of *Woodfordia fructicosa* flowers and *Gardenia gummifera leaves in* dermatitis lesion induced by formaldehyde in rats. However, there are no established scientific reports for their potential in dermatitis.

Methodology: For induction of dermatitis lesion, 0.1 ml formaldehyde (37%) was topically applied for 10 days on the dorsal surface of the skin of Wistar albino rats. Therapeutic effect of phytomedicine was evaluated after the induction of dermatitis lesion. Formulated gel and suspension were treated once daily for 16 days and analyzed for macroscopic features (severity index), histological study (epidermal thickness and other features) and biochemical estimation (hydroxyproline content).

Results: Percent reduction in the severity index in macroscopic study, decreased epidermal thickness in microscopic study and increased hydroxyproline content in biochemical estimation were observed in formulation treated dermatitis lesion as compared to vehicle treated animals.

Conclusion: The preliminary *in vivo* study concludes that extractive phytoconstituents of *Woodfordia fructicosa* flowers and *Gardenia gummifera* leaves might be a novel therapeutic approach in treating contact dermatitis as prepared formulations possess potent anti-dermatitis activity.

INTRODUCTION

There is a wide range of dermatological conditions that include inflammatory skin disorders ranging in severity from mild skin rash to severe dermatitis.[1] Dermatitis describes a specific type of inflammation of the skin. Contact dermatitis describes inflammation which is caused by contact with something in the environment.[2] Contact dermatitis represents a non-specific dermal reaction to direct action of an irritant or an allergen. Due to variable ways of its induction, the disease was categorised into two principal forms: allergic contact dermatitis (ACD) and contact dermatitis induced by an irritant (irritant contact dermatitis, ICD).[3-5] Allergic contact dermatitis develops only in individuals who have developed a specific sensitivity/allergy to a substance. Examples of these substances include nickel, rubber, hair dyes and perfumes or preservatives used in some creams and cosmetics. Irritant contact dermatitis develops when the skin is in contact with irritant substances like detergents and solvents that strip the skin of its natural oils. The main way of treating contact dermatitis is to identify its cause (irritant or allergen) and then to remove it or reduce contact with it. People with very severe contact dermatitis may need other treatments such as steroids, cyclosporin, methotrexate, azathioprine or alitretinoin.[2]

Many studies involving both experimentally controlled and occupational exposures have demonstrated that

formaldehyde causes irritation of the eyes, nose and throat, headaches, direct skin irritation, and skin sensitization resulting in dermatitis and uriticaria.[6,7] Flavonoids, triterpenoids and polyphenolic compounds are well known as potent antioxidants and for their anti-inflammatory, antiproliferative, immunomodulatory and free radical scavenging activities[8,9] These characteristics of polyphenolic phytoconstituents may be beneficial for the treatment of diseases with multiple etiologies such as psoriasis and dermatitis.

Woodfordia fructicosa (Kurz.) (family Lythraceae) is a straggling leafy shrub and distributed abundantly throughout the north India as well as in the majority of the East Asian countries. Pharmacological claims of *Woodfordia fructicosa* dried flowers can be ascribed for its important bioactive phytoconstituents such as flavonoids, sterols, anthraquinones, saponins and tannins.[10-11]Preclinical data from various studies indicates, that dried *Woodfordia fructicosa* flower extract possess anti-pyretic, anti-inflammatory , anti-tumor , anti-viral, immunomodulatory, anti-fertility, antibacterial, hepatoprotective, anti-hyperlipidemic, antidiabetic, bronchoprotective , wound healing and anti-asthmatic activity. [12]

Gardenia gummifera (family- Rubiaceae) is geographically distributed in India (Konkan region, North Kanara & Malabar Coast), Burma and Bangladesh. *Gardenia gummifera* is claimed to have a number of medicinal properties which include anthelmintic, antispasmodic, carminative, diaphoretic, expectorant, potentiation of pentobarbitone induced sleep, antiepileptic, peripheral and central analgesic, cardiotonic, antioxidant and anti-hyperlipidemic.[13] It is also useful in dyspepsia, flatulence for cleaning foul ulcers and wounds and to keep off flies from wounds in veterinary practice. The present study aimed to study the effects of newly developed herbal formulations (topical and oral) from extract of *Woodfordia fructicosa* and *Gardenia gummifera in* dermatitis lesion induced by formaldehyde in rats.

