International Journal of Pharmacy Teaching & Practices 2013, Vol.4, Issue 4, 858-862.

Evaluation of the Anti-Inflammatory Activity of *Desmodium Triangulare* (Retz.) Merr. Root

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Research Article

Please cite this paper as R.S.Jayaseelan[1*], Fijesh P.Vijayan[1], Brindha[2], Suresh .V[1] Jose Padikkala[2]. Evaluation of the Anti-Inflammatory Activity of *Desmodium Triangulare* (Retz.) Merr. Root. IJPTP, 2013, 4(4), 858-862.

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Abstract

Desmodium triangulare, belonging to the family fabaceae is a medicinal plant.use in the present study , the anti-inflammatory effect of *Desmodum. triangulare* was studied using both model acute and chronic inflammatory models.carrageenan and dextran and induced acute paw edema . formalin induced chronic paw edema in swiss albino mice. Diclofenac at a dose of 10mg /kg body weight severe as standard drug .the anti-inflammatory activity estimated volumetrically by measuring the mean mice with the help of a vernier caliper at different time of paw intervels. The administration of *Desmodium .triangulare.*extract at dose 50,250mg /kg body weight given by oral administration. inhibited by carrageenan after 3 hour 50mg/kg(0.29±0.007)(47.06) 250mg kg(0.20±0.018)(64.70%)dextran with reduced after 5hour inhibition50mg/kg (0.27±0.244)(30.84%)250mg/kg (0.235±0.274)(53.86)formaline reduced the 6th day interval 50mg(1.64±0.72)(49.38%) 250mg/kg (1.15±0.47)(64.50%) of inhibition activity..The experimental data demonstrated that *Desmodium.triangulare*.extract possess remarkable anti-inflammatory activity.

Keywords: *Desmodium triangulare, Anti-inflammatory, carrageenan, dextran, formaldehyde-induce edema*

Introduction

Medicinal plant is believed to be an important source of new chemical substances with potential therapeutic effects. Herbalism is a traditional medicinal or folk medicine practice based on the used plant and plant extracts.(Acharya *et al*.,2008) many plant synthesize substance that are use full synthesis and maintenance of the health human for animals these include the aromatic substances. Most of which are phenol are there oxygen-substituted derivates such as tannis. Many herbs spices of used to human spices used to human season food yield used the medicinal compounds (lai 2004; tapsell,2006) Inflammation is a physiologic process in response to tissue damage resulting from microbial pathogen infection, chemical irritation, and/or wounding (Philip *et al*.,2004). At the very early stage of inflammation, neutrophils are the first cells to migrate to the inflammatory sites under the regulation of molecules produced by rapidly responding macrophages and mast cells prostationed in tissues (Coussens and, Werb2002)(Nathan2002).As the inflammation progresses, various types of leukocytes, lymphocytes, and other inflammatory cells are activated and attracted to the inflamed site by a signaling network involving a great number of growth factors, cytokines, and chemokines (Coussens and, Werb2002)(Nathan2002),Although it is a defense mechanism, the complex events and mediators involved in the inflammatory reaction can aggravate many diseases.(Sosa *et al*.,2002) The current management of inflammatory diseases is limited to the use of anti-inflammatory drugs whose chronic administration is associated with several adverse effects. Therefore, development of newer and more anti-inflammatory drugs with lesser side effects is necessary.

Material and Method

Animals

Male Swiss albino mice of 8-10 week old weighing 25-28 g, were selected from inbred group maintained under standard condition of temperature (25±5) and humidity. Animals were provided with food and water *ad libitum.* All experiments in this study were carried out with the prior approval of the Institutional Animal Ethics Committee (IAEC) and were conducted strictly adhering to the guidelines of Committee for the Purpose of Control and Supervision of Experiments

on Animals (CPCSEA) constituted by the Animal Welfare division of government of india.

