Research article

Anti - inflammatory activity of ten indigenous plants in carrageenan induced paw oedema in albino rats

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(Received: April 2021 Revised: August 2021 Accepted: August 2021)

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ABSTRACT

Introduction and Aim: Inflammation is a symptom associated with many diseases, can be treated with steroidal and non-steroidal anti-inflammatory drugs, which can cause severe side effects when used as long-term treatments. Plants have the ability to synthesize a wide verity of phytochemical compounds as secondary metabolites which shows anti-inflammatory activity. The anti-inflammatory activity of crude extracts of leaves of *Jasminum grandiflorum* (Jg), *Vinca rosea* (Vr), *Azadirachta indica* (Ai), *Lawsonia inermis* (Li), *Nerium indicum* (Ni), *Calotropis gigantea* (Cg), *Tectona grandis* (Tg), *Andrographis paniculata* (Ap), *Tabernaemontana corymbosa* (Tc) *and Marsedinia volubilis* (Mv) as well as alcoholic extracts of leaves of Cg, Tg and Ap were evaluated in Wistar rats.

Methods: Acute anti-inflammatory activity of crude extracts of ten indigenous plants were studied by calculating the volume changes in the hind paw after injecting carrageenan in rats comparing with Ibuprofen which was used as a standard drug in this study. Anti-inflammatory effect of alcoholic extract of leaves of Ap, Tg and Cg (200mg/kg body weight) also studied.

Results: The groups treated with crude extract & alcoholic extract of Cg, Tg and Ap showed significant reduction in the oedema compared to control and other plant extracts. The result was very significant in Ap treated rats.

Conclusion: Plants are one of the most important sources of medicines. In our study, the anti-inflammatory activity of ten indigenous plants could be due to presence of naturally occurring antioxidant flavonoids**.** The leaves of Ap are very rich in flavonoids, could be the reason for significant anti-inflammatory activity found in Ap treated rats.

Keywords: Indigenous plants, carrageenan, anti-inflammatory, *Andrographis paniculata*, rats

INTRODUCTION

Inflammation can be defined as the "reaction of vascularized living tissue to local injury." The main objective of inflammation is to dilute, delimit and possibly eliminate the foreign particles, microorganisms or antigens. It also helps in clearing the damaged site of dead cells and thereby initiating the way for wound repair (1). The healing process begins during the early phase of inflammation but usually reaches completion after the injurious influence had been neutralized. Acute inflammation is of relatively short duration lasting for a few minutes to several hours or even few days. The local clinical signs of acute inflammation are heat, redness, swelling and pain. When the noxious stimulant cannot be destroyed or eliminated by a process of acute inflammation, there will be development of signs and symptoms of sub-acute inflammation (2). These symptoms are treated with steroidal and non-steroidal anti-inflammatory drugs, which can cause severe side effects when used as long-term treatments.

India is richly endowed with diverse medicinal plants with anti-inflammatory activities that have been shown to be effective in the treatment of inflammatory conditions in traditional medicine.

Plants are one of the most important sources of medicines. Since ancient time's medicinal plants have been used to treat different ailments due to their accessibility, availability, inherited practice, economic feasibility, and perceived efficacy. Research on plants with inflammatory activities is one of the developing areas in modern biomedicine

In our study, the anti-inflammatory activity of Crude extracts of 10 indigenous plants viz., *Jasminum grandiflorum* (Jg), *Vinca rosea* (Vr), *Azadirachta indica* (Ai), *Lawsonia inermis* (Li), *Nerium indicum* (Ni), *Calotropis gigantea* (Cg), *Tectona grandis* (Tg), *Andrographis paniculata* (Ap), *Tabernaemontana corymbosa* (Tc) *and Marsedinia volubilis* (Mv) (Figure 1) and alcoholic extracts of *Andrographis paniculata*, *Calotropis gigantea* and *Tectona grandis*, were studied in rats using carrageenan induced rat hind paw oedema model.

