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Topic:- Phytochemical investigation and Pharmacological evaluation of *Amorphophallus paeoniifolius* for immunomodulatory action.

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Abstract:- Human health is very much dependent on functional status of gastrointestinal (GI) tract. Ayurvedic system, since its inception had immense rationale of relying on gut health for overall wellbeing. Majority of human population worldwide suffers of GI disorders of various types and magnitude on account of a dysregulated GI system. The GI system comprises the GI tract, associated glands and a diverse microbial community of probiotics, commensals and pathogens, collectively called as gut microbiota.

In fact, gut microbes have co-evolved with humans and other living beings. For achieving and maintaining gut health, the gut microbiota and the GI barrier remain pivotal. Scientific evidences indicate that the enteric microbiome present within every individual, possess a unique combinations of gut microbes that could define the status of that individual either healthy or diseased. The peak population profiles of these gut microbes keeps on changing in response to diet, age, disease and medical intervention. A number of epidemiological studies have suggested that a dysregulated GI system is caused mainly due to imbalances within the gut microbiota

Keywords:- Immunomodulation, *Amorphophallus Paeoniifolius*, Ayurvedic System, Antianaphylatic Activity, Broncho Alveolar Levage, Carrageenan

1. Introduction:-

Immune responses for Infectious and Non-Infectious Diseases

Changes in the micro-environmental conditions lead to alterations in the biochemical reaction network that disrupts the balance between the effector cell populations and favours the progression of the disease. This immune-suppression is observed very frequently in the cases of chronic infections (e.g., Chronic *Leishmania* infection) and Cancer. The following sections deal with the changes in the immune-regulatory network during these diseased conditions.

2 Plant Profile

Elephant foot yam (*Amorphophallus paeoniifolius*) was one of the important staple food in the 1940s and 1950s in java. Araceae or aroids are family of monocotyledonous flowering plants which are very familiar to everyone but surprisingly little known, in this family the flower are born on a characteristic hood like spathe and central fleshy spike known as spadix.

Amorphophallus paeoniifolius is a strong herbaceous plant with erect, solitary stem usually 1 to 2.5 m in height and having leaves at the top which is highly dissected. The life cycle of the plant is usually 4 to 6 years. At the end of the plant life cycle, a large terminal inflorescence is produced, the underground stem is large globose with a depression on top it is called corm and at the end of each season it produces small cormels.

3. Review of Literature

AK Ghosh *et. al.* 2010 demonstrated Methanol extract of Amorphophallus paeoniifolius has prominent anti-inflammatory activity while the chloroform extract has milder activity. 3 hours after the carrageenan injection, the methanol extract at the dose of 200 and 400 mg/ kg produced 37.5% and 45.83% inhibition when compared to the control group.

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4. Research Envisaged & Objectives of the study

- To explore the immunomodulatory activity in the corm of *Amorphophallus paeoniifolius.*
- To explore phytochemicals present in *Amorphophallus paeoniifolius.*

5 Plan of work

- Detailed literature survey.
- Identification, collection of Plant.
- Authentication of Plant.
- Extraction of Plant.
- Evalution & Identification of Phytochemicals :-
- ✓ Qualitative Chemical Evalution
- ✓ Determination of Nutritional content
- ✓ Determination of mucilage content
- Estimation of Chemical Constituents :-
- Pharmacological Studies

- Models used

A. *In- vitro* models

i.Alteration of Histamine Release from MAST cell

ii.Plaque formation colony test

B. In-vivo models

i.Antianaphylatic Activity (Scholtz-Dale Reaction)

ii.Broncho alveolar levage and lung histology

- Compilation of Data
- Summary & Conclusion

6. MATERIALS AND METHODS

Table 6.1:- Lists of Various Materials Used

Se. No.	Materials	Source
1	Anesthetic ether	Karnataka Fine Chemicals, Bangalore
2	Alcohol	Gauri Industries limited, Mandia
3	Pet. Ether	Karnataka Fine Chemicals, Bangalore
4	Chloroform	Karnataka Fine Chemicals, Bangalore
5	NaOH	Karnataka Fine Chemicals, Bangalore
6	Oxalic acid	Nice Chemicals Pvt. Ltd, Cochin
7	Toffer's Reagent	Nice Chemicals Pvt. Ltd, Cochin
8	Phenol Reagent	Nice Chemicals Pvt. Ltd, Cochin
9	90% Alcohol	Gauri Industries limited, Mandia
10	Conc. HCl	Karnataka Fine Chemicals, Bangalore
11	Phenolphthelien	Merck, Mumbai
12	Dexamethasone	Medly Pharmaceuticals Ltd, Daman

6.2 Plant Authentication

The plants Amorphophallus paeoniifolius were authenticated by Dr. Pushpendra Kumar Khare, Department of Botany, Govt. Maharaja PG College, Chhatarpur (M.P.). A voucher specimen no. is Bot/GMC/2025/154.

