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Hydroalcoholic Extract of *Plumbago Zeylanica* Linn root bark exhibit Analgesic and Anti-Inflammatory activities in Experimental Rat models

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ABSTRACT

The root bark of *Plumbago zeylanica* Linn has potent analgesic and anti-inflammatory activity which has been used in many formulations of Indian system of medicine in treating Cancer. Since less information is available on the effects of hydro alcoholic extract of *Plumbago zeylanica* on inflammation and nociception, the present study was designed. To study the analgesic and anti-inflammatory activity of the hydro alcoholic extract of root bark of *Plumbago zeylanica* Linn. Soxhlet extraction of root bark powder done using 85% Methanol and Water 15% to obtain test drug. Analgesic effect was studied using hot plate and tail immersion analgesia in rat. Anti-inflammatory activity was investigated through *in vitro* Human Red Blood Cell Membrane protective activity and, Carrageenan induced rat paw edema and Complete Freund's Adjuvant induced chronic inflammatory model in rat. Analgesic activity of the extract was studied by hot plate and tail immersion method revealed significant activity at 350 mg/kg b.wt dosage on comparing with weak analgesic standard drug Paracetamol. Studies of extract on both acute and chronic model of inflammation induced by Carrageenan and Complete Freund's Adjuvant in rat showed moderate anti -inflammatory action at primary phase of inflammation at the dosage of 250 mg/kg b.wt comparable with standard Indomethacin.

Keywords: *Plumbago zeylanica* Linn, Hydroalcoholic extract, Analgesic, Anti-inflammation, Carrageenan, Complete Freund's Adjuvant

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INTRODUCTION

Plumbago zeylanica. Linn (Plumbaginaceae) commonly known as Ceylon Lead Wort, *Chitraka* in Sanskrit and *Plumbago zeylanica* in Tamil is an herb found in Southern Peninsular region of Asia. In Indian system of medicine, root bark of this plant is used for treating chronic inflammatory ailments. Taste, nature and division of this plant belong to hot[1]. Root has the property in increasing digesting power and promotes appetite[2]. Hakims use this root in rheumatism and splenic enlargement[2]. Ayurveda mentioned the root for dyspepsia, piles, anasarca, and diarrhea and skin diseases[2]. The root bark is used in treating colic pain, deranged vatham, apoplexy, zymotic fever, rheumatic pain and headache[2]. It is also used in the preparation of calcination of copper[2]. The root of this plant is mixed with sesame oil known as *Chitramool ennai* is used in treating migraine and all types of headache on using as oil bathing[3]. The root bark has the properties such as antiperiodic and diaphoretic action[1]. The dose of administration of the root bark powder is up to 2 g[1]. A pill called *Chittramoola kuligai* prepared by grinding equal amount root bark of this plant, *Pooram* (Mercuric subchloride) and *Omam* is used in pain management of Cancer treatment[1]. For arthritis, oil prepared from the root called *Kodiveli thylam* is externally applied[1]. The roots stick cause dilation of cervix of uterus and used as abortifacient[1]. Acetone and ethanolic extract of leaves of *P. zeylanica* have an antifertility effect by interrupting the estrous cycle in rats by prolonging diestrous stage[4]. The phytochemical constituents present in the roots are plumbagin, dihydroserone, elliptinone, droserone, nisoshinanolone, plumba zeylanone, zeylanone, 3 – chloro plumbagin, iso zeylinone, 3,3’ – bi plumbagin[3]. New guanidine alkaloids plumbagines and plumbagosides isolated from *P. zeylanica*[5]. In 1889, Fluckiger isolated Plumbagin from the root of this plant which causes skin irritation[2]. Keien ko in 1931 mentioned Plumbagin stimulates central nervous system in small doses whereas in large doses Plumbagin cause paralysis which leads to respiratory failure[2]. Plumbagin, a naptho-quinone isolated from the *P. zeylanica* inhibit cell growth and induces apoptosis in human breast cancer[6] and in lung cancer[7]. Plumbagin has anti diabetic effect in streptozotocin induced diabetes in rats by causing glucose homeostasis[8]. Plumbagin inhibits T cell proliferation and suppress the NF kappa B activation and shows immunomodulatory effect in mice[9]. The ethanolic extract of the root of *P. zeylanica* decreased the testicular cell population causing antispermatogenic action which can be utilized for fertility control[10]. Ethanolic extract of the root enhance the spontaneous ambulatory activity in rat brain by increasing dopamine and homavanilic acid level[11].

