

EFFECT OF *GYNANDROPSIS PENTAPHYLLA* LEAVES ON MILK INDUCED EOSINOPHILIA AND LEUCOCYTOSIS IN MICE

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Article Received on
05 Sept. 2017,

Revised on 25 Sept. 2017,
Accepted on 15 October 2017

DOI: 10.20959/wjpr201714-9938

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ABSTRACT

Gynandropsis pentaphylla syn. *Cleome pentaphylla*, (Capparadiaceae) is commonly known as 'Pandharitilvan and Safedhulhul'. *G. pentaphylla* is a annual, erect, branched plant Various parts of the plant are reported as rubefacient, counter-irritant, anthelmintic, in neuralgia, headache and otalgia. Terpenes, β -carotene, sterols, fatty acids, flavonoids, glycosides and alkaloids were reported from various part of plant. The seeds are anthelmintic and rubefacient and used internally for expulsion of round worms and externally as counter irritant and in headache. The seeds and leaves of *G. pentaphylla* have immunosuppressant, anthelmintic, antifungal and antimicrobial activity.

Objective of present work is to evaluate different extracts of *G.pentaphylla* for preliminary phytochemical screening and for milk induced eosinophilia and leucocytosis in mice. Different extract shows presence of alkaloids, tannins, flavonoids and Saponins. All the extracts were found to be significantly effective against eosinophilia and leucocytosis in mice. The aqueous and methanolic extracts shows prominent inhibition when compare with standard drug dexamethasone.

KEYWORDS: *Gynandropsis pentaphylla*, asthma, leucocytosis, milk, eosinophilia.

INTRODUCTION

Gynandropsis pentaphylla Linn (Capparadiaceae) is also known as *Cleome pentaphylla*. *G. gynandra* is commonly known as Pandharitilan or Safedhulhul². It is an erect, rather showy, glandular, pubescent, annual shrub, 1-3 feet high, commonly found in waste places in Tropical Countries and in warmer parts of the India.^[1,3]

Leaves are 3-5 foliolate, leaflets are sub-sessile, elliptic-obovate, obtuse, acute or acuminate, cuneate at base, pubescent on both sides with sub entire margin. Flowers are white, viscid-pubescent with lanceolate sepals and seeds are dark brown.^[1,3]



Fig. 1 *Gynandropsis pentaphylla*.

In Ayurveda, it is reported that roots has a hot sharp taste, removes ‘vata’, stomachic good in ascites, tumors, ulcers, pain, earache, spleen enlargement and biliary fevers. In Sushruta, it is reported that the leaves are applied externally to boils to prevent the formation of pus. The bruised leaves are rubefacient and vesicant; expressed juice is popular for local application in otalgia, curing earache and sometime curing headache. The pounded leaf is applied as counter-irritant in rheumatism, neuralgia and headache and in stiff neck. The seeds are anthelmintic and rubefacient and employed internally for expulsion of round worms and externally as counter irritant and in headache.^[1,3] The *G. pentaphylla* having prominent antifungal and antimicrobial activity^[4] it also possess anthelmintic^[5] and immunosuppressant effect.^[6] The aqueous and alcoholic extracts of leaf show antibacterial activity.^[7,8] The plant extract possess potent mosquito repellent activity^[9] and prominent larvecidal effect.^[10] The whole plant extract contains different flavonoids like flavone apegenin 4 and flavonols 1-3, 5-6. These flavonoids show prominent anticancer activity against murine P388 lymphocytic leukemia cell lines.^[11]

The plant essential oil of seeds contains glucosinolate Glucocapprin^[12], Carvacrol, trans-phytol, linalool, trans-2-methylcyclopentanol, m-cymene, β -caryophyllene, nonalal, 1- α -terpineol, β -cyclocitral, nerol, trans-geraniol, β -ionone, trans-geranylacetone and nerolidol.^[13]

Seeds also contains dammarane triterpenoid mainly Cleogynol.^[14] The leaves contain methyl glucosinolates^[15], Lupeol, β -sitosterol, kaempferol and rutin^[16] and β -carotene.^[17] The seeds contains proteins, lipids, fatty acids-oleic and linoleic acid^[18] and 5, 7-dihydroxychromone, 5-hydroxy-3, 7, 4'-trimethoxy flavone and luteolin.^[19] Flowers of *G. pentaphylla* contains rutin.^[20] The whole plants contains Glucoiberine, glucocapparine, neoglucobrassicin and glucobrassicin.^[21]

MATERIAL AND METHOD

Plant Material collection: The fresh, matured leaves of *G. pentaphylla* were collected from Ahmednagar district of Maharashtra, cleaned and dried at room temperature in shade, away from direct sunlight. The dried leaves were coarsely powdered in grinder. The powder was sieved through 60-120 mesh to maintain the particle size and stored in airtight container for further study. The plant was authenticated in "Botanical Survey of India, Pune." and a sample voucher specimen of plant was deposited for future reference.