6.3 IAEC Approval for Animal Studies

In this study, wistar albino rats of either sex (male and female) weighing between 185 to 225 gm were used randomly. Institutional Animal Ethics Committee approves the experimental protocol; Proposal no. is DIPS/IAEC/M/25/04. Animals were maintained in standard circumstances in an animal house which has been approved by Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA).

Albino rats were used in this experiment was obtained from the Animal House Facility, Daksh Institute of Pharmaceutical Science, Chhatarpur MP.

The animals were in-housed in Poly propylene cages and maintain at 25˚C ± 2˚C less than 12 hrs light / dark cycles and were nourish ad libitum with paradigm pellet diet and had liberated entrance to water. The animals were procured from standard diet supplied by Godrej Agrovet Ltd. Pune. The composition of the diet has been protein (10%), Arachis oil (4%), Fibers (1%), Calcium (1%), Vitamin A 1000 IU/gm and Vitamin D 500 IU/gm.

6.4 Extraction of Plants

The tuber/corm of *Amorphophallus paeoniifolius* were shadow dry and compact to coarse fine particles in a motorized dicer. The powdered products obtain were then subjected to extraction in batch by various solvents in a soxhlet extractor. The different extracts found were evaporated at 45°C to get a semisolid mass. The extracts thus get were exposed to phytochemical study.

6.5 Preliminary phytochemical investigations:

All the extracts of corm were subjected for systematic preliminary qualitative phytochemical investigations.

1) Tests for alkaloids:

The various extracts were basified with ammonia and extracted with chloroform. The resulted chloroform solution was acidified with dilute hydrochloric acid. The acid layer was used for testing the alkaloids.

2) Tests for Tannins and Phenolic compounds:

The test solution was prepared by dissolving extract in ethanol or aqueous ethanol solution and added few drops of the following reagents.

- Ferric chloride test: Deep blue black colour
- One drop of NH_4OH, excess 10% $AgNO_3$ solution, Heated for 20 min. in boiling water bath. White precipitate observed, then dark silver mirror deposits on wall of test tube.

3) Tests for flavanoids:

The flavanoids are basically derived from parent moiety called flavones. Naturally flavonoids occur in the free form as well as glycosides along with sugars. Hence the plant extracts are hydrolyzed and subjected to flavonoidal detection.

- *Shinoda test*: To the ethyl acetate fraction a few fragments of magnesium ribbon and concentrated hydrochloric acid was added. Appearance of magenta colour after few minutes indicates the presence of flavonoids

4) Tests for sterols:

The extract was dissolved in chloroform, filtered and filtrate was tested for sterols.

- *Salkaowski test:* Few drops of concentrated sulphuric acid was added to the chloroform solution, shaken and allowed to stand, appearance of red colour in lower layer indicates the presence of sterols**.**

5) Tests for Glycosides:

The test solution was prepared by dissolving extract in ethanol or hydro alcohol and subjected to the following tests.

Tests for cardiac Glycosides:

- *Baljet's test*: The test solution treated with sodium picrate gives yellow to orange colour.

6) Coumarin glycosides:

Extract made alkaline, showed blue/green fluorescence under uv light.

7) Tests for saponins:

The test solution was prepared by dissolving extract in ethanol or aqueous ethanol solution and the following test were done.

- *Haemolysis test:* To 2 ml of 1.8% sodium chloride solution in two test tubes, 2 ml distilled water was added to one test tube and to other 2 ml of 1% extract was added. Few drops of blood was added to both test tubes and gently mixed, observed under microscope. If the haemolysis observed in the tube containing the extract it indicates the presence of saponins.

8) Tests for triterpenoids:

The chloroform extract was prepared and used to test the presence of triterpenoids.

- *Salkaowski test*: Few drops of concentrated sulphuric acid was added to the chloroform solution, shaken and allowed to stand for some time, appearance of golden yellow color indicates the presence of triterpenoids.

9) Tests for Proteins:

The test solution was prepared by dissolving extract in ethanol or aqueous ethanol solution and the following test were done.

- Millon's test: The solution treated with Millon's reagent. The resulted white precipitate formed turns to red precipitate and upon dissolving forms red coloured solution.

10) Tests for Amino acids:

The test solution was prepared by dissolving extract in water or hydroalcohol and the following tests were carried-out.

- Ninhydrin test: Test solution with Ninhydrin solution gives blue colur.

11) Tests for fats and fixed oils:

The filter paper gets permanently gets stained with fats and oils.

12) Tests for carbohydrates:

Small quantities of extracts were dissolved in little amount of distilled water and filtered separately. The filtrate was used to test the presence of carbohydrates as follows.