MATERIALS AND METHODS

Plant materials

The root barks of *Plumbago zeylanica* were collected from TAMPCOL farm, Chennai and got authentication from the botany department of Captain Srinivasa Murthi Drug Research Institute, Dept. of AYUSH, Chennai, India.

Extract preparation

The collected plants were shade dried for a period 10 days and the root barks were separated. The dried barks were coarsely powdered using a pulverizer. The powder was stored in an airtight container until further use. The coarsely powdered plant drugs were subjected to successive soxhlet extraction with solvents of 85% of Methanol / 15% of Water and stored in the refrigerator. The extract was concentrated *in-vaccuo* and percentage of yields was calculated.

Animal procurement and maintenance

Wistar Albino rats of either sex, weighing 120-180 g were purchased from The Tamil Nadu Veterinary University Animal House, Chennai, India and they were acclimatized in SASTRA University Animal University, Tanjore, India at 21 - 23ºC. Animal ethical guidelines of CPCSEA, Ministry of Animal Husbandry and Welfare, Govt. of India were strictly followed for the care and maintenance of procured animals. The animals were fed on standard rodent pellet and RO water was provided *ad libitum.* The animals were kept for overnight fasting before experimentation.

Analgesic activity evaluation

The analgesic activity was evaluated by measuring the reaction time to thermal stimuli. The extracts were administered orally before the exposure to thermal stimuli. Two methods were employed for giving thermal stimuli. 1) Eddy's Hot plate method[12]. 2) Tail immersion method[13].

Eddy's Hot plate method

The experimental design done for this method is illustrated in table 1. In this method, all the rats were placed on the hot plate which was maintained at 55±1°C and the time taken to lick the hind paw or jumping response was considered as the reaction time with cut off time being 60 min. The readings were taken at 1, 2, 3 and 4 h for all groups after the above treatment. The basal reaction time measured in the same manner at 0 h before the treatment[12].

Tail Immersion method

The experimental design done for this method is illustrated in table 1. The rat tails dipped in a water bath at 55±1° up to 3 cm. The time taken by the rats to withdraw the tail clearly out of

water was considered as the reaction time and the readings were taken every 15 min and continued up to 60 min after the drug administration[13].

Table 1: Treatment protocol for analgesic studies by both hot plate and tail immersion method

Group	Treatment	Vehicle	Frequency	Route of administration
Group I	Nil	5% Tween 80, 2ml	Single	Oral
Group II	Paracetamol, 200 mg/kg b.wt	5% Tween 80, 2ml	Single	Oral
Group III	HAE, 150 mg/kg b.wt	5% Tween 80, 2ml	Single	Oral
Group IV	HAE, 250 mg/kg b.wt	5% Tween 80, 2ml	Single	Oral
Group V	HAE, 350 mg/kg b.wt	5% Tween 80, 2ml	Single	Oral

Note: HAE – Hydroalcoholic extract of *P. zeylanica.* Each group consisted six rats (n=6)

Anti –inflammatory studies

In vitro determination of Human Red Blood Cell Membrane Stabilization

The method[14] adopted here was followed according to Trnavsky & Swingle, 1974. Fresh human blood was collected and mixed with equal volume of sterilized Alsevier solution (2% Dextrose, 0.8% Sodium citrate, 0.05% Citric acid and 0.42% Sodium chloride) and used within 5 h. 2 ml Hyposaline (0.25%), 1 ml Phosphate buffer (0.15 M, pH 7.4) and 0.5 ml HRBC (1%) were taken in the test tubes. The contents in all tubes were incubated at 37°C for 30 min and centrifuged. For control, 1 ml isosaline (0.85%) was used. The intensity of colour developed in the supernatant which was due to the presence of Hb, was measured at 560 nm using an UV Spectrometer. Initially, we conducted experiment to compare the membrane protecting activity of various NSAIDs. As Ibuprofen showed better membrane protecting activity, we used Ibuprofen as standard. The standard drug and test extract were taken in concentrations ranging from 10 to 200 µg in order to determine the optimum concentration for evaluation. HRBC membrane protecting activity is represented as percentage by using the formula

