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Biochemistry

EFFECT OF PLUMBAGO ZEYLANICA ETHYL ACETATE EXTRACT IN PREVENTION OR TREATMENT OF ARTHRITIS USING ADJUVANT INDUCED ARTHRITIC RAT MODEL

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ABSTRACT PZE-6, a freeze dried ethyl acetate fraction, purified from the roots of Plumbago zeylanica, its anti-inflammatory and anti-arthritic effects on disease development was assessed in comparison with indomethacin and cyclophosphamide in rats with adjuvant-induced arthritis (AIA). PZE-6 fraction significantly suppressed the arthritis by decreasing clinical score and delayed type hypersensitivity response (DTH). Normalized hematological parameters were also observed after the treatment with PZE-6 in AIA rats. In addition, we have conducted experiments to investigate the effect of PZE-6 on the prevention of adjuvant induced arthritis and simultaneously determined its effect on established AIA.

KEYWORDS : Rheumatoid arthritis, Plumbago zeylanica, Adjuvant arthritis, arthritic score, anti-arthritic activity, Freund's adjuvant.

Introduction

Rheumatoid arthritis is a painful and crippling systemic disease for which there is no permanent cure. The best experimental model for studying rheumatoid arthritis in humans is the adjuvant-induced arthritis in rats Trentham et al. (1977). Conventional treatment of rheumatoid arthritis has been directed towards either the inflammatory aspect of the disease via agents, which inhibits the production of inflammatory mediators such as prostaglandins, or towards the immune component of the disease via agents, which modify certain immune responses. Though these agents assuage the symptoms of the disease they have considerable side effects. Hence, the search for better therapeutic agents, with minimal side effects to alleviate the symptoms of the disease is continued.

Plumbago zeylanica was selected, as it is easily cultivated in gardens and distributed throughout India Chopra et al. (1956), and the extracts of P. zeylanica have been used in China and other Asian countries as folk medicine for alleviation of cancer, rheumatoid arthritis and dysmenorrheal Itoigawa et al. (1991). A number of naphthoquinones, flavonoids, anthocyanins and β-sitosterol have been purified from plant Dinda et al. (1995). Chloroform extract showed antibacterial activity Chakraborty and Patil (1997). We propose to test the anti-arthritic and anti-inflammatory activity of P. zeylanica on adjuvant arthritis model.

Induction of Arthritis: Briefly, 6-8 weeks old rats (10/group) of three different strains (wistar, Fischer, and Lewis) were immunized by subcutaneous injection of 0.1 ml of Freund's complete adjuvant (CFA) (0.2 mg of heat killed Mycobacterium in 0.05 ml of paraffin oil) into the footpad of the right hind paw on day 0. The rats were found to develop adjuvant induced arthritis (AIA) by 7 days. Control rats were injected similarly with 0.1 ml of incomplete Freund's adjuvant (IFA).

Preparation of PZE-6 from ethanol extract of plant: The alcohols soluble (AS) were separated by vacuum filtration using a rotavapor. The resultant residue was freeze dried to remove the traces of alcohol. AS were further separated into different fractions by using silica gel column chromatography with different organic solvents of increasing polarity. The vacuum evaporated fractions were further separated into six fractions and were designated as PZE-1, PZE-2, PZE-3, PZE-4, PZE-5 and PZE-6 with 5-30% ethyl acetate in benzene. These fractions were freeze dried and dissolved in olive oil for the current investigation (Wen-Zhen et al., 1995) Treatment protocols: In the first experiment, prevention of adjuvant arthritis by PZE-6 (freeze dried ethylacetate fraction) was studied. First control group (n=10) was injected with 1 ml of water subcutaneously daily for 13 days beginning on day 0. PZE-6 (20 mg/kg) was dissolved in 1 ml of olive oil injected subcutaneously into the second group. Cyclophosphamide (2 mg/rat) and indomethacin (2 mg/rat) were suspended in 0.3% Carboxymethyl cellulose and treated the third and fourth group of adjuvant arthritic rats from day "O" to day 13.