- *Molisch's test*: The test solution was treated with Molisch reagent (alcoholic solution of α-nephthaol) and concentrated sulphuric acid was added along the sides of the test tube to form a layer. A reddish violet ring shows the presence of carbohydrates.

13) Tests for Gum and Mucilages:

Hydrolyze the above test solution using dilute hydrochloric acid and performed Felhing's or Benedict's test as mentioned earlier. Gums and mucilage are present if red colour develops. Presence of mucilage is tested with addition of ruthenium red solution to get rose to pink colour.

14) Tests for lactones

The test solution was prepared by dissolving extract in ethanol or aqueous ethanol solution and the following test were carried-out.

- *Legal test*: The test solution was dissolved in pyridine and a mixture of sodium nitroprusside and sodium hydroxide was added. Deep red colour indicates the presence of lactones.

15) Tests for vitamins:

- ***Vitamin* A:** Dissolved a quantity equivalent to 10 to 15 units in 1ml of chloroform and added 5ml of antimony chloride solution, the formation of a transient blue colour immediately indicates the presence of vitamin A.

6.5. Chromatographic studies

Thin Layer Chromatography (TLC) studies were carried out to confirm the presence of different phytocostituents detected in the qualitative chemical investigation of the extracts. Much attention was given for the TLC analysis of methanolic extract which is preferred in the present work for *in vitro* and *in vivo* pharmacological screening.

TLC is accepted as a separation method for the phytoconstituents in an extract at analytical laboratory levels after suitable sorbents became available for self resolution on thin layer plates. Qualitative initial screening of the extracts is routinely performed and the presence of ubiquitous compounds such as plant sterols and certain phenolics can be ascertained at an early stage by running the appropriate standard alongside of test extract.

Separation of phytoconstituents by TLC, a mode of liquid chromatography, is effected by application of the extract as a spot or thin line onto a sorbent layer that has been applied to a backing plate. The solvent front that migrates up the plate through the sorbent by capillary action resulting in the resolution of phytoconstituents due to their differential adsorption / partition co-efficient with respect to both mobile and stationary phases. Each separated compound has same migration time but different distances.

The mobile phase may be of single solvent or mixture of solvents and the sorbents may be Silica Gel, Cellulose, Polyamide, and Alumina etc. Among these Silica Gel (type 60) is most commonly used. For the present work Silica Gel 60 GF_{254} precoated sheets as sorbent system was employed to analyze the extracts.

The information provided by finished chromatography includes 'Migration behavior', given in the form of the Retardation Factor (R_f) value using the formula;

$$\text{Retardation factor (Rf)} = \frac{\text{Distance traveled by the solute}}{\text{Distance traveled by the solvent}}$$

6.6 Dose & Group:- Animals were divided into 5 groups and each group contain 6 animals GROUP I receives normal Saline Group II receives standard drug while Group III, IV, V receives 100 mg/kg b.w., 150mg/kg b.w., 200mg/kg b.w. respectively.

6.7 In Vitro Models:-

a. Alteration of Histamine Release from MAST Cell

1. Cell Preparation

- Harvest mast cells (e.g., by peritoneal lavage or enzymatic dispersion of tissue) and determine cell count and viability.

- Resuspend cells in an appropriate buffer, such as HEPES-buffered salt solution (HBSS) containing calcius and magnesium, to the desired concentration.
- For IgE-mediated release studies, cells are often sensitized with IgE antibodies overnight before the experiment.

2. Stimulation and Treatment

- Aliquot cell suspensions into reaction tubes or multi-well plates.
- For studies involving inhibitors, pre-incubate the cells with the test compound for a specified time (e.g., 10-30 minutes) before adding the secretagogue.
- Add the secretagogue (e.g., anti-IgE or calcium ionophore) to the wells to induce histamine release.
- Include control samples:
  - **Vehicle control:** Cells with buffer alone (for spontaneous release measurement).
  - **Positive control:** Cells with a known potent secretagogue (e.g., high concentration of calcium ionophore) to ensure cell responsiveness.
  - **Total histamine control:** An aliquot of cells lysed (e.g., by boiling or detergent like Triton X-100) to determine the total cellular histamine content.
- Incubate the cells at 37°C for a specific period (typically 15-30 minutes for degranulation, longer for *de novo* synthesis studies).

3. Termination and Sample Processing

- Terminate the release reaction by adding ice-cold buffer or placing the tubes/plates on ice.
- Centrifuge the samples to separate the cell-free supernatant from the cell pellet.
- Carefully collect the supernatant, which contains the released histamine, and store at -20°C until assay.

