



Original Article

ANTI-INFLAMMATORY AND EFFECT ON CENTRAL NERVOUS SYSTEM OF *ALSEODAPHNE ANDERSONII* IN EXPERIMENTAL ANIMAL MODELS

Indu Dhillon¹, Atul Kaushik*¹ & Jeevan Jyoti Kaushik²

Affiliated to:

¹Department of Pharmaceutical Chemistry, GRD (PG) Institute of Management and Technology, 214-Rajpur, Dehradun, 248009 Uttarakhand, India

²Department of Life Sciences, CSJM, Kanpur University, Kanpur, 208024 India.

ABSTRACT

Alseodaphne andersonii (King ex Hook. f.) Kosterm. (Lauraceae), a plant growing in the Himalayan region, has been selected for the current study. The leaf extracts were screened for anti-inflammatory activity and effect on central nervous system in animal models using acute carrageenan paw oedema method and by using actophotometer respectively. Both the extracts significantly ($P < 0.05$) suppressed the paw oedema induced by carrageenan in rats at the dose level of 500mg/kg. CNS stimulant effect of the extract at all doses level is much higher when compared to the control and standard caffeine (a known stimulant). Differences between means were assessed by one way analysis of variance (ANOVA), followed by Dunnett's test using sigma stat software. Stimulant effect was significant ($p < 0.0001$ at all doses tested) and this was also found to be dose dependent. More studies are required to achieve the proper role of *Alseodaphne andersonii* extract to find out more specific biochemical, pharmacological and molecular aspects of the targeted molecules within that may have the broadest implication to society.

KEY TERMS - *Alseodaphne andersonii*, carrageenan, CNS stimulant, inflammation, leaf extract.

*Corresponding author:

Dr. Atul Kaushik, Head, Department of Pharmaceutical Chemistry
GRD (PG) Institute of Management and Technology,
Rajpur, Dehradun, 248009, India
E.-Mail: atul.kaushik29@gmail.com



1.0 INTRODUCTION

Plant and plant extracts have been used since the dawn of civilization by man. The use of ethno botanical preparations for various reasons justified or not, is still continued by various cultures around the world⁵. Many anti-inflammatory and CNS stimulant drugs have been used clinically for the treatment of inflammation and drowsiness¹. Therapies with these drugs are effective, but sometimes there are adverse effects and compliance can be low³. In view of this, *Alseodaphne andersonii* (King ex Hook. f.) Kosterm. (Lauraceae), a plant growing in the Himalayan region, has been selected for the current study. The plant is reported to be used as timber in Yuan-Nan⁸ Only five gamma lactones were isolated from *A. andersonii*⁴. Lack of scientific data with respect to the pharmacological properties of the *Alseodaphne andersonii* encouraged for the evaluation of for anti-inflammatory activity. The purpose of the present study was to investigate its use as a remedy for drowsiness (sedation) and inflammation, the actophotometer was used to check its effect on stimulation in animal models and using carrageenan – induced paw oedema method respectively. This is the first report of this kind on this plant.

2.0 METHODS

2.1 Sample collection

The leaves of *Alseodaphne andersonii* (King ex Hook. f.) Kosterm. (Lauraceae) were collected from, Forest Research institute, Dehradun (U.K.)

India. and authenticated by Dr. Prashant chaddha, Scientist, Botanical Survey of India (BSI), Dehradun (U.K.), India, on 07-01-2008 with letter Ref. No. BSI/NC- 9 (/2007-08/Tech/1011). Authentic sample was deposited in the department of pharmacognosy of the institute.

2.2 Processing of sample

The fresh leaves of the plant were dried at room temperature (25-35 °C) for 20-25 days. The dried leaves were powdered in a grinder and weighed before used for calculating the yield (w/w).