METHODS

Plant materials and preparation of extracts

The flowers of *Woodfordia fructicosa* and leaves of *Gardenia gummifera Linn* were collected in the month of February 2014 from the Swarn Jayanti Park, near Sarvadharm colony, Bhopal (Madhya Pradesh). Both the plants were authenticated by Department of Botany, Safia College Bhopal. The voucher specimen for *W. Fructicosa* (460/Bot/Saif/14) and *G. Gummifera* (503/Bot/Saif/14) has been deposited in the herbarium section of Department of Botany, Safia College, Bhopal for future and further reference. The flowers of *Woodfordia fructicosa* and leaves of *Gardenia gummifera* were dried under shade in college laboratory. It was pulverized to coarse powder. These powders of plants were used for extraction. The ethanolic extract was prepared by soxhlation method by taking 200 g of powdered and extracting with 500mL of ethanol for four days.[14] Extracts were filtered; filtrates were evaporated at room temperature to dryness. The extracts were used to prepare gel and suspension. Formulation development of topical (gel) was done by the method of Misal G et al [15] and Oral (Suspension) was done by the method of Sanaa S et al. [16]

The animal experimental protocol was approved by the Institutional Animals Ethical Committee (IAEC), Sapience Bioanalytical Research Lab. (Reg. no. 1413/PO/E/S/11/CPCSEA), Bhopal, India. Protocol Approval Number is SBRL/IAEC/ June 2014/14. Male and female Wistar albino rats (120-140g) were provided by Sapience Bioanalytical Research Lab. The animals were housed in standard conditions of temperature (25±2^{0}C) and 12:12 hour light-dark cycle. The rats were fed with commercial diet and water *ad Libitum*. The animals were acclimatized to the laboratory conditions for a minimum period of seven days prior to commencement of treatment.

Acute dermal toxicity

The acute dermal toxicity test of extracts was determined according to the Organisation for Economic Cooperation and Development (OECD) guidelines 402.[17] Adult Wistar rats of either sex were used. Nine rats were divided in three groups, each group comprised of three rats. Approximately 24 hours before the test, 5% hairs of the body were removed from the dorsal area of the trunk of the test animals by using hair removal cream. Group I animals were considered as control, group II and group III animals received topically 1000 mg/kg body weight (limit test) of ethanolic extract of *Woodfordia fructicosa* flowers and *Gardenia gummifera* leaves respectively. All animals were monitored for 14 days for changes in fur, eyes, behaviour and toxic reactions. The extracts were safe up to the topical dose of 1000 mg/kg and from results suitable dose was chosen for further activity.

Acute oral toxicity

Acute oral toxicity study was evaluated as per OECD guidelines 425 on Wistar albino rats. [18] Nine animals were

divided in three groups, each group comprises three animals. Group I animals were considered as control, group II and group III animals received 1000 mg/kg body weight (limit test) of ethanolic extract of *Woodfordia fructicosa* flowers and *Gardenia gummifera* leaves respectively by gavage using oral canula. The extracts were safe up to the oral dose of 1000 mg/kg and from results suitable dose was chosen for further activity.

Evaluation of anti-dermatitis activity

Preliminary induction of dermatitis lesions

A report described the dermal effects (erythema and increased skin thickness) of different concentration of formaldehyde in Guinea pigs.[19] Some modifications in this reported study were applied for induction of skin lesions (like contact dermatitis) in Wistar albino rats. Two Wistar rats weighing 130 g were used. Hair on the dorsal skin was removed using hair removing cream. A 5% body surface area was treated with 0.1ml formaldehyde (37%). Animals were monitored for observing any change on the skin, any appearance of skin lesion and other different behaviours.

