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Evaluation of Anti-Inflammatory and Analgesic Activity of Bay Leaf in Rat

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ABSTRACT: Inflammation associated with pain, biological proteins lose their biological activity and function when denatured. Denaturation of protein is a cause of inflammation or pain. Medicinal plants are still a valuable source of new pharmaceutical products and natural products still remain as one of the best reservoir of new structural-typed bioactive compounds. Plant drugs and herbal formulations are frequently considered to be less toxic and free from side effects. Anti-inflammatory agents of plant origin used in traditional medicine are important. The attributed anti-inflammatory effects of these plants are due to their ability to restore the function of tissues. Hence, treatment with herbal drugs has an effect on protecting prostaglandin synthesis and smoothing inflammation and analgesic levels.

Indian bay leaf or malabathrum (Cinnamomum tamala, Lauraceae) ethanol and water extract used for the activity in mice carrageenan- induced hind paw edema method, and the result are show the reaction time for jump response from the hot plate method was determined, and the results were compared with control group. The result of the study shows that the standard drug was showing the maximum analgesic activity and the test compound (100 mg/kg of extract) was more near to the standard compound.

KEY WORD:- Inflammation, bay leaf, ethanol extract, paw edema, alkaloids, NSAIDs,

I. INTRODUCTION:

Inflammation may be a complex process which is related to with pain and involves occurrences such as: increase of protein denaturation and membrane alteration and also the increase of vascular permeability, as a part of the investigation on the mechanism of the anti-inflammatory activity, ability of plant extract to inhibit protein denaturation was studied. The mechanisms of inflammation involve a series of events during which the metabolism of arachidonic acid plays a important role[1]. It will be metabolized by the Cyclooxygenase pathway to prostaglandins and thromboxane A2, or by the 5-lipoxygenase pathway to hydroperoxy-eicosatetraenoic acids and leukotrienes (LT's), which are essential biologically active mediators in a very kind of inflammatory events. Inhibition of 5-LOX and COX-leads to decreased production of LTs and PGs, such a drug would have the potential to produce anti-inflammatory and analgesic effects with a reduction within the GI side effects. Inflammatory processes also involve reactive oxygen species started by leukocyte activation.

Inflammatory disorders include rheumatoid arthritis, osteoarthritis, inflammatory bowl diseases, retinitis, multiple sclerosis, psoriasis and atherosclerosis (Delves & Riott 2000). Non-steroidal anti-inflammatory drugs (NSAIDs) are commonly prescribed for the treatment of acute and chronic inflammation, pain and fever. Inflammation is divided into two basic patterns, acute inflammation and chronic inflammation[2]. Pain motivates the individual to withdraw from damaging situations, to protect a damaged body part while it heals, and to avoid similar experiences in the future Simple pain medications are useful in 20% to 70% of cases. [3-5]

Medicinal plants are still a valuable source of new pharmaceutical products and natural products still remain one of the best reservoirs of new structural-typed bioactive compounds[6]. It is estimated that only 15% of higher plants have been investigated for potentially useful in biological activity. [7-8]

Anti-inflammatory agents of plant origin used in traditional medicine are important. The attributed anti-inflammatory effects of these plants are due to their ability to restore the function of tissues by causing a decrease in cell mediators. Hence, treatment with herbal drugs has an effect on protecting prostaglandin synthesis and smoothing inflammation and analgesic levels. In general, there is very little biological knowledge on the specific modes of action in the treatment of inflammation, but most of the plants have been found to contain substances like phenolics, glycosides, alkaloids,

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terpenoids, flavonoids etc., that are frequently implicated as having anti-inflammatory effects. Thus, this study aimed at assessing the anti-inflammatory activity of the bay leaf extract using carrageenan-induced hind paw edema in mice.

II. MATERIAL AND METHODS

Experimental Work: Ratio of bay leaf to solvent: The experiments being carried out using the equipment set up. The ratio of bay leaf to solvent was 50 gm. sample to 300ml of solvent was used Solvents used: Ethanol, Water of AR grade.

Extraction time: The term extraction time is used for the duration of time it took for experiment to run. In this research, the experiments were carried out from 12 to 14 hrs of extraction time.

Raw material selection and analysis Selection of bay leaf extraction: The technological feasibility of both fresh and dry bay was assessed to obtain extract. Fresh bay and dried is used for the experimental purpose. The fresh and dried bay leaves were crushed and powdered into fine size. Bay leaf was extracted by soxhlet extraction.