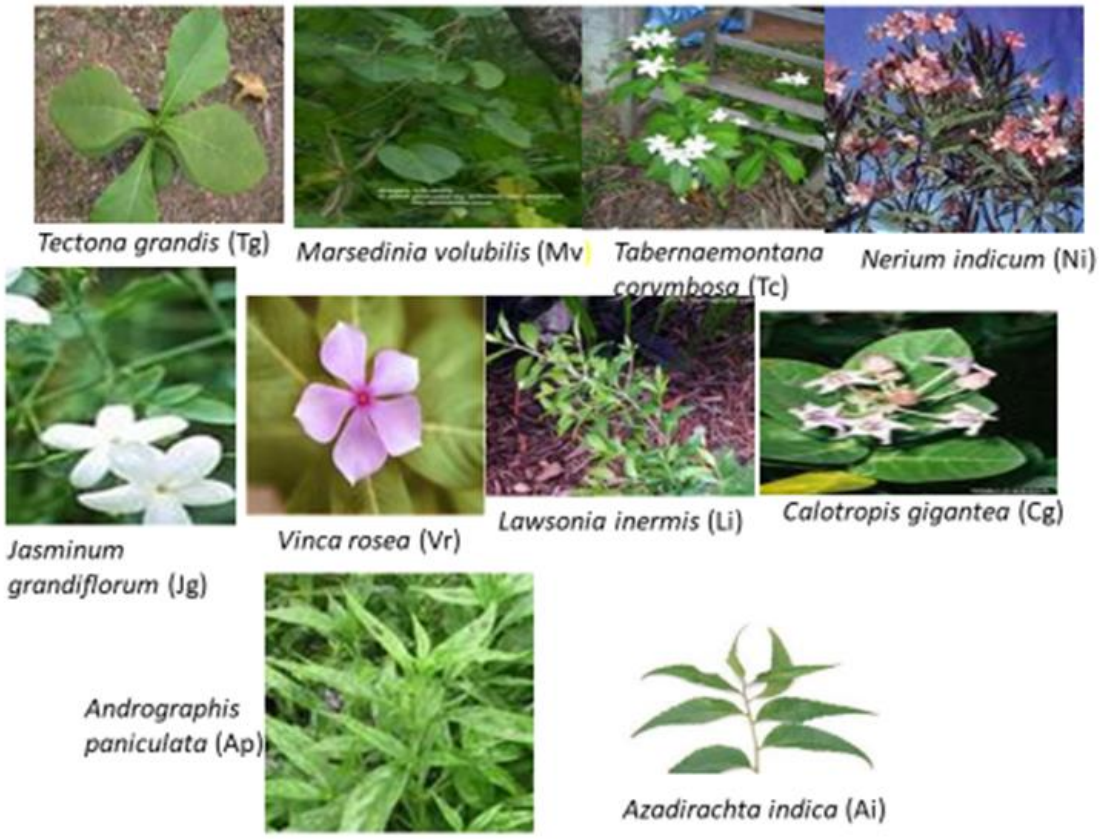

Fig. 1: List of selected indigenous plants

MATERIALS AND METHODS

Animals

Healthy adult albino rats of either sex (150-250 g) of Wistar strain were selected for this experiment. The animals were procured and maintained in the Central animal facility, Kasturba Medical College, Manipal Academy of Higher Education. These animals were maintained under controlled conditions of temperature ($28^0 \pm 10^0$C temperature and 50 ± 5% humidity) and light in animal house having free access to water and standard pallet diet. All the experiments were performed in accordance with the approval and guidelines of Animal Ethics Committee of Kasturba Medical College Manipal, Manipal Academy of Higher Education.

The plants which are used for screening were collected in and around Udupi and Manipal during September–January and identified by Prof. Aravinda Hebbar, department of botany, M.G.M. College, Udupi.

Experimental design

Preparation of crude extract

Fresh leaves of the plants were collected, and the leaves were crushed with a mortar and pestle. The expressed juice was centrifuged. In our experiment, crude extracts of 10 indigenous plants were used. The volume of juice obtained for each plant is expressed as ml/ 100gms of leaves. Dose used in the study is 2ml/ kg body weight.

Preparation of alcoholic extract

Leaves were collected, dried in the shade, and powdered. The powder was used for preparation of extract. Leaf powder (75 g) was extracted with 700ml of 95% ethanol in a Soxhlet apparatus at 60-75°C (3). Extract was concentrated.

Acute edema model- Carrageenan induced paw edema model used.
Route of drug administration – Oral, 1 hour before inducing edema.
For screening of crude extract of 10 indigenous plants- 8 rats of either sex per group was used.
Group 1-control, treated with normal saline, group 1I – standard drug- Indomethacin. Group III-XI1-treated with crude extract of Jg, Ai, Li, Ni, Vr, Mv, Tc, Cg, Tg and Ap respectively.
Dosage: 2ml/kg body weight.

