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COMBINATORIAL EFFECT AND EVALUATIONS OF PHARMACOLOGICAL, PHYTOