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Anti-Asthmatic, Anti-Inflammatory and Antioxidant Activity of Ethanolic and Hot Water Extract of the Rhizomes of the Plant *Alpinia calcarata*

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KEYWORDS

Alpinia calcarata, anti-asthmatic, anti-inflammatory, anti-oxidant.

A B S T R A C T

The aim of the paper is to evaluate the anti-asthmatic, anti-inflammatory and anti-oxidant activity of the plant *Alpinia calcarata* rhizomes. *In vivo* anti-asthamtic studies are done by histamine induced bronchoconstriction in guinea pigs and also done by milk induced leukocytosis and eosinophilia. The anti-inflammatory studies are done by protein denaturation and rabbit red blood cell membrane stabilization method. Anti-oxidant studies are done by hydrogen peroxide scavenging and reducing power assay. The ethanolic and hot water extract of the plant rhizomes are used for the study.Primary phytochemical screening of the ethanolic extract plant revealed the presence of carbohydrate, reducing sugar, alkaloid, flavonoids and phenolic compounds. The hot water extract of the plant rhizomes revealed the presence the alkaloid, flavonoid, carbohydrate and phenolic compound. *Alpinia calcarata* Roscoe (Family: Zingiberaceae), it is a rhizomatous perennialherb, which is commonly used in the traditional medicinal systems in Sri Lanka. *Alpinia calcarata* is cultivated in tropical countries, including india, Srilanka and Malaysia. Experimentally, rhizomes of *Alpinia calcarata* are shown to possess antibacterial, antifungal, anthelmintic, antinociceptive, anti-inflammatory, antioxidant, aphrodisiac, gastro protective, and antidiabetic activities.

Introduction

This research article emphasizes on traditionally used clinically potential plant *Alpinia calcarata* roscoe. *Alpinia calcarata* Roscoe (Zingiberaceae) is a rhizomatous plant widely used as systemic medicinal sources in Sri Lanka. The mature rhizomes are branched and dense with a light to dark brown color. The leaf of the plant is simple, alternative, 25-32 cm long, 2.5-5 cm broad. The flowers are irregular, bisexual and pendanculate. Terminal densed flowers are found in panicles 8.5cm long. *A. calcarata* is cultivated in tropical countries including India, Sri Lanka and Malaysia.

Plant Description

Rhizomatous perennial herb with a nontuberous rootstock, stems slender, about 75 cm tall; leaves simple, alternate, 25-32 cm long and 2.5-5 broad, lanceolate, acuminate, long-pointed, glabrous on both surfaces and shining on the upper surface, scantily hairy along the margin, petioles sheathing; flowers pinkish white, irregular, bisexual, in pendunculate, terminal, dense flowered panicles 8.5 cm long, two flowers together at each node, one opening earlier than the other, each bearing a pair ofbracteoles, the inner one smaller than the outer, bracteoles oblong, papery white, each flower about 4cm long, pedicels short, hairy; sepals 3, fused into a campanulate tube lcm long, pubescent outside, glabrous inside, apices rounded; petals 3, fused at base but segments free tinged with pink, segments oblong-spathulate, pubescent outside, lateral narrow; staminodes 3, fused at base with the stamen into a tube adnate to corolla, two basal staminodes reduced to minute filaments, the larger one petaloid, 3 cm by 2.3 cm ovale, yellow with vinous red streaks, emarginated, apex frilled and darker, glabrous and shining on both surfaces; stamen J, anther tubular, style passing through, filament flat, 1.5 cm long, anther 0.8 cm long, style 3.5 cm long, tinged pink, hairy towards the apex, stigma swollen; ovary inferior, 3 mm long, strongly pubescent, 3-locular with ovules in each loculus on a central axis, capsules not seen.This study evaluate the anti-asthmatic,anti-inflammatory and anti-oxidant activity of the plant.