100 – O.D of treated drug sample / O.D of Control x 100

***In vivo* Carrageenan induced acute hind paw inflammation**

All animals were treated according to the design mentioned in table 2. Then, Carrageenan induced acute paw oedema was induced by injecting 0.1 ml of 1% (w/v) Carrageenan solution (Sigma) prepared in normal saline in sub plantar region of the left hind paw of all rats [15]. The volume paw was measured at 0, 1, 2, & 3 h using Vernier Caliper after the administration of Carrageenan. The percentage of paw edema inhibition calculated by the formula A – B / A x 100. Where, A denotes the mean increase in paw edema of Control group, B denotes the mean increase in paw edema of standard / test groups.

Table 2: Treatment protocol for anti-inflammatory studies on Carrageenan induced acute inflammation

Group	Treatment	Vehicle	Frequency	Route of administration
Group I	Nil	5% Tween 80, 2ml	Single	Oral
Group II	Indomethacin 10 mg/kg b.wt	5% Tween 80, 2ml	Single	Oral
Group III	HAE, 150 mg/kg b.wt	5% Tween 80, 2ml	Single	Oral
Group IV	HAE, 250 mg/kg b.wt	5% Tween 80, 2ml	Single	Oral
Group V	HAE, 350 mg/kg b.wt	5% Tween 80, 2ml	Single	Oral

Note: HAE – Hydroalcoholic extract of *P. zeylanica.* Each group consisted six rats (n=6)

***In vivo* Complete Freund's adjuvant induced chronic inflammation**

This study was performed according to the method[16] of Newbould, 1963. The right foot of each rat was injected subcutaneously with 0.05 ml of Complete Freund's Adjuvant agent (CFA, Sigma). All animals were treated according to the design mentioned in table 3. After challenging the animals with CFA, the acute inflammatory response became evident in the injected hind paw within 24 h. The swelling increased linearly till 4th day and the plateau thereafter until day 12. The immune mediated response starts at around day 12. The control paw exhibited swelling continued to increase and reached a maximum on 18th or 21st day after CFA administration. The swelling expected to subside to some extent after 35-40 days after CFA administration. Physical observations such as paw volume and body weight were observed. The paw volume was measured daily using Vernier Caliper and Plethysmograph. The percentage of edema inhibition calculated by the formula

$$\frac{\text{Paw volume of control group} - \text{Paw volume of standard / test group}}{\text{Paw volume of control group}} \times 100$$

Hematological parameters and biochemical parameters were analyzed. The concentration of oxidative stress markers such as Lipid peroxide, Glutathione, Glutathione peroxidase and Catalase were analyzed. Lipid peroxides (Thiobarbituric Acid Reactive Substances – TBARS) in tissues were assayed by the method of Yagi[17]. The colour formation with Thiobarbituric acid (TBA) was used as index. Reduced glutathione (GSH) was estimated by the method of Ellman[18] in which yellow colour developed when dithionitro-bis-benzoic acid (DTNB) added to to the compounds sulfhydryl groups. Glutothione peroxidase (GPx) estimated by the method of Rotruck et al, 1973 in which H2O2 reduced to water whereas organic hydroperoxides reduced to alcohol at the expense of GSH[19]. The activity of Catalase (CAT) was determined by the method of Sinha[20]. In this assay, Dichromate in acetic acid heated in the presence of hydrogen peroxide converted to perchromic acid and then to chromic acetate. The formed chromic acetate was

measured at 620 nm.

