

An Experimental Study on the Anti inflammatory Effect of Roots with that of Leaves of Nirgundi (*Vitex negundo* Linn, Family Verbenacea)

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Abstract

Nirgundi (*Vitex negundo* Linn, Family Verbenaceae) is a widely used medicinal plant, and therefore a fastly depleting one due to indiscriminate collections by the local herbalists and habitat destruction. Almost all its parts are used medicinally, especially roots and leaves. Anti inflammatory effect of this plant is clearly mentioned in ayurvedic books (nighantus) and its root and leaf extracts are proven effective experimentally. There is an increased demand for herbal drugs, due to the side effects of the currently available anti inflammatory drugs. For this reason, as well as to encourage the use of alternate plant parts, the current study was conducted.

Aim of the study was to compare the anti inflammatory effect of kwatha (decoction) of roots and leaves of Nirgundi in acute inflammation by carrageenan induced hind paw oedema in albino rats. Comparison of change in paw volume of the test groups with control at 1hr, 3hrs and 5hrs after inducing inflammation by injecting Carrageenan, is used as the assessment criteria. From the results it can be concluded that leaves of Nirgundi showed a better anti inflammatory effect than its roots in carrageenan induced acute inflammation. Also both roots and leaves showed mild anti inflammatory effect in acute stage of Inflammation.

Keywords

Nirgundi, Vitex negundo Linn, Anti inflammatory



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INTRODUCTION

The dawn of 20th century has witnessed increasing realization about the importance of medicinal plants at the global level. Demand for medicinal plants is increasing in both developing and developed countries, due to the growing recognition of natural products being non narcotic, having comparably less side effects, easily available at affordable prices and sometimes the only source of healthcare available to poor. This increased demand has led to an increased pressure on the natural resources. While the demand for medicinal plants is growing, some of them are increasingly being threatened in their natural habitat. Consequently, due to less availability of medicinal plants and to meet the ever-growing demand of these plants, there is a gap in demand and supply that may lead to adulteration and substitution of genuine material. The concept of substitution prevailed ages back and in ayurveda we can find this in the treatise of Bhavaprakasa and Yogaratnakara.

In India, the govt in its 11th five year plan, the medicinal plant board proposed many thrust areas for researches. It include studies like substitution of plant parts like roots, leaves, bark, flowers, fruit, wood etc coming from the same plant to avoid destructive harvesting as far as possible.

Nirgundi (*Vitex negundo* Linn, Family Verbenaceae) is a plant with a large number of medicinal properties. Almost all its parts are used as medicine especially roots and leaves. Anti-inflammatory (sophahara) effect of its root and

leaf extracts are proven experimentally.^{1,2}The anti-inflammatory effect of Nirgundi is clearly mentioned in the nighantus.³⁻⁶ Roots are mainly used for kwatha (decoction) preparations and leaves for gudikas, tailas etc. Since many yogas containing Nirgundi are currently produced in large scale, this wide harvesting of the plant for its root will leads to its destruction in the near future. Already its scarcity is reported from various parts of the world. It is mainly due to destructive harvesting and improper land use. Considering the anti inflammatory effect of roots and leaves and their wide use as medicine, leaf is selected as an alternate plant part to compare with its root. This study is an attempt to evaluate and compare the Anti-inflammatory effect of kwatha of roots and leaves of Nirgundi in acute inflammation by carrageenan induced hind paw oedema and it is particularly beneficial, since it can promote substitution of plant parts in medicinal use and thus minimize the destruction of the flora.

Phytochemical studies

All the phytochemical studies were performed in the Drug standardization located of Govt Ayurveda college, Trivandrum. A comparison of various physical and physico chemical parameters and different extractive values were done using the decoction of roots and leaves of the plant Nirgundi. Results are given in table 1-table 5 below.

Table 1 Results of Quantitative Evaluation

Experiment	%(root)	%(Leaf)
Moisture content	8%	7%
Volatile oil content	Nil	Trace
Total ash	3%	5.5%
Water insoluble ash	2.6%	4.5%
Acid insoluble ash	.8%	2%
Fiber content	59.43%	19.16%
Cold water soluble extractive	29.16%	20.72%
Alcohol soluble extractive	6.6%	20%

Table 2 Results of Successive solvent extraction

Solvent	Percentage(root)	Percentage(leaf)
Petroleum ether	.7%	5.76%
Cyclohexane	.3%	1.32%
Acetone	3.7%	18.08%
Alcohol	4.03%	7%

Table 3 Results of Qualitative chemical analysis of alcoholic extract

Chemical constituent	Root	Leaf
Steroid	+	+
Flavonoid	+	+
Phenol	+	+
Alkaloid	+	+
Tannin	+	+
Saponins	-	-

Table 4 Rf values of visualized spots of alcoholic extract of leaf

Solvent system	No of spots	Rf Value
Toluene-Ethyl acetate	4(UV)	0.305,0.388, 0.518,0.657
Benzene-Acetone	6(UV)	0.230,0.433, 0.5486,0.672, 0.752,0.929

Table 5 Rf values of visualized spots of alcoholic extract of the root

Solvent system	No of Spots	Rf Value
Toluene-Ethyl acetate	5 (UV)	0.114,0.190, 0.352,0.561, 0.628
Benzene-Acetone	5 (UV)	0.158,0.457, 0.514,0.672, 0.822

EXPERIMENTAL STUDY

Acute inflammation by Carrageenan induced hind paw oedema was studied using the kwatha of Nirgundi(both roots and leaves). Separate Kwathas were made according to the method mentioned in the kwatha vidhi of Ayurveda Pharmacopoeia of India (API)⁷. Since the therapeutic equivalent rat dose of kwatha is too large compared to rat stomach capacity (2ml/100 g body weight), the filtered liquid was again concentrated over low flame to 1/2 of the filtered volume (20ml root kwatha, 24 ml leaf kwatha). The effective dose for the test drug was calculated using the table constructed by Paget G.E and Barne J.M (1964) in evaluation of drug activities by fixing the Human dose for Kwatha as 96ml (2palam) based on the Kwatha vidhi of Sarngadhara samhitha.⁸ Based on this the Effective dose* of the study drug was taken as 0.864ml/100g body weight. (X=1.728 ml).

