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Evaluation of analgesic and Anti-inflammatory effects of aerial parts of *Breynia rhamnoides* Muell. Arg

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Abstract

The plant *Breynia rhamnoides* Muell. Arg (family-Phyllanthaceae/Euphorbiaceae) was reported to have numerous potent constituents and the same plant was used for many traditional medicines. Phytochemical analysis of ethanolic extracts of *Breynia rhamnoides* revealed the presence of many active compounds such as flavonoids, glycosides, phenolic compounds, terpenoids, steroids and saponins. Present work describes the extraction and evaluation of analgesic and anti-inflammatory activities of aerial part extract of *Breynia rhamnoides*. Acute toxicity studies were performed as per OECD-423 guidelines. Toxicity signs and symptoms were not observed. Anti-inflammatory activity was evaluated by carrageenan-induced paw edema model and analgesic activity by Eddy's hot plate method and Acetic acid induced writhing method in Wistar albino rats. The extract exhibited significant anti- inflammatory and peripheral analgesic activity.

Keywords: *Breynia rhamnoides*, anti-inflammatory, carrageenan, analgesic

1. Introduction

Breynia rhamnoides is a pretty evergreen shrub or a small tree up to 3m in height with horizontal, flexuous, bifarious branches and angular branchlets occurring throughout the tropical parts of India including the Andaman Islands. It is also frequently planted as an ornamental hedge in gardens. Bark yellowish grey, rough., leaves distichous, elliptic-ovate, dark brown or black when dry, flowers small, greenish yellow or pink, male in few-flowered fascicles, female solitary., berries globose, dull red, purple or white. Decoction of the root is employed in the Philipines as mouth wash for tooth ache. The leaf juice is given to women after child birth to prevent haemorrage. The dried leaves are powdered and smoked like tobacco for relief in tonsillitis. Treatment of edema, diabetes and dental caries [1-2]. Inflammation is a complex response of vascular tissue to harmful stimuli caused by injury, infection, environmental agents, malignancy and cellular changes. The classic inflammatory symptoms: calor-warmth, dolor-pain, rubor-redness, and tumor-swelling [3]. Inflammation may be acute or chronic, depending on the nature of the stimulus and the effectiveness of the initial reaction in eliminating the stimulus or the damaged tissues [4]. Pain can also be elicited by inflammation. Progress has been made in elucidating the role of various endogenous substances such as prostaglandins and peptides in the inflammatory process. Most of the non-steroidal anti-inflammatory agents have analgesic activity [5]. Effective pain treatment should be an integral part of medical practice, but good analgesia also facilitates recovery from injury or surgery, aids rapid recovery of function, and may minimize chronic pain and disability. Analgesics are divided into two types :a) Morphine and related compounds. b) The antipyretic and anti-inflammatory agents [6].

Analgesic and anti- inflammatory drug abuse has become a major problem in our country due to the over-the-counter sale of such drugs and these are causing not only gastritis, gastric ulcers, gastro-intestinal tract bleeding and renal damage, but a number of other problems too. Research on medicinal plants and natural products like *Breynia rhamnoides* may provide basis for invention of some safe, cheap and effective agents [7].

2. Materials and Methods

2.1 Plant Materials

The *Breynia rhamnoides* plants were collected in the month of March, 2012 from Thottilpalam in Kozhikode district of Kerala, India. The leaves were identified and authenticated by the botanist Mr. Rogimon P. Thomas, Department of Botany, C.M.S. College Kottayam. A voucher specimen (No. 266) was preserved at C.M.S. College, Kottayam.

2.2 Preparation of extract

Shade dried and powdered aerial parts (100g) of *Breynia rhamnoides* was soaked in rectified spirit in a one litre round bottom flask. After soaking it for one day, it was refluxed with ethanol 95% (500ml) for 3 hours and the clear solution was decanted off. The extraction was repeated thrice. The combined extracts were concentrated to a semisolid consistency. Thus total ethanolic extract (TEE) was obtained.

2.3 Phytochemical Screening:

The *Breynia rhamnoides* was tested for the presence of glycosides, flavonoids, phenolics, steroids, terpenoids and reducing sugars by qualitative methods [8].