In the therapeutic study, the rats were injected with M. tuberculin suspension. The symptoms of adjuvant arthritis usually take from 14 to 21 days to develop. After 21 days, treatment was initiated with 20 mg/kg suspensions of PZE-6 subcutaneously daily from day 21 through day 38.

Assessment of adjuvant arthritis

Clinical disease score: The incidence of arthritis was defined as the number of rats that had clinical evidence of arthritis within 14 days after the induction of disease. The severity of clinical disease was scored from 0-3 as follows: no swelling or limitation in movement=0; distinct swelling of the distal hind limb joints but no limitation of movement=1; moderate swelling of hind limb and fore limb joints and minor restriction of mobility=2; and marked swelling in all limbs and significant limitation in mobility=3. The arthritic score for each rat was the sum of the score for each of the four paws. The maximum arthritis score was the highest score of an individual rat during the entire course of the disease.

Cutaneous DTH: A small portion of the hair on the back of the animals was shaved and 50 µl of MT in oil or oil alone was injected intradermally 72 hours prior killing. After the rats were sacrificed, on day 14 skin thickness was measured using calipers.

Prevention and Therapeutic study

The rats were anesthetized with ether on paw-measuring days. Their hind paw volumes were determined by dipping them into a fluid-filled cell up to the anatomical hairline Jones et al. (1982). Day 0 measurements were taken 6 hours after the paws were injected with M. tuberculin adjuvant or oil alone. This initial measurement was used as a reference from which units of edema were calculated in the prevention study. These units of edema were calculated by subtracting the day 0 volumes from those measured on days 7, 14, and 21. The base-line values for calculating units of edema in the regression study were measured on day 21. Day 21 values were viewed as 0 units of edema. The hind paws were measured on days 0, 21, 28, 35, and 38 for this study.

The animal's body weight was measured on days 0 and 21 in both studies and day 38 in the regression study. The change in weight during the experiments was calculated by subtracting from day 38 weights in the therapeutic experiment. The change in edema was divided by the change in weight to obtain a relative change in edema. This served to rule out any gain in paw volume caused by weight gain. A CU-5 Medical Land Camera was used to photograph representative rats on days 21 (prevention study).

Statistical analysis

Mean paw volumes and body weights were recorded for all animals. Standard errors were determined by using the formula SE = E d2/N (N - 1). The deviation of individual values from the mean is Ed2, and N - 1 represents the degrees of freedom.

Results

The immunosuppressive fraction (PZE-6) isolated from P.zeylanica was tested for antiarthritic activity, using the standardized adjuvant arthritis (AA) assay in Lewis rats. The Photographs in Fig-1, illustrate the difference in paw edema between the rats receiving PZE-6 and

olive oil (control). PZE-6 fraction significantly suppressed the arthritis by decreasing clinical score (0-3) and delayed type hypersensitivity response (DTH) as shown in Fig-3. Furthermore, PZE-6 also normalized the hematological parameters like WBC count, erythrocyte sedimentation rate (ESR), and hemoglobin (Hb) concentration in AIA rats as mentioned in Table-1.

The effect of PZE-6 was studied on prevention of arthritis. Previous studies shown AIA induced by injecting CFA and day 0 paw volume measurements were taken. Arthritic rats were daily treated for 13 days beginning on day 0. An increase in the volume of the right hind paw is an inflammatory response in the presence of the adjuvant, whereas the swelling of the left hind paw is an autoimmune response, against the animal's own cartilage. Mycobacterium tuberculin (MT) present in the CFA is similar to cartilage Currey and Ziff (1968). So the immune response attacks both. Results in the Table-2 demonstrate that in PZE-6 treated rats, the edema in the right hind paw (inflammation) 60% less than the adjuvant controls on day21. The left hind paw (immune) was 42% less than that of controls. Arthritic rats treated with drugs like, Indomethacin and cyclophosphamide inflammatory and immunosuppressive drugs respectively could not show any inhibition of neither inflammation nor immune response.

