



Anti inflammatory Activity of Methanolic Extract of Whole Plant of *Euphorbia nivulia* on Carrageenin induced Edema in Wistar Rats.

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Received: 10-01-2017; Revised: 30-01-2017; Accepted: 14-02-2017.

ABSTRACT

Euphorbia nivulia is an herbal extensively used in Indian system of medicine belongs to family Euphorbiaceae. It is a traditional medicinal plant having broad pharmacological activities. The objective is to evaluate anti-inflammatory activity of methanolic extract of whole plant of *Euphorbia nivulia* by using different animal models. Adult wistar rats of either sex weighing 150-250gms were randomly divided into 8 groups (n=6); control (vehicle), Standard (Ibuprofen 25mg/kg) and methanolic extract (200,400,600 mg/kg) and Aq extract (200,400,600 mg/kg). Each rat was fed orally with the respective drug 1hr prior to the administration of the phlogistic agent. The in vivo anti-inflammatory activity was studied using carrageenan induced paw edema, acetic acid induced vascular permeability in mice, xylene-induced ear edema swelling in mice, PMA induced ear edema in mice as acute inflammatory evaluation methods and Cotton pellet induced granuloma in rats as cronic evaluation method. Anti-inflammatory activity is expressed as Percent Inhibition. The Percent Inhibition with the control, Standard (Ibuprofen) and the test compounds methanolic extract (200,400,600 mg/kg) and Aq extract (200, 400, 600 mg/kg), in the carrageenan induced paw edema model were% inhibition of standard is 80% and most effectively responded dose at methanolic extract 600mg at 85%, in the acetic acid induced paw edema model were p.s.b. value of standard is 114.06 and most effectively responded dose at methanolic extract 600mg at 121.35, in the xylene induced paw edema model were% inhibition of standard 62%and most effectively responded dose at methanolic extract 600mg at 58%, in the PMA induced paw edema model were% inhibition of standard 82.44% and most effectively responded dose at methanolic extract 600mg at 78.65%, in the cotton pellet induced paw edema model were% inhibition of standard 66.66% and most effectively responded dose at methanolic extract 600mg at 72.33%. The *Euphorbia nivulia* methanolic extract and Aq extract also shows considerable anti-inflammatory activity in comparison to Ibuprofen, with minimal side effects. Hence *Euphorbia nivulia* whole plant powder can be used as a potential adjuvant with the conventional anti-inflammatory drugs for the therapy of inflammation.

Keywords: *Euphorbia nivulia*, carrageenan, acetic acid, xylene, cotton pellet, formalin.

INTRODUCTION

Traditional medicine occupies a central place among rural communities of developing countries for the provision of health care in the absence of an efficient primary health care system. The traditional medicine mainly depends on plant bio-diversity and related knowledge of their use as herbal medicines. In the recent years there has been a tremendous interest in the developed countries for herbal medicines and dietary supplements for a new deal in healthcare in which the old remedies feature strongly. Antioxidant effects of herbal compounds and their role in health and disease(s) in particular is a strongly emergent field. It is generally accepted that free radicals play an important role in the development of tissue damage and pathological events. In addition to the protective effects of the endogenous antioxidant defense system, natural products with antioxidant activity could retard the oxidative damage of a tissue by increasing those defenses.

Inflammatory diseases including different types of rheumatic diseases are very common throughout the world. Although rheumatism is one of the oldest known diseases and affects a large population, no substantial progress has been made in achieving a permanent cure.

Most clinically important steroidal or non-steroidal chemical agents for the treatment of inflammation-related diseases have various moderate to severe adverse effects. The disadvantage of these drugs rests in their toxicity and reappearance of symptoms after discontinuation and a long-term treatment is required in chronic disease. Most of the Euphorbiaceae plants are rich in alkaloids, flavonoids, terpenoids, and glycosides. Several studies on these plants and their constituents have shown to display wide range of biological actions such as gastro protective, anti-inflammatory, antibacterial, antiviral, anticancer and anti-allergic activities. The herbs *Euphorbia nivulia* is herbaceous dissent plant, grown in various parts of India. These species are grown widely in tropical forests and wastelands in most parts of south India. various parts of *Euphorbia nivulia* have been extensively used in alternative systems of medicine in different forms such infusions, decoctions, poultice, either alone or in combination with other herbs for the treatment of inflammation, ulcers, asthma, rheumatism, arthritis, neuropathy, fever, skin diseases, headache. Vegetation is one of the precious gifts of nature where plants are intimately related to human being. People have been utilizing plants as medicine since the earliest period of civilization. The use of plants for curing human diseases is an ancient practice, in which