2.4 Animals

Male Wister albino rats weighing 150-200gm were used for anti-inflammatory and analgesic activities. They were maintained under standard environmental conditions and were fed with standard pellet diet with water *ad libitum*.

2.5 Preparation of the drug for the experimental study:

Ethanolic extract and standard drug Diclofenac sodium were administered in the form of suspension in 0.5% CMC solution was used for the study.

2.6 Acute Toxicity Studies

Acute oral toxicity studies were performed as per OECD-423 guidelines (No: 025/MPH/UCP/CVR/13). 3 female Wistar albino rats were used for the study. The extract was administered orally at the dose of 2000 mg/kg and observed at half an hour intervals for 4 hr, then after 24 hr. There were no mortality and no signs of toxicity.

2.7Anti-inflammatory Activity

2.7.1 Carrageenan-induced paw edema model

The animals were divided into four groups of six animals each, so a total of 24 animals were used and divided according to the following manner:

Group 1: Positive control rats (Inflammatory control)
Group 2: Inflammation induced rats, given Diclofenac sodium (75mg/kg - orally)
Group 3: Inflammation induced rats, given ethanolic extract (200mg/kg- orally)
Group 4: Inflammation induced rats, given ethanolic extract (400mg/kg-orally)

The animals were starved overnight. Approximately 1ml of 1% carrageenan in saline was injected into the plantar side of the right hind paw of the rat. The standard drug and extract were administered 30 min before the carrageenan injection. In this study diclofenac was taken as standard anti-inflammatory agent. The paw was marked with ink at the level of the lateral malleolus and immersed in mercury up to this mark. The paw volume was measured plethysmographically immediately after injection, 1, 2 and 3hr after carrageenan administration [5]. The percentage inhibition of paw edema was calculated by using the following formula;

Percentage of edema inhibition = [(Vc - Vt) / Vc] ×100

Vc- Volume of edema in control group
Vt- volume of edema in treated group

2.8 Analgesic Activity

2.8.1 Eddy's hot plate method

The rats were divided into 4 groups, 6 animals in each group. The hot plate which was commercially available consists of an electrically heated surface. The temperature was controlled between 55° to 56 °C. That was a copper plate or a heated glass surface. The maximum cut off time was 15 seconds. The control group was treated with vehicle: standard group was treated with Pentazocine 5mg/kg and test groups were treated with 200 and 400 mg/kg orally. The animals were placed on the hot plate and the time until either licking or jumping occurs was recorded by a stop-watch. The latency was recorded before and after 0, 30, 60, 90 and 120 min following oral administration of the standard or the test compound[5].

2.8.2 Acetic acid induced writhing method

In this method, pain was produced by the administration of 1% v/v of acetic acid. The rat was placed in separate boxes under observation immediately after acetic acid injection and numbers of abdominal constrictions were counted over a period of 20 min. The experimental protocols were given below:

Group 1- served as control group and was treated with 2%w/v saline solution (1mL/100g body weight of animals).
Group 2- treated with diclofenac (75mg/kg, orally).
Groups 3- treated with alcoholic extract of Breynia rhamnoides (200mg/kg)
Group 4- treated with alcoholic extract of *Breynia rhamnoides* (400mg/kg)

2.9 Statistical analysis

Statistical analysis was performed by one way ANOVA followed by Dunnet multiple comparison test in graph pad prism version 6 software. The result was expressed as Mean± SEM to show differences in groups.

3. Results

Preliminary phytochemical screening showed the presence of glycosides, flavonoids, phenolics, steroids, terpenoids and reducing sugars. The plant extract did not exhibit any mortality up to the dose level 2000mg/kg. The ethanolic extract of aerial parts of *Breynia rhamnoides* was evaluated for anti-inflammatory and analgesic activity. The ethanolic extracts of *Breynia rhamnoides* at the doses of 200 and 400 mg/kg significantly reduced the carrageenan induced edema. The maximum percentage inhibition of extract and Diclofenac sodium were noticed at the 3rd hr of carrageenan administration. Extract and Diclofenac sodium showed significant inhibition of 50.82% and 73.74% at doses of 400mg/kg and 75mg/kg respectively.