Evaluation of acute oral toxicity of *Desmodium triangulare* extract

Acute oral toxicity studies were done according to OECD guidelines (Guidelines no: 423). 15 animals are randomly selected and divided in to 5 groups (3 animals each), marked to permit individual identification, and kept in their cages for at least 5 days prior to dosing to allow for acclimatisation to the laboratory conditions. The extracts at doses 5, 50, 300 and 2000 mg/kg body weight were used for the study. The extract was administered in a single dose by gavage using a stomach tube or a suitable intubation canula. Animals were observed individually for mortality and mordability, after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours, and daily thereafter, for a total of 14 days. The additional observations such as changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous systems, and somatomotor activity, behaviour pattern and attention to observations of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma.fare Division of Government of India.

Evaluation of Anti-Inflammatory Activities

Carrageenan induced acute inflammation

The anti-inflammatory activity was evaluated by the carrageenan-induced paw edema test in the mice. Male Swiss albino mice (22–28 g) were injected subplantarly into right hind paw with 0.02 ml of 1% suspension of carrageenan in 0.9 % normal saline.. Paw volume was measured 1h prior and for 5 h after carrageenan administration using a Vernier Caliper. The 70% methanol extract of *D. triangulare.* at dosages 50 mg/kg body and 250 mg/kg was administered (oral) 1 h prior to carrageenan injection. Diclofenac (10 mg/kg) was used as standard reference drug. The control group received equivalent volume of the vehicle. The percentages of inhibition were calculated according to the following formula.

Percent inhibition = [(V_T − *Vo*) control − (V_T − V_o) treated group/(V_T − V_o)] × 100

Were, VT - Paw oedema at various time intervals

V_o - Initial paw oedema

Group 1: Control, injected with 1% carrageenan.

Group 2: Standard, administered with 10 mg/ml Diclophenac + 1% carrageenan injection

Group 3: The 70% methanol extract of *D.triangulare* 50 mg/kg body weight + 1% carrageenan injection.

Group 4: The 70% methanol extract of *D.triangulare* 250 mg/kg body weight + 1% carrageenan injection.

Dextran induced acute inflammation

The inflammatory was induced by using dextran in Swiss albino mice. All the animals were injected subplantarly into right hind paw with 0.02 ml of 1% suspension of dextran in 0.1% carboxy methyl cellulose. Paw volume was measured 1h prior and for 5 h after dextran administration using a vernier caliper. The 70% methanol extract of *D.triangulare.* extract at dosages 50 mg/kg body and 250 mg/kg was administered (oral) 1 h prior to dextran injection. Diclofenac (10 mg/kg) was used as standard reference drug. The control group received equivalent volume of the vehicle. The percentages of inhibition were calculated according to the following formula.

Percent inhibition = [(V_T − *Vo*) control − (V_T − V_o) treated group / (V_T − V_o)] × 100

Were, V_T - Paw oedema at various time intervals

V_o - Initial paw oedema

Group1: Control, injected with 1% dextran.

Group 2: Standard, administered with 10mg/ml Diclophenac + 1% dextran injection

Group 3: The 70% methanol extract of *D. triangulare.*extract 50 mg/kg body weight + 1% dextran injection.

Group 4: The 70% methanol extract of *D.triangulare* extract 250 mg/kg body weight + 1% dextran injection.

Formalin induced acute inflammation

The inflammatory was induced by using formalin in Swiss albino mice. All the animals were injected subplantarly into right hind paw with 0.02 ml of 1% solution of formalin. Paw volume was measured 1h prior and for 6 days after formalin administration using a vernier caliper. The 70% methanol extract of *D.triangulare.* extract at dosages 50mg/kg body and 250 mg/kg was administered (oral) 1 h prior to dextran injection. Diclofenac (10 mg/kg) was used as standard reference drug. The control group received equivalent volume of the vehicle. The percentages of inhibition were calculated according to the following formula.

Percent inhibition = [(V_T − *Vo*) control − (V_T − V_o) treated group / (V_T − V_o)] × 100

Were, V_T - Paw oedema at various time intervals

V_o - Initial paw oedema

Group1: Control, injected with 1% formalin

Group 2: Standard, administered with 10 mg/ml Diclophenac + 1% formalin injection

Group 3: The 70% methanol extract of ***D.Triangulare*** extract 50 mg/kg body weight + 1% formaline injection.