interest has been revived at modern age. People of remote villages and tribal areas are dependent upon the practice of folk medicines. In recent times, focus on plant research has increased all over the world and a large body of evidence has been collected to show immense potential of medicinal plants used in various traditional systems. Ethno botanically plant latex has a great potential with respect to its medicinal value. Latex has been reported to occur in 12000 plant species belonging to 900 genera. A common feature that can be found in the latex of the *Euphorbiaceae* is the presence of noticeable digestive enzyme activity. *Euphorbia* is a large genus consisting of about over 2000 species in the world. Approximately 195 species of *Euphorbia* have been recorded from India

MATERIALS AND METHODS

Acute Anti-inflammatory studies

(a) Carrageenan-induced paw oedema in rats

Oedema was induced by injecting 0.1ml of carrageenan (1% w/v) in normal saline into the sub planter region of the left hind paw, after 1 hr of extract / standard drug administration orally. The volume of paw was measured with pleythesmometer after 1 hr, 2hr, 3hr, and 5hr of carrageenan injection. Results were determined as the percent inhibition of oedema and were compared with the control.

(b) Acetic acid-induced vascular permeability in mice

The mice were dosed orally with the test substances suspended in 0.3% carboxy methyl cellulose solution (CMC) 30 min before the injection of 0.7% acetic acid-saline solution (i.p., 0.1 ml/10 g b.w). 4% pontamine sky blue (10 ml/kg) was also injected by tail vein after 30 minutes of acetic acid injection. After 20 min, the mice were sacrificed and then the pigment exuded to abdominal cavity was washed with 10 ml of distilled water and centrifuged (3000rpm for 10 min). The absorbance of supernatants was measured at 580 nm using UV-vis spectrophotometer. The vascular permeability effects were expressed in terms of dye amount per 30 g weight of mouse, which leaked into the peritoneal cavity. The results were compared with positive control aspirin.

(c) Xylene induced ear oedema

Swiss albino mice were selected for the study and divided into groups of six each. One hour after intraperitoneal injection of the extract, 0.03 ml of xylene was applied to the anterior and posterior surfaces of the right ear. The left ear was considered as control. Thirty minutes after xylene application, mice were killed by euthanasia within an atmosphere of ether and both ears were removed. The earlobes were punched and weighed. The increase in weight of the right ear punch over that of left indicated the oedema.

(d) PMA induced mouse ear oedema in mice

Oedema was induced by topical application of 2.5 µg phorbol-12-myristate-13-acetate (PMA) in 20µl acetone

to the right ear of mice. The left ear served as control and received only acetone. The hydro alcoholic extract was dissolved in acetone before PMA application. After 4 h, mice were subjected to light anesthesia by ether and then killed by cervical dislocation. The earlobes were punched and weighed. The increase in weight of the right ear punch over that of left indicated the oedema and was measured.

Chronic Anti-inflammatory studies

(a) Cotton pellet-induced granuloma in rats

Male Wistar albino rats were used for this study. Granulomatous lesions were induced by surgically implanting two cotton pellets (50 mg) subcutaneously in the dorsal region of the rats, one near each axilla. Plant extract (250 and 500mg/kg body wt.) or vehicle (5 ml/kg body wt.) were given orally once daily for 7 days. On 8th day, the rats were anesthetized under light ether, and the pellets covered by granulomatous tissue were dissected and dried at 60°C to a constant weight. The mean weights for different groups were determined, and compared with the control.

(b) Formalin-induced paw oedema

The animals were pre-treated orally with hydro alcoholic extracts suspended 0.3% CMC (250 and 500 mg/kg body weight). After treatment with plant extracts, the animals were injected with 0.05 ml of 2% formalin (in 0.9% aq. NaCl) into the plantar surface of the left hind paw. The volumes of the paws were measured before the induction and also at various time intervals after the injection. Oedema was expressed as an increase of foot pad thickness in relation to the initial value. Positive control group was treated orally with indomethacin (50 mg/kg).