The extract exhibited very minute analgesic activity in central analgesia, which was evaluated by eddy's hot plate method. In the acetic acid induced writhing test, the analgesic activity of ethanolic extract of *Breynia rhamnoides* showed significant analgesic effect at the doses of both 200 and 400 mg/kg. The maximum percentage inhibition obtained at the dose of 400mg/kg (47.02%) was comparable to that of the standard Diclofenac sodium at the dose of 75mg/kg (63.28%).

Table 1: Anti-inflammatory activity study using Carrageenan induced paw edema model

Treatment	Mean paw edema volume in M±SEM				Percentage (%) inhibition			
	0hr	1hr	2hr	3hr	0hr	1hr	2hr	3hr
Control	2.250± 0.0763	2.750± 0.0885	2.90± 0.2107	2.983± 0.3167	-	-	-	-
Diclofenac sodium	2.00± 0.6325	1.817± 0.1302**	1.00± 0.1751***	0.7833± 0.0872***	11.11	33.92	65.51	73.74
TEE (200mg/kg)	2.200± 0.06325	2.263± 0.1515	2.150± 0.3585	1.683± 0.3229*	2.22	21.96	25.86	43.58
TEE (400mg/kg)	2.117± 0.0872	2.117± 0.2845	1.850± 0.3603*	1.467± 0.3293**	5.91	23.01	36.20	50.82

Values are Mean±SEM, n=6. ANOVA followed by multiple comparison Dunnet test. *P<0.05, **P<0.01, ***P<0.001 was considered as significant when compared to positive control

Table 2: Analgesic activity by Eddy's hot plate method

Treatment	Dose	Reaction time in seconds (Mean±SEM)					Percentage inhibition (60 min)
		0 min	30min	60min	90min	120min	
Control	Normal saline	4.5± 0.4282	5± 0.3651	5± 0.3651	4.883± 0.3073	4.667± 0.4216	-
Pentazocine	5mg/kg	4.167± 0.3073	8± 0.8563**	13.67± 0.2108***	13.50± 0.4282***	12.83± 0.6540***	63.42%
TEE	200mg/kg	4.167± 0.3073	4.66± 0.333	5.167± 0.4216	4.33± 0.333	4.33± 0.333	3.23%
TEE	400mg/kg	3.833± 0.3073	4.33± 0.2108	5.451± 0.4773	4.5± 0.3416	4.33± 0.333	8.27%

Values are Mean±SEM, n=6. ANOVA followed by multiple comparison Dunnet test. *P<0.05, **P<0.01, ***P<0.001 was considered as significant when compared to positive control

Table no. 3 Analgesic activity by acetic acid induced writhing method

Sl No.	Treatment	Dose	No. writhing movement (20 min) Mean±SEM	Percentage inhibition
1	Control	1.5 ml of 1%v/v acetic acid	19.50±2.045	-
2	Diclofenac sodium	75mg/kg	7.167±0.6009***	63.28%
3	TEE	200mg/kg	13±0.9309*	33.33%
4	TEE	400mg/kg	10.33±2.186**	47.02%

Values are Mean±SEM, n=6. ANOVA followed by multiple comparison Dunnet test. *P<0.05, **P<0.01, ***P<0.001 was considered as significant when compared to positive control