Table 3: Treatment protocol for anti-inflammatory studies on Complete Freund's induced chronic inflammation

Group	Treatment	Vehicle	Period	Route of administration
Group 1	Nil	5% Tween 80, 2ml	1-45 days	Oral
Group 2	Indomethacin 10 mg/kg b.wt	5% Tween 80, 2ml	1-45 days	Oral
Group 3	Indomethacin 10 mg/kg b.wt	5% Tween 80, 2ml	16-45 days	Oral
Group 4	Indomethacin 10 mg/kg b.wt	5% Tween 80, 2ml	31-45 days	Oral
Group 5	HAE, 250 mg/kg b.wt	5% Tween 80, 2ml	1-45 days	Oral
Group 6	HAE, 250 mg/kg b.wt	5% Tween 80, 2ml	16-45 days	Oral
Group 7	HAE, 250 mg/kg b.wt	5% Tween 80, 2ml	31-45 days	Oral
Group 8	No experiment induction and treatment done. Animal kept normal			

Note: HAE – Hydroalcoholic extract of *P. zeylanica.* Each group consisted six rats (n=6)

Statistical analyses

All the values have been expressed as Mean ± S.D. Analyses were done using Student's T test by SPSS software.

RESULTS AND DISCUSSION

Analgesic activity

The results of analgesic activity by both hot plate and tail immersion method of hydro alcoholic extract of *Plumbago zeylanica* on are summarized in Table 4 & 5. No significant activity was observed in all doses studied at $P < 0.001$ and $P < 0.01$ levels. However, a significant activity was found in Groups IV and V at $P < 0.05$ level. The thermal stimuli induced in the hot plate and tail immersion test is specific for central analgesic activity. At the higher dose 350 g/kg b.wt of the extract *P. zeylanica* prolonged the hot plate latency at 2 h comparable with the standard weak analgesic drug Paracetamol. The ability of the extract to prolong the latency suggests that the extract have weak analgesic activity at the higher dose.

Table 4: Evaluation of Analgesic activity of Hydroalcoholic extract on rats by hot plate method

Groups	Response time in seconds				
	0 h	1h	2h	3h	4h
Group I - Control	3.3±1.41	4.0±0.50	4.3±0.57	4.4±0.58	2.3±0.50
Group II – Paracetamol 200 mg/kg	4.2±1.73	5.9±1.03	7.5±0.57	6.5±1.84	5.3±1.26
Group III – HAE 150 mg/kg	4.0±1.41	5.5±1.59	5.7±1.59	5.6±1.71	4.8±1.25
Group IV – HAE 250 mg/kg	4.5±1.73	5.4±0.75	6.5±1.00	5.6±1.71	5.6±0.64
Group V – HAE 350 mg/kg	3.8±1.73	6.2±0.95	7.8±1.70	3.9±0.90	5.6±0.58

Note: $^{*}P < 0.01$, $^{**}P < 0.001$. HAE – Hydroalcoholic extract of *P. zeylanica.*

Table 5: Evaluation of Analgesic activity of Hydroalcoholic extract on rats by tail immersion method

Groups	Response time in seconds				
	0 min	15 min	30 min	45 min	60 min
Group I - Control	4.3±0.5	4.0±0.8	3.8±0.5	5.3±0.96	4.0±0.8
Group II – Paracetamol 200 mg/kg	3.8±0.96	4.3±1.5	4.4±1.1	5.3±1.3	5.1±0.3
Group III – HAE 150 mg/kg	2.8±0.5*	4.3±0.5	3.5±1.0	4.3±0.96	4.9±0.9
Group IV – HAE 250 mg/kg	2.6±0.5*	3.5±1.0	4.8±1.3	4.1±0.9	4.0±0.0
Group V – HAE 350 mg/kg	3.1±0.3	4.3±0.5	4.0±0.8	4.4±1.1	4.1±0.9