Anti-dermatitis activity of Topical formulations

A total 25 Wistar rats weighing around 120-140g were used. Dermatitis lesions were induced in animals as mentioned in preliminary induction protocol. After induction, animals were divided into five groups comprising of 5 animals in each group as follows.

Group I: Normal control (no induction)

Group II: Disease control (Vehicle treated dermatitis lesion)

Group III: Induced dermatitis lesion treated with 0.04% w/w Tretinoin Microsphere gel (Unknown Standard) once daily for 16 days.

Group IV: Induced dermatitis lesion treated with 0.1% gel of extract of *Woodfordia fructicosa* once daily for 16 days.

Group V: Induced dermatitis lesion treated with 0.1% gel of extract of *Gardenia gummifera* once daily for 16 days.

Anti- dermatitis activity of oral formulations

In the experiment, a total of 20 rats were used. The rats were divided into 4 groups comprising of 5 animals in each group as follows:

Group I: Normal control (no induction)

Group II: Disease control (Vehicle treated dermatitis lesions)

Group III: Induced dermatitis lesion treated with suspension of extract of *Woodfordia fructicosa* (100mg/kg body wt., p.o.) once daily for 16 days.

Group IV: Induced dermatitis lesion treated with suspension of extract of *Gardenia gummifera* (100mg/kg body wt., p.o.) once daily for 16 days.

Macroscopic Examination

Animals were evaluated by severity index (severity score) of dermatitis lesions every second day. A visual (by naked eyes) scoring system was developed based on Severity Index (SI). SI was scored on a scale from 0 to 3: 0- none (clear); 1- mild (redness); 2- moderate (redness and mild erythema); 3- severe (redness and severe erythema).

Histopathological Examination

At the end of study, animals were anaesthetized using ketamine. Specimens of skin (tissues) were collected and preserved in glass vials containing 10% formalin solution. Longitudinal sections of skin specimen (about 5µm thickness) were prepared by microtomy and stained with hematoxylin-eosin dye for histological examination. All of histological procedures were done by Pathology Department of Peoples Medical College, Bhopal. The thickness of the cellular part of epidermis was determined using a calibrated ocular micrometer and all measurements were adjusted for magnification optics.

Biochemical Estimation: The hydroxyproline content in each sample was analyzed by the method of Woessner et al.[20]

Statistical analyses: All the values are expressed as mean ± standard error of mean (S.E.M.) and analyzed for ANOVA and posthoc Tukey-Kramer Multiple Comparisons Test by employing statistical software, Graph Pad Prism 7.

RESULTS

Acute dermal toxicity

The extracts were safe up to the dose of 1000 mg/kg. There were no changes in fur, eyes and behaviour of treated animals as well as no toxic reactions determined and from results suitable dose (0.1%w/w) was chosen for each gel for further studies.

Acute oral toxicity

Animals were observed individually for any toxicity sign of gross changes like convulsion, tremor, circling, depression and mortality after dosing for 24 hours with special attention given during the first four hours and thereafter, 24 hours. All observations were systematically recorded with individual records being maintained for

each animal. Administered dose was found tolerable (no death found) and from results suitable dose (100mg/kg body weight) was chosen for each suspension for further studies.

Macroscopic Examination

Preliminary induction of skin lesions like contact dermatitis

Apparent skin lesions (lesions like dermatitis) were observed on day 10. Finally 0.1ml formaldehyde was applied for induction of skin lesions like contact dermatitis (dermatitis lesions) for further study. The results are shown in Table 1 and Figure 1.

Topical formulations

The results of the topical formulations on severity index are given in Table 2. Results reveal that both test groups (IV and V) and standard group (III) showed day to day decrease in severity index.