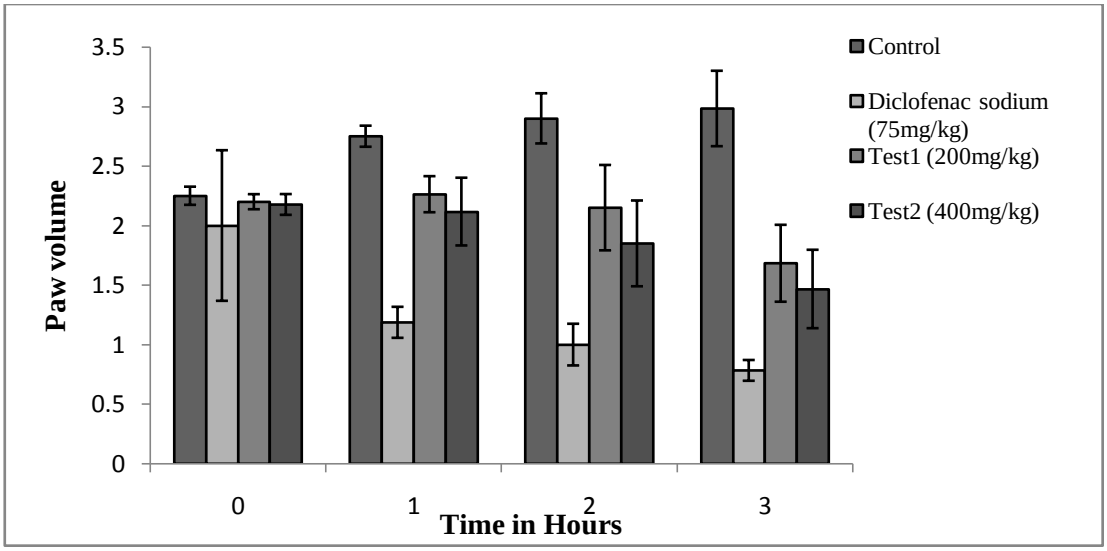

Figure 1: Anti-inflammatory activity by Carrageenan induced paw edema model

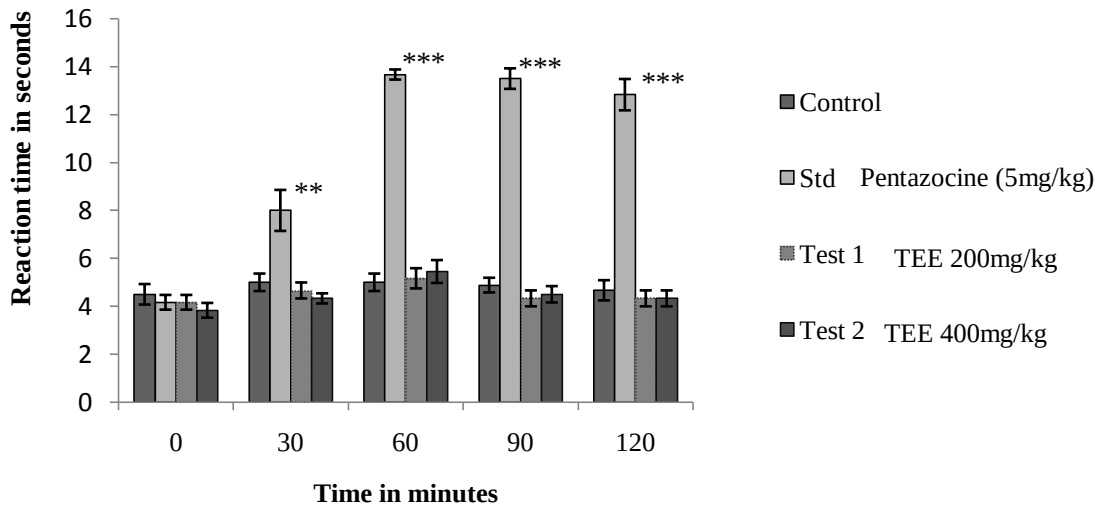

Figure 2: Analgesic activity by Eddy's hot plate method

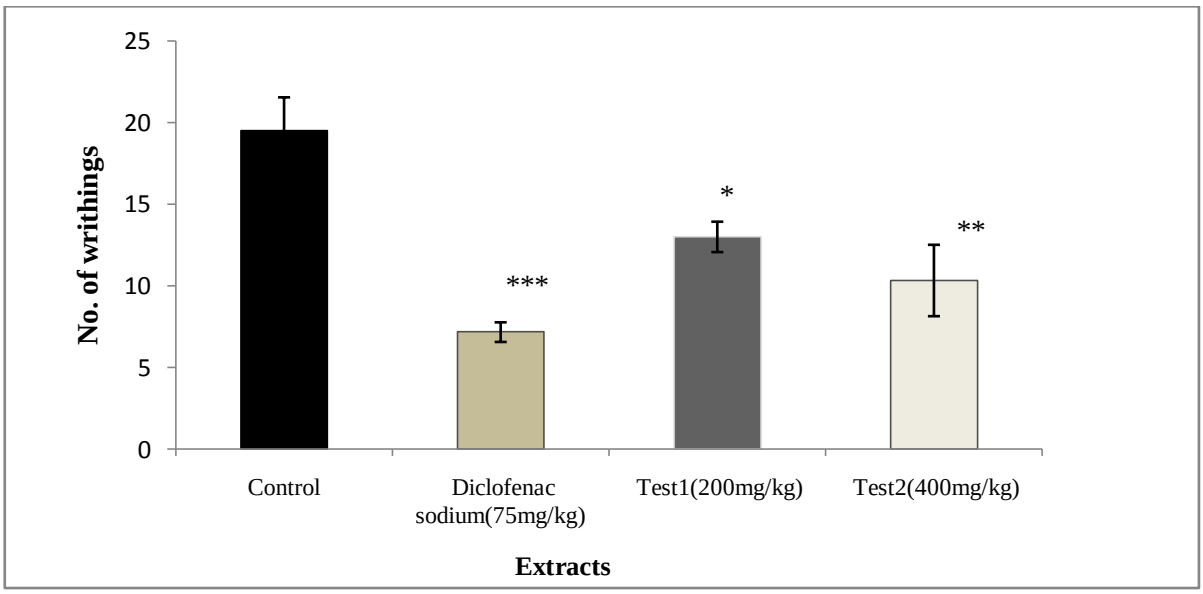

Figure 3: Analgesic activity by acetic acid induced writhing method

4. Discussion

In the present study, the aerial parts of *Breynia rhamnoides* were evaluated for anti-inflammatory and analgesic activities. The extracts were found to be rich in bioactive secondary metabolities including phenolics, flavonoid, terpenes, steroids, gylcosides and carbohydrates in preliminary phytochemical screening. Acute oral toxicity studies were performed and mortality was not observed up to 2000mg/kg.

Carrageenan-induced inflammatory process is believed to be biphasic. The initial phase seen at the 1st hr is attributed to the release of histamine and serotonin. The second accelerating phase of swelling is due to the release of prostaglandin, bradykinin and lysozyme. It has been reported that the second phase of edema is sensitive to both clinically useful steroidal and non-steroidal anti-inflammatory agents. The anti-inflammatory activity exerted by TEE of *Breynia rhamnoides* suggests that it may be due to inhibition of kinnin, prostaglandin, bradykinin and lysozyme synthesis.

The ethanolic extracts of *Breynia rhamnoides* at the doses of 200 and 400 mg/kg significantly reduced the carrageenan induced edema. The maximum percentage inhibition of extract and diclofenac sodium was observed at 3 hr of carrageenan administration. Extract and diclofenac sodium showed significant inhibition of 50.82% and 73.74% at doses of 400 mg/kg and 75mg/kg respectively.