Group 4: The crude methanol extract of *D.triangulare* extract 250 mg/kg body weight + 1% formalin injection.

Statistical Analysis

The values are presented as mean ± SD. Differences between group's means were estimated using a one way analysis of variance followed by Dunnett test, using GraphPad Instat Software. The results were considered stastically significant when *P<0.05*.

Results

Table: 1, Effect of D. *triangulare* extract on Carrageenan induced paw edema in swiss albino.

Groups	Initial	0th hour	1st hour	2nd hour	3rd hour	4th hour	5th hour	24th hour
Control	**0.20 ± 0.019**	**0.32 ±0.012**	**0.34± 0.015**	**0.37±0.011**	**0.37±0.034**	**0.33 ±0.035**	**0.30 ±0.028**	**0.22 ±0.010**
Diclofenac	**0.20 ± 0.018**	**0.35±0.012****	**0.38±0.008****	**0.37±0.011**	**0.27±0.011****	**0.24±0.022****	**0.22±0.021****	**0.20±0.010**
D.triangulare	**0.20 ± 0.013**	**0.32±0.011**	**0.32±0.011**	**0.33±0.013****	**0.29±0.007****	**0.26±0.011***	**0.22±0.017****	**0.21±0.010**
D.triangulare	**0.20 ± 0.018**	**0.33±0.012**	**0.34±0.012**	**0.30±0.043****	**0.26±0.032****	**0.23±0.025****	**0.22±0.0148****	**0.20±0.013**

Values are Mean ± SD, for 6 animals in each group. * *P < 0.01*; ** *P < 0.05* when compared to control.

Table: 2, Inhibition of carrageenan induced paw edema volume in mice by *D. triangulare* treatment on 3rd hour.

Groups	Doses	Initial Paw thickness (cm)	Paw thickness on 3rd hour (cm)	Increase in paw thickness (cm)	% of inhibition
Control	------	0.20 ± 0.019	**0.37±0.034**	0.170015	—
Diclofenac	10 mg/kg body weight	0.20 ± 0.018	**0.27±0.011**	0.06993	58.86
D.triangulare	50 mg/kg body weight	0.20 ± 0.013	**0.29±0.007**	0.08999	47.06
D.triangulare	250 mg/kg body weight	0.20 ± 0.018	**0.26±0.032**	0.0600	64.70

Values are Mean ± SD, for 6 animals in each group. * *P < 0.01*; ** *P < 0.05* when compared to control.

Table: 3, Effect of *D.triangulare* extract on dextrone induced paw edema in swiss albino.

Groups	Initial	0th hour	1st hour	2nd hour	3rd hour	4th hour	5th hour	24th hour
Control	0.17±0.011	0.30±0.085	0.32 ± 0.0282**	0.31 ±0.151	0.30 ±0.011	0.25± 0.280	0.23 ± 0.257	0.21±0.011
Diclofenac	0.18±0.0205	0.31±0.0934	0.32±0.0830**	0.27±0.356	0.24±0.0237	0.25±0.221	0.27±0.283	0.20±0.0205
D.triangulare	0.18±0.01491	0.31±0.04123	0.31±0.1475**	0.29±0.321	0.27±0.0214	0.27±0.244	0.253±0.133	0.22±0.01461
D.trianngulare	0.20±0.0290	0.322±0.1299*	0.314±0.08616**	0.279±0.1888**	0.260±0.0325	0.235±0.274	0.219±0.2036	0.21±0.0290

Values are Mean ± SD, for 6 animals in each group. * *P < 0.01*; ** *P < 0.05* when compared to control.

Table: 4, Inhibition of dextrone induced paw edema volume in mice by *D. triangulare* treatment on 3rd hour .