Table 1: Macroscopic observations in preliminary induction of dermatitis lesions by formaldehyde in rats

Day	Severity Index		Average
	Animal 1	Animal 2	
0	0	0	0
2	1	1	1
4	1	1	1
6	2	2	2
8	2	2	2
10	3	3	3

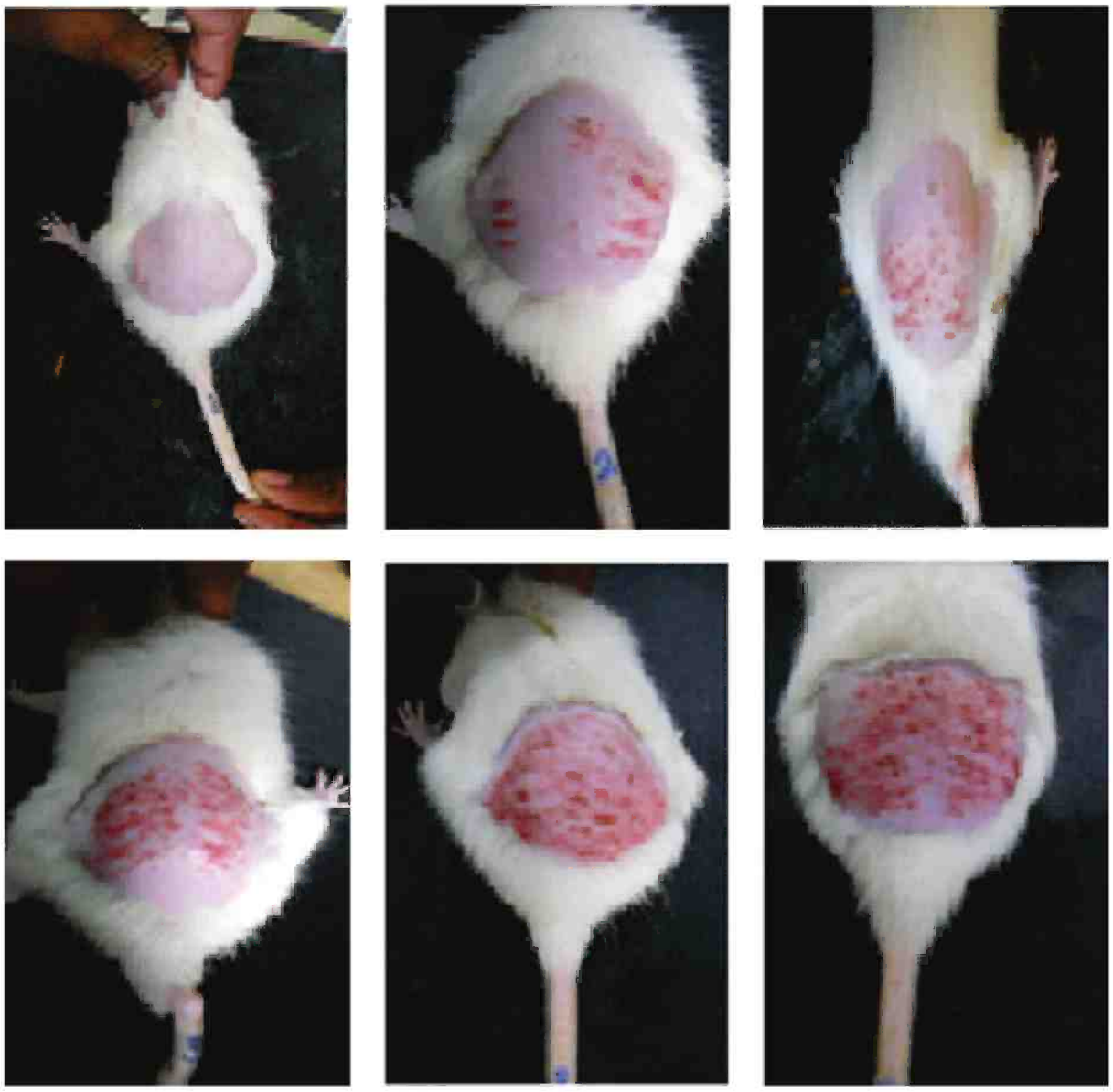


Figure 1: Preliminary induction of dermatitis lesions by formaldehyde in rats.