Extraction: The powdered leaves of *G. pentaphylla* (500 gm) were extracted by soxhlet extraction process by using petroleum ether, chloroform, ethyl acetate and ethanol as solvent. The marc remained were subjected for aqueous extract preparation by means of decoction. The extracts thus obtained were further concentrated using rotator evaporator to yield the extracts.

Animals

Male Swiss albino mice weighing between 25-30 g were procured from National Toxicological Center, Pune. The mice were housed at least one week in the animal house under standard laboratory conditions of temperature ($22 \pm 2^{\circ}\text{C}$) relative humidity ($60 \pm 5^{\circ}\text{C}$) and light (12 hours light and dark cycle). Animals were fed with pellet and water *ad libitum*. The animal ethical committee of the institute approved all the protocols of the study.

Preliminary phytochemical screening

The Preliminary phytochemical Screening of the extracts was done for detection of various secondary metabolites like alkaloids, glycosides, tannins, flavonoids, Saponins and steroids.^[22]

Acute toxicity study

Acute toxicity studies were carried out using acute toxic class method as per guidelines.^[22, 23] Healthy adult albino mice of either sex, starved overnight, were divided into different groups of six mice in each group. Each group was injected orally with increasing doses (50, 100, 200, 400, 800, 1500, 2000, 3000 and 4000mg/kg) of petroleum ether, chloroform, ethyl acetate, methanol and aqueous extracts of *G. pentaphylla* leaves and observed for a period of 48 hrs for any mortality.

Milk induced leucocytosis in Mice

Mice were divided into seven groups, five animals in each group. Blood samples were collected from the retro orbital plexus. The total leucocytes were counted for animals of each group before the administration of drugs and 24 hours after milk injection. The previously boiled and cooled milk was given to the all animal at a dose of 4 ml/kg, s. c. the blood of each animal were sucked in WBC pipette upto the mark and further diluted with WBC diluting fluid. Fluid was shaken properly to mix the blood and WBC diluting fluid and kept aside for few minutes. The Neubaur's chamber was charged with above fluid and total leucocyte count was done. The group I were treated with vehicle (5ml/kg. i.p.) and served as control. Group II-VI was treated with petroleum ether, chloroform, ethyl acetate, methanol and aqueous extracts of leaves of *G. pentaphylla* at a dose of 150 mg/kg, i. p. and group VII was treated with dexamethasone at a dose of 50 mg/kg, i. p. One hour after administration of different extracts each animal was injected with milk. Difference in total leucocytes count before and after 24 hours of drug administration was calculated.^[25]

Table 1 Effect of leaves of *G. pentaphylla* on Milk induced Leucocytosis in mice.

Groups	Before	After	Difference
Group I	6592 ± 350.80	11061 ± 388.40	4469 ± 180.90
Group II	6880 ± 229.87	9957 ± 270.5	3077 ± 120.30
Group III	6696 ± 253.69	9071 ± 218.6	2375 ± 190.87
Group IV	6542 ± 159.2	8892 ± 352.4	2350 ± 159.80
Group V	6781 ± 268.89	8951 ± 221.60	2170 ± 103.90
Group VI	6332 ± 320.2	7822 ± 430.4	1468 ± 129.80
Group VII	6739 ± 294.2	7926 ± 384.4	1187 ± 98.80

Milk-induced Eosinophilia in mice

Mice were divided into seven groups, five animals in each group. Blood samples were collected from the retro orbital plexus. The total eosinophil count was done for animals of each group before the administration of drugs and 24 hours after milk injection. The

previously boiled and cooled milk was given to the all animal at a dose of 4 ml/kg, s. c. The blood of each animal were sucked in WBC pipette upto the mark and further diluted with eosin solution. The eosin solution facilitates destruction of all corpuscles except eosinophiles. Fluid was shaken properly to mix the blood in the pipette and kept aside for few minutes. The Neubaur's chamber was charged with above fluid and total eosinophil count was done. The group I were treated with vehicle (5ml/kg. i.p.) and served as control. Group II-VI was treated with petroleum ether, chloroform, ethyl acetate, methanol and aqueous extracts of leaves of *G. pentaphylla* at a dose of 150 mg/kg, i. p. and group VII was treated with dexamethasone at a dose of 50 mg/kg, i. p. One hour after administration of different extracts each animal was injected with milk. Difference in total eosinophil count before and after 24 hours of drug administration was calculated.^[25]

Table 2 Effect of of *G. pentaphylla* leaves on Milk induced Eosinophilia in mice.

