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AN IMMUNO MODULATORY PROFILE OF AQUEOUS EXTRACT OF *OXALIS CORNICULATA* LINN. IN ALBINO WISTAR RATS AND SWISS ALBINO MICE

Mr. Priyadarshini Karre[1], Ms. Yerravalli Priyanka[2], Mrs. Sayeeda Tabassum[3]

Assistant professor[1,2,3]

Department of Pharmacology,

Global College of Pharmacy, Hyderabad. Chilkur (V), Moinabad (M), Telangana- 501504.

ABSTRACT: Objective and Background: Using Albino wistar rats and Swiss albino mice, we aimed to examine the immunological activities of an aqueous extract of Oxalis corniculata. The edible and medicinal plant Oxalis corniculata plays an essential function in the food sector and might potentially play a major part inmedical services. Subjects and methodology: Oxalis corniculata whole plant aqueous extract was given orally at doses of 200 mg/kg/day and 400 mg/kg/day body weight, respectively. Tests for immunomodulatory action included carbon clearance, cyclophosphoamide neutropenia, delayed type hypersensitivity, and haemagglutination antibody titre. The immunostimulating agents cyclophosphamide andlevamisole are used. Remarks, Discoveries, and Final Thoughts: Flavonoids, alkaloids, polysaccharides, terpenoids, sterols, proteins, vitamins, and minerals are just a few of the many chemical components that give oxalis corniculata its immunomodulatory pharmacological properties. The plant is also highly valuable to the food industry. Analysis and Significance: Through interference with immune system functions, immuno-modulation can change an organism's immune system. When this process improves the immune system, the drug is called an immunostimulative, and it mainly means that it stimulates both specific and non-specific systems, such as granulocytes, macrophages, complement specific T-lymphocytes, and other effecting substances. Therefore, more research into the mechanisms of action is necessary prior to considering Oxalis corniculata for additional therapeutic applications. Research in both laboratory and living organism settings, including animal models and cell culture experiments, has confirmed the bioactivities of Oxalis corniculata extracts and compounds. The discovery lends credence to its potential in pharmaceuticals and other therapeutic applications, and calls for further investigation into and use of the substance.

Keywords: Immunomodulation, Oxalis corniculata, Albino wistar rats

INTRODUCTION:

By interfering with its functions, immunomodulation changes an organism's immune system.

As a consequence of this interference, additional immuno-stimulants or immunosuppressants are produced. To control the immune system, one needs an immuno-modulator. An immunomodulator aids in the optimization of the immune response by regulating and normalizing this process. While immunomodulators do not often increase immunity, they do normalize it, and this is leading to their rising popularity in the natural health community throughout the globe. Efforts to modify the immune system must be directed with this perspective in mind. in order to create a safe and effective immunomodulator for therapeutic usage and to effectively treat a variety of immune system-related illnesses. Immuno- modulators are biological modifiers; exert their effects by enhancing host defense mechanism against illness. Pathogenesis 1 may result from an immune mechanism imbalance, which is itself the result of a delicate balance between effector and regulatory cells.

Thanks to both long-established traditional practices and modern scientific understanding, herbal therapy has rapidly integrated into mainstream health care. The therapeutic efficacy and safety of medicinal plants have been the

subject of increased scientific investigation due to their rising popularity. It is thought that several medicinal plants might boost the body's inherent immunity against diseases.

The creeping wood sorrel, or Oxalis corniculata, is a kind of weed that thrives in damp environments and can be common in places like gardens, wastelands, roadsides, and hedges. Procumbent yellow sorrel, also known as sleeping beauty, is acidic, prefers full sun, and has a life cycle that is both perennial and annual, much like the common yellow wood sorrel. It has a wide geographical distribution and does best in loamy soil that drains well. The plant's life cycle is annual in colder regions because of the low overwintering temperature, but perennial in warmer regions. In a typical environment, this plant will reach a height of 18–20 cm on horizontal stolons.

