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Antianaphylactic Activity of Hydro Alcoholic Extract of *Phyllanthus Virgatus* Leaves

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Abstract: Allergic disease, such as anaphylaxis is a hypersensitivity reaction initiated by immunological mechanisms. The present investigation was undertaken to evaluate the effect of hydroalcoholic extract of leaves of *Phyllanthus Virgatus* on experimental anaphylactic conditions. The antianaphylactic activity of alcoholic extract of *Phyllanthus Virgatus* with two different doses of 100mg/kg and 200mg/kg was studied by using different animal models such as effect on passive cutaneous anaphylaxis using rat, by measuring leakage of Evans blue dye in skin, passive paw anaphylaxis using rat by measuring the paw volume by plethysmometer and allergic pleurisy model by measuring the total and differential leukocyte count. Treatment with alcoholic extract of *showed* a dose dependent beneficial effect on a dose-dependent beneficial effect was observed on leakage of Evans blue dye in skin challenged with antigen. Anaphylactic activity of alcoholic extract of *Phyllanthus Virgatus* may be possibly due to its membrane stabilizing potential, inhibition of antigen induced histamine release, and inhibition of release of various inflammatory mediators.

Key words: *Phyllanthus Virgatus*, antianaphylactic, allergic pleurisy model, Evans blue dye in skin, passive paw anaphylaxis.

Introduction

Allergic disorders are in rise every year and are stated as an endemic disease of the 21st century. Some of the allergic disorders, which may be caused by an allergen originating from immune system, environment, and by genes, are asthma, eczema, hay fever, anaphylaxis, autoimmune disorders etc [1].

Anaphylaxis is an acute multi-system severe type I hypersensitive reaction. Anaphylactic reactions requiring hospital treatment appear to be increasing along with the years. Anaphylaxis refers to a rapidly developing and serious allergic reaction that affects a number of different areas of the body at one time. Severe anaphylactic reactions can be fatal. Most people experience allergy symptoms only as a minor annoyance. However, a small number of people are susceptible to a reaction that can lead to shock or even death.

Anaphylaxis is often triggered by substances that are injected or ingested and thereby gain access into the blood stream. An explosive reaction involving the skin, lungs, nose, throat, and gastrointestinal tract can then result. Although severe cases of anaphylaxis can occur within seconds or minutes of exposure and be fatal if untreated, many reactions are milder and can be ended with prompt medical therapy. Anaphylactic shock is anaphylaxis associated with systemic vasodilatation which results in lowering of blood pressure. It is also associated with severe bronchoconstriction.

The rate of anaphylaxis appears to be increasing. The rate in the 1980s was 21 per 100,000 per year while in the 1990s it had increased to 50 per 100,000 per year. The risk is greatest in young people and females.

The trigger in the young is usually food related while in adults, medications and insect venoms are more common causes.

The present investigation was undertaken to evaluate the effect of hydroalcoholic extract of roots of *Phyllanthus Virgatus* on experimental anaphylactic conditions. The antianaphylactic activity of alcoholic extract of *Phyllanthus Virgatus* with two different doses of 100mg/kg and 200mg/kg was studied by using different animal models such as effect on passive cutaneous anaphylaxis using rat, by measuring leakage of Evans blue dye in skin, passive paw anaphylaxis using rat by measuring the paw volume by plethysmometer and allergic pleurisy model by measuring the total and differential leukocyte count.

Materials and Methods

Plant material:

The dried leaves of *Phyllanthus Virgatus* in the present study were plant collected from the Sri Venkateswara University, Tirupathi and identified and authenticated taxonomically by Dr.K.Madhayachetty.the plant was chopped into small pieces and dried in tray drier under controlled conditions

Animals:

Swiss albino mice (20-25g) and Wistar rats (150-200) were procured and.The animals were maintained under standard laboratory conditions i.e., 12hours dark and 12 hours light cycle at a temperature of $27\pm 1^{\circ}\text{C}$. The humidity is 60%. Pellet diets and water was available *ad libitum*.

Chemicals Required

Freund's complete adjuvant solution, Compound 48/80 (N-methyl-p-methoxyphenethylamine & formaldehyde.) Egg albumin, Formaldehyde, Phosphate buffer saline containing EDTA, Evans blue

Plant Extraction Procedure:

The leaves were dried under shade and coarsely powdered and passed through 40 mesh sieve. The powdered material (500g) was extracted with ethanol using Soxhlet apparatus for about 48 h. The extracts were filtered and concentrated in vacuum under reduced pressure using rotary flash evaporator and dried in the dessicator

Methods

The following methods were used for the present study.

Anaphylaxis Screening Model

1. Passive cutaneous anaphylaxis model
2. Passive paw anaphylaxis model
3. Allergic pleurisy model

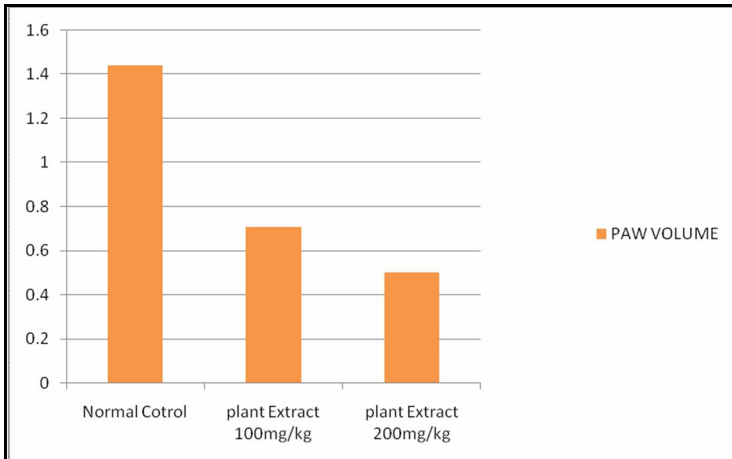
Statistical Analysis: All the values were expressed as Mean \pm SEM. All the data was analysed using one-way analysis (ANOVA) followed by Dunnet's test. $P < 0.05$ was considered significant.