The experimental protocols were approved by institutional Animal Ethics Committee and a written permission from Institutional Animal ethics committee, Govt. Ayurveda College, Thiruvananthapuram (order no:9/1AEC/AVC/2011,dated: 11/04/2011) was taken to carry out and complete this study and standard guidelines were followed for the

maintenance and use of the experimental animals. The study was conducted in the animal house of Govt. Ayurveda College, Thiruvananthapuram

Healthy albino rats of both sexes weighing between 150-250 g were selected for the study. A sample of 35 animals were selected and divided into seven groups, each containing five animals. They were acclimatized for a period of 7 days prior to performing the experiment. The animals were provided with free supply of standard laboratory pelleted rat feed and water *ad libitum*

The acclimatized albino rats were weighed and grouped into 7 (Group A, B, C, D, E, F and G) with 5 animals in each group. Selection was done randomly so as to assure equal distribution of sex, body weight etc. in each group. Then the animals were marked on tail for proper identification and kept each group in separate cages. Each cage was labelled separately for group identification. The dose for each animal was calculated as per the body weight and was tabulated.

➤ **Group A/GA** : Inflammation induced but receiving only distilled water (Control group)

➤ **Group B/GEL** : Inflammation induced & leaf kwatha in Effective dose

➤ **Group C/GHL** : Inflammation induced & leaf kwatha in half of the effective dose

➤ **Group D/GDL** : Inflammation induced & leaf kwatha in double of the effective dose

➤ **Group E/GER** : Inflammation induced & root kwatha in Effective dose

➤ **Group F/GHR** : Inflammation induced & root kwatha in half of the effective dose

➤ **Group G/GDR** : Inflammation induced & root kwatha in double of the effective dose

Group B to Group G were termed as the Test groups.

The animals of the test groups were over night fasted without restricting water in the previous day of experiment. The tibio tarsal junction of right hind paw was marked with permanent marker and the initial paw volumes of the right hind paw was measured by Plethysmograph. The drugs were administered orally in the calculated doses using a feeding cannula to all groups except Group A, which was kept as the control group. After 1 hr 0.1 ml of Carrageenan (1% Carrageenan solution w/v) was injected in the plantar region of the right hind paw of each animal using a tuberculin syringe. Paw volumes were

measured using a Plethysmograph 1 hr after injection, 3 hrs and 5 hrs respectively^{9, 10}.

STATISTICAL ANALYSIS AND RESULTS

Statistical comparisons between the control and test groups were made using Two way ANOVA (Analysis Of Variance). Mean paw volume of control and test groups before and after inducing inflammation was calculated. Using One way ANOVA mean change in paw volume of test groups were compared with that of control in 1st hour, 3hrs and 5hrs after inducing inflammation and values are expressed as mean±sd. Percentage change in paw volume of control and test groups were calculated. Using Two way ANOVA a paired comparison between control and test groups was done and the values are expressed as mean±se. Among the root kwatha treated groups only double dose treated group showed a better anti inflammatory effect when compared to control. It showed the effect from the 1st hr onwards. 3rd hr onwards effective dose treated group showed values almost equal to control. 5th hr also it had the same action. Half dose treated group always showed an increased value during the observation. Even though double dose treated group showed anti inflammatory effect, it is not significant when compared with control. From the results it can be concluded double dose of root kwatha showed anti-inflammatory effect in acute inflammation by

carrageenan induced hind paw oedema in albino rats.

Among the leaf kwatha treated groups, all groups showed anti inflammatory effect from 3rd hr after carrageenan injection. Double dose treated groups showed almost similar activity at 3rd & 5th hrs. But half dose and effective dose showed better anti inflammatory effect at 5th hr. But these groups also showed no significance when compared to control. From the results it can be concluded that different doses of leaf kwatha showed anti inflammatory effect in acute inflammation by carrageenan induced hind paw oedema in albino rats.

A paired comparison of control and test groups was done at 1st hr, 3 hrs and 5 hrs after inducing inflammation to find out the significant difference between them. Paired comparison between the control and test groups showed no significance at 1st hr, 3 hrs and 5 hrs of observation. But the paired comparison between root kwatha and leaf kwatha treated group showed significance from third hr onwards.

Combining the results of these two comparisons, it can be stated that different doses of Nirgundi leaf kwatha showed better anti inflammatory effect than its root kwatha when compared to control in acute inflammation by carrageenan induced hind paw oedema. Also results tend to suggest that even though Nirgundi leaf kwatha and root kwatha possess anti-inflammatory effect, it is very mild in the acute stage of Inflammation.

CONCLUSION

Nirgundi (*Vitex negundo* Linn) leaf kwatha shows better anti-inflammatory effect than its roots in carrageenan induced acute inflammatory condition. From this it can be concluded that Nirgundi leaves can substitute with its roots in acute inflammatory conditions if necessary. Since Kwatha of roots and leaves of Nirgundi shows mild anti inflammatory effect in the acute stage of Inflammation, further studies in different experimental models are necessary. Also study can be repeated in sub acute and chronic inflammation in experimental models. Comparative studies can be conducted in formulations like Sinduvara eranda by preparing the medicine using leaves and roots of Nirgundi separately. Similar studies can be done in Red Endangered Threatened (RET) plants for finding potent substitutes and sustainable plant parts.

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