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IN VITRO STUDIES OF THE USE OF SOME MEDICINAL HERBALS LEAVES AGAINST ANTIDEPRESSANT, ANALGESIC ACTIVITY, AND ANTI-INFLAMMATORY ACTIVITY

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The aim of present study was to assess the anti-inflammatory activity of polyherbal formulation of leaves of *Annona squamosa, Azadiracta indica.* The mature green leaves of *Annona squamosa, Azadiracta indica* were collected and authenticated. Extractions of dried leaves and rhizome were carried out with ethanol in soxhlet apparatus. The polyherbal formulation showed the significant anti-inflammatory activity comparable to the standard drug Indomethacin against carrageenan induced rat paw edema method. The polyherbal formulation reduced the inflammation induced by carrageenan by 53.0% and 47.0% on oral administration at 100 mg/ kg and 200 mg/kg respectively as compared to the control treated group an analgesic activity comparable to the standard drug tramadol HCl.

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1. Introduction

Annona squamosa (Family: Annonaceae) is a semi-evergreen shrub or small tree reaching 6–8 meters (20–26 ft) tall native to the tropical Americas. It is used as insecticide, antiovulatory, haematinic, sedative, stimulant, expectorant and abortifacient [1]. The main active constituents are liriodenine, moupinamide, anonaine, squamosamide, sachanoic acid[3]. *Azadirachta indica* (Family: Meliaceae) is a fast-growing tree that can reach a height of 15-20 m, rarely to 35-40 m, native to Bangladesh, India, Myanmar and Pakistan. According to Ayurevedic text it is used for anthelmintic, antifungal, antidiabetic, antibacterial, antiviral, anti-infertility, sedative and skin disease[1]. The main active constituents of the plant are nimbin, nimbinin, nimbidin, limocinol, limocinone, azadirol, naheedin, azadironolide, limbocinin [2,4].

2. Experimental and result discussions

The leaves of *Azadiracta Indica*, *Annona squamosa* were collected from Guna (M.P). The plant authenticated by comparing with the herbarium voucher specimen. The material was air dried under shade, pulverized by a mechanical grinder and passed through a 40 mesh and then stored in airtight containers. The powdered leaves and rhizome (250 g) were extracted with

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ethanol for 24 h using a soxhlet extractor. This ethanolic extract was concentrated to dryness under reduced pressure and controlled temperature (50-60^{o}C) to yield solid masses.

Table I. Anti-inflammatory activity

Compounds	Average Change in paw volume after 2hours (Mean± SEM)	% Inhibition of paw edema after 3 hours (Mean)	Average Change in paw volume after 5 hours (Mean ± SEM)	% Inhibition of paw edema after 5hours (Mean)
Control	2.86 ± 0.02	--	1.96 ± 0.02	--
01	2.73 ± 0.04**	25.11	1.86 ± 0.02**	33.41
02	2.46 ± 0.02*	36.51	1.63 ± 0.02*	24.37
03	2.66 ± 0.03*	13.25	1.80 ± 0.03*	26.66
04	2.53 ± 0.02*	68.37	1.66 ± 0.02*	51.25
-05	2.63 ± 0.02*	46.74	1.76 ± 0.02*	40.83
Indomethacin	2.20 ± 0.03*	66.74	1.40 ± 0.03*	58.33
One way ANOVA F df P	73.45 12,18 <0.001		36.20 26,15 <0.001	
n =6 in each group. *P<0.001, **P<0.01 compared to control. The results were analyzed for statistical significance using one –way ANOVA followed by Dunnet's test. A P value < 0.05 was considered significant.				

Anti-inflammatory activity

Healthy inbred Wister albino rats of either sex, (150-180 g) were selected and housed in polypropylene cages at a well-ventilated, temperature-controlled (30±1^{o}C) animal room with food and water ad libitum. Animals were periodically weighed before and after experiments. Animals were divided in four groups of 6 animals each. The control group receives vehicle orally, while other groups receives test drug and standard drug respectively .The animals were treated with drugs by oral route and subsequently one hour after treatment, 0.1ml of 1% suspension of carageenan in normal saline was injected to the sub planter region of left hind paw to induce edema. The paw volume was measured initially at 1, 3 and 5 hours after carageenan injection using plathismometer .The difference between the initial and subsequent reading gave the actual edema volume which was compared with control. The difference of average values between treated animals and control group is calculated for each time interval and evaluated statistically [6]. The percent inhibition is calculated using the formula as follows- %edema inhibition = [1-(Vt/ Vc)*] 100. Vt and Vc are edema volume in the drug treated and control groups, respectively.