In the established phase, the rats were injected with MT suspension, the symptoms of adjuvant arthritis usually taken from 14 to 21 days to develop. After 21 days, treatment was initiated with PZE-6 (20 mg/kg) daily from Day 21 to 38. On the contrary, as seen in Table-3, PZE-6 showed anti-inflammatory activity (36%), immune response (31%) in disease developed rats. Indomethacin exhibits 16% immune response inhibition, whereas Cyclophosphamide inhibited 25% inflammatory response.

Discussion

PZE-6 was efficacious in preventing joint inflammation when treatment was started before but not after onset of joint inflammation. Furthermore PZE-6 significantly suppressed the arthritis by decreasing paw volume, clinical score and delayed type hypersensitivity reaction. A significant suppression of arthritis by PZE-6 was also observed in a dose dependent manner. Moreover, 20 mg/Kg of PZE-6 was found to inhibit the development of inflammation in AIA rats. The dose of PZE-6 above 20 mg/kg was found to be lethal to the rats. The root of P. zeylanica was used as indigenous medicine to improve digestion and was also being used for piles, anascara, diarrhoea, rheumatism, and skin disease Chopra (1949). The extracts from other parts of the plant like leaves, stem did not show significant anti-arthritic activity as of the root extract. The modulator ability of Plumbagin (5-dihydroxy-2-methyl-1, 4-Naphtho quinone), a natural product from P. zeylanica, was studied on peritoneal macrophages in BALB/c mice. The macrophage functions evaluated were bactericidal activity, hydrogen peroxide and superoxide anion release Abdul and Ramchander (1995). P. zeylanica was studied for its effect on the development of antibiotic resistance using antibiotic sensitive strains of Escherichia coli and Staphylococcus aureus Durga and Sridhar (1992). Anthraquinones isolated from Aloe vera exhibited anti-inflammatory and antiarthritic activities Davis et al. (1986). PZE-6 was also found to be effective anti-inflammatory and influence the T-cell responses Aparanji et al. (2005).

Conclusion

The anti-inflammatory and anti-arthritic activities of the PZE-6 were tested to determine if they could prevent the formation of arthritis. It may also have therapeutic applications in various autoimmune diseases.

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Table -1:

Hematological Parameter	Normal	Arthritic	Treated (PZE-6)
1. Haemoglobin (gm %)	10.1	6.4	9.5
2. Total Leucocytes/Cu.mm	3,000	5,550	3,600
3.Polymorphs	29%	30%	28%
4. Lymphocytes	69%	64%	70%
5.Monocytes	8%	7%	8%
6.Eosinophils	2%	5%	2%
7. E. S.R. (1st hr)	4	8	4

Table -2: Effect of PZE-6 on Prevention of arthritis

Protocol	Aqueous	Cyclophosphamide	Indomethacin	PZE-6
Treatment (mg/Kgx13) Days0 to12		2	2	20
Number of rats	10	10	10	10
Final body weight (gm)	156±3.21	122±2.64	139±9.5	119±5.56
Edema of hind paws (Volume units±SE) Day 7				
Left	0.87±0.01	0.86±0.005	0.72±0.03	0.62±0.01
Right	1.87±0.01	1.77±0.02	1.65±0.04	1.67±0.01
Day14				
Left	0.9±0.01	0.83±0.02	0.81±0.02	0.78±0.01
Right	1.67±0.01	1.98±0.01	1.52±0.02	1.72±0.01
Day 21				
Left	0.80±0.01	0.62±0.02	0.56±0.01	0.71±0.01
Right	1.23±1.01	1.10±0.02	1.26±0.01	1.60±0.02
Percent Inhibition Day 21				
Left		-22.5	-30	42.25
Right		-10.57	2.69	60.4

Initial body weight, 170-185 g.Symbols: +/-, standard error; and the negative value (-, minus) means swelling.
Adjuvant arthritis with 0.1 mg/kg H2O x 13, day 0-12.
Percent difference from aqueous adjuvant controls.