Note: *P < 0.01, **P < 0.001. HAE – Hydroalcoholic extract of *P. zeylanica.*

Anti-inflammatory activity

From the results of table 6, it was observed that the extract exhibit maximum HRBC membrane protecting activity 63% at 100 microgram concentration. The result of table 7 show the extract exhibit maximum activity at 250 mg/kg b.wt dosage and found to be 30.4 % of inhibition of paw edema induced by Carrageenan at the above dose. The injection of Carrageenan leads to formation of paw edema in the rat is the biphasic event. The first phase is due to release of histamine and serotonin lasting for one hour. The second phase is due to release of prostaglandins, proteases and lysosomes[21,22]. Carrageenan injection in the rat paw induces bradykinin liberation that leads to produce prostaglandins responsible for the formation of inflammatory exudates[23]. The mechanism of anti-inflammatory activity of hydroalcoholic extract *P. zeylanica* might be due to prostaglandins inhibition.

The injection of Complete Freund's Adjuvant leads to development of edema which peaks 15-22nd day of the experimental period in the various groups studied. Paw edema and volume are the physical observation of the inflammation in primary and secondary phase. The determination of paw edema is a simple, sensitive and quick procedure for evaluating the degree of inflammation and assessing therapeutic drugs. The changes in paw volume and duration of edema in three different treatment periods are represented in table 8, 9, 10 & 11. The standard Indomethacin has produced a maximum of 29.22% inhibition when administered from day 1 to day 45 and minimum activity during 16 – 45 and 31 – 45 days treatment periods. The hydroalcoholic extract exhibit 16.9% maximum edema inhibition when administered during the primary response period of disease. This might be due to the effect of extract on the primary events of inflammatory process. The extract *P. zeylanica* treated group showed significant inhibition in paw edema while treating continuously for 45 days in rat but not so as maximum edema inhibition activity of Standard drug Indomethacin. The extract *P. zeylanica* might be suggesting as a moderate anti-inflammatory drug causing suppressive effect on Th-1 helper cells[24]. In the present study, the body weight change of rats was estimated as one of the observations to assess

the response of extract in the treatment of chronic inflammation. The body weight changes and average weight gain or loss are calculated and represented in table 12, 13, 14 & 15. The body weight of the control animals decreases for a period of 3 weeks and the overall growth are affected. The animals have gained only 10.71% weight at the end of experiment. This might be due to the adjuvant injection. The standard drug Indomethacin treated group has projected an overall weight gain of 14-16%, which is more than control group. The extract treated group has registered significant weight gain at the end of treatment. The result from the studies revealed the decrease of body weight during the course of experimental period in all groups due to the alteration in the metabolism. But at the end of the treatment, the extract *P. zeylanica* group improved the loss of body weight when compared to the control group. This effect might be due to inhibition of muscle wasting and improvement against inflammation which leads to increase in intestinal absorption of nutrients. During inflammation, body weight will be decreased due to less intestinal absorption of nutrients[25]. The results of table 16 show the role of extract as antioxidant. Indomethacin and extract lowered the lipid peroxidation significantly when compared to the control groups. The extract improves the level of glutathione and glutathione peroxidase. The CAT activity in liver and kidney of control groups are found to decrease due to oxidative stress but increase in Indomethacin group. The extract has affected the enzyme in liver alone. The haematological parameters, liver function tests and renal parameters are found no significant variations due to the administration of hydroalcoholic extract.

Table 6: HRBC Membrane Stabilizing Activity of different standard drugs and Extract

Sample	**% Protection**				
Concentration in µg	**20**	**40**	**60**	**80**	**100**
Nimsulide	31.44	34.68	42.87	43.88	50.25
Mefenemic acid	45.16	51.46	53.79	54.90	55.30
Indomethacin	34.70	37.50	41.35	51.34	57.53
Piroxicam	26.39	32.35	39.83	42.66	48.23
Ibuprofen	43.16	43.28	61.69	67.94	67.45
HAE	52.00	57.20	61.27	62.61	63.04