Preliminary phytochemical study: The bay leaf extracts were evaluated for the presence of alkaloids, steroids, and tannins flavonoids reducing sugars and saponin.

S. No	Test for	Reagent/ Test	Observation	Result
1	Alkaloids	Mayer's	White ppt	+
		Wagner's	Brown color	+
		Dragondroff's	Orange-red ppt	+
		Molish	Purple Color	+
2	Carbohydrate	Benedict	Orange ppt	-
		Fehling's	Red-Orange	+
3	Tannins	$FeCl_3$	Green Color	+
4	Saponins	General	Reddish color	+

EVALUATION OF ANTI-INFLAMMATORY ACTIVITY:

Acute oral toxicity of bay leaf: Prihapsara et al. **reported** the results of the acute toxicity test showed median lethal dose (LD50) values from SNEDDS from ethyl acetate extract of bay leaf 1409.30 mg/kg BW.

To evaluate the anti-inflammatory activity of bay leaf one model have been selected .i.e. **Carrageenan induced paw edema in rats** :Labeling, grouping of animals and procedure for the evaluation of anti-inflammatory activity by For this study the 200 gm of albino Sprague Dawly rats (Either sex) were taken. There have been three groups of rats. One for the control group, one group represented the positive control or standard group and one group was for test compound. There have been 5 rats in each group. The tail of rats was labeled for every group i.e. for control, standard, and test group.

Procedure: Male or female Sprague-Dawley rats with a weight between 100 and 150g are used. The animals are starved over night. To insure uniform hydration, the rats receive 5ml of water by stomach tube (controls) or the test drug dissolved or suspended within the identical volume. After 30 minutes the rats are administered by a sub-cutaneous injection of 0.05ml of 1% solution of carrageenan into the plantar side. It is measured by plethysmo graphically immediately after injection. This process again 3 and 6h, and eventually24h after challenge.

EVALUATION: the rise of paw volume after 3 h is calculated and as percentage compared with the quantity measured given immediately after injection of the irritant for each animal. Effectively treated animals show much less edema. The difference of average values between treated animals and control groups is calculated for each time measure and statistically evaluated.

Eddy's hot plate method: For this study the 200 gm of albino Sprague Dawly rats were taken. There have been three groups of rats. One for the control group, one group represented the positive control or standard group and one group was for test compound.

Procedure: The paws of rats are very sensitive to heat at temperatures which are not damaging the skin. The responses are jumping, withdrawal of the paws and licking of the paws. The time until these responses occur is prolonged after administration of centrally acting analgesics, whereas peripheral

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analgesics of the acetylsalicylic acid or phenyl-acetic acid type do not generally affect these responses. The method originally described by Woolfe and Mac Donald (1944) has been modified by several investigators. The following modification has been proven to be suitable. The hot plate, which is commercially available, consists of a electrically heated surface. The temperature is controlled for 55° to 56°C. This can be a copper plate or a heated glass surface. The animals are placed on the hot plate and the time until jumping occurs is recorded by a stop-watch. The latency is recorded before and after 20, 60 and 90min following oral administration of the standard and the tests compound.

III. RESULT

ethanolic extract of bay was collected as the test compound for the evaluation of anti inflammatory activity in rats.

Evaluation of anti-inflammatory activity: Carageenan induced paw edema in rat: The experiment was performed using the plethysmometer and the following results were obtained.

Group	Mean Change in paw volume (ml) at different time interval (n=5)			
	0h	1h	2h	3h
Control (No drug only carageenan)	0.58	0.92	1.16	1.35
Standard+ Carageenan (Diclofenac sod)	0.52	0.78	0.878	0.975
Test + Carageenan (100 mg /kg)	0.61	0.84	0.92	1.1

Table 2 : Observed readings for the Jump response obtained from Eddy's hot plate

Treatment/ Groups	Mean (n=5) Reaction time at different time points for jump response				
	0.5 hrs	1 hrs	1.5 hrs	2 hrs	3 hrs
Control	7.4 ± 4.5	7.6±0.45	7.0 ±0.56	8.01 ±0.36	8.50 ±0.1
Standard(50 mg/kg)	17.5 ± 0.81	19.05±1.59	20.5 ±2.1	21.08 ±1.4	23.3 ±3.6
Test (100 mg/kg)	5.3 ± 0.80	5.5±0.72	5.8 ±0.82	6.05 ±0.7	6.20 ±0.09