$$\frac{\text{Change in hind paw vol. aqueous adjuvant}}{\text{Change in body weight}} \quad \frac{\text{Change in test paw vol.}}{\text{Change in body weight}} = \text{Relative change in volume.}$$

Table - 3 Effect of PZE-6 on established Arthritis

Protocol	Aqueous	Cyclophosphamide	Indomethacin	PZE-6
Treatment (mg/Kgx13) Days0 to12		2	2	20
Number of rats	10	10	10	10
Final body weight (gm)	191±1.15	135±0.57	153±3.05	123±3.05
Edema of hind paws (Volume units±SE) Day 28				
Left	0.94±0.02	1.08±0.03	0.93±0.01	1.12±0.03
Right	2.05±0.03	1.63±0.04	2.17±0.03	2.32±0.05
Day35				
Left	1.07±0.01	1.15±0.03	1.08±0.02	1.22±0.02
Right	2.44±0.04	2.02±0.02	2.86±0.02	2.67±0.01
Day 38				

Left Right	0.48±0.04 0.74±0.03	0.56±0.05 0.46±0.13	0.45±0.03 0.93±0.05	0.63±0.04 1.01±0.01
Percent Inhibition Day 38				
Left Right		16.66 -37.83	-6.4 25.55	31.04 36.35

Initial body weight, 170-185 g.Symbols: +/-, standard error; and the negative value (-, minus) means swelling.Adjuvant arthritis with 20 mg/kg, H2O day 21-38.Percent difference from aqueous adjuvant controls.

$$\frac{\text{Change in hind paw vol. aqueous adjuvant}}{\text{Change in body weight}} \Big/ \frac{\text{Change in test paw vol.}}{\text{Change in body weight}} = \text{Relative change in volume.}$$

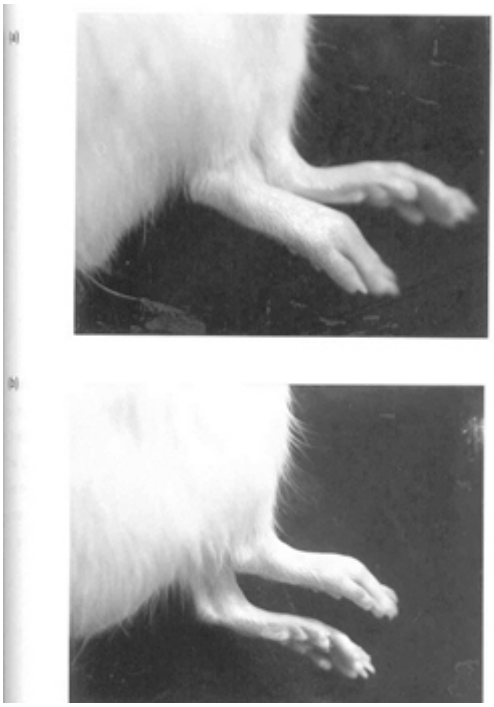

Figure-1: The difference in paw edema between the rats receiving PZE-6 and olive oil (control).

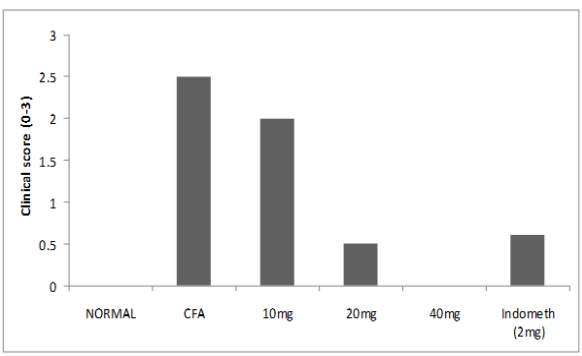

Figure-2: Five groups of Lewis rats (10/group) were injected with 0.1 ml of Freund's complete adjuvant on day 0 to induce AIA. First group (control) was treated with olive oil alone. The four groups of rats were treated with PZE-6 (10, 20, 40 mg) or Indomethacin daily from day 0 to day13. Normal group did not receive any treatment. Clinical score (0-3) was measured on day 14.