2.3 Preparation of leaf extracts and qualitative analysis

The dried powdered material of *A. andersonii* was extracted using soxhlet apparatus with methanol and water in a sequence of polarity. The extracts were concentrated under vacuum to obtain semi-solid mass and qualitative phytochemical tests showed that methanol and water extracts tested positive for carbohydrates, reducing sugars, alkaloids, glycosides, flavonoids, sterols and saponins. All the extracts were stored in a clean glass bottles for further pharmacological studies.

2.4 Anti-inflammatory activity

2.4.1 Chemicals

Methanol, Plethysmometer, Carrageenan, Indomethacin, tween 20.

2.4.2 Animals used

30 Wister Rats of either sex, weighing 300-400 g were procured from Animal House, GRDIMIT, Dehradun (U.K.). The animals were housed in cages under standard laboratory conditions (12:12 hour light/dark cycle at 25± 20C). They had free access to standard commercial diet and water. The



animals were divided into six groups of 5 each. The 1st group served as the control group, the 2nd, 3rd, 4th and 5th groups were used as test groups and the 6th group was the standard group.²

2.4.3 Ethical approval

The animal experiment was carried out as per the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) norms, mentioned by the NIH guidelines wide our college Regn No: 1145/a/07/CPCSEA, Dated; 02 Jan 2008 and Project resolution No. 7.

2.4.4 Preparation of doses

The control group received the vehicle (25% tween 20 and 2.5% DMSO); groups 2nd and 3rd were treated with methanol extract at different dose level (250 and 500mg/kg, p.o, respectively); groups 4th and 5th were treated with water extract at the dose of 250 and 500mg/ kg, p.o. respectively and group 6th with Indomethacin (10mg/kg, p.o.), as a standard anti-inflammatory agent².

2.4.5 Evaluation

0.1 ml of 1% carrageenan in 0.9% NaCl was administered into the plantar surface of the right hind paw of the animals¹³. The experimental groups, control group (2.5% DMSO and 2.5% tween 20), and standard group (10mg /kg Indomethacin p.o.) were given either the control drug or test compounds orally, an hour prior to the administration of the carrageenan. Before injection of carrageenan, the average Volume (Vo) of the right hind paw of each rat was calculated from 3 readings that did not deviate

more than 3%. After injection of the phlogistic agent, readings (Vt) were obtained for each rat was at 30, 60, 120, 180, 240 min. with the aid of a plethysmometer. The edema was expressed as an increase in the volume of paw.

The edema was expressed as an increase in the volume of paw. The experimental results were expressed as the mean \pm standard error of mean (S.E.M.).

The percentage inhibition for each rat and each group was obtained as follows:

$$\% \text{ of Inhibition} = \frac{(\text{Vt} - \text{Vo}) \text{ control} - (\text{Vt} - \text{Vo}) \text{ treated} \times 100}{(\text{Vt} - \text{Vo}) \text{ control}}$$

Differences between means were assessed by one way analysis of variance (ANOVA), followed by Dunnett's test using sigma stat software. P<.05 was considered significant.

2.5. CNS stimulant activity

Effect of water extract of *Alseodaphne andersonii* was studied in mice using the digital actophotometer (Space- 001 Lab, Maharashtra, India). The continuous beam of light falls on photoelectric cells. When the reading is considered zero any cut of in the continuity of light by the animal, is recorded on a digital counter in the forms of counts. Depending on the CNS stimulant action of the drug, the animals show increased locomotors activity⁶.

2.5.1 Animals used

20 Albino mice (25-30g) of either sex were procured from the animal house (Reg. No.: 1145/a/07/CPCSEA) of the institute. The animals were housed in cages under standard laboratory



conditions (12:12 hour light/dark cycle at 25 ± 2 OC). They had free access to standard commercial diet and water. The animals were divided into four groups of five animals each. The group 1 served as the control group, the 2nd, 3rd, groups were used as test groups and the group 4 was the standard group.