Materials and Methods

Plant material: The plant was collected from pathanamthitta district and plant material was authenticated by Dr kavitha department of botany, specimen no: 106

Preparation of extract: Rhizomes of the plant were air dehydrated and crinkled into powdered form. The crushed powder was extracted with 70% ethanolin soxhlet apparatus. The ethanolic extract was stored in 5^0c to get viscous mass. Also hot water extract was also prepared. Fresh *Alpinia calcarata* rhizomes were cut in to small pieces and air dried for 5-6 days and were boiled for 4 hour with distilled water.

Experimental animals: Guinea pig of either sex weighing 180-350 gm and albino mice of either sex 25-40 gm and albino rats rats weighing 120-180 are used for the study

Acute toxicity studies: Acute toxicity studies were implemented on Albino rats of either sex selected by sampling technique. The animals were fasted for 4hrs with free access to water only. The ethanolic extract of *Alpinia calcarata* was administered orally with varying doses. The mortality was experimented for three days. If mortality was observed in 2/3 or 3/3 of animals, then dose administered was considered as a toxic dose. However, if the mortality was observed in one rat out of three animals then the same dose was repetitive to confirm the toxic effect. If mortality was not observed, then procedure repeated with higher doses.

Anti-inflammatory Activity

Protein-denaturation method

A solution of 0.2% w/v of BSA was prepared in a Tris Buffer Saline and pH was adjusted to 6.8 using glacial acetic acid .Test drug of 100µg/ml conc. was prepared using ethanol as solvent. 50µl (0.05ml) of each extract was transferred to test tubes using 1ml micropipette. 5ml of 0.2% w/v BSA was added to the entire above test tubes. The control consists of 5ml of 0.2% w/v BSA solution with 50µl of alcohol. The test tubes

were heated at 72ºC for 5 minutes and then cooled for 10 minutes. The absorbance of these solutions was determined by using a UV-VIS Double beam spectrophotometer (ELICO SL 244) at a wavelength of 660nm. Each experiment was carried out in triplicate and the mean absorbance was recorded. The percentage of inhibition of precipitation was determined on a percentage basis relative to control using the formula.

Percentage of inhibition of denaturation=

$$\frac{absorbance\ of\ control - absorbance\ of\ sample}{absorbance\ of\ control} \times 100$$

The Rabbit Red Blood Cells (RRBC) Membrane Stabilization Method

Preparation of red blood cells suspension (RBC suspension)

The fresh whole blood rabbit blood (5 ml) was collected from the marginal ear vein to syringe containing sodium citrate to prevent clotting .The tubes were centrifuged at 3000 rpm for 10 minutes and were washed three times with equal volume of normal saline .the volume of the blood was measured and reconstituted as 10% v/v suspension with normal saline .

Membrane Stabilization Test By Hyotencicity-Induced Haemolysis

The reaction mixture consists of 1 ml of test sample of different concentration (25,50,100,200,400) in normal saline and 0.5 ml of 10% RBC suspension ,1 ml of 0.2 M phosphate buffer ,1 ml hypo saline were incubated at 37 c for 30 minutes and centrifuged at 3000 rpm for 20 minutes and the haemoglobin content of the supernatant solution was estimated spectrometrically at 560 nm. Diclofenac sodium was used as standard and a control was prepared without extract the percentage of RBC haemolysis and membrane stabilization or protection was calculated by using the formula

% Haemolysis=

$$\left(\frac{optical\ density\ of\ test\ sample}{optical\ density\ of\ control}\right) \times 100$$

$$\%PROTECTION = 100 - \%\ haemolysis$$

Antioxidant Activity

Hydrogen-Peroxide Scavenging Assay

A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). Extracts (100 µg/mL) in distilled water were added to a hydrogen peroxide solution (0.6 mL, 40mM). Absorbance of hydrogen peroxide at 230 nm was determined 10 minutes later against a blank solution containing the phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging of both PLANT extracts and standard compounds were calculated:

% Scavenged [H2O2] =

[(AC – AS)/AC] x 100

Where AC is the absorbance of the control and AS is the absorbance in the presence of the sample