Groups	Before	After	Difference
Group I	539 ± 3.63	668 ± 24.32	129 ± 20.35
Group II	370 ± 9.65	463 ± 14.57	93 ± 10.20
Group III	349 ± 4.58	427 ± 10.14	78 ± 9.68
Group IV	342 ± 7.49	409 ± 6.83	67 ± 3.84
Group V	428 ± 5.74	482 ± 8.59	54 ± 7.38
Group VI	353 ± 10.40	410 ± 7.80	57 ± 7.60
Group VII	332 ± 8.2	378 ± 6.4	46 ± 5.80

Statistical significance

All observations were presented as mean ± SEM. The data was analysed by one way ANNOVA followed by Newman keuls' test. P<00.5 was considered as significant.

RESULTS AND DISCUSSIONS

Preliminary phytochemical screening: Preliminary phytochemical screening of all the extracts shows presence of different chemical constituents. Petroleum ether extract showed the presence of steroids. Chloroform extract showed presence of steroids and alkaloids while Ethyl acetate extract showed positive test for steroids, saponins, alkaloids, flavonoids and tannins. Steroids, alkaloids, saponins, flavonoids and tannins were found to be present in methanol extract. The aqueous extract showed presence of alkaloids, glycosides, flavonoids, tannins and Saponins.

Acute Toxicity Study: The LD₅₀ value of all the extracts when given orally in mice was found to be more than 4000 mg/kg body weight.

Milk induced leucocytosis in Mice

In the present study it was observed that all the extracts showed prominent effect on decrease in leucocyte count. The mice treated with aqueous extract (1468 ± 129.80) and methanolic extract (2170 ± 103.90) showed prominent decrease in leucocyte count as compare to control group (4469 ± 180.90). All the extract shows less activity as compare to standard drug dexamethasone (1187 ± 98.80).

Milk-induced Eosinophilia in mice

This study reveals that 24 hours after the administration of milk, mice treated with methanol extract (54 ± 7.38) and aqueous extract (57 ± 7.60) shows prominent decrease in eosinophil count as compare to control (129 ± 20.35) and standard drug dexamethasone (46 ± 5.80).

In the present study the petroleum ether, chloroform, ethyl acetate, methanol and aqueous extracts of leaves of *G. pentaphylla* at a dose of 150 mg/kg, i. p. were evaluated for milk induced leucocytosis and eosinophilia as a models of asthma. The administration of milk by parenteral route causes prominent increase in leucocytes count and eosinophil count immediately after 24 hr of administration.^[26] In asthmatic condition of patients the leukocyte count and eosinophil count increased and hypersensitivity reactions takes place.^[27,29] Excessive stress durin the asthmatic condition also increase the leukocyte count and eosinophil count.^[30] The aqueous and methanolic extract causes significant inhibition in the leucocytes as well as eosinophil count, this shows that both the extracts might be effective against type I hypersensitivity reactions in management of asthma.

Flavonoids ans saponins have bronchodilator and smooth muscle relaxant effect by inhibition of basophil histamine release and neutrophil β - glucuronidase release and thereby possess in vivo anti allergic effect.^[31,33]

The preliminary phytochemical study indicated that the aqueous and methanolic extracts show presence of Flavonoids and Saponins. The inhibition of leucocytes and eosinophiles might be due to effect of Saponins and Flavonoids.

CONCLUSION

All the present study shows presence of different constituents in all the extracts. The aqueous and methanolic extract shows prominent decrease in leucocyte and eosinophil count of mice,

which indicates the leaves of *G. pentaphylla* are having antiallergic effect and it might be useful in treatment of asthma.

ACKNOWLEDGMENT

The authors are very much thankful to Mr. M. C. Mirchandani, Principal, Ideal College of Pharmacy and Research, Kalyan, Maharashtra for providing me good laboratory facilities and equipments for performing the research work.

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