For further screening, alcoholic extracts of Cg, Tg and Ap - 8 rats of either sex per group was used. Group 1 - control, treated with normal saline, Group 1I – standard drug, Group III-V -treated with Cg, Tg and Ap respectively. Dosage :200mg/kg body weight,

Hind paw oedema model

In this wound model, acute anti-inflammatory activity is studied by calculating the volume changes in the hind paw after injecting carrageenan. In this wound model, % inhibition of oedema was measured. This was studied by the rat paw edema method by intraplantar injection of 0.05ml of 1% carrageenan solution into the right hind paw of the rat to induce edema. The zero-hour paw volume was measured using a water plethysmograph immediately after

carrageenan injection. The paw volume was again measured after 3hrs. The difference between the 3hr and 0 hr values gives the edema volume due to acute inflammation caused by the carrageenan injection. The % inhibition of edema was calculated by

$$\% \text{ Reduction in paw edema} = \frac{Vc - Vt}{Vc \times 100}$$

Vc = mean edema volume in control group
Vt = mean edema volume in drug treated animal.

Statistical analysis

Data obtained from all the above experiments were correlated and analyzed by one way Analysis of Variance (ANOVA) followed by Bonferroni's post-test wherever applicable using statistical software package, Graph Pad in Stat (GPIS) 1990: version 1.13. Values of $p<0.05$ were considered statistically significant.

RESULTS

The rats treated with standard (Indomethacin), % inhibition of oedema was 62.96%.

The groups treated with crude extracts of Vr, Ai, Li, Cg, Tg and Ap showed significant reduction in the oedema (48.14%; 48.14%; 46.29; 48.14%; 48.14% and 51.85% respectively) compared to control group. But it was not very significant compared to standard treated rats (Fig. 1).

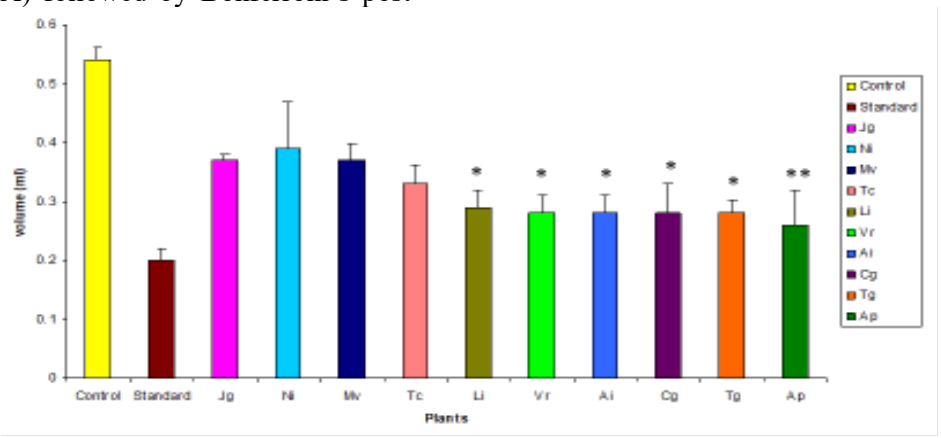

Fig. 1: Effect of standard drug and various indigenous drugs on acute inflammation.
Jg- *Jasmimum grandiflorum*; **Ai**- *Azadirachta indica*; **Li**- *Lawsonia inermis*; **Ni**- *Nerium indicum*; **Vr**- *Vinca rosea*; Mv- *Marsedinia volubilis*; **Tc**- *Tabernaemontana corymbose*; **Cg**- *Calotropis gigantea*; **Tg**- *Tectona grandis*; **Ap**- *Andrographis paniculate*. *p<0.05, **p<0.01, ***p<0.001 v/s control.

There was a significant decrease in oedema in alcoholic extracts of Cg (48.14%), Tg (46.3%) and Ap (51.9%) treated rats compared to control. Out of these 3 plants, significant result was found in Ap treated rats. From the above screening studies, crude and alcoholic extracts of Ap have shown significant anti-inflammatory activity as compared to other plants (Fig. 2).