4. Histamine Quantification and Analysis

- Measure the histamine concentration in the collected supernatants using a sensitive method, most commonly an **ELISA** or **fluorometric assay**.
- Calculate the percentage of histamine release using the following formula:

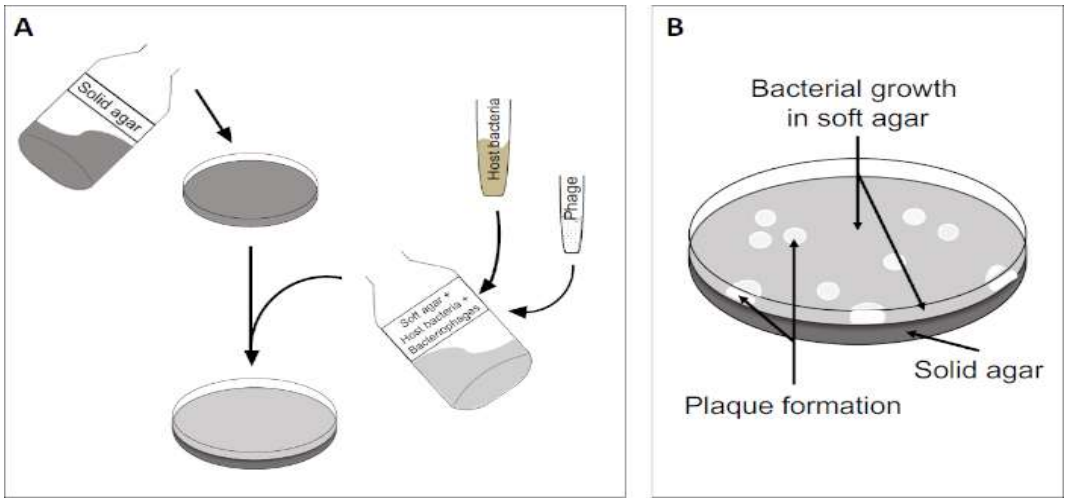
$$\% \text{ Histamine Release} = \frac{\text{Histamine in supernatant}}{\text{Total cellular histamine}} \times 100$$

- Compare the percentage release in treated samples to that of controls to determine the effect of the test compound on histamine release using statistical analysis.

b. Plaque Formation Colony Test

1. **Prepare Host Cells**: Grow a confluent monolayer of susceptible host cells in a multi-well plate (for animal viruses) or an actively growing broth culture of bacteria (for bacteriophages).
2. **Serial Dilution**: Prepare a series of 10-fold dilutions of the virus/phage stock in a sterile diluent (e.g., tryptic soy broth or cell culture medium). Use a fresh pipette tip for each dilution to ensure accuracy and prevent cross-contamination.
3. **Infection/Adsorption**:
   - **For bacteriophages**: Add a small volume of the diluted phage sample to tubes containing a measured amount of the host bacterial culture. Briefly incubate at room temperature or 37°C to allow the phages to adsorb (attach) to the bacteria.
   - **For animal viruses**: Wash the cell monolayer and add the diluted virus inoculum to each well. Gently rock the plates to ensure even coverage, and incubate for a short period (e.g., 1 hour) to allow the virus to adsorb to the cells.
4. **Overlay with Semi-Solid Medium**:
   - **For bacteriophages (Double Agar Layer Method)**: Add the infected bacteria to molten soft agar (kept at ~45°C in a water bath) and mix gently. Quickly pour the mixture over a pre-poured, solidified "bottom agar" plate.
   - **For animal viruses**: Aspirate the inoculum, and carefully add a semi-solid overlay medium (e.g., agarose mixed with culture medium) to the wells. The overlay should be warm enough to be liquid but not so hot as to kill the cells.
5. **Incubation**: Allow the overlay to solidify, then invert the plates (to prevent condensation from dripping onto the agar) and incubate at the appropriate temperature (e.g., 30°C for phages, 37°C for animal viruses) for a period of time sufficient for plaque development (typically 6 hours to several days, depending on the agent).
6. **Fixation and Staining**: Once plaques are visible, the cell monolayer can be fixed (e.g., with formaldehyde or ethanol) and stained (e.g., with crystal violet or neutral red) to enhance the contrast between the living cells (which take up the stain) and the clear plaques (zones of cell lysis).
7. **Count Plaques and Calculate Titer**: Count the number of discrete plaques on plates with an ideal number of plaques (typically between 5 and 100 plaques for optimal accuracy). The original virus concentration (titer) is calculated using the following formula:

$$\text{Titer (PFU/mL)} = \frac{\text{Number of plaques}}{\text{Dilution Factor} \times \text{Volume plated}}$$



c. Antianaphylatic Activity:-

The Wistar rats of either sex were injected intraperitoneally with 0.2 mL, 10% egg albumin, 0.2 mL of bordetella pertusis vaccine on day 1, 3, and 5. After 21 days of first immunization, blood was collected from orbital plexus under light ether anesthesia. The blood was allowed to clot and serum was separated by centrifugation at 1 500 rpm. The separated serum was stored at 20 °C until it was used for the experiment.