Table 2: Effect of topical formulations on macroscopic features in formaldehyde induced dermatitis lesions in rats

Groups	Severity Index							
	Day 4	% Decrease	Day 8	% Decrease	Day 12	% Decrease	Day 16	% Decrease
I	0 ± 0.0	-	0±0.0	-	0±0.0	-	0±0.0	-
II	3.4±0.24	-	2.6±0.24	-	1.6±0.24	-	0.6±0.24	-
III	2.4±0.24	29.41	1.8±0.2	30.76	1.2±0.2	25.0	0±0.0	100
IV	3.2±0.2	5.88	2.4±0.24	7.69	1.4±0.24	12.5	0.4±0.24	33.33
V	2.8±0.2	17.64	2.2±0.2	15.38	1.2±0.2	25.0	0.2±0.2	66.66

Table 3: Effect of oral formulations on macroscopic features in Formaldehyde induced dermatitis lesions in rats

Groups	Severity Index							
	Day 4	% Decrease	Day 8	% Decrease	Day 12	% Decrease	Day 16	% Decrease
I	0±0.0	-	0±0.0	-	0±0.0	-	0±0.0	-
II	3.6±0.24	-	2.8±0.2	-	1.8±0.2	-	0.8±0.2	-
III	3.2±0.2	11.11	2.2±0.2	21.42	1.6±0.24	11.11	0.6±0.24	25.0
IV	3.0±0.0	16.66	2.0±0.0	28.57	1.4±0.24	22.22	0.4±0.24	50.0

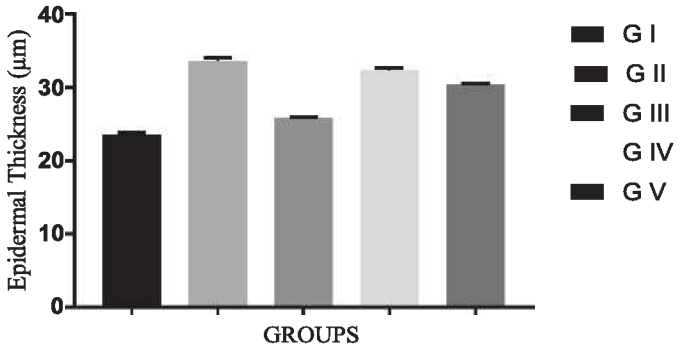


Figure 2: Effect of topical formulations on epidermal thickness in formaldehyde induced dermatitis lesions in rats.

Oral formulations

The results of the oral formulations on severity index are given in Table 3. Results reveal that both test groups (III & IV) showed day to day decreases severity index as compared to group II (vehicle treated).

Epidermal Thickness

Topical formulations

The results of the topical formulations on epidermal thickness are given in Table 4 and Figure 2. The microscopic evaluation revealed significant reduction ($p<0.001$) in epidermal thickness.

Oral formulations

The results of the oral formulations on epidermal thickness are given in Table 5 and Figure 3. The microscopic evaluation revealed significant reduction ($p<0.001$) in epidermal thickness.

Table 4: Effect of topical formulations on epidermal thickness in formaldehyde induced dermatitis lesions in rats

Groups	Treatment	Epidermal thickness (µm)
I	Normal Control	23.5±0.35
II	Vehicle treated	33.6±0.43[a]
III	Standard	25.8±0.25[a,b]
IV	Test-1	32.4±0.29[a]
V	Test-2	30.4±0.18[a,b]

[a] Highly significant (HS) difference in compare to normal control (Group-I)
[b] Highly significant (HS) difference in compare to vehicle treated (Group-II)

Table 5: Effect of oral formulations on epidermal thickness in formaldehyde induced dermatitis lesions in rats