Note: HAE – Hydroalcoholic extract of *P. zeylanica.*

Table 7: Effect of Hydroalcoholic extract on Carrageenan induced Rat Paw Edema

Groups	**Increase in Paw edema (Mean ± SD) in mm**	**% Inhibition of Paw edema**
Group I – Control 5% Tween 80	2.0±0.14	-
Group II – Indomethacin 10 mg/kg	0.99±0.1	50.2
Group III – HAE 150 mg/kg	1.58±0.25	20.8
Group IV – HAE 250 mg/kg	1.39±0.12*	30.42
Group V – HAE 350 mg/kg	1.75±0.15	12.96

Note: *P < 0.01, **P < 0.001. HAE – Hydroalcoholic extract of *P. zeylanica.*

Table 8: Effect of Hydroalcoholic extract in Complete Freund's adjuvant induced Paw Edema (1 – 45 days Treatment)

Days	Control	Indomethacin 10 mg/kg		HAE 250 mg/kg	
	P.V	P.V	P.I	P.V	P.I
1	4.27 ± 0.08	4.32 ± 0.11	-	4.22 ± 0.09	-
8	6.3 ± 0.43	6.2 ± 0.33	1.59	6.8 ± 0.47	-
15	6.68 ± 0.51	6.38 ± 0.41	4.49	6.27 ± 0.05	1.50
22	6.7 ± 0.87	6.08 ± 0.25	9.25	6.15 ± 0.09	5.22
29	6.7 ± 0.46	5.85 ± 0.19*	12.69	5.83 ± 0.05*	8.96
36	6.63 ± 0.32	4.78 ± 0.19*	27.90	5.64 ± 0.16*	13.12
43	6.45 ± 0.39	4.52 ± 0.11*	29.92	5.26 ± 0.13*	16.90

Note: P.V – Paw volume in mm; P.I – Percentage Inhibition as compared to Control; Mean ± S.D, P<0.05. HAE – Hydroalcoholic extract of *P. zeylanica.*

Table 9: Effect of Hydroalcoholic extract in Complete Freund's adjuvant induced Paw Edema (16 – 45 days Treatment)

Days	Control	Indomethacin 10 mg/kg		HAE 250 mg/kg	
	P.V	P.V	P.I	P.V	P.I
1	4.27 ± 0.08	4.17 ± 0.08	-	4.18 ± 0.13	-
8	6.3 ± 0.43	6.48 ± 0.14	-	7.23 ± 0.52	-
15	6.68 ± 0.51	6.8 ± 0.15	-	7.22 ± 0.80	-
22	6.7 ± 0.87	6.2 ± 0.12	7.46	6.15 ± 0.51	8.21
29	6.7 ± 0.46	6.0* ± 0.16	10.45	6.38 ± 0.27	4.77
36	6.63 ± 0.32	5.82* ± 0.24	12.22	6.03 ± 0.19	9.05
43	6.45 ± 0.39	5.47 ± 0.29	15.19	5.74* ± 0.08	11.01

Note: P.V – Paw volume in mm; P.I – Percentage Inhibition as compared to Control; Mean ± S.D, P<0.05. HAE – Hydroalcoholic extract of *P. zeylanica.*

Table 10: Effect of Hydroalcoholic extract in Complete Freund's adjuvant induced Paw Edema (31 – 45 days Treatment)

Days	Control	Indomethacin 10 mg/kg		Extract 250 mg/kg	
	P.V	P.V	P.I	P.V	P.I
1	4.27 ± 0.08	4.15 ± 0.09	-	4.15 ± 0.05	-
8	6.3 ± 0.43	6.7 ± 0.32	-	7.23 ± 0.05	-
15	6.68 ± 0.51	6.95 ± 0.29	-	7.28 ± 0.12	-
22	6.7 ± 0.87	6.82 ± 0.29	-	6.88 ± 0.27	-
29	6.7 ± 0.46	6.37 ± 0.16	4.92	6.35 ± 0.16	5.22
36	6.63 ± 0.32	6.02 ± 0.14	9.20	6.0 ± 0.34	9.50
43	6.45 ± 0.39	5.68 ± 0.31	11.94	5.7 ± 0.23	11.63

Note: P.V – Paw volume in mm; P.I – Percentage Inhibition as compared to Control; Mean ± S.D, P<0.05.