Table II. Analgesic Activity

Compound	Pre drug Reaction time in sec (Mean ± SEM)	Post Drug reaction time in seconds (Mean ± SEM)			
		30 Min. (Mean±SEM)	60 Min. (Mean±SEM)	90 Min. (Mean±SEM)	180 Min. (Mean±SEM)
Control	6.36 ± 0.13	5.35 ± 0.12	5.89 ± 0.11	4.45 ± 0.10	3.33 ± 0.09
01	5.46 ± 0.30*	4.20 ± 0.20	6.03 ± 0.27*	5.73 ± 0.36*	4.86 ± 0.36**
02	6.24 ± 0.04	4.91 ± 0.02**	6.51 ± 0.08*	6.56 ± 0.20*	6.01 ± 0.01*
03	6.04 ± 0.05	4.05 ± 0.06	7.13 ± 0.05*	6.82 ± 0.34*	6.04 ± 0.04*
04	6.05 ± 0.06**	4.80 ± 0.07**	5.04 ± 0.08*	5.29 ± 0.15*	5.45 ± 0.11*
05	5.79 ± 0.07**	4.91 ± 0.04**	5.73 ± 0.10*	5.99 ± 0.02*	5.91 ± 0.06*
Tramadol HCl	6.22 ± 0.18	6.16 ± 0.05*	7.97 ± 0.15*	9.44 ± 0.06*	9.39 ± 0.06*
One –way ANOVA F df P	7.75 21,45 P<0.01	26.16 66,85 P<0.001	55.89 76,85 P<0.001	61.21 16,11 P<0.001	83.30 16,25 P<0.0001
n= 6 in each group.*P<0.001,**P<0.05 compared to control. The results were analyzed for statistical significance using one –way ANOVA followed by Dunnet's test. A P value < 0.05 was considered significant					

Analgesic activity

Analgesic activity was measured by tail flick method using the radiant type analgesiometer. Basal reaction time to radiant heat were taken by placing the tip of the tail on the radiant heat source. Swiss albino mice (25-30 g) of either sex were divided into different groups (control, test and standard) containing six animals each. For each animal, the tail flick reaction time was obtained thrice before drug administration and mean was used as pre drug reaction time. After the administration of drug, the tail flick reaction times were measured at 30 minutes, 60 minutes, 90 minutes and 180 minutes. The test and standard drug were given intraperitoneally, while the control group received only vehicle. The animals were administered a 30 mg/kg (body weight) dose of the test drugs and 22.8 mg/kg (body weight) dose of standard drug (tramadol HCl).

Table III. Antidepressant activity.

Compounds	Number of mobile phase in pretreatment period (Mean±SEM)	Number of mobile phase in post treatment period (Mean±SEM)	% Increase in mobile phase as compare to pre treatment
Positive Control	22.30±1.20	23.00±0.50	9.69
Negative Control	211.50±0.20	22.50±0.80	16.08
1	19.50±1.38	14.00±1.57	33.33
2	17.67±0.80	11.50±0.84	35.68
3	21.67±0.98	18.50±1.17	25.68
4	20.50±0.67	15.66±0.61*	26.57
5	1 9.50±0.42	22.33±0.66**	33.31
Fluoxetine HCl	19.00±0.73	30.50±0.99*	30.00

n=6 in each group, **p<0.05, **p<0.001 compared against control group.

Tail suspension test in mice

Antidepressant activity was measured by the tail suspension test in mice[7]. Balb/cj mice (30-35 g of body mass) of both sexes were divided into different groups (control, test and standard) containing six animals each. The animals were housed in standard polypropylene cages under standard laboratory conditions (12:12 hour light/dark cycle at 25±3^{0}C).They had free access to standard commercial diet and water. The ethical guidelines for the investigations of animals used in experiments were followed in all tests. In this study, the animals were administered 30 mg kg^{-1}(body mass) dose of the test drug and 15 mg kg^{-1}(body mass) dose of standard drug fluoxetine hydrochloride. The test and standard compounds were suspended in 10% tween-20 suspension and administered intraperitoneally 30 minutes prior to testing. The control group animals, however received the same volume of vehicle (10% tween-20 suspension).Test mice were suspended on the edge of a shelf 58cm. above a table top by adhesive tape placed approximately 1cm from the tip of tail. The duration of immobility is reported for a period of 5 minutes and this time were divided into 20 phases and each phase consist of 15 sec. mice were considered immobile when they hang passively and completely motionless for at least 10-12 seconds out of 15 seconds. The results are reported in table **1** and were analysed for statistical significance using students "t" test followed by. A P value < 0.05 was considered significant.

3. Statical analysis

The results of these experiments are expressed as means±sem of six animals in each group. The data was subjected to one-way ANOWA and the values of $p \leq 0.01$ were considered statistically significant.

4. Conclusion

Polyherbal formulation possesses potent anti-inflammatory activity and analgesic activity.as it inhibits maximum edema at 5 hrs, which was comparable to that of standard Indomethacin. Since, serotonin, histamine and prostaglandins are the major mediators of inflammation, anti inflammatory effect of polyherbal formulation could be due to inhibition of either their synthesis or release possibly due to inhibition of the enzyme cycloxygenase leading to inhibition of prostaglandin synthesis at third stage of inflammation.Based on the results of the present study, it can be concluded that polyherbal formulation showed significant anti-inflammatory activity and analgesic activity .

References

[1] J. D. Kirtikar and Basu BD. Indian Medicinal Plants, Published by Lalit Mohan Basu; Leader Road, Allahabad, India, **2066-2070** (536), 2423 (1994).
[2] C. K. Kokate, A. P. Purohit."The text book of Pharmacognocy, Published by Nirali Prakashan, Pune, 581-594, 165, 2003.
[3] Yang Y et al. New cyclic peptides from the seeds of Annona squamosa l. and their anti-inflammatory activities, Journal of agricultural and food chemistry, **56**, 386 (2008).
[4] Koley KM and Lal J. Anti-inflammatory activity of Azadirachta indica (neem) leaves, British Journal of Pharmacology, **65**, 524 (1994).
[5] L. Steru, R. Chermat, B.Thierry and P. Simon, Tail suspension test: A new method for screening antidepressants in mice. Psychopharm. **85**, 367 (1985).