RESULTS

(a) Effect of *Euphorbia nivulia* on Carrageenan induced paw edema in rats

The effects of 50% methanolic extract of *Euphorbia nivulia* at three dose levels (200 mg/kg, 400mg/kg and 600 mg/kg) in carrageenan induced rat paw edema was studied. The results revealed that all extracts tested at three dose levels (200 mg/kg, 400mg/kg and 600 mg/kg) showed significant protection in the acute inflammation induced by carrageenan. In particular, at 400 mg/kg both the extracts were capable of producing 83-88% protection. The onset of action in all the extracts tested was observed more than 50% at the end of 2hrs itself.

Further, the results when compared with 0 Hr reading of same group at specific dose levels tested (200 mg/kg, 400mg/kg and 600 mg/kg), it was observed that methanolic extract of *Euphorbia nivulia* started showing the protection after 2 hrs at 200 mg/kg and 600mg/kg whereas 400 mg/kg showed at the end of 1 hr itself and continued till the end of 5 hrs and quite comparable with that of Ibuprofen.



Table 1: Effect of *Euphorbia nivulia* on Carrageenan induced paw edema in rats

Treatment	Paw Volume (ml) and % inhibition of paw edema								
	0 Hr	1 Hr		2 Hr		3 Hr		5 Hr	
Control (CMC)	0.11 ± 0.008	0.66 ± 0.03 ^{###}	-	0.95 ± 0.04 ^{####}	-	1.18 ± 0.02 ^{####}	-	1.51 ± 0.07 ^{####}	-
Ibuprofen (25 mg/kg)	0.11 ± 0.010	0.21 ± 0.08	66	0.17 ± 0.006	80	0.15 ± 0.006	85	0.13 ± 0.02	93
EN methanol extract (200 mg/kg)	0.12 ± 0.017	0.41 ± 0.03 [#]	38	0.37 ± 0.03 [#]	61	0.31 ± 0.04	74	0.225 ± 0.04	85
EN methanol extract (400 mg/kg)	0.13 ± 0.007	0.36 ± 0.04 [#]	46	0.32 ± 0.03	67	0.27 ± 0.02	77	0.18 ± 0.02	88
EN methanol extract (600 mg/kg)	0.13 ± 0.008	0.22 ± 0.02	77	0.19 ± 0.006	85	0.18 ± 0.02	88	0.14 ± 0.02	92
EN Aq extract (200 mg/kg)	0.19 ± 0.007	0.53 ± 0.04 ^{###}	21	0.47 ± 0.03 ^{###}	50	0.4 ± 0.03 [#]	66	0.31 ± 0.03	80
EN Aq extract (400 mg/kg)	0.13 ± 0.01	0.47 ± 0.03 ^{###}	30	0.41 ± 0.03 [#]	56	0.34 ± 0.02	72	0.25 ± 0.02	83
EN Aq extract (600 mg/kg)	0.14 ± 0.02	0.33 ± 0.02	48	0.28 ± 0.02	75	0.21 ± 0.02	81	0.16 ± 0.02	89

Values are expressed as mean ± S.D; n=6; [#]P<0.05, ^{###}P<0.01, ^{####}P<0.001 when compared to 0 Hr reading

(b) Effect of *Euphorbia nivulia* on acetic acid induced vascular permeability in mice

The intraperitoneal injection of acetic acid caused squirming and increased the capillary permeability that was measured by direct estimation of plasma-bound dye (Pontamine Sky Blue) which has leaked into the peritoneal cavity. The study revealed that all extract of *Euphorbia nivulia* (200 mg/kg, 400mg/kg and 600 mg/kg) significantly decreased the vascular permeability. Moreover, EN AQ extract 600 significantly (P<0.001) decreased the vascular permeability when compared to EN AQ extract 200 and 400. The results were tabulated in table 2.