Groups	Treatment	Epidermal thickness (µm)
I	Normal Control	22.2±0.4
II	Vehicle treated	38.0±0.41[a***]
III	Test-1	33.8±0.25 [a***,b***]
IV	Test-2	24.0±0.35 [a*,b***]

*$p<0.05$, ***$p<0.001$
[a] Significant difference in compare to normal control (Group-I)
[b] Significant difference in compare to vehicle treated (Group-II)

Hydroxyproline Content

Topical formulations

The results of the topical formulations on hydroxyproline content are given in Figure 4. The biochemical evaluation revealed no significant reduction in hydroxyproline content. The hydroxyproline content of the test group IV

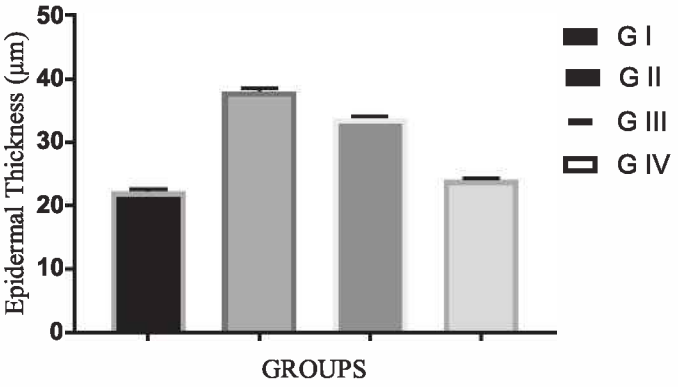


Figure 3: Effect of oral formulations on epidermal thickness in formaldehyde induced dermatitis lesions in rats.

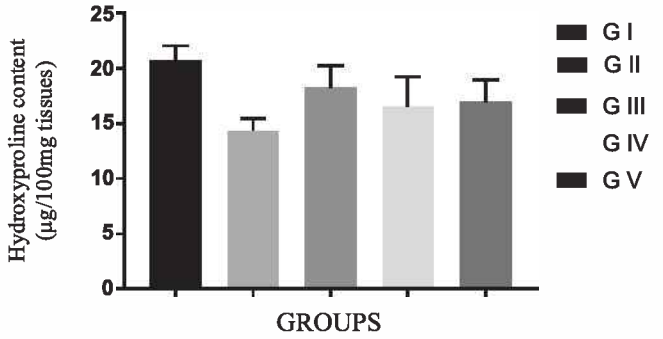


Figure 4: Effect of topical formulations on hydroxyproline content in formaldehyde induced dermatitis lesions in rats.

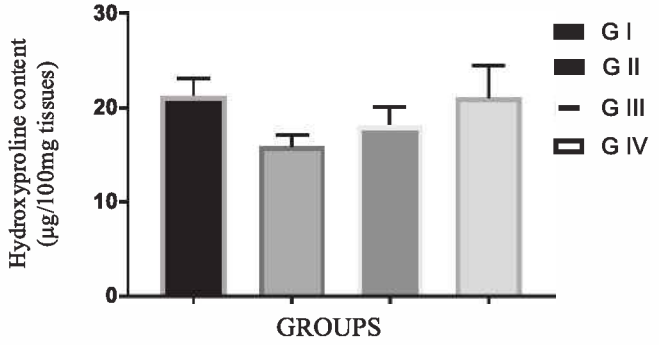


Figure 5: Effect of oral formulations on hydroxyproline content in formaldehyde induced dermatitis lesions in rats.

and V was found to be almost equal respectively which was slightly higher than that of vehicle treated group (II) and lesser than that of standard group (III).

Oral formulations

The results of the oral formulations on hydroxyproline content are given in Figure 5. The biochemical evaluation revealed no significant reduction in hydroxyproline content.Results of histopathological examinations depicting elongation of rete ridges and capillary loop dilation are shown in Figure 6 and 7.