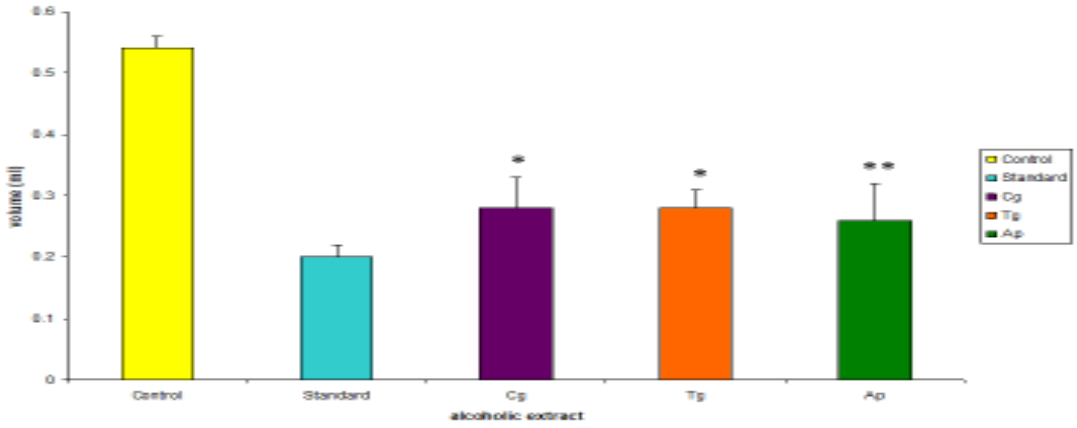

Fig. 2: Effect of standard and alcoholic extracts of Cg, Tg, and Ap on acute inflammation
Cg- Calotropis gigantea; **Tg**- Tectona grandis; **Ap**- Andrographis paniculate. *p<0.05, **p<0.01 v/s control.

DISCUSSION

Inflammation is a part of the body's immune response. Inflammation and tissue damage are due to the liberation of free radicals (4, 5). Plants have can synthesize a wide variety of phytochemical compounds as secondary metabolites which shows anti-inflammatory activity. In our initial screening study, the ten indigenous plants, *Jasminum grandiflorum* (Jg), *Vinca rosea* (Vr), *Azadirachta indica* (Ai), *Lawsonia inermis* (Li), *Nerium indicum* (Ni), *Calotropis gigantea* (Cg), *Tectona grandis* (Tg), *Andrographis paniculata* (Ap), *Marsedinia volubilis* (Mv) and *Tabernaemontana corymbosa* (Tc) were mentioned in Ayurvedic textbooks and folklore medicine. These plants were selected on the basis of

earlier reports and local reputation on anti-inflammatory activity. There was a significant decrease in oedema induced by carrageenan in groups treated with crude extracts of Ap, Cg, Tg, Vr, Li and Ai, compared to control. There are many plants having antiinflammatory activity. Some of the plants which have shown antiinflammatory activity, *Andrographis paniculata* (6), *Ocimun sanctum* (7), *Loasa speciosa* (8), *Hemigraphis colorata* (9), *Bergenia ciliate sternb* (10), *Hyptis suaveolels* (11), *Hibiscus esculantus* (12) and *Benincasa hispida* (13) were reported.

It has been reported that in acute inflammation, chemical mediators like 5-hydroxy tryptamine (5-HT), histamine, bradykinin and PGE_1 are released. In Ai and Cg treated rats, it has been shown that the anti-inflammatory activity is probably due to decrease in 5-HT and PGE_1 (14 & 15). It was also reported, the acute inflammation is due to the releasing of chemical mediators like 5-hydroxy tryptamine (5-HT), histamine, bradykinin and PGE_1 in groups treated with *Andrographis paniculata* (6). Most of the researchers concluded their study by mentioning that the anti-inflammatory activity may be due to inhibition of the enzyme cyclooxygenase leading to inhibition of prostaglandin synthesis (16). Recent studies suggested the anti-inflammatory activity could be due to presence of naturally occurring antioxidant flavonoids (17) which scavenges the free radicals produced during inflammation.

Out of the 10 indigenous plants screened, the crude extracts of Cg, Tg, and Ap have shown significant results than other plants. Hence, further screening studies were also done with the alcoholic extracts of Ap, Cg and Tg plants.

Rats treated with alcoholic extract of Ap has shown significant anti-inflammatory activity by decreasing %inhibition of oedema compared to alcoholic extracts of Cg and Tg.

Andrographis paniculata Nees is a medicinal plant belonging to the family of Acanthaceae, commonly called as Kalmegh is reported to be widely used by tribals all over India (18). Diterpenoids and flavonoids are the main chemical constituents of Ap and these compounds are believed to be responsible for the biological activities of the plant (19). Oral administration of extracts of Ap showed significant anti-inflammatory activity in pathogen induced PID rats (20). It was reported that extracts of Ap have anti-inflammatory, antioxidant properties (21, 22).

CONCLUSION

Natural products are an alternative source of new compounds with anti-inflammatory activity. Plants are one of the most important sources of medicine as they have gained importance and popularity in recent years because of their safety, efficacy and cost effectiveness. In our study, the anti-inflammatory activity of these indigenous plants could be due to presence of naturally occurring antioxidant flavonoids.

CONFLICT OF INTEREST

There is no conflict of interest

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