Groups	Doses	Intial Paw thickness (cm)	Paw thickness on 3rd hour (cm)	Increase in paw thickness (cm)	% of inhibition
Control	------	0.17±0.011	0.30 ± 0.011	0.13	—
Diclofenac	10 mg/kg body weight	0.18±0.0205	0.24 ± 0.237	0.05981	53.99
D. triangulare	50 mg/kg body weight	0.18±0.01491	0.27± 0.0214	0.0899	30.84
D.triangulare	250 mg/kg body weight	0.20±0.0290	0.260 ± 0 .0325	0.05997	53.86

Values are Mean ± SD, for 6 animals in each group. * *P < 0.01*; ** *P < 0.05* when compared to control

Acute oral toxicity studies

The acute oral toxicity of the methanolic extract of Desmodium triangulare was carried out by guidelines as OECD 423 – guidelines (Acute toxic class method). The acute toxicity studies revealed that LD_{50} > 2000mg/kg for this extract.

Hence, the biological dose was fixed at 3 levels, of methanolic extract of *desmodium* extract *triangulare* 50, and 250 mg/kg of body weight for the animal.

Carrageenan induced paw edema

The carrageenan induced acute inflammation. The sub plantar injection of carrageenan into the mice hind paw elicited an inflammation (swelling and erythema) and a time-dependent increase in paw oedema that was maximal at 3th hour after carrageenan. In the control group, the paw thickness increased by diclofenac treated in-3th hour after injection of carrageenan (0.27±0.011)(58.86) (*P* < 0.001). The inflammatory response to subplantar carrageenan, i.e. edema, was significantly reduced *by D triangulare* at doses of 50mg/kg and 250mg/kg given orally 1 hour prior to carrageenan, but the time course of the anti-oedema effect varied among two different doses. Thus, *at D triangulare* doses of 250mg/kg, the inhibition of oedema formation by the drug was treated at and 3rd hour time points post-carrageenan and PTX given at the above doses reduced the paw oedema response

by(0.29±0.007)(47.06) or by 0.20±0.018(64.70)%, respectively, 3rd hour following carrageenan.(Tab1,2)

Dextran induced paw edema

In the control group, the paw thickness increased to 0.307±0.011, three hours after injection of dextran, representing increase in paw thickness. The oedema response was significantly reduced at 3rd hour by

Table: 5, Effect of D. *triangulare* extract on formaline induced paw edema in swiss albino.

Groups	Initial	0th day	1st day	2nd day	3rd day	4th day	5th day	6th day
Control	0.204± 0.0250	*0.304± 0.0134*	*0.336 ± 0.1140*	0.372 ± 0.013	0.392 ± 0.0216	0.39 ± 0.00707	0.38 ± 0.0254	0.378 ± 0.0178
Diclofenac	0.202±0.1303	*0.304±0.0134*	*0.33±0.0158*	0.352±0.0228	0.372±0.0286	0.352±0.0189**	0.317±0.0170**	0.275±0.02081**
D.triangulare	0.212±0.0447	*0.30±0.0141*	*0.334±0.0151*	0.358±0.0148	0.356±0.0114*	0.374±0.0114	0.33±0.0070**	0.31±0.0070**
D.triangulare	0.206±0.2073**	*0.298±0.0164*	*0.334±0.0207*	0.348±0.0164	0.354±0.02607*	0.332±0.01483**	0.306±0.01483**	0.27±0.0158**

Values are Mean ± SD, for 6 animals in each group. * *P < 0.01*; ** *P < 0.05* when compared to control.

Table : 6, Effect of *D. triangulare* extract on formaline induced paw edema in swiss albino.

Groups	Initial	0th day	1st day	2nd day	3rd day	4th day	5th day	6th day
Control	0.204± 0.0250	*0.304± 0.0134*	*0.336 ± 0.1140*	0.372 ± 0.013	0.392 ± 0.0216	0.39 ± 0.00707	0.38 ± 0.0254	0.378 ± 0.0178
Diclofenac	0.202±0.1303	*0.304±0.0134*	*0.33±0.0158*	0.352±0.0228	0.372±0.0286	0.352±0.0189**	0.317±0.0170**	0.275±0.02081**
D.triangulare	0.212±0.0447	*0.30±0.0141*	*0.334±0.0151*	0.358±0.0148	0.356±0.0114*	0.374±0.0114	0.33±0.0070**	0.31±0.0070**
D.triangulare	0.206±0.2073**	*0.298±0.0164*	*0.334±0.0207*	0.348±0.0164	0.354±0.02607*	0.332±0.01483**	0.306±0.01483**	0.27±0.0158**

Values are Mean ± SD, for 6 animals in each group. * *P < 0.01*; ** *P < 0.05* when compared to control.