Runner or decumbent, with hairy or smooth texture, the stem roots at nodes. Taproots and subterranean rhizomes are present in some of the roots. The stems are thin and silky, and the leaves alternate and cluster at the joints and ends of the stalks. For a brief period in the early sunshine, the clusters of yellow flowers that bloom in clusters at the base of each leaf open one by one. When the seeds are ready to be sown, they are released from their little pod. It can withstand dry conditions and poor, compacted soils because to its taproot and fibrous secondary roots. Rheumatic and antifungal properties have long been associated with this plant. Pharmacological studies on this plant have focused on its antifungal, antioxidant, antibacterial, anti-inflammatory, antidiabetic, diuretic, analgesic, and wound healing capabilities.

Seeds are dispersed when flowers are left to self-pollinate, which allows the Oxalis corniculata plant to reproduce regardless of whether the corolla is removed or not. There occurs an abrupt breaking along the abaxial axis and an inside-out transformation of the seeds, which are packed in smooth turgid arid.

5. Because of this, seed dispersion may occur up to 2 meters away from the parent plant. Shortly after one seed pops out of its capsule, the other seeds begin to scatter in a seemingly endless chain. Its sticky seeds attach to just about everything 6.

MATERIAL AND METHODS:

Plant Material: The fresh whole plant *Oxalis corniculata* were collected from the local market ofand vegetation gardens of Bhopal MP India.

Preparation of Extract: Dried coarsely powdered tubers of *Oxalis corniculata* (400g) were defatted with water at for 72 hrs using maceration process. The crude brown residue mass of extract was then concentrated, stored and preserved (2-8 °C). The Percentage yield of extract (4.8w/w) was found on dry wet basis.

Experimental Animals: Albino mice (Swiss) of either sex were used. The animals were fed with standard pellet diet, water and maintained under standard environment condition employed. They were housed under standard conditions (22 ± 45 °C with 12 h of light/dark cycle). All experimental protocols were approved by Institutional Animal Ethical Committee (Protocol Approval Reference No. PBRI/IAEC/PN-411) Pinnacle Biomedical Research Institute Bhopal MP. India (CPCSEA Registration No. 1283/PO/c/09/CPCSEA).

Drugs and Chemicals: All the drugs and chemicals were of analytical grade while the other drugs were procured from Levamisole (Lupin Pharmaceutical Mandideep), Cyclophosphamide (Lupin pharmaceutical, Mandideep), Colloidal carbon (Indian ink, camel India Pvt. Ltd.).

Acute Toxicity Studies: Acute toxicity studies were performed according to organization for economic cooperation and development (OECD) guidelines, received draft guidelines 425, received from CPCSEA, Ministry of social justice and empowerment, Government of India.

Mice weighing between 20-25gm in groups of five were used (n = 5). The animals were fasted for 4 hr. with free access to water only. The aqueous extract was administered orally in doses of 200 and 400mg/kg to different groups of mice and rats and observed over 48 hours for mortality and physical/ behavioral changes. The experiments were performed after the experimental protocols had been approved by the Institutional Animal Ethical committee [7].

Haemagglutination Antibody (HAT) Titre: Animals were injected i.p. 0.2 ml of 5×10^9 SRBC on day 0. Test sample will be administered to animals on -3, -2, 0, 2, 3 days. Control group received equal volume of vehicle. Blood samples were collected from retro orbital plexus on day 7. Two –fold dilutions of serum samples made in 25µl volumes of normal saline containing 0.1% suspension of SRBC in BSA in V bottom haemagglutination plates were added 25µl of 0.1% suspension of SRBC in BSA saline. After through mixing SRBC were allowed to settle at room temperature for 90 min until controls wells through mixing SRBC were allowed to settle at room temperature for 90 min until control wells showed small buttons of cells (negative pattern) [8].