Results

Table no: 1 Effect of Alcoholic Extract of *Phyllanthus Virgatus* on Passive cutaneous anaphylaxis

S.No	Group	Amount of Dye Leaked
1	Normal Control	1.123 \pm 0.02575
2	Plant Extract 100mg/kg	0.8083 \pm 0.01822**
3	Plant Extract 200mg/kg	0.3983 \pm 0.01639**

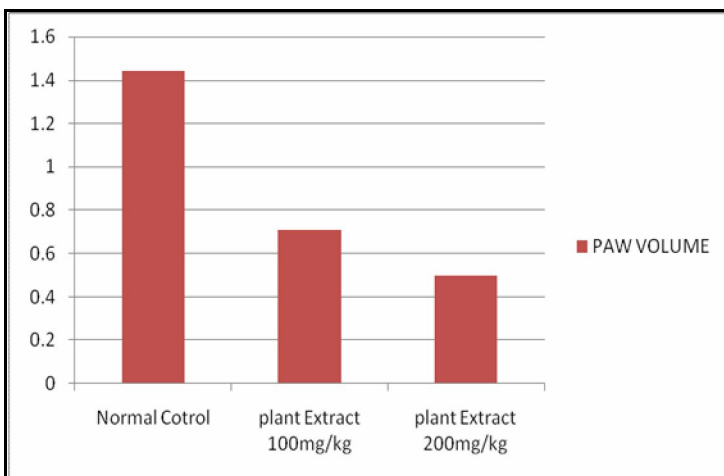
Values (ng/ml) are expressed am mean \pm SEM (n=6). Value comparisons were made between Normal control Vs Group II, III, (* $p < 0.01$). ** - value is highly significant



Phyllanthus Virgatus produced a significant dose dependent decrease in the amount of Evans blue dye leaked at a site. Standard drug also produces significant decrease in the amount of Evans blue dye leaked at site.

Table no:2. Effect of Alcoholic Extract of *Phyllanthus Virgatus* on Passive paw anaphylaxis.

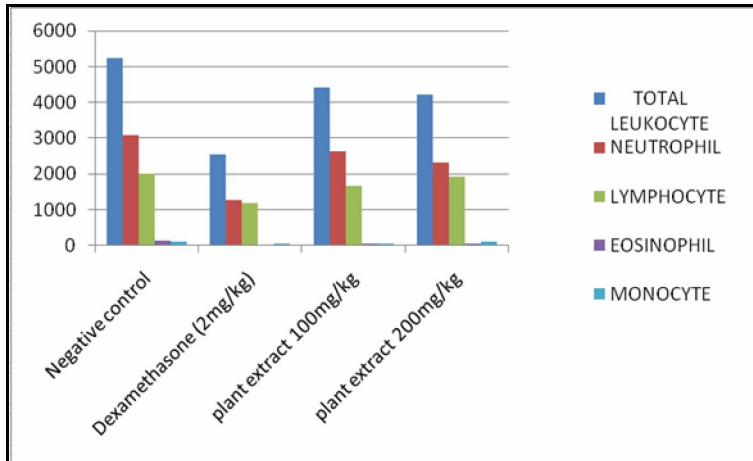
S.NO	GROUP	PAW VOLUME
1	Normal Cotrol	1.440±0.02033
2	Plant Extract 100mg/kg	0.7083±0.01078**
3	Plant Extract 200mg/kg	0.4983±0.01515**



Phyllanthus Virgatus produced a significant dose dependent decrease in the paw volume induced by antiserum. Standard drug also produces significant decrease in the paw volume induced by antiserum.

Table no: 3. Effect of Alcoholic Extract of *Phyllanthus Virgatus* cell counts in allergic pleurisy

Group	Total Leukocyte	Neutrophil	Lymphocyte	Eosinophil	Monocyte
Negative control	5225±383.78	3072.75±264.3	2002±108.44	145±27.54	111.75±5.63
Dexamethasone (2mg/kg)	2550±413.32*	1273.5±202.3*	1182.25±187.9*	32.75±9.3*	61.5±20.36*
Plant extract 100mg/kg	4425±968.14*	2630.5±761.2*	1679.5±219.2*	51.75±8.87*	63.25±12.92*
Plant extract 200mg/kg	4223±259.41*	2319.25±277.2	1938±160.67*	67±14.29*	100.75±15.96*



Accumulation of total and differential leukocytes was observed in sensitized as compared to the control group. Dexamethasone pre-treatment significantly inhibited the influx of total and differential leukocytes as compared to the sensitized group. The oral pre-treatment with plant extracts markedly inhibited the eosinophil accumulation as compared to the control group and this inhibition was selective for decrease in the number of eosinophil.

Conclusion

The result of the study showed that the alcoholic extract of *Phyllanthus Virgatus* is found to be effective in various experimental models of anaphylaxis. It showed significant reduction in paw volume induced by various inflammatory mediators in a dose dependent manner. The reduction in eosinophil infiltration in egg albumin sensitized mice shows antianaphylactic activity by the treatment of alcoholic extract of *Phyllanthus Virgatus*

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