Rats (6 per group) were divided into five groups. The first 3 groups received oral doses of 10, 25 and 50 mg/kg of the compound 1. The 4th and 5th groups were treated orally with indomethacin (10 mg/kg) as a reference drug and saline (10 mL/kg) as control, respectively. The animals were dosed for seven consecutive days.

2h after last dose of drug administration (on 7^{th} day), rats were passively sensitized into left hind paw with 0.1 mL of the undiluted serum. The contralateral paw received an equal volume of saline. 24 h after sensitization, the rats were challenged in the left hind paw with 10 mg of egg albumin in 0.1 mL saline. The hind paw volume was measured after 30 min by volume displacement method using mercury column plethysmometer. The % inhibition was calculated by using the formula: (C-T/C)×100.

d. Broncho-alveolar levage and lung histology in mice

Albino mice of either sex were divided into seven groups containing five animals each (n=5).All animals were sensitized by an intraperitonial injection of 1ml alum precipitate antigen containing 20µg of ova albumin and 8mg of alum suspended in 0.9% of sodium chloride solution. A booster injection of this alum-albumin mixture was administered 7 days later. Non sensitized animal were injected with alum only (Group I). Seven days after (15 days) the second injection, animal was exposed to aerosolized oval albumin (1%) for 30 min. Animals belonging to groups II, III, IV, V, VI received drug combination T1, T2, T3, T4, T5. Animals of group VII, as positive control group received dexamethasone (0.27mg/kg p.o.) 5 hr before antigen challenge. The mice were sacrificed at the end of study (24hr after sensitization) and trachea catheter was inserted in trachea.

7. **Result & Discussion**

Phytochemical screening:-

Table No.7.1 shows the result Phytochemical Present in Aqueous Extract of tubers of Amorphophallus paeoniifolius

Se. No.	Phytoconstituents	AE
1.	Sterols	+
2.	Saponins	-
3.	Tannins	+ + +
4.	Flavonoids	+ +
5.	Carbohydrates	+ +
6.	Starch	+ +
7.	Protein and amino acids	+ +
8.	Alkaloids	+
9.	Volatile oil	-
10.	Fixed oil / Fat	+
11.	Coumarins	+
12.	Triterpenoids	+
13.	Glycosides	-
14	Vitamins	+

a) TLC profile of Aqueous extract

TLC profile of the aqueous extract showed three spots after acid spray and heated up to 110^0C , where as single spot was seen, when observed under UV light at 365 nm, before acid spray using Toluene : Ethyl acetate (93:07) as mobile phase. The colour of the spots and R_f values are recorded in the following table.

Table No.7.2 shows the result of TLC Profile of Aqueous Extract of tubers of *Amorphophallus paeoniifolius*

Extract	Adsorbent	Solvent system	Observation / R_f values	
			Under UV light 365 nm	After acid spray and heated at 110°C
70% Hydro alcoholic extract	Silica Gel $60GF_{254}$ Precoated sheets	Toluene**:** Ethyl acetate (93:07)	1 spot **:** 0.27 (deep blue)	3 spots **:** 0.13, 0.27, 0.92

Alteration of Histamine Release from MAST Cell

Table No.:- 7.3 shows the result Histamine Release from MAST Cell of Aqueous Extract of tubers of *Amorphophallus paeoniifolius*

Se. No.	Treatment	Amount / Volume of Histamine Release (microliter)
1	Normal Saline / Buffer	6058 ±12.069
2	Std Dexamethasone	3343 ±18.682**
3	Test Group I (100 mg/kg b.w.)	4060 ±57.879**
4	Test Group II (150 mg/kg b.w.)	3530 ±25.495**
5	Tets Group III (200 mg/kg b.w.)	3520 ±46.368**