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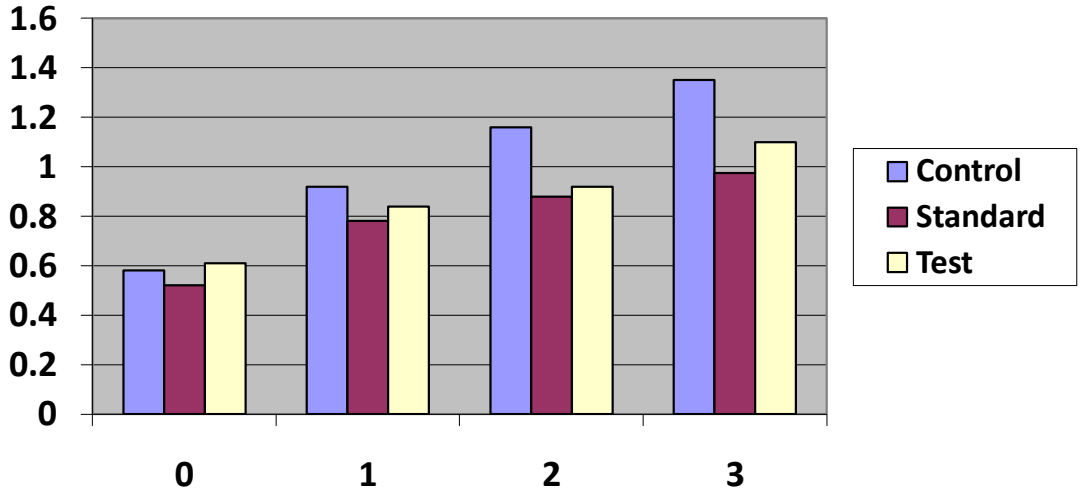

Fig 1: Comparative results from paw edema method (Graph plotted against Time v/s change in volume)

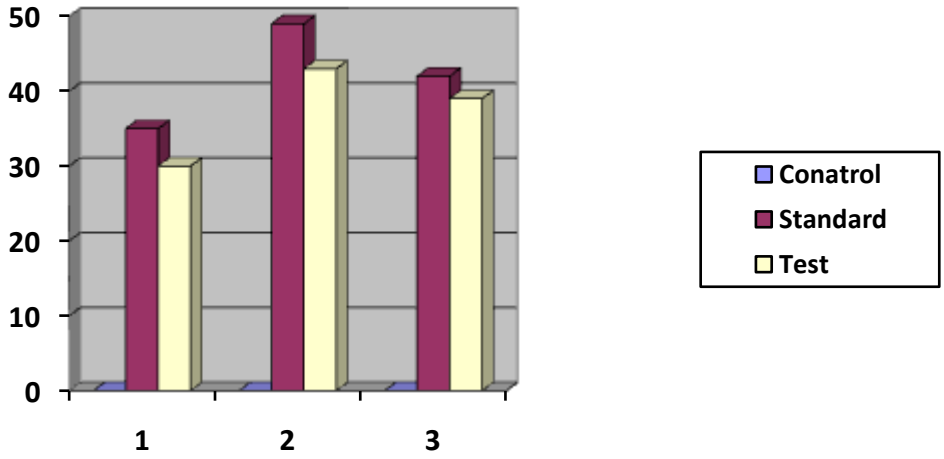

Fig 2: Anti-inflammatory activity (% inhibition)

IV. CONCLUSION:

In the present study, results indicate that the ethanol extracts of bay leaf possess anti inflammatory properties. These activities may be due to the strong occurrence of polyphenolic compounds such as carbohydrates, glycosides, saponins, alkaloids, tannins, steroids, and phenols, The extract fractions serve as free radical inhibitors or acting as primary oxidants and inhibited the heat induced albumin denaturation, proteinase activity and stabilized the Red Blood Cells membrane. In the present study the anti-inflammatory activity of bay was evaluated in rats with the help of one of the most important pharmacological screening method that is carrageenan induced paw edema in rat. The data was obtained by the model and compared. For the evaluation of anti-inflammatory activity of test compound, standard drug diclofenac sodium was used and for the test compound 100 mg/kg of the ethanolic extract of bay leaf was used. The change in paw volume was determined.The reaction time for jump response from the hot plate method was determined and the results were compared with control group. The results of the study shows that the standard drug was showing the maximum analgesic activity and the test compound (100 mg/kg of extract) was more near to the standard compound .

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