Reducing Power Assay

The reducing power of the extract was determined by the method .1 ml of the extract solution (25,50,100,200 and 400) was mixed with 2.5 mlphosphate buffer (0.2 M ,PH 6.6) AND 2.5 ML of potassium ferricyanide (k2 fe (cn)6) 10 g/l. then the mixture was incubated at 50 c for 20 minutes.A portion (2.5 ml) of trichloro

acetic acid (15%) was added to the mixture, which was then centrifuged at 3000 rpm for 10 minutes. Finally 2.5 ml of the supernatant solution was mixed with 2.5 ml of distilled water a\nd 0.5 ml ferric chloride (Fecl3, 0.1%) and absorbance was measured at 700 nm in uv visible spectrophoptometer. The experiments were performed in triplicate. Increased absorbance of the reaction mixture indicates stronger reducing power.

Invivo anti ashmatic study

Milk induced leukocytosis and eosinophilia

Mice were divided into five groups with six in each group. Blood samples were collected from retro-orbital plexus. Group I served as control and received carboxy methyl cellulose solution, groups II-IV received EAPL at (100-150 mg/kg i.p.), group V received dexamethasone at 50 mg/kg i.p. All the groups injected boiled and cooled milk (4 mL/kg, s.c.) 30 min after treatments. Total leukocyte and eosinophile count was done in each group before administration of test compound and 24 h after milk injection. Difference in total leukocytes and eosinophile count before and after 24 h drug administration was calculated

Histamine aerosol induced broncho-constriction in guinea pigs (in-vivo)

Histamine was dissolved in distilled water to prepare 0.2% w/v solution. Experimentally bronchial asthma was induced in guinea pigs by exposing histamine aerosol by an ultra-sound nebulizer in an aerosol chamber (30 x 15 x 15cm) made of Perspex glass. The required time for appearance of preconvulsive dyspnoea produced by the histamine was noted for each animal. Each animal was placed in the histamine chamber and exposed to 0.2 % histamine aerosol. The preconvulsion time (PCT), i.e. the time of aerosol exposure to the start of dyspnoea leading to the appearance of convulsion, was noted. As quickly as the preconvulsion dyspnoea (PCD) was recorded, the animals were removed from the chamber and positioned in fresh air for recover. This time for preconvulsive dyspnoea was recorded as basal value. Guinea pigs were then allowed to recover from dyspnoea for 2 days. After that, the animals were allotted to four different groups of 4-5 animals per group. Animals in group 1 served as control and received distilled water. The animals of group 2 and 3 were given, by oral intubation, 200 and 500mg/kg of the plant extract, respectively, while group 4 received the standard drug - Chlorpheniramine maleate, intraperitoneally. After receiving the drugs, all the animals were again exposed to histamine aerosol in the chamber, one hour, four hours and 24 hrs, to determine pre convulsive time (PCT).

Results and Discussion

Anti-inflammatory

Inhibition of protein denaturation

Denaturation of proteins is a well documented cause of inflammation. As part of the investigation on the mechanism of the anti-inflammation activity, ability of different solvent plant extract protein denaturation was studied. It was effective in inhibiting heat induced protein denaturation. Diclofenac sodium a standard anti-inflammatory agent posses maximum % inhibition 91.6 at concentration 400 µg/ml. The ethanolic and hot water extract of the plant *Alpinia calcarata* rhizomes posses significant % inhibition activity at concentration 200µg/ml and 400µg/ml.So the plant posses significant anti-inflammatory activity at that concentration

Table.1 Ethanolic extract of the plant *Alpinia calcarata* rhizomes

Sl no	Concentration	Absorbanc	% inhibition
1	25	1.28±0.05	14
2	50	0.578±0.03	61.6
3	100	0.382±0.002	74.63
4	200	0.189±0.01	87.4
5	400	0.172±0.002	88.57