Table 11: Maximum % Edema Inhibition by the Hydroalcoholic extract during the different treatment periods

Period of Treatment	Maximum % Edema Inhibition	
	Indomethacin 10 mg/kg	HAE 250 mg/kg
1 – 45 days	29.92	16.9
16 – 45 days	15.19	11.01
31 – 45 days	11.94	11.63

Note: HAE – Hydroalcoholic extract of *P. zeylanica.*

Table 12: Effect of Hydroalcoholic extract in body weight of Complete Freund's adjuvant Rat model (1 – 45 days Treatment)

Days	Normal	Control	Indomethacin 10 mg/kg	HAE 250 mg/kg
1	110.56 ± 1.72	125.83 ± 9.36	125.59 ± 4.65	109.04 ± 6.48
8	111.53 ± 2.96	114.09 ± 10.99	116.89 ± 7.52	103.48 ± 8.63
15	117.13 ± 15.41	112.52 ± 13.99	125.18 ± 14.40	113.53 ± 16.12
22	125.77 ± 19.68	124.58 ± 10.99	133.30 ± 18.05	124.73 ± 20.63
29	131.57 ± 22.99	127.83 ± 12.40	133.47 ± 18.67	119.18 ± 20.77
36	142.62 ± 27.78	137.32 ± 10.92	140.10 ± 22.36	120.93 ± 20.09
43	143.3 ± 31.74	139.31 ± 13.85	146.51 ± 21.52	121.89 ± 22.62

Note: HAE – Hydroalcoholic extract of *P. zeylanica.* Values are expressed as Grams Mean ± S.D

Table 13: Effect of Hydroalcoholic extract in body weight of Complete Freund's adjuvant Rat model (16 – 45 days Treatment)

Days	Normal	Control	Indomethacin 10 mg/kg	HAE 250 mg/kg
1	110.56 ± 1.72	125.83 ± 9.36	125.58 ± 4.65	158.77 ± 7.98
8	111.53 ± 2.96	114.09 ± 10.99	121.73 ± 12.87	140.68 ± 6.08
15	117.13 ± 15.41	112.52 ± 13.99	119.87 ± 9.34	152.05 ± 8.23
22	125.77 ± 19.68	124.58 ± 10.99	127.45 ± 10.47	160.74 ± 9.86
29	131.57 ± 22.99	127.83 ± 12.40	129.69 ± 10.17	162.61 ± 10.15
36	142.62 ± 27.78	137.32 ± 10.92	130.37 ± 11.16	166.32 ± 14.59
43	143.3 ± 31.74	139.31 ± 13.85	133.07 ± 12.65	174.86 ± 16.54

Note: HAE – Hydroalcoholic extract of *P. zeylanica.* Values are expressed as Grams Mean ± S.D

Table 14: Effect of Hydroalcoholic extract in body weight of Complete Freund's adjuvant Rat model (31 – 45 days Treatment)

Days	Normal	Control	Indomethacin 10 mg/kg	HAE 250 mg/kg
1	110.56 ± 1.72	125.83 ± 9.36	178.66 ± 3.71	178.66 ± 6.61
8	111.53 ± 2.96	114.09 ± 10.99	157.77 ± 3.83	157.77 ± 11.25
15	117.13 ± 15.41	112.52 ± 13.99	157.14 ± 13.83	157.14 ± 12.38
22	125.77 ± 19.68	124.58 ± 10.99	138.97 ± 15.30	138.97 ± 17.30
29	131.57 ± 22.99	127.83 ± 12.40	150.36 ± 17.84	150.36 ± 16.04
36	142.62 ± 27.78	137.32 ± 10.92	153.95 ± 19.17	153.95 ± 15.0
43	143.3 ± 31.74	139.31 ± 13.85	159.27 ± 22.47	159.27 ± 14.22

Note: HAE – Hydroalcoholic extract of *P. zeylanica.* Values are expressed as Grams Mean ± S.D

Table 15: Body weight changes (% Weight gain or loss) by the Hydroalcoholic extract during the different treatment periods