Delayed Type Hypersensitivity (DTH): Animals were sensitized with 0.1ml of 10% SRBC (1×10^8 cells) at day zero. Test sample will be administered
-3 days to +3 days of SRBC immunization (administration of test sample may change as per protocol). On day 7, animals will be challenged with 1×10^8 SRBC cells, intradermally into the left footpad of each animal, while PBS (pH 7.4) will beinjected into right hind paw. The increase in foot pad thickness (FPT) will be measured 24 hours after SRBC challenge by digital vernier caliper [9].

Cyclophhosphoamide Neutropenia: Cyclophos- phamide induced neutropenia. Swiss albino mice received the drug or vehicle orally for 5 days. On 5^{th} day, neutropenic dose of cyclophosphamide (200mg/kg, s.c) was injected and this day was labelled as day zero. Blood was collected; the total leukocyte count (TLC) and DLC were performed prior to and on day 3 after injection of cyclophosphamide. The TLC and neutrophil counts (%) in treated groups were compared with the values of the control group [10].

Carbon Clearance Test: Test samples were administered for five days. On day six, all the groups were given 0.1ml of carbon ink suspension through the tail vein. Blood was collected from retro orbital plexuses of individual animals at 0 and 15 minutes immediately after the injection carbon suspension. Blood (25µl) was lysed with 2ml of 0.1% sodium carbonate and the absorbance was measured spectrophotometrically at 675nm for determination of optical densities [11].

RESULTS:

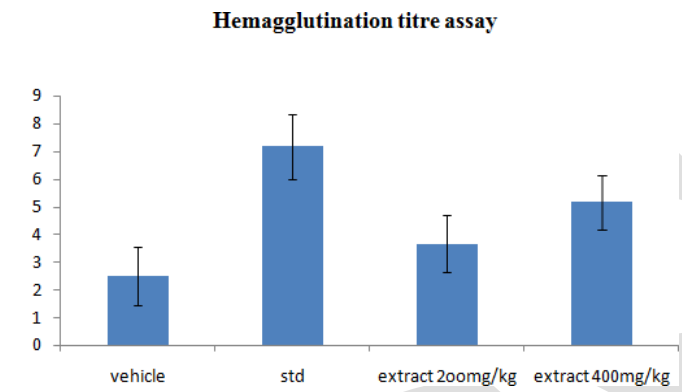
Hemaggulutination Antibody Titre: Hemaggu- lutination antibody titre assay is an important investigation of components acting on humoral immunity. Antibody molecules, a product of lymphocytes and plasma cells, are cellular to humoral immune response, IgG and IgM are the major immunoglobins which are involved in the complement activation, opsonisation, neutralization of toxins. In 200mg/kg of *Oxalis corniculata* extract treated animals titre valve was to be non significant 3.66 ± 1.032. In the 400mg/kg *Oxalis corniculata* extract treated animals titre value was found to be more 5.16 ± 0.983 shown in **Table 1** and **Graph 1.**

TABLE 1: HEMAGGLUTINATION ANTIBODY TITRE

S. No	Treatment	Titre value
1.	Vehicle	2.5 ± 1.048
2.	Standard	7.16 ± 1.16
3.	Extract 200mg/kg	3.66 ± 1.032^{ns}
4.	Extract 400mg/kg	5.16 ± 0.983*

Values are expressed as Mean ± SD at n = 6, one way ANOVA followed by Benferroni's 't' test. **p < 0.001,*p <

as level of significance and ^{ns}p, 0.001 as non significantcompare vehicle group.