Table.2 Hot water extract of the plant *Alpinia calcarata* rhizomes

Sl no	Concentration	absorbance	% inhibition
1	25	1.38±0.04	8.36
2	50	0.656±0.03	56.3
3	100	0.568±0.001	62.2
4	200	0.289±0.002	80.2
5	400	0.188±0.001	87.5

Table.3 Standard (diclofenac sodium)

Sl no	Concentration	absorbance	% inhibition
1	25	0.854±0.02	43.2
2	50	0.578±0.002	61.6
3	100	0.434±.0.001	70.8
4	200	0.289±0.002	80.8
5	400	0.133±0.121	91.6

Fig.1 Inhibition of protein denaturation

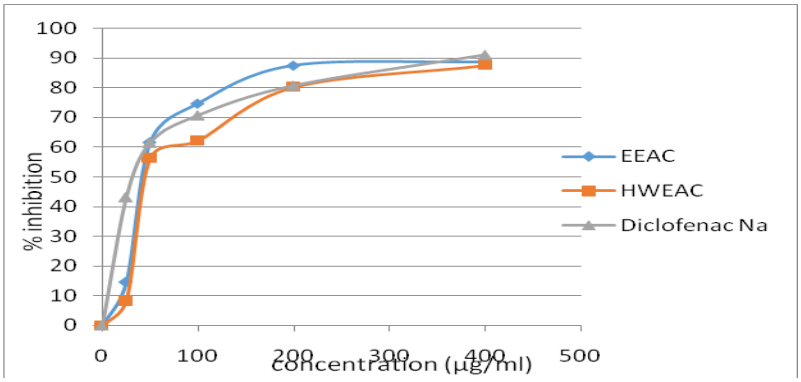


Table.4 Ethanolic extract of the plant *Alpinia calcarata* rhizomes

Sl no	Concentration	Absorbance	% inhibition
1	25	0.632±0.0005	17.16
2	50	0.539±0.00052	29.5
3	100	0.474±0.0056	38.04
4	200	0.414±0.0005	46
5	400	0.357±0.00056	53.3

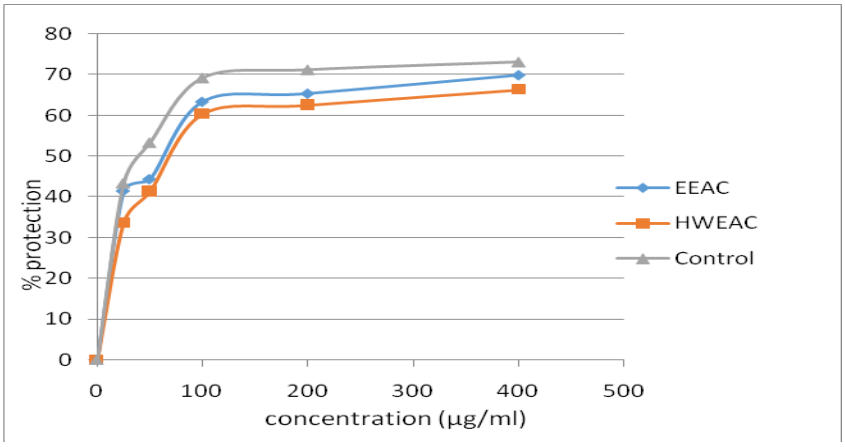
Table.5 Hot water extract of the plant *Alpinia calcarata* rhizome

Sl no	Concentration	Absorbance	% inhibition
1	25	0.648±0.004	15.09
2	50	0.554±0.0003	27.4
3	100	0.488±0.002	36.05
4	200	0.438±0.003	42.6
5	400	0.386±0.0023	49

Table.6 Standard (hydrogen peroxide)

Sl no	Concentration	Absorbance	% inhibition
1	25	0.568±0.003	25.5
2	50	0.489±0.002	36.9
3	100	0.388±0.004	49.16
4	200	0.355±0.001	53.5
5	400	0.312±0.004	58.4