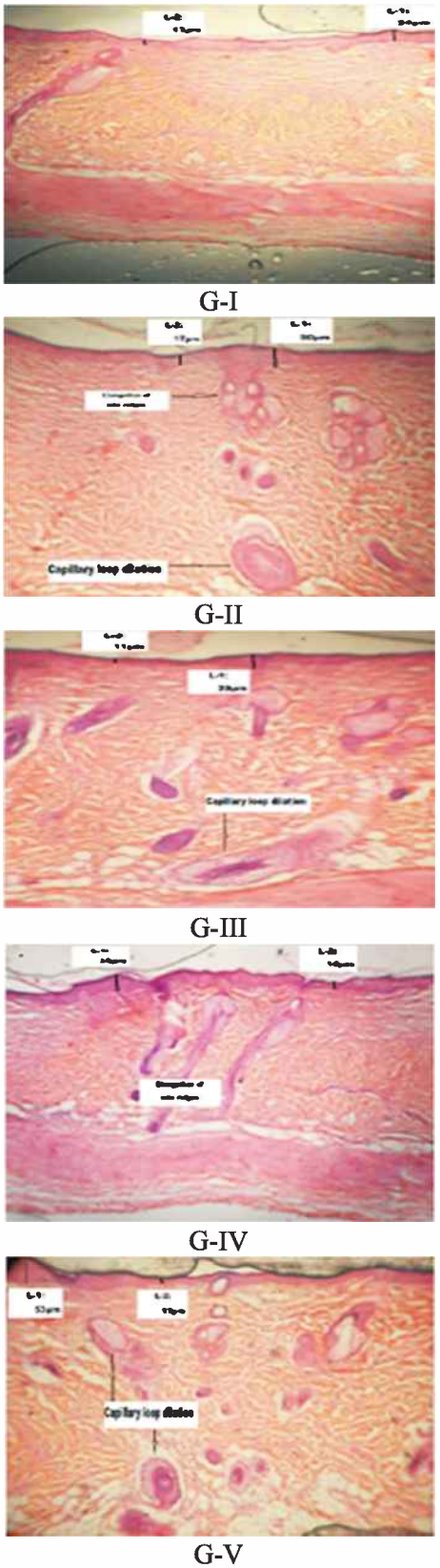
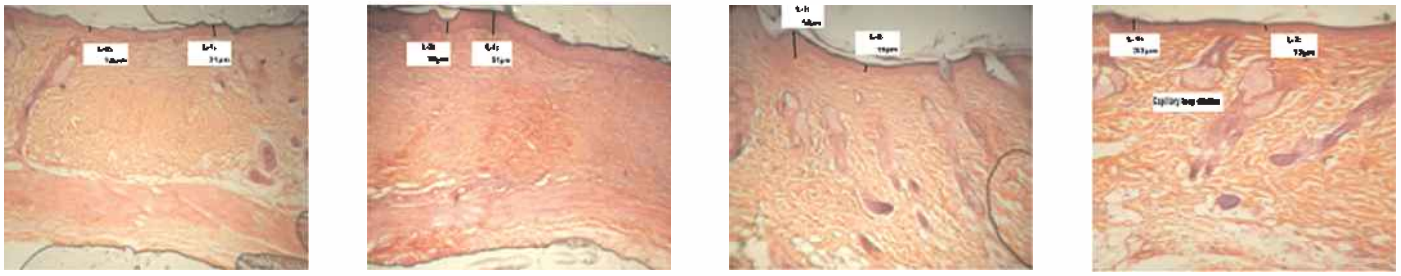


Figure 6: Effect of topical formulations on histopathological features in formaldehyde induced dermatitis lesions in rats.



G-I G-II G-III G-IV

Figure 7: Effect of oral formulations on histopathological features in formaldehyde induced dermatitis lesions in rats.