2.5.2 Preparation of doses

The control group 1 received the vehicle; groups 2 and 3 were treated with water extract at different dose level (25 and 50 mg/kg respectively); and group 4 with caffeine (standard CNS stimulant agent) as 10mg/kg dissolved in normal saline (0.9% NaCl). All the groups were given 0.2ml of the respective drug by the I.P. route.

2.5.3 Evaluation

Group 1 received vehicle and served as control, group 2 and 3 received 25 mg/kg and 50 mg/kg (0.2 ml i.p.) of aqueous extract respectively and used as test groups, group 4 received (0.2 ml i.p.) of 10mg/kg caffeine. The basal activity scores of all the animals were recorded 2 days before the start of study using a photoactometer. On the day of the study all animals were given their assigned treatments, 30 minutes after the treatment each animal was retested for activity scores for 10 min and the difference in the activity scores were compared with the control scores⁶. The mean score for each group as determined was compared with each other. The test groups were compared with the standard and the control group.

2.5.4 Statistical evaluation

Differences between means were assessed by one way analysis of variance (ANOVA), followed by Dunnett's test using sigma stat software. $P < 0.05$ was considered significant.

3.0 RESULTS

3.1 Anti inflammatory activity

The effects of extracts of *A. andersonii* on paw edema induced by carrageenan are shown in table 1. Treatment with methanol and aqueous extracts of *A. andersonii* produced a diminished inflammation in rat hind paw when challenged with carrageenan. Percentage inhibition observed in aqueous extract treated groups were more from that observed with methanol extract treated groups at higher dose level (Table 1). This indicates that the extracts exhibited dose dependent effect on inflammation i.e. 50% and 65.62% inhibition at doses level of 250 mg/kg and 500 mg/kg respectively in methanol extract and 59.37% and 70.3% inhibition at doses level of 250 mg/kg and 500 mg/kg respectively in aqueous extract. Differences between means were assessed by one way analysis of variance (ANOVA), followed by Dunnett's test using Sigma stat software. $P < 0.05$ was considered significant.

3.2 Effect on CNS activity

In vivo methods using intact animals are considered to be the best method for investigating the action of drugs on the CNS. To obtain meaningful results regarding the effect of *Alseodaphne andersonii* leaf extract (aqueous) on the CNS in mice at different dose level, effect on CNS was noted with the help of actophotometer⁶.



Table 1 Comparative study of anti-inflammatory activity of leaf extracts of *a. Andersonii* vs indomethacin.

Treatment	Dose (mg/kg)	Inflammation Volume (ml) Mean + S.E.M.					% Inhibition at 4 hrs
		30 min	1 hr	2 hr	3hr	4hr	
Control (Normal Saline)	0.0	0.15 ± 0.0100	0.22 ± 0.0487d	0.4 ± 0.199	0.52 ± 0.0489	0.64 ± 0.0478	-
Methanol Extract	250	0.07 ± 0.0120	0.16 ± 0.0244	0.18 ± 0.0199	0.26 ± 0.0399	0.32 ± 0.0489a	50.00 %
	500	0.08 ± 0.0122	0.1 ± 0.0273b	0.14 ± 0.0244	0.16 ± 0.0584	0.22 ± 0.0199	65.62 %
Water Extract	250	0.11 ± 0.0099	0.19 ± 0.0100	0.21 ± 0.0244	0.23 ± 0.0224	0.26 ± 0.0204	59.37 %
	500	0.08 ± 0.0122b	0.14 ± 0.0244b	0.18 ± 0.0204	0.18 ± 0.0204	0.19 ± 0.0053	70.3 %
Indomethacin	10	0.08 ± 0.0122d	0.09 ± 0.0099c	0.16 ± 0.0244	0.18 ± 0.0199	0.19 ± 0.0245	70.31%

Values expressed as mean + S.E.M, n = 5 in each group pa<0.01, Pb <0.05, Pc 0.005, Pd < 0.1 compared with control

The activity was determined separately for each group (Table 2). After observing the CNS effect on different groups, it is clearly indicating that aqueous leaf extract of *Aseodaphne andersonii* significantly increased the locomotor activity. Aqueous extract at the dose level of 50 mg/kg

was showing an excellent CNS stimulant effect (150.2 ± 2.495) with respect to the standard group (137.6 ± 5.767), but the 25mg/kg dose level (137.8 ± 3.595) was fewer stimulants as compared to standard caffeine 10 mg/kg (Table 2).