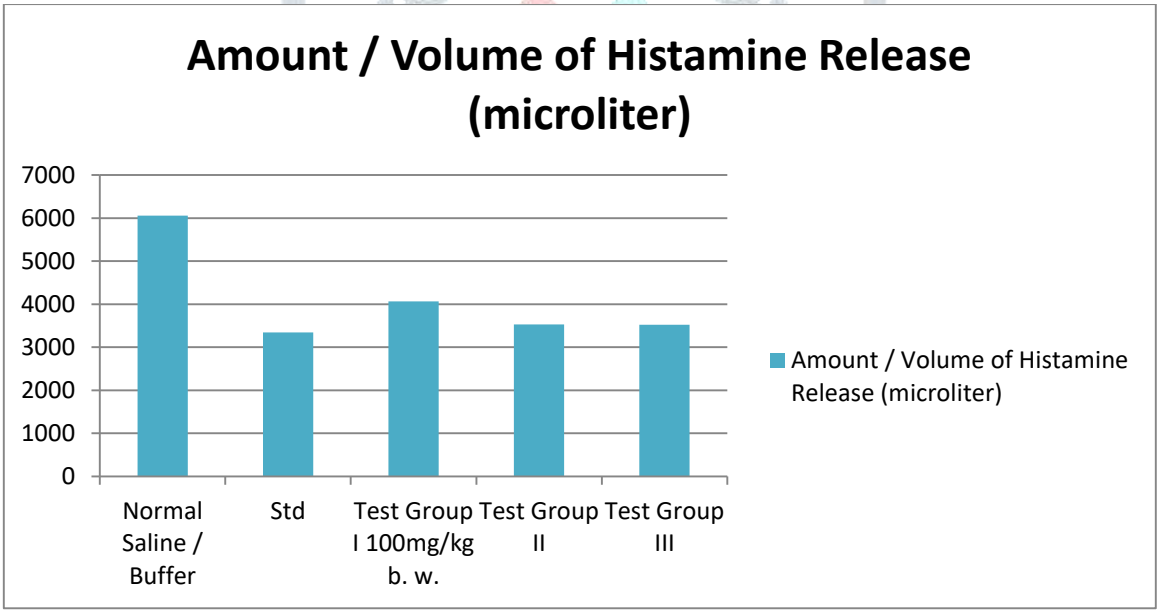


Figure:- 7.1 Histamine Release from MAST Cell of Aqueous Extract of tubers of Amorphophallus paeoniifolius

Plaque formation Colony Test

Table No 7.4 Plaque formation Colony of Aqueous Extract of tubers of Amorphophallus paeoniifolius

Se. No.	Treatment	No. of Colonies	
		Total Colonies	Medium & Big Colonies
1	Normal Saline / Buffer	812±25.495	403±12.455
2	Std Dexamethasone	485±16.434**	246±10.434**
3	Test Group I (100 mg/kg b.w.)	629±25.235	312±20.235
4	Test Group II (150 mg/kg b.w.)	526±12.495**	266±12.905**
5	Tets Group III (200 mg/kg b.w.)	525±18.495**	258±9.475**