Weeks	1 – 45 days				16 – 45 days		31 – 45 days	
	N	C	I	E	I	E	I	E
1	0.87	-9.33	-6.93	-5.06	-3.85	-18.09	-10.78	-20.89
2	5.94	-10.58	-0.33	4.09	-5.72	-6.72	-4.45	-21.52
3	13.75	-0.99	6.14	4.09	1.87	1.97	-2.37	-39.69
4	18.99	1.58	6.28	14.29	4.11	3.85	0.15	-28.31
5	28.99	9.13	11.55	9.24	4.79	7.56	0.51	-24.71
6	29.61	10.71	16.66	10.83	7.49	16.10	14.66	-19.39

Note: (-) sign indicates % weight loss, N indicates Normal, C indicates Control group, I indicates Indomethacin group 10 mg/kg, E indicates Extract group 250 mg/kg

Table 16: Effect on Oxidative stress by the Hydroalcoholic extract during the different treatment periods

Groups	TBARS		GSH		GPx		CAT	
	Liver	Kidney	Liver	Kidney	Liver	Kidney	Liver	Kidney
Normal	0.47 ± 0.05	0.29 ± 0.04	25.85 ± 0.39	26.67 ± 2.65	0.1863 ± 0.0007	0.1122 ± 0.0001	17.60 ± 1.59	15.24 ± 1.81
Control	0.82 ± 0.03*	0.34 ± 0.03	19.74* ± 2.45	25.72 ± 3.99	0.1068 ± 0.0001	0.1124 ± 0.0001	11.23 ± 0.72	12.49 ± 0.56*
Indo 1	0.47 ± 0.09*	0.40 ± 0.10	25.23*± 2.93	27.56 ± 1.38	0.1407 ± 0.0001*	0.1125 ± 0.0157	16.43 ± 1.24*	15.40 ± 1.41*
Indo 2	0.52 ± 0.14*	0.47 ± 0.04	25.60*± 1.40	28.33 ± 5.04	0.1405 ± 0.0001*	0.1125 ± 0.0001	16.00 ± 2.94*	14.87 ± 1.10*
Indo 3	0.54 ± 0.12*	1.13 ± 0.13	21.64* ± 1.89	25.52 ± 5.90	0.1409 ± 0.0001*	0.1121 ± 0.0004	16.94 ± 0.43*	15.58 ± 1.41
Ext 1	0.61 ± 0.10*	0.71 ± 0.36	21.86* ± 0.59	22.96 ± 4.29	0.1324 ± 0.0002*	0.1117 ± 0.0001	15.14 ± 0.22*	12.07 ± 1.16
Ext 2	0.60 ± 0.366	0.53 ± 0.4	23.97 ± 2.86	22.69 ± 1.39	0.1249 ± 0.0001*	0.1119 ± 0.0003	15.67 ± 0.22*	12.07 ± 1.16
Ext 3	0.73 ± 0.51	0.26 ±0.03	29.80* ± 6.90	27.09 ± 3.39	0.1405 ± 0.0004*	0.1123 ± 0.0001	16.75 ± 1.84*	12.75 ± 0.91

Note: Values are expressed as Mean ± S.D; *P < 0.05, TBARS – Thiobarbituric acid reactive substances, GSH – Reduced Glutathione, GPx – Glutathione Peroxidase, CAT – Catalase; Indo 1, 2, 3 – Indomethacin 10 mg/kg, 1-45, 16-45, 31-45 days treatment respectively; Ext 1, 2, 3 – Hydroalcoholic extract 250 mg/kg, 1-45, 16-45, 31-45 days treatment respectively.

CONCLUSION

The authors concludes that the hydroalcoholic extract of the root bark of *P. zeylanica* have shown the presence of Polyphenol, Phytosterol and Plumbagin which may be the possible chemical constituents responsible for its moderate analgesic and anti-inflammatory activity at the single dose of 350 mg/kg b.wt and 250 mg/kg b.wt dose for 45 days treatment respectively in

experimental rats.

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