53.99 % 0.25±0.221 (*P* < 0.01) in mice receiving diclophenac (10 mg/kg, i.p.) I hour before dextran (Table 3,4). *D triangulare* given at two different doses (50mg/kg and 250mg/kg) 1 hour before dextran had a anti-oedema effect. *D triangulare* produced significant and dose dependent decrease in paw edema, 50mg /kg lower dose (0.27±0.244)30.84 % at higher dose (250 mg/kg) reduced the inflammatory effect of dextran by (0.235±0.274) 53.86 % at 3rd hour.in addition of d triangulare extract is anti-oedema effect.

Formalin induced paw edema

In the experiment designed to delineate the effect of the *D triangulare* extract on formalin induced oedema, the extract given at doses of 50 mg /kg and 250mg/kg showed marked anti-oedema effect at 6th day reducing oedema by 43.75% and 63.33%, respectively.(Tab.5,6)

Discussion and Conclusion

The anti-inflammatory activity was evaluated by both acute (carrageenan and dextran) and chronic (formalin) models in Swiss mice. The clinical treatment of inflammatory diseases is dependent on nonsteroidal or steroidal chemical therapeutics (Rainsford, 2007). However, long-term administration of NSAID may induce gastro-intestinal ulcers, bleeding, and renal disorders (Robert, 1976; Peskar, 1977; Tapiero *et al*., 2002). Likewise, the use of steroidal antiinflammatory agents also causes multiple side effects (Schäcke *et al*., 2002; Reinke *et al*., 2002). Therefore, developing new agents with more powerful antiinflammatory activities and with lesser side effects will be of great interest. . the carrageenan inflammatory model basically reflects the actions of prostaglandins(Brooks and day1991), (Gryglewski 1981) histamine, serotonin and kinnins that are involved in the early stage of carragenin induced oedema (Vane and Booting, 1987). Prostaglandin which is known to mediate the second phase of carragenin induced inflammation (Vane and Booting, 1987). The second phase occurs three hour after carrageenan injection. In this phase the macrophages in carrageenan inserted dermal tissue relese much interleukin-1 (IL-1) to induce accumulation of polymorphic nuclear cells (PMNs) into the inflammatory area. The activated PMN then release the lysozomal enzymes active oxygen species. This significant decrease of paw edema by *D triangulare* extract reveals the inhibition of these enzymatic networks.

The dextran-induced oedema is a well-known experimental model for acute inflammation dextran causes of degeneration of mast cells and release of several and inflammation mediator such as histamine and serotonin (vinegar *et al.,1969)* The dextran model also showed reduction in paw edema with the administration of extract at doses 50 mg/kg and 250 mg/kg. Formalin induced paw edema is one of the most suitable test procedure to screen anti-inflammatory agents. In *D. triangulare* extract treated groups of animals a decrease in paw edema is found which revealed the protective activity of the extract in chronic inflammatory disorder Formalin induced paw edema is also one of the most suitable test procedure to screen chronic anti-inflammatory agents as rats it closely resembled human arthritis

(Greenwald, 1991). *D. triangulare* extract treated with reduced the chronic inflammation

Summary

The administration of the extract reduced carageenan and dextran induced acute inflammation and formalin induced chronic inflammation in dose dependent in animal model.Therefore the extract can be used as anti-inflammatory agents.

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AUTHORS' CONTRIBUTIONS

Authors contributed equally to all aspects of the study.

PEER REVIEW

Not commissioned; externally peer reviewed.

CONFLICTS OF INTEREST

The authors declare that they have no competing interests.