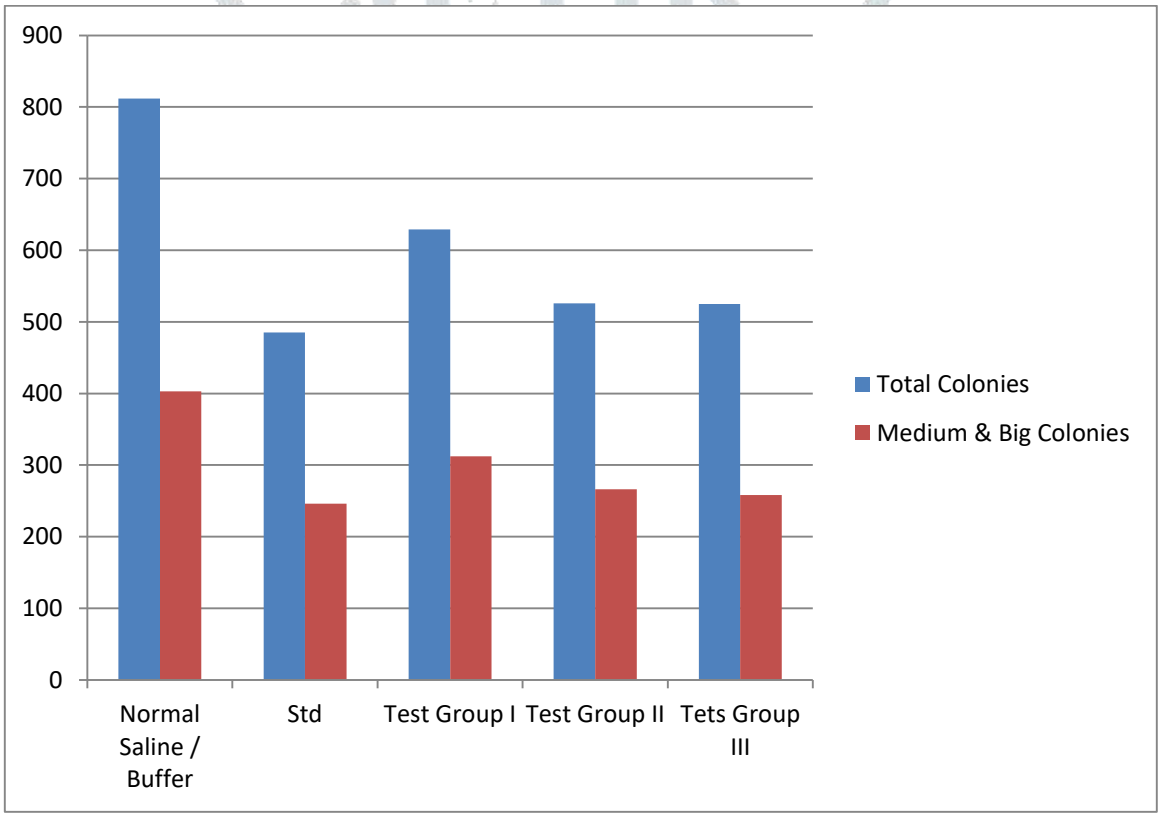


Figure:- 7.2 Histamine Release from MAST Cell of Aqueous Extract of tubers of Amorphophallus paeoniifolius

Antianaphylatic Activity:-

Table 7.5:- Antianaphylatic activity (Paw Edaema) of Aqueous Extract of tubers of Amorphophallus paeoniifolius

Groups	Treatment	Difference in Paw Edaema			
		1 hr	2 hr	3 hr	4 hr
I	Normal Saline/ Buffer	0.63 ±0.05	0.69 ± 0.02	0.72 ± 0.05	0.77 ± 0.04
II	Std (Dexamethasone)	0.16 ±0.06**	0.12 ±0.03**	0.09 ±0.01**	0.11 ±0.05**
III	Test Group I (100 mg/kg b.w.)	0.61 ±0.04	0.26 ±0.05**	0.08 ±0.05**	0.02 ±0.01**
IV	Test Group II (150 mg/kg b.w.)	0.29 ±0.07**	0.11 ±0.05**	0.09 ±0.02**	0.03 ±0.01**
V	Tets Group III (200 mg/kg b.w.)	0.20 ±0.05**	0.24 ±0.11**	0.28 ±0.05*	0.10 ±0.02**

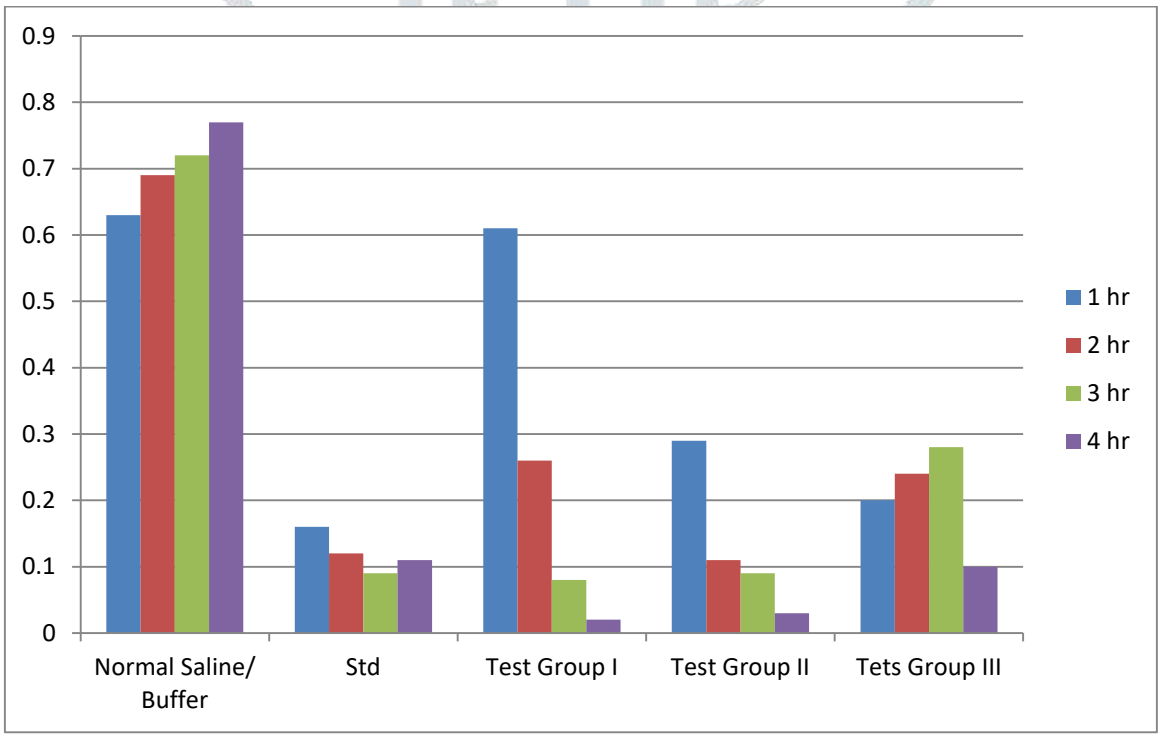


Figure:- 7.3 Antianaphylatic activity (Paw Edaema) of Aqueous Extract of tubers of *Amorphophallus paeoniifolius*