Table 2 Comparative study of CNS stimulant activity of leaf extracts of *a. Andersonii* vs caffeine.

S.No.	Group	Dose	Mean ± S.E.M	Stimulant
1	Control	1% Tween 20	103 ± 4.405	NS
2	Standard	10 mg /kg Caffeine	137.6 ± 5.767	+++
3	Test1	25 mg/kg Aqueous extract	137.8 ± 3.595	+++
4	Test 2	50 mg/kg Aqueous extract	150.2 ± 2.495	+++

+++ = Strong Stimulant, ++ = Stimulant, NS = Not Showing, Result shows a statistically significant difference ($P < 0.001$)

The results were dose dependent and statistically significant. As the score scale, was not isomorphic to the arithmetic scale the mean value and standard error was used to analyze difference between groups of mice. The differences in the

mean values among the treatment groups are greater than would be expected by chance; there is a statistically significant difference ($P < 0.001$). To isolate the group or groups that differs from the others use a multiple comparison procedure.



All pair wise multiple comparison procedures (Dunnett's Method) were also performed for authentication of the results.

3.3 Qualitative analysis

Both the extracts were analyzed for various phytoconstituents, qualitative phytochemical tests showed that methanol and water extracts tested positive for carbohydrates, reducing sugars, alkaloids, flavonoids, and saponins (Table 3).

Table 3 Qualitative chemical analysis of leaf extracts of *Alseodaphne andersonii*.

S. No.	Test	Observation	Pet. ether	Chloroform	Acetone	Methanol	Water
1	Test of Alkaloids						
	(a) Mayer's test	Cream	-	-	-	+	+
	(b) Hager's test	Yellow	-	-	-	+	+
	(c) Wagner's test	Brown	-	-	-	+	+
	(d) Dragendorff's Test	Brown	-	-	-	+	+
2	Test for carbohydrates						
	(a) Molisch Test	Violet Ring	-	-	+	+	+
	(b) Benedict's Test	Red	-	-	+	+	+
	(c) Fehling's Test	Brick -Red	-	-	+	+	+
3	Test for steroids						
	(a) Salkowski's Test	Red	-	-	-	-	-
4	Test for Saponins	Foam	-	-	-	+	+
5	Test for Phenolic Compounds and Tannins						
	(a) Dil FeCl ₃ Test	Deep- blue	+	+	+	+	+
	(b) Lead acetate Test	White	+	+	+	+	+

4.0 DISCUSSION

Folkloric treatment of inflammation of various etiologies, using medicinal plants, is well known to masters of the art of traditional medicine practice. The medicinal plants are suggestive of high therapeutic potency in disease conditions especially in inflammatory disorders⁷. Caffeine, a mild stimulant is the most widely used psychoactive drug in the world¹⁰. It increases nor-epinephrine secretion and enhances neural activity in numerous brain areas. Many of its effects are believed to occur by means of

competitive antagonism at adenosine receptors. Tolerance occurs rapidly to the stimulating effects of caffeine, thus a mild withdrawal syndrome has been produced. Many anti-inflammatory and CNS stimulant drugs have been used clinically for the treatment of inflammation and drowsiness¹. Therapies with these drugs are effective, but sometimes there are adverse effects and compliance can be low³. In view of this, *Alseodaphne andersonii* (King ex Hook. f.) Kosterm. (Lauraceae), a plant growing in the Himalayan region, has been selected for the