DISCUSSION

Many herbal remedies individually or in combination have been recommended in various medical expositions for the cure of different diseases. Plants are among the most important and common sources of potentially valuable new drugs. Much work has been carried out on herbal treatment of various diseases, but still there is need to explore more. Herbal remedies are promising in the management of dermatological conditions including psoriasis, dermatitis. Our previous study concluded that extractive Phytoconstituents of *Woodfordia fructicosa* flowers and *Gardenia gummifera* leaves have potent anti-psoriatic activity in UV radiation induced psoriasis in experimental rats. [21]

Skin sensitization (allergic contact dermatitis) is induced only by direct skin contact with formaldehyde solutions in concentrations higher than 2%. The lowest patch test challenge concentration in an aqueous solution reported to produce a reaction in sensitized persons was 0.05% formaldehyde.[22] The potency of formaldehyde as a contact allergen is demonstrated by the observation that occluded dermal exposure of guinea pigs to 5% formaldehyde for 3 weeks sensitized 70% of the animals to later dermal challenges with 1% formaldehyde.[23] For induction of skin lesions (like contact dermatitis) in this study, Wistar albino rats was treated with 0.1ml formaldehyde (37%) and monitored for appearance of skin lesion and other different behaviours. Apparent skin lesions (lesions like dermatitis) were observed on day 10 (Table 1 and Figure 1). Vehicle treated groups showed more severity index as compared to formulation treated group (Table 2 and 3). The microscopic evaluation revealed significant reduction ($p<0.001$) in epidermal thickness, which showed the effectiveness of the test formulations of both extracts against dermatitis lesion induced by formaldehyde (Table 4 and 5; Figure 2 and 3).

Given the high reactivity, volatility and aqueous solubility of formaldehyde and its rapid metabolism by cells, it is likely that dose-response relationships for dermal irritation from acute exposure may not be widely different from relationships for intermediate-and chronic-duration exposures. This hypothesis is supported by the results from inhalation exposure studies in rats indicating that exposure concentration is more important than exposure duration in determining the extent and severity of formaldehyde-induced epithelial lesions in the upper respiratory tract.[24] Nevertheless, additional animal studies comparing dose-response relationships for skin irritation for acute, intermediate and chronic exposure durations may be useful in estimating concentrations that will not damage the skin with repeated exposures. The potency of formaldehyde as a contact allergen is demonstrated by the observation that occluded dermal exposure of guinea pigs to 5% formaldehyde for three weeks sensitized 70% of the animals to later dermal challenges with 1% formaldehyde.[25]

The pathogenesis of allergic contact reactions requires that effector T lymphocytes are recruited into the sites of dermal exposure and this involves complex cell–matrix interactions regulated by adhesion molecule and integrin–receptor interactions with directional guidance supplied by relevant cytokines and chemokines.[26,27] The focus here, however, is consideration of which T cell phenotypes affect the reaction. The accepted view was that allergen-specific Th1 cells play the predominant role. However, other cell types may be of equal or greater importance.[28]

No significant result of hydroxyproline content were obtained in study but comparing with vehicle treated group (disease control group), the herbal formulation treated group showed higher hydroxyproline content values (Figure 4 and 5). Histopathological features like

elongation of rete ridges and capillary loop dilation were observed and compared with normal control group (Figure 6 and 7).Percent reduction in the severity index, decreased epidermal thickness and increased hydroxyproline content observed in formulation treated dermatitis lesion investigation reveals that prepared formulations possess potent anti- dermatitis activity.

CONCLUSION

Prepared topical and oral herbal formulations from ethanolic extract of *Woodfordia fructicosa* flowers and *Gardenia gummifera* leaves decreases severity index, epidermal thickness as well as other histopathological features and also increased hydroxyproline content. From the preliminary *in vivo* study we concluded that extractive Phytoconstituents of *Woodfordia fructicosa* flowers and *Gardenia gummifera* leaves might be novel therapeutic approach in treating contact dermatitis. Further research is warranted to elucidate the specific phytoconstituent/s involved.

Conflicts of interest: The authors declare that they have no competing interests.

Acknowledgements: Authors are thankful to Truba Institute of Pharmacy and Sapience Bio analytical Research Lab. Bhopal, India for providing necessary research facilities for the successful completion of research work.

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