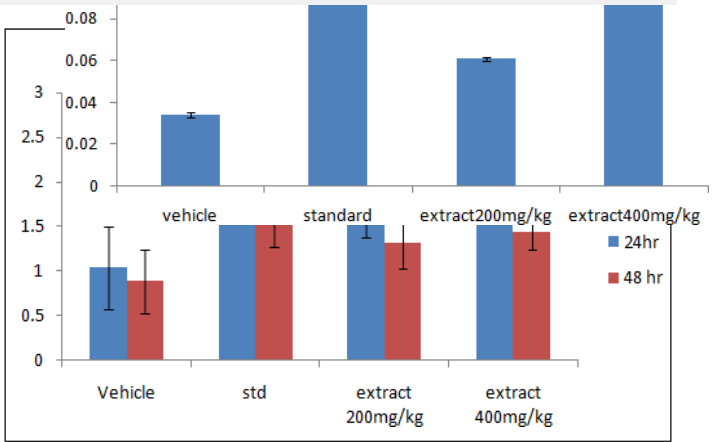
GRAPH 1: HEMAGGLUTINATION TITRE ASSAY

Delayed Type Hypersensitivity: In 200mg/kg of *Oxalis corniculata* extract treated animal paw

thickness was found 1.69 ± 0.314 at 24 hrs and 2.18 ± 0.411 in 400mg/kg *P. Oleracea* (p < 0.001) as compared to vehicle treated at 1.04 ± 0.462 24 hrs and 0.89 ± 0.36 at 48 hrs shown in **Table 2** and **Graph 2**.

TABLE 2: DELAYED TYPE HYPERSENSITIVITY

S. no	Treatment	Difference paw diameter (mm) 24hr	48hr
1.	Vehicle	1.04±0.462	0.89±0.36
2.	Standard	2.36±0.284	1.57±0.294
3.	Extract 200mg/kg	1.69±0.314*	1.32±0.287^{ns}
4.	Extract 400mg/kg	2.18±0.411**	1.44±0.198*



GRAPH 2: DELAYED TYPE HYPERSENSITIVITY

Carbon Clearance Test: The increase in carbon clearance index reflects the enhancement of the phagocytic function of mononuclear macrophages and non- specific immunity. Macrophages are innate immune cells with well established roles in primary responses, inflammation and repair. In 200 mg/kg of *Oxalis corniculata* extract treated animals titre value was found to be more 0.0061 ± 0.0009** (p < 0.001) as compared to vehicle treated animals at 0.034 ± 0.0012 even in standard drug treated animals titre value was significantly more 0.117 ± 0.0019.

In 400mg/kg of *Oxalis corniculata* extract treated animals titre value was found to be more 0.089 ± 0.0023** (p < 0.001) as compared to vehicle treated animals at 0.034 ± 0.0012 even in standard drug treated animals titre value was significantly more 0.117 ± 0.0019.

TABLE 3: CARBON CLEARANCE

S. No.	Treatment	Phagocytotic index
1.	vehicle	0.034 ± 0.0012
2.	standard	0.117 ± 0.0019
3.	Extract 200mg/kg	0.0061 ± 0.0009**
4.	Extract 400mg/kg	0.089 ± 0.0023**

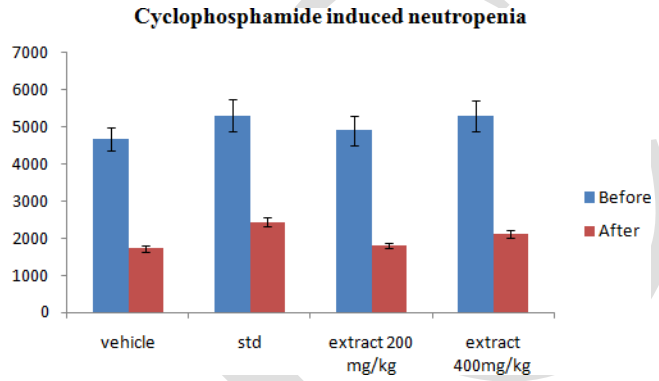
GRAPH 3: CARBON CLEARANCE

Cyclophosphoamide Induced Nutropenia:

The percentage reduction in neutrophil count was found to be and in control and OSE groups respectively. The low and high dose of aqueous extract of *Oxalis corniculata* (AEOC) demonstration 40.00% and 48.23% reduction in neutrophille count compared to initial values. In 400mg/kg extract the value was 2119 ± 102.11 ** ($p < 0.001$).