Fig.2 Membrane stabilization method



Rabbit red blood cell membrane stabilization method

% protection of each samples are calculated. Standard drug diclofenac sodium show significant % protection. The hot water and ethanolic extract of the plant at concentration 200 and 400 µg/ml show significant % protection. The plant *Alpinia calcarata* rhizomes posses significant anti-inflammatory activity

Antioxidant activity

Hydrogen peroxide scavenging activity

The scavenging ability of hot water and ethanol extracts of *Alpinia calcarata* on hydrogen peroxide was studied by using ascorbic acid as standard. The rhizomes of the plant extracts were capable of scavenging hydrogen peroxide in an amount dependent manner. 100 µg/ml of hot water and ethanol extracts of the plant exhibited 36.05% and 38.04% scavenging activity on hydrogen peroxide. On the other hand, using the same amounts standard drug exhibited 49.16% hydrogen peroxide scavenging activity. Also plant extract of ethanol and hot water at concentration 400 µg/ml posses 53 and 49% scavenging activity.The standard drug posses 58.4% scavenging activity.The plant *Alpinia calcarata* rhizomes posses significant activity.

Table.7 Ethanolic extract of the plant *Alpinia calcarata* rhizomes

Sl no	Concentration	Absorbance	% inhibition
1	25	0.632±0.0005	17.16
2	50	0.539±0.00052	29.5
3	100	0.474±0.0056	38.04
4	200	0.414±0.0005	46
5	400	0.357±0.00056	53.3

Table.8 Hot water extract of the plant *Alpinia calcarata* rhizome

Sl no	Concentration	Absorbance	% inhibition
1	25	0.648±0.004	15.09
2	50	0.554±0.0003	27.4
3	100	0.488±0.002	36.05
4	200	0.438±0.003	42.6
5	400	0.386±0.0023	49

Table.9 Standard (hydrogen peroxide)

Sl no	Concentration	Absorbance	% inhibition
1	25	0.568±0.003	25.5
2	50	0.489±0.002	36.9
3	100	0.388±0.004	49.16
4	200	0.355±0.001	53.5
5	400	0.312±0.004	58.4

Fig.3 Hydrogen peroxide sacavenging assay

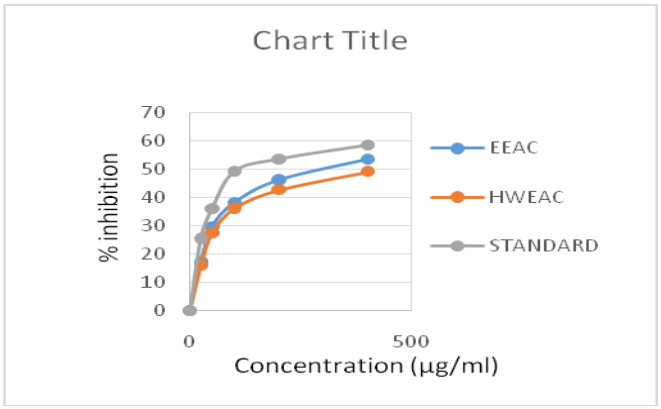


Table.10 Ethanolic extract of the plant *Alpinia calcarata* rhizomes

Sl no	Concentration	Absorbance
1	25	0.782±0.32
2	50	0.891±0.41
3	100	1.3±0.32
4	200	1.4±0.42
5	400	1.56±0.82

Table.11 Hot water extract of the plant *Alpinia calcarata* rhizome

Sl no	Concentration	Absorbance
1	25	0.713±0.24
2	50	0.783±0.31
3	100	0.812±0.12
4	200	1.1±0.22
5	400	1.3±0.21