Table 2: Effect of *Euphorbia nivulia* on acetic acid induced vascular permeability in mice

Group	Amount of P.S.B (µg/30g b.wt)
Normal	97.96 ± 4.72
Control	247.11 ± 12.14
Asprin (100 mg/kg)	114.06 ± 6.63 ^{***}
EN methanol extract (200 mg/kg)	152.23 ± 6.01 ^{***}
EN methanol extract (400 mg/kg)	132.85 ± 4.66 ^{***}
EN methanol extract (600 mg/kg)	121.35 ± 6.23 ^{***} €€€
EN AQ extract (200 mg/kg)	217.33 ± 8.43 ^{**}
EN AQ extract (400 mg/kg)	188.25 ± 5.78 ^{***} †††
EN AQ extract (600 mg/kg)	162.33 ± 3.78 ^{***} †††

(c) Effect of *Euphorbia nivulia* on xylene-induced ear edema swelling in mice

The circular sections of mice ears were cut and measured. The results showed that both extracts of

Euphorbia nivulia (200 mg/kg, 400mg/kg and 600 mg/kg) significantly (P<0.001) inhibited the ear swelling. Further, the EN methanol extract 600 showed the equipotent activity as that of EN methanol extract 200 and 400. Moreover, EN Aq extract 600 was significantly (P<0.001) inhibited the ear swelling when compared to EN Aq extract 200 and 400 showing the dose dependent activity. The results were tabulated in table 3.

Table 3: Effect of *Euphorbia nivulia* on xylene-induced ear edema swelling in mice

S.No	Treatment	Ear Swelling (mg)	Inhibition (%)
1	Control (CMC)	30.38 ± 2.34	-
2	Ibuprofen (25 mg/kg)	11.87 ± 1.46 ^{***}	62
3	EN methanol extract (200 mg/kg)	17.43 ± 1.91 ^{***}	43
4	EN methanol extract (400 mg/kg)	15.06 ± 1.05 ^{***}	51
5	EN methanol extract (600 mg/kg)	13.08 ± 2.45 ^{***} €	58
5	EN AQ extract (200 mg/kg)	24.85 ± 2.26 ^{***}	17
6	EN AQ extract (400 mg/kg)	18.20 ± 1.47 ^{***}	40
6	EN AQ extract (600 mg/kg)	15.20 ± 3.73 ^{***} †††	50

Values are expressed as mean ± S.D, n=6; ^{***}P<0.001 when compared to control group; €P<0.05 when compared to EN methanol extract 200 and 400; ^{†††}P<0.001 when compared to EN AQ extract 200 and 400; One way ANOVA was applied followed by Turkey's multiple comparison tests

(d) Effect of *Euphorbia nivulia* on PMA induced ear edema in mice

The punched ear lobes were weighed after PMA application. The results showed that both extracts of *Euphorbia nivulia* (200 mg/kg, 400 mg/kg and 600 mg/kg) significantly inhibited the ear swelling and shown dose dependent activity. Further, the EN Aq extract 600 showed the equipotent activity as that of EN methanol extract 600. The results were tabulated in table 4.

Chronic Anti-inflammatory studies**(a) Effect of *Euphorbia nivulia* on Cotton pellet induced granuloma in rats**

The effect of methanolic extracts of *Euphorbia nivulia* (200 mg/kg, 400mg/kg and 600 mg/kg) in cotton pellet induced granuloma formation in rats was studied and the percentage inhibition of granuloma was calculated. The study revealed that treatment of rats with all the extracts of *Euphorbia nivulia* (200 mg/kg 400 mg/kg and 600 mg/kg) showed significant ($P < 0.001$) reduction in weight of cotton pellet induced granuloma.

Table 4: Effect of *Euphorbia nivulia* on PMA induced ear edema in mice

S.No	Treatment	% Inhibition
1	Indomethacin	82.44
2	EN ethanol extract (200 mg/kg)	32.12
3	EN ethanol extract (400 mg/kg)	59.94
4	EN ethanol extract (600 mg/kg)	78.65
5	EN Aq extract (200 mg/kg)	33.82
6	EN Aq extract (400 mg/kg)	60.52
7	EN AQ extract (600 mg/kg)	66.66

Values are expressed as mean \pm S.D; n = 6

Moreover, *Euphorbia nivulia* extracts showed the anti-inflammatory effects dose dependently. Although, the groups treated with EN Aq extract has less efficacy when compared to the EN methanol extract treated groups, the activity of EN AQ extract 500 is equipotent to EN methanol extracts of 200 and 400. The results were provided in table 5.

(b) Effect of *Euphorbia nivulia* on paw edema induced by formalin in mice.