TABLE 4: CYCLOPHOSPHOAMIDE INDUCED NUTROPENIA

S. no.	Treatment	TLC (cell/mm^3) Before	After
1.	vehicle	4680±312.18	1730±91.36
2.	standard	5308±431.44	2433±123.81
3.	Extract200mg/kg	4913±391.46^{ns}	1806±84.89^{ns}
4.	Extract 400mg /kg	5292±418.44^{ns}	2119±102.11**



GRAPH 4: CYCLOPHOSPHOAMIDE INDUCED NUTROPENIA

DISCUSSION: The immune system is a complex system, to protect the host from invading and to eliminate diseases. Immunomodulators are being used as an adjuvant in conditions of immunodeficiency in cancer and other immunodeficiency syndrome [12]. In this present study, *Oxalis corniculata* Linn. showed increasing antibody production. It may be the release of mediators of hypersensitivity reactions and tissue responses to these mediators in the target organs by *Oxalis corniculata*. Organoleptic evaluation of any extract provides a significant data to standardize an extract. Even though enhances of human error are much more in this part. Further phytochemical investigations were performed to a certain presence and or absence of a range of bioactive components in extract [13]. It was observed that aqueous extract of *Oxalis corniculata* was devoid of the most of the phytochemical classes it was found to be rich in saccharides, omega-3 fatty acid, glycosides [14].

Acute oral toxicity revealed of the aqueous extract of *Oxalis corniculata* of the whole plant was not found having any acute toxicity. Basic endeavour of the present study was to evaluate Immunomodulatory effect of aqueous extract of *Oxalis corniculata*. This effect was ascertained on the basis of effect on the cellular immunity, humoral immunity, neutropenia and phagocytosis. In the extract treaded animals paw thickness was found to be more ($p < 0.05$). As compared to vehicle treated animals paw thickness was notably more ($p < 0.05$). This confirmed that extract was modulating cellular immunity [15].

The delayed type hyper sensitivity (DTH) that was measured this experiment has only some measure component sensitization, release of cytokinins and inflammation [16]. DTH reaction is characterized by invasion of non - specific inflammatory cells, in which the macrophages is a determine participants. It is a type IV hypersensitivity reaction that developed when antigen activates sensitized TDTH cells. These cells generally appears to be a TH_I The Cyclophosphoamide induced nutropenia model concentrates on the protective effects against Cyclophosphoamide induced myelosuppression in the experimental animals [17].

Both low and high doses of AEOC caused decrease in the Cyclophosphoamide induced nutropenia suggesting that it attenuates the effect of Cyclophosphoamide on the haemopoetic system [18]. Immunomodulatory agents can enhance or inhibit the immunological responsiveness of an organism by interfering with its regulatory mechanisms. They may selectively activate either cell-mediated or humoral immunity by stimulating either TH1 or TH2 type cell response, respectively [19].

Immunomodulatory agents that are free from side effects and which can be administered for long duration to obtain a continuous immune activation are highly desirable for the prevention of diseases. There is a variety of naturally and chemically derived compound discovered with the Immunomodulatory activity such as Levamisole, cyclophosphamide [20]. The role of phagocytosis is primary the removal of microorganism and foreign bodies, but also the elimination of dead or injured cells. Phagocytic defects are associated with varied pathological condition in humans [21].

CONCLUSION: The results of the present study suggest that the aqueous extracts of *Oxalis corniculata* may be beneficial in the treatment of impaired immunity. DTH requires the specific recognition of a given antigen by activated T lymphocytes, which subsequently proliferate and release cytokines. These in turn increase vascular permeability, induced vasodilatation, macrophage accumulation and activation, promoting increased phagocytic activity and increased concentration of lytic enzyme for more effective killing.

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