Fig.4 Reducing power asssay

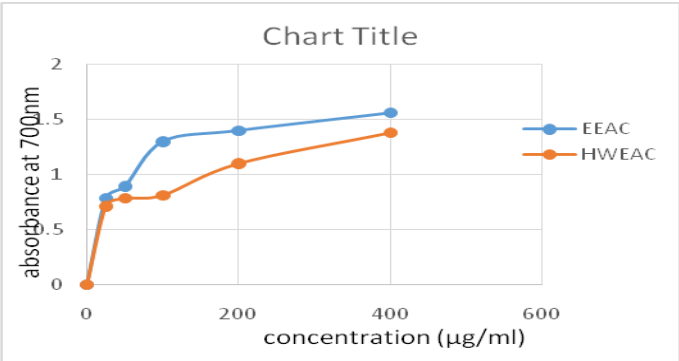


Table.12 Effect of rhizomes of *Alpinia calcarata* on histamine induced bronchoconstriction in guinea pig

Group	Latent period of convulsion			
	Before	1 hr	4 hr	24 hr
Chlorephenaramine maleate (1 mg/kg)	18.46±0.08	60.2±0.05	68.2±0.01	36.5±0.06
Alpinia calcarata ethanolic extract(100 mg/kg)	16.71±0.06	29.6±0.04	39.3±0.03	28.2±0.003
Alpinia calcarata ethanolic extract(200 mg/kg)	15.71±0.06	30.5±0.06	40.3±0.04	28.4±0.001
Alpinia calcarata hot water extract(100 mg/kg)	16.45±0.07	27.6±0.01	36±0.05	28.6±0.06
Alpinia calcarata hot water extract(200 mg/kg)	16.9±0.06	29.6±0.06	39±0.01	29.6±0.04
Control(carboxy methyl cellulose	16.3±0.02	18.3±0.05	18.6±0.05	18.4±0.002

Table.13 Effect of rhizomes of *Alpinia calcarata* on histamine induced bronchoconstriction in guinea pig

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Chlorephenaramine maleate(1 mg/kg)	18.46±0.08	60.2±0.05	68.2±0.01	36.5±0.06
Alpinia calcarata ethanolic extract(100 mg/kg)	16.71±0.06	29.6±0.04	39.3±0.03	28.2±0.003
Alpinia calcarata ethanolic extract(200 mg/kg)	15.71±0.06	30.5±0.06	40.3±0.04	28.4±0.001
Alpinia calcarata hot water extract(100 mg/kg)	16.45±0.07	27.6±0.01	36±0.05	28.6±0.06
Alpinia calcarata hot water extract(200 mg/kg)	16.9±0.06	29.6±0.06	39±0.01	29.6±0.04
Control(carboxy methyl cellulose	16.3±0.02	18.3±0.05	18.6±0.05	18.4±0.002

Table.14 Milk induced leukocytosis and eosinophilia

Groups	Drug dose	Difference in no of leucocytes before and after treatment(Cu.mm)
Standard(Dexamethosone)	50 mg/kg	600±22
Alpinia calcarata ethanolic extract	100 mg/kg	2580±18
Alpinia calcarata ethanolic extract	200 mg/kg	1280±26
Alpinia calcarata hot water extract	100 mg/kg	2920±19
Alpinia calcarata hot water extract	200 mg/kg	1960±20
Control(carboxy methyl cellulose)	0.2%	4100±31

Table.15 Milk induced eosinophilia

Groups	Drug dose	Difference in no of eosinophilic count before and after treatment(Cu.mm)
Standard(Dexamethosone)	50 mg/kg	38±3
Alpinia calcarata ethanolic extract	100 mg/kg	82±5
Alpinia calcarata ethanolic extract	200 mg/kg	53±2
Alpinia calcarata hot water extract	100 mg/kg	91±6
Alpinia calcarata hot water extract	200 mg/kg	64±1
Control(carboxy methyl cellulose)	0.2%	118±12