The effect of methanolic extracts of *Euphorbia nivulia* (200 mg/kg 400mg/kg and 600 mg/kg) in formalin induced paw edema in mice was studied and the paw thickness was measured. The study revealed that treatment of mice with all the extracts of *Euphorbia nivulia* (200 mg/kg 400mg/kg and 600 mg/kg) showed significant ($P < 0.001$) reduction of paw thickness. Further, the extracts showed dose dependent activity in reducing the paw edema. Moreover, the activity of EN Aq extract 600 is equipotent to EN methanol extracts of 200 and 400. The results were detailed in table 6.

DISCUSSION

Inflammation is a protective attempt by the organism to remove the injurious stimuli and to initiate the healing process. Inflammation can be classified as either acute or chronic. Acute inflammation is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes (especially granulocytes) from the blood into the injured tissues. A cascade of biochemical events propagates and matures the inflammatory response, involving the local vascular system, the immune system, and various cells within the injured tissue. Prolonged inflammation, known as chronic inflammation, leads to a progressive shift in the type of cells present at the site of inflammation and is characterized by simultaneous destruction and healing of the tissue from the inflammatory process. Anti-inflammatory drugs inhibit different stages of inflammation.

One way ANOVA was applied followed by Dunnet's test

Euphorbia nivulia is a dessert plant with enormous properties for curing and preventing diseases. Various studies have been performed with this plant for its antibacterial, antioxidant, antiulceric, antimalarial, antidiabetic, anti-inflammatory, antilipidemic, anti-cancer and immuno modulatory properties. phytochemical studies are conducted for determination of active constituents in this plant.

Table 5: Effect of *Euphorbia nivulia* on Cotton pellet induced granuloma in rats

S. No	Treatment	Increase in weight of pellet (mg)	% inhibition
1	Control (CMC)	122.66 \pm 5.77	0
2	Ibuprofen (25 mg/kg)	40.96 \pm 5.00***	66.66
3	EN methanol extract (200 mg/kg)	87.65 \pm 4.17**	28.40
4	EN methanol extract (400 mg/kg)	47.35 \pm 5.23**	61.29
5	EN methanol extract(600 mg/kg)	42.44 \pm 8.38***	82.33
6	EN AQ extract (200 mg/kg)	95.2 \pm 9.15**	22.31
7	EN AQ extract (400 mg/kg)	75.44 \pm 4.46**	38.34
8	EN AQ extract (600 mg/kg)	52.77 \pm 7.64**	58.54

Values are expressed as mean \pm S.D; n=6; *** $P < 0.001$ when compared to control group; One way ANOVA was applied followed by Turkey's multiple comparison test.

In studies conducted previously, the diterpenes of



Euphorbia nivulia was found to possess significant anti-inflammatory activity against carrageenan and other mediator-induced paw edema in rats. The results of anti-inflammatory activity of *Euphorbia nivulia* support the dual inhibition of arachidonate metabolism as indicated by its activity in inflammation models. Carrageenan induced paw edema has been used to study acute and sub-acute phases of inflammation. It is a widely used irritant or a phlogistic agent.

Table 6: Effect of *Euphorbia nivulia* on paw edema induced by formalin in mice

S. No	Treatment	Edema (mm X 10 ⁻²)
1	Control (CMC)	36.28 ± 3.52
2	Indomethacin (50 mg/kg)	23.35 ± 2.61***
3	EN methanol extract (200 mg/kg)	33.00 ± 1.66*
4	EN methanol extract (400 mg/kg)	29.45 ± 2.61**
5	EN methanol extract (600 mg/kg)	24.56 ± 1.78***
5	EN AQ extract (200 mg/kg)	32.12 ± 2.32**
6	EN AQ extract (400 mg/kg)	26.6 ± 1.78**
7	EN AQ extract (600 mg/kg)	25.5 ± 5.83**

Values are expressed as mean ± S.D; n=6; ***P<0.001 when compared to control group

CONCLUSION

In conclusion, the methanolic extract and Aq extract of *Euphorbia nivulia* inhibited edema produced by carrageenan, acetic acid, xylene, formalin and PMA. *Euphorbia nivulia* was found to be a potent anti-inflammatory agent in these tests. Although further experiments are required to establish these sesqui terpenoid compounds as potential therapeutic agents, some of the beneficial effects ascribed in traditional medicine to whole plant of *Euphorbia nivulia* could be related with the anti-inflammatory effects.

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Source of Support: Nil, **Conflict of Interest:** None.