current study. The Methanol and aqueous leaf extracts of *A. andersonii* exhibited the anti-inflammatory activity compared to that of indomethacin. *A. andersonii* contains some physiological active molecules, which were responsible for the anti-inflammatory activity. Further work on the types of phytoconstituents isolation of bioactive compound can reveal the exact potential the plant to inhibit the several types of inflammation and encourage in developing a novel new anti-inflammatory drug in future. After screening of leaf extracts of *A. andersonii* has revealed that the leaf possesses potent anti-inflammatory effect in the models of acute inflammation. These extracts may have inhibited the release of pro-inflammatory mediators of acute inflammation such as histamine and prostaglandin. Thus, these extracts may exert anti-inflammatory effect by inhibiting the synthesis of prostaglandin. More studies are required to achieve the proper role of *A. andersonii* extract to find out more specific biochemical, pharmacological and molecular aspects of the targeted molecules within that may have the broadest implication to society.

5.0 ACKNOWLEDGEMENT

The author are highly thankful to S. Raja Singh Chairman GRD(PG) Institute of Management and Technology, Dehradun, India, for providing requisite facilities for the research work.

6.0 REFERENCES

1. Agharakar, S.P. (1991). Medicinal Plants of Bombay Presidency. Pb. I.

Scientific Publishers, Jodhpur, India: 208-209.

2. Dimo, T., Agathe, L.F., Nguielefack, t. B., Asongalem, E A., Kamtchouing, P. (2006). Anti-inflammatory activity of leaf extract of *Kalanchoe crenata* Andr. Indian Journal of Pharamcol, Vol-38: 115-119.
3. Goel, R.K., Pathak, N.K., Biswas M., Pandey, V.B., Sanyal, A.K. Effect of lapachol, a naphthaquinone isolated from *T. Grandis*, on experimental peptic ulcer and gastric secretion. J. Pharm. Pharmacol., 1987, 39 (2): 138-140.
4. Lee SS, Chang SM, Chen CH. Chemical constituents from *Alseodaphne andersonii* J Nat Prod. 2001, 64(12):1548-51.
5. Massoud, M., Jalipour, H. and Salehian, P. Toxicity of pegamum harmala review and a case report. Iran J. of Pharmacol. Therao. 2002, 1: 1-4
6. Nikajoo, L.T. Central nervous system depressant activity of alcohol and aqueous root extracts of *Pergularia daemia* (forsk.) Chiov *Pharmacologyonline*, 2009, 1: 119-124.
7. Okoli, C. O., Akah, P. A. and Ezugworie, U.. Antinflammatory activity of extracts of root bark of *securidaca longipedunculata* fres polygalaceae) Afr. J. Trad. CAM 2005, 2 (3): 54 – 63.
8. Parcha, V. Kaushik, A., Kaushik, J., RAWAT, M.S.M. AND BISWAS,



- S.A.S. Pharmaceutical Biology. Evaluation of Antimicrobial Potential of *Alseodaphne andersonii*. Leaf Extracts against Pathogenic Bacteria. 2007, Vol. 45, No. 1: 60-63.
9. Raymond I. Ozolual AND Peter Alonge. Aqueous and ethanol leaf-extracts of *Piliostigma thonningii* (Schum) increase locomotor activity in Sprague-Dawley rats African Journal of Biotechnology 2008, Vol. 7 (2): 073-076.
10. Silverman K, Evans SM, Strain EC, Griffith RR Withdrawal syndrome after the double-blind cessation, caffeine consumption. N. Engl. J. Med. 1999, 327: 1109-1114
11. Trease GE, Evans WC (1978). Pharmacology 11th Ed. Bailliere Tindall Ltd, London. pp 60-75.
12. Varier, P.S. INDIAN MEDICINAL PLANT: A compendium of 500 species Vol.V. Pbl. Orient Longman, Hyderabad, India: 1997, 245-248.
13. Winter, C.A., Risley, E.A., Nuss, G.W., Carrageenan induced edema in Hind paw of rat as an assay for anti inflammatory drugs. 1962, Proc Soc expt Biol Medical. 544-47.