Table 7.6: Broncho alveolar levage and lung histology in mice

	Group	Total count / microliter	PMN	LYM	Mon
I	Normal Saline/ Buffer	6058 ±12.069	55.200 ±0.8602	60.200 ±0.7348	9.200 ±0.3742
II	Std (Dexamethasone)	3843 ±18.682**	40.200 ±0.7348**	40.000	2.400 ±0.2449**

				±0.7071**	
III	Test Group I (100 mg/kg b.w.)	4060 ±57.879**	49.000 ±1.000**	43.800 ±1.241**	2.400 ± .2449**
IV	Test Group II (150 mg/kg b.w.)	3530 ±25.495**	42.400 ±0.9274**	49.600 ±2.205**	2.600 ±0.2449**
V	Tets Group III (200 mg/kg b.w.)	3520 ±46.368**	45.000 ± 1.000**	48.600 ±1.166**	2.600 ±0.2449**

Table 7.6 (Countinue…): Broncho alveolar levage and lung histology in mice

	Group	**Eos**	**Bas**	**Macrophages /HPF**	**Epithelial cells**
I	Normal Saline/ Buffer	7.000 ±0.3162	4.800 ±0.3742	6.200 ±0.3742	6.600 ±0.2449**
II	Std (Dexamethasone)	2.000 ± 0.3162**	1.400 ±0.2449**	3.400 ±0.2449**	2.600 ±0.2449*
III	Test Group I (100 mg/kg b.w.)	3.000 ±0.3162**	1.400 ±0.2449**	4.600 ±0.2449**	3.000 ±0.3162**
IV	Test Group II (150 mg/kg b.w.)	2.200 ±0.3162**	1.700 ±0.2000**	4.600 ±0.2449**	2.200 ±0.2000**
V	Tets Group III (200 mg/kg b.w.)	2.800 ±0.2000**	2.000 ±0.3162**	4.000 ±0.3162**	2.000 ±0.3162**

PMN : Polymorpho nuclear leukocyte

LYM : Lymphocyte

Mon : Monocytes

Eos : Eosinophils

Bas : Basophils

8. SUMMARY AND CONCLUSION

Phytochemical analysis of the extract revealed that extract contains the Carbohydrates, flavanoid, saponin, tannin/polyphenol and fat, phyto-sterols and tri-terpenoids. From the acute toxicity study of the hydro-alcoholic extract was establish to be non-lethal up to doses up to 2000 mg/kg body weight of the animals.

Immuno-modulatory action was screened by In-Vitro (Alteration of Histamine Release from MAST cell, Plaque formation colony test) and In-Vivo (Antianaphylatic Activity (Scholtz-Dale Reaction), Broncho alveolar levage and lung histology).

The Aqueous Extract of tubers of *Amorphophallus paeoniifolius* exhibited pronounced Immuno-modulatory effect in a dose-dependent manner following oral pre-treatment on Plaque formation colony test compare with the positive standard.

Though the action is important but relatively it is smaller than the Dexamethasone 2 mg/kg.

In conclusion, aqueous extract of tubers of *Amorphophallus paeoniifolius* possessed significant anti- Immuno-modulatory activity against different models. The extract has potential effect on the reduction of Anti-histaminics action than the other effects. The above effects of it may also be due to the presence of tannins and flavanoids in the extract.

9. REFERENCES

1. Thomas SC. Medicinal plants culture, utilization and phytopharmacology, Li. United States: CRC Press; 1995. p. 119-54.

2. Evans WC, Trease GE. Trease and Evans pharmacognosy. 15th ed. China: W.B. Saunders; 2002. p. 3. Mukerjee PK. Quality control of herbal drugs. 1st ed. New Delhi: Business Horizons Publication; 2002. p. 2-24.

4. Baker JT, Borris RP, Carte B, Cordell GA, Soejarto DD. Natural product drug discovery and development: New perspective on international collaboration. J Natl Prod 1995;58:1325-57.

5. Trichopoulos D, Willett WC. Nutrition and cancer. Cancer Causes Cont. 1996;7:3-4.

6. Block G. The data support a role for antioxidants in reducing cancer risk. Nutr Rev 1992;50:207-13.

7. Rogers AE, Zeisel SH, Groopman J. Diet and carcinogenesis. Carcinogenesis 1993;14:2205-17.

8. Dorai T, Aggarwal BB. Role of chemopreventive agents in cancer therapy. Cancer Lett 2004;215:129-40.