Antiinflammatory Assays of Extracts of Medicinal Plants

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Ethyl acetate and methanol extracts of five medicinal plants have been screened for *in vivo* anti-inflammatory activity in albino rats. Methanol extract of *Aegle marmelos* and ethyl acetate extract *Atalantia monophylla* showed significant antiinflammatory activity at a dose of 100 mg/kg.

Aegle marmelos (Rutaceae) is used in traditional medicine treatment. Essential oil isolated from leaves has shown to possess antifungal activity[1]. Alkaloids and coumarins have been reported from this plant[2]. *Bridelia retusa* (Euphorbiaceae) has exhibits antiviral and hypotensive properties in pharmacological studies[3]. An infusion of the leaves of *Phyllanthus maderaspatensis* (Euphorbiaceae) is used in headache. Its seeds possess laxative, carminative and diuretic properties[4]. Essential oil of *Toddalia asiatica* (Rutaceae) showed antimicrobial[5] and antiinflammatory[6] activities. Leaves of *Atalantia monophylla* (Rutaceae) exhibited antiviral activity[7] In this study, we report the antiinflammatory activity of ethyl acetate and methanol extracts of these plants.

Leaves of *A. marmelos*, *B. retusa*, and *P. maderaspatensis* were collected from Indian Institute of Technology campus, Mumbai. Leaves of *A. monophylla* and *T. asiatica* were collected from Native Medicare Centre, Kalpakkarn, Tamilnadu. All the plant material was authenticated at Department of Botany, Ramniranjan Jhunjhunwala college, Ghatkopar, Mumbai. The plant material was shade dried, powdered and extracted with ethyl acetate and methanol successively at room temperature. The extracts were concentrated to dryness under reduced pressure and controlled temperature (40-50°).

Colony bred Wistar rats weighing between 160-220 g were used to assess antiinflammatory activity. All the animals were maintained under controlled standard animal house conditions with 24 h fasted and water *ad libitum*. Doses of ethyl acetate and methanol extracts of *A. marmelos*, *B. retusa*, *P. maderaspatensis*, and *T. asiatica* and ethyl acetate extract of *A. monophylla* (100 mg/kg) were prepared in 1% Tween-80, suspended in distilled water and administered orally to the animals with the help of a catheter.

Determination of antiinflammatory activity is based on plethysmographic measurement of oedema produced by sub-plantar injection of formalin in the hind paw of the rat. The method described by Wilhelmi and Domenjoz[8] as modified by Sisodia and Rao[9] was used for measuring the paw volume. For this study, Wistar rats of either sex weighing between 160-220 g were used and divided into 11 groups of 6 each. The first group served as control and received vehicle only (1% w/v solution is prepared in distilled water) Tween-80. Second group of animals received standard phenylbutazone (100 mg/kg body weight, orally). And the third to eleventh group received the various extracts of plants at a dose of 100 mg/kg, orally. A mark was made on both the hind paws (right and left) just beyond tibio-tarsal junction, so that everytime the paw could be dipped in the mercury column up to the mark to ensure consistent paw volume. After 30 min, 0.1 ml of formalin (1% w/v) was injected into the planter region of left paw of the rats of all the above groups. The right paw served as reference to non-inflammed paw for comparison, which was treated as control. The paw volume was measured both in control as well as in treated animals including standard animals at 0,1, 2, 3 and 4 h of interval. The percent increase in oedema over the initial reading was also calculated. This increase in oedema in animals treated with standard drugs, and various extracts of plants were compared with increase in oedema of untreated i.e. control animals at the corresponding intervals of 0,1, 2, 3

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TABLE 1: ANTIINFLAMMATORY ACTIVITY OF VARIOUS PLANT EXTRACTS.

Group	Average weight (g)	Dose (mg/kg)	Mean Value ± SE of oedema				
			0 h	1 h	2 h	3 h	4 h
Control (Tween 80)	170	-	0.54 (±0.038)	0.55 (±0.045)	0.57 (±0.035)	0.56 (±0.068)	0.54 (±0.056)
Standard (Phenylbutazone)	185	100	0.55 (±0.021)	0.20* (±0.025)	0.20* (±0.035)	0.18* (±0.042)	0.14* (±0.078)
A. mermalosa							
E.A.	206	100	0.54 (±0.026)	0.35* (±0.062)	0.31* (±0.067)	0.31* (±0.045)	0.21* (±0.02)
MeOH	208	100	0.53 (±0.026)	0.26* (±0.028)	0.26* (±0.026)	0.218* (±0.034)	0.16* (±0.08)
A. retusa							
E.A.	210	100	0.54 (±0.026)	0.52* (±0.068)	0.48* (±0.058)	0.46* (±0.047)	0.39* (±0.028)
MeOH	215	100	0.52 (±0.026)	0.29* (±0.032)	0.29* (±0.038)	0.28* (±0.042)	0.27* (±0.045)
P. madaraspetensis							
E.A.	212	100	0.52 (±0.021)	0.47* (±0.058)	0.45* (±0.043)	0.42* (±0.028)	0.28* (±0.038)
MeOH	220	100	0.54 (±0.021)	0.32* (±0.048)	0.29* (±0.045)	0.28* (±0.035)	0.27* (±0.018)
T. asiatica							
E.A.	210	100	0.53 (±0.036)	0.51* (±0.031)	0.56* (±0.034)	0.48* (±0.042)	0.26* (±0.05)
MeOH	215	100	0.52 (±0.032)	0.37* (±0.048)	0.38* (±0.052)	0.31* (±0.032)	0.21* (±0.04)
A. monophylla							
E.A.	216	100	0.52 (±0.021)	0.41* (±0.038)	0.42* (±0.042)	0.38* (±0.036)	0.17* (±0.020)