The hot plate test was selected to investigate central analgesic activity because of its several advantages particularly the sensitivity to strong analgesic and limited tissue damage. The extract exhibited very minute analgesic activity in central analgesia, which was evaluated by eddy's hot plate method.

Acetic acid induces pain by enhancing the levels of endogenous substances like PGE_2 and PGF_2 in the peritoneal cavity. This indicates that acetic acid acts indirectly in the stimulation of nociceptive neurons by the release of endogenous mediators. Thus, acetic acid was used to screen compounds for peripheral analgesic activity. In the present study, the diclofenac sodium and ethanolic extract of *Breynia rhamnoides* treated groups showed a significant analgesic effect compared to that of control group. The percentage inhibition by TEE at the dose of 400 mg/kg (47.02%) was comparable to that of the standard diclofenac sodium at the dose of 75mg/kg (63.28%).

Prostaglandins and bradykinins were suggested to play an important role in pain. Bioactive secondary metabolites including phenolics, flavonoids, steroids, terpenoids and glycosides were reported to be inhibitors of prostaglandin synthesis. So these bioactive molecules may be responsible for the analgesic effect produced by *Breynia rhamnoides*.

5. Conclusion

The aerial parts of *Breynia rhamnoides* have potent analgesic and anti-inflammatory activites. The activities might be due to the presence of bioactive secondary metabolites.

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