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

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ABSTRACT: *Gynandropsis pentaphylla* syn. *Cleome pentaphylla*, (Capparadiaceae) is commonly known as 'Pandharitilvan and Safedhulhul.' *G. pentaphylla* is an 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 the plant. The seeds are anthelmintic and rubefacient and used internally for the expulsion of round worms and externally as a counter irritant and in headache. The seeds and leaves of *G. pentaphylla* have immunosuppressant, anthelmintic, antifungal and antimicrobial activity. The objective of present work is to evaluate different extracts of *G. pentaphylla* for preliminary phytochemical screening and milk induced eosinophilia and leucocytosis in mice. The different extract shows the 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.

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

In Ayurveda, it is reported that roots have 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 sometimes curing headache.

The pounded leaf is applied as counter-irritant in rheumatism, neuralgia, and headache and stiff neck. The seeds are anthelmintic and rubefacient and employed internally for the expulsion of round worms and externally as a counter irritant and in headache^{1, 2, 3}. The *G. pentaphylla* is having prominent antifungal and antimicrobial activity⁴ it also possesses anthelmintic⁵ & immunosuppressant effect⁶.

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The aqueous and alcoholic extracts of leaf show antibacterial activity^{7, 8}. The plant extract possesses potent mosquito repellent activity⁹ and prominent larvicidal effect¹⁰. The whole plant extract contains different flavonoids like flavone apigenin 4 and flavonols 1-3, 5-6. These flavonoids show prominent anticancer activity against murine P388 lymphocytic leukemia cell lines¹¹.



FIG. 1: GYNANDROPSIS PENTAPHYLLA

The essential plant oil of seeds contains glucosinolate glucocapparin¹², carvacrol, trans-phytol, linalool, trans-2-methylcyclopentanol, α -cymene, β -caryophyllene, nonalal, 1- α -terpineol, β -cyclocitral, nerol, trans-geraniol, β -ionone, trans-geranylacetone, and nerolidol¹³. Seeds also contain dammarane triterpenoid mainly cleogynol¹⁴. The leaves contain methyl glucosinolates¹⁵, lupeol, β -sitosterol, kaempferol and rutin¹⁶ and β -carotene¹⁷. The seeds contain proteins, lipids, fatty acids-oleic and linoleic acid¹⁸ and 5, 7-dihydroxychromone, 5-hydroxy-3, 7, 4'-trimethoxy flavone and luteolin¹⁹. Flowers of *G. pentaphylla* contains rutin²⁰. The whole plants contain glucoiberine, glucocapparine, neoglucobrassicin and glucobrassicin²¹.

MATERIALS AND METHODS:

Plant Material Collection: The fresh, matured leaves of *G. pentaphylla* were collected from Ahmednagar district of Maharashtra, cleaned and dried at room temperature in the 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 using 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 ± 2 °C) relative humidity (60 ± 5 %) and light (12 h 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 steroids²².

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 4000 mg/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 h 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 was sucked in WBC pipette up to the mark and further diluted with WBC diluting fluid. The fluid was shaken properly to mix the blood and WBC diluting fluid and kept aside for a few minutes.

The Neubaur's chamber was charged with the above fluid, and total leucocyte count was done. The group I were treated with vehicle (5 ml/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. The difference in total leucocytes count before and after 24 h of drug administration was calculated²⁵.

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 h 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 was sucked in WBC pipette up to the mark and further diluted with eosin solution. The eosin solution facilitates the destruction of all corpuscles except eosinophils. The fluid was shaken properly to mix the blood in the pipette and kept aside for a few minutes.

The Neubaur's chamber was charged with the above fluid, and total eosinophil count was done. The group I were treated with vehicle (5 ml/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. The difference in total eosinophil count before and after 24 h of drug administration was calculated²⁵.

TABLE 2: EFFECT 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 were analyzed by one way ANOVA followed by Newman keuls' test. P<00.5 was considered as significant.

RESULTS AND DISCUSSION:

Preliminary Phytochemical Screening:

Preliminary phytochemical screening of all the extracts shows the presence of different chemical constituents. Petroleum ether extract showed the presence of steroids. Chloroform extract showed the presence of steroids and alkaloids while ethyl

acetate extract showed a 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 the 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 a prominent effect on the decrease in leucocyte count. The mice treated with an aqueous extract (1468 ± 129.80) and methanolic extract (2170 ± 103.90) showed prominent decrease in leucocyte count as compared to the control group (4469 ± 180.90). All the extract shows less activity as compared to standard drug dexamethasone (1187 ± 98.80).

Milk-Induced Eosinophilia in Mice: This study reveals that 24 h after the administration of milk, mice treated with methanol extract (54 ± 7.38) and aqueous extract (57 ± 7.60) shows a prominent decrease in eosinophil count as compared 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 model of asthma. The administration of milk by parenteral route causes a prominent increase in leucocytes count and eosinophil count immediately after 24 h of administration²⁶. In the asthmatic condition of patients, the leukocyte count and eosinophil count increased, and hypersensitivity reactions take place^{27, 28, 29}. Excessive stress during the asthmatic condition also increases the leukocyte count and eosinophil count³⁰. 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 the management of asthma.

Flavonoids and 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, 32, 33}. The preliminary phytochemical study indicated that the aqueous and methanolic extracts show the presence of flavonoids and saponins. The inhibition of leucocytes and eosinophils might be due to the effect of saponins and flavonoids.

CONCLUSION: All the present study shows the presence of different constituents in all the extracts. The aqueous and methanolic extract shows a

prominent decrease in leucocyte and eosinophil count of mice, which indicates the leaves of *G. pentaphylla* have an anti-allergic effect, and it might be useful in the treatment of asthma.

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CONFLICT OF INTEREST: Nil

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