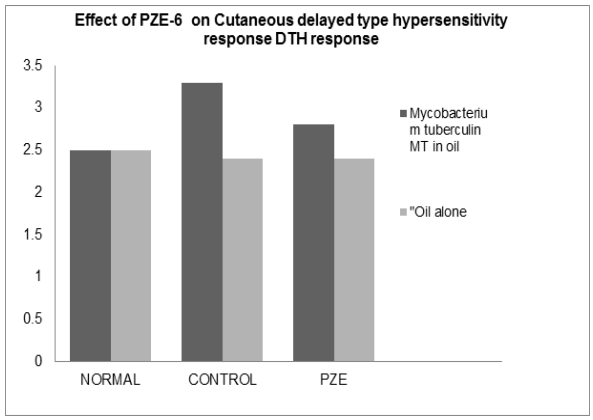

Figure-3: Effect of PZE-6 (20 mg/kg) treatment on skin thickness after cutaneous challenge with Mycobacterial antigens in mineral oil (CFA). Two groups of rats were injected with 0.1ml of Freund's complete adjuvant (CFA) and other two groups of rats were injected with 0.1ml of incomplete Freund's adjuvant (IFA). Two groups (one from CFA injected + one from IFA injected) were treated with PZE-6 from day 0 to 13. After the rats were killed on day 14, skin thickness was measured using calibrated skin fold calipers. Normal group did not receive any injection. The group that received olive oil alone was served as control.

References

1. Abdul KM and Ramchender RP (1995). Modulation effect of Plumbagin (5-hydroxy, 2-methy 1, 4-naphthoquinone) on macrophage functions in BALB/c mice. Potentiation of macrophage bactericidal activity. Immunopharmacology. 30: 231-235.
2. Aparanji P, Veerendra kumar B, Prasanna Kumar S, Sreedevi K, Rao DN, Rama Rao Athota (2005). Induction of anti-inflammtory and altered T-cell proliferative responses by the ethanol root extract of Plumbago zeylanica in adjuvant-induced arthritic rats. Pharm. Biol. 43: 784-789.
3. Chakraborty RR, Patil AT (1997). Preliminary phytochemical and antimicrobial studies of Plumbago Zeylanica. Indian Jl. of Nat. Prod. 13: 3-7.
4. Chopra RN, Nayar SL, Chopra BN (1956). Glossary of Indian medicinal plants. Council of scientific and industrial research, New Delhi, India.
5. Currey H, Ziff MJ (1968). Suppression of adjuvant disease in the rat by heterologous antilymphocyte globulin. J Exp Med. 127: 185-190.
6. Davis, Robert H, Agnew, Patrick S, Shapiro, Eugene (1986). Antiarthritic Activity of Anthraquinones found In Aloe. Jl. of American Podiatric Medical Assoc. 76: 2-6.
7. Dinda B, Das SK, Hajra AK (1995). Naphthoquinones from the roots of Plumbago rosea Linn. Indian J. Chem. 34: 525-532.
8. Durga R, Sridhar PH (1992). Antimutagenic activity of Plumbagin in Ames Salmonella typhimureum test. Ind J. Med. 96: 143-146.
9. Itoigawa M, Takeya K, Furukawa H (1991). Cardiotonic action of plumbagin on guinea pig papillary muscle. Planta Med. 57: 317-323.
10. Jones SA, Kennedy AJ, Roberts NA (1982). Assessment of drugs for activity in established type II collagen arthritis. Agents actions. 12: 5-12.
11. Trentham DE, Townes AS, and Kang AH (19771). Autoimmunity to type II collagen: an experimental model of arthritis. J. Exp. Med. 46: 857-859.
12. Wen-Zhen GU, Randal C, Neal B (1995) Isolation, purification and characterization of immunosuppressive compounds from Tripteygium triptolide and tripdiolide.Int.J Immunopharmacol 17: 351-355.