Each group consisted of 6 animals. E.A. denotes ethyl acetate extract and MeOH stands for methanol extract. Asterisk denotes significant difference from controls at $P \leq 0.001$.

and 4 h. Thus, the percent inhibition of oedema at known intervals in treated animals was used for the purpose of calculating the percent inhibition of oedema of the control. Similarly, percent inhibition of oedema for standard and various extracts of plants were calculated. The results of antiinflammatory activity are given in Table 1.

Among the various extracts tested for antiinflammatory activity (Table 1) methanol extract of *A. marmelos* and ethyl acetate extract of *A. monophylla* showed significant activity comparable to the standard drug whereas the ethyl acetate extract of *A. marmelos* and methanol extract of *T.psiatica* exhibited moderate activity. The remaining extracts except ethyl acetate extract of *B. retusa*, were less active. The results were significant ($p<0.001$) using Student's t test.

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Spectrophotometric Determination of Rofecoxib in Pharmaceutical Dosage Forms

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A colorimetric method has been developed for the determination of rofecoxib. Rofecoxib produces a yellow color chromogen in alkaline solution (pH 10-12), which exhibits a λ_{max} at 355 nm. The system followed Beer-Lambert's Law in the concentration range of 5-40 μg/ml. The method is simple, economic, convenient, reproducible, precise and free from interference by excipients.

Rofecoxib, {4-[4-(methylsulfonyl) phenyl]-3-phenyl-2-(5H)-furanone} is comparatively a new non-steroidal antiinflammatory drug[1], that is active at a low dose[2] and has less gastric toxicity[3]. It inhibits the activity of the enzyme, cyclooxygenase, which is responsible for the formation of prostaglandins in normal physiological conditions[4,5]. Prostaglandins are involved in mediating inflammation, swelling, pain and fever. It is preferred over conventional NSAIDs[6] as they may lead to serious GI complications such as ulcer, severe bleeding and perforation, resulting in hospitalization and even death[7]. It is available in tablets and the analytical methods reported for its determination are based mainly on HPLC[8,9]. Therefore, the aim of the present investigation is to develop a simpler and cheaper analytical method as compared to HPLC methods for the determination of rofecoxib in various formulations. Rofecoxib produces a yellow chromogen in alkaline solution (pH 10-12), which exhibits λ_{max} at 355 nm. The yellow colored chromogen is monitored spectrophotometrically for the determination of drug in different formulations.

A Shimadzu UV-2101 PC, UV/VIS spectrophotometer was used for all absorbance measurements. Rofecoxib was obtained as a gift sample from Ranbaxy Laboratories, New Delhi.

Rofecoxib (25 mg) was accurately weighed and dissolved in 20 ml of methanol in a volumetric flask. The final volume was made up with 0.5 M sodium hydroxide solution to 100 ml to obtain a concentration 250 μg/ml. This stock solution was used to prepare various standard solution of drug.

Aliquots of stock solution of rofecoxib (0.2-1.6 ml, 250 μg/ml) were transferred into a series of 10 ml of volumetric flasks and volume was made up to the mark with 0.5 M sodium hydroxide solution. The absorbances of the chromogen were measured at 355 nm against the reagent blank solution (prepared similarly without using drug).

The rofecoxib content in two marketed brands of rofecoxib tablets were determined. Ten tablets of rofecoxib were taken and finely powdered by trituration. A powder equivalent to 25 mg of drug was weighed accurately and transferred into a 100 ml volumetric flask. Methanol (20 ml) was added to the flask, sonicated for 20 min and then diluted to volume with 0.5 M sodium hydroxide solution. The resultant was filtered through Whatman filter paper no 41. Filtrate (10 ml) was then transferred into a 100 ml volumet-

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