Fig.5 latent period of convulsion

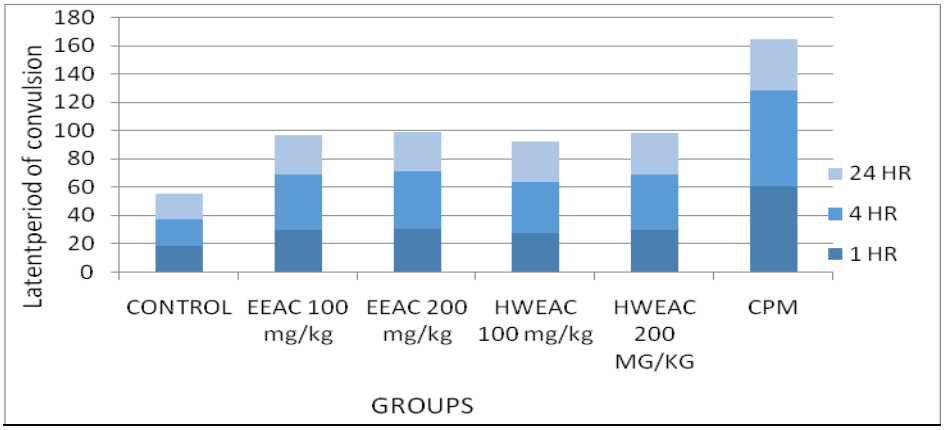


Fig.6 % protection

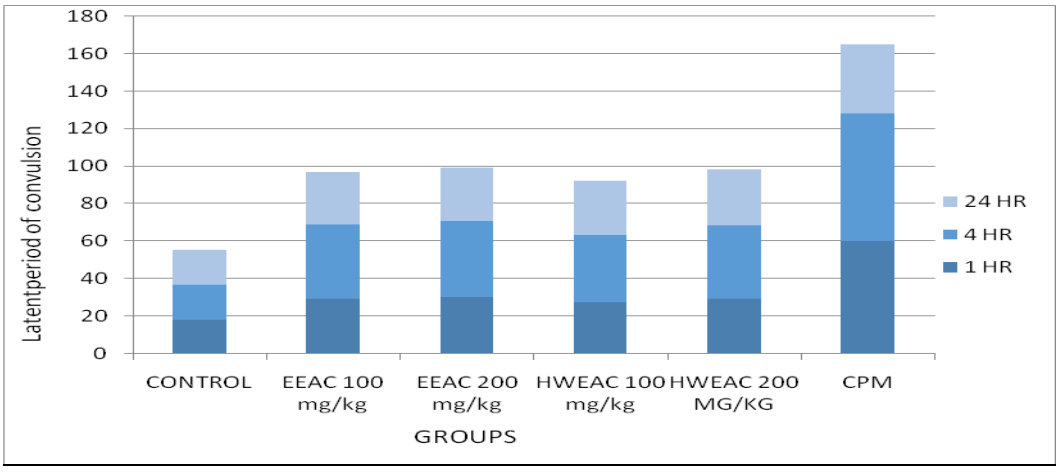


Fig.7 Difference in number of leucocytes

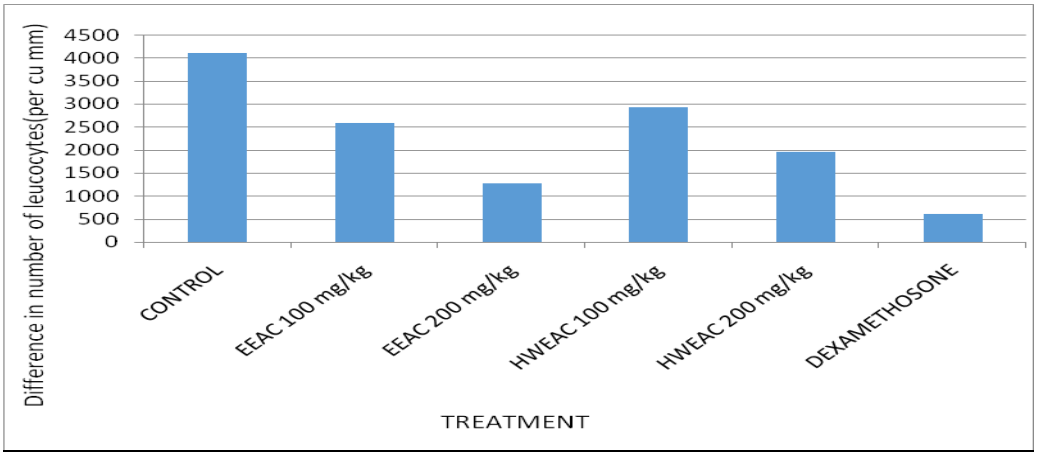
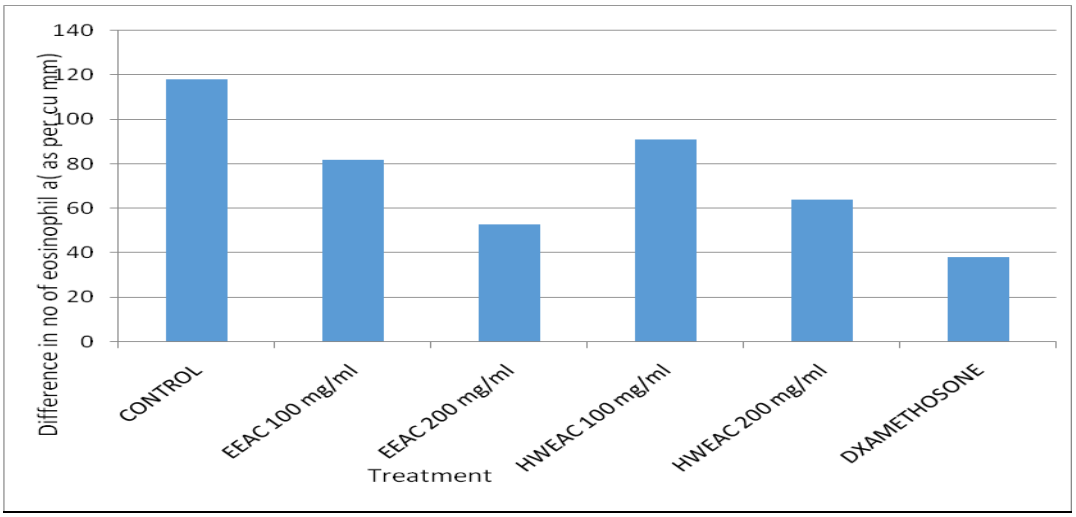


Fig.8 Difference in no of eosinophil



Reducing power assay

The reducing ability of the extract served as a significant indicator of its potential antioxi dant activity.The reducing power of the plant increased concentration dependently.

Aniti asthmatic activity

Histamamine aerosol induced bronchoconstriction in guinea pig

Bronchoconstriction induced by Histamine is an immunological model of antigen induced airway obstruction.

Histamine when inhaled causes hypoxia and leads to spasm in Guinea pigs and causes very strong smooth muscle contraction and capillary dilation in cardiovascular system. Bronchodilators can delay the occurrence of these symptoms.

The study resulted in deep-rooted the bronchodilator properties of the plant, justifying its claiming in the treatment of asthma. The ethanolic and hot water extract of the plant expressively extended the latent period of spasms followed by exposing to histamine aerosol at the dose 400 mg/kg which showed the protection respectively

60.79 and 58.56 % at time 4 hour as compred to the standard drug chlorpheniramine maleate 1mg/kg which untaken maximum protection of 73.3 at time 4 hour.

Milk induced leukocytosis and eosinophilia

Maximum increase in difference of leukocytes(4100±31) and eosinophil (118±12) was observed in control group. The groups of mice pretreated with 200 mg/kg of ethanolic and hot water ectract showed significant inhibition of milk induced leukocytosis and eosinophilia as compared to the The standard drug dexamethasone.

Conclusion

Drugs effective in asthma are steroidal in nature. The plant *Alpinia calcarata* posses significant anti-asthmatic effect. Also the plant rhizomes posses significant anti-oxidant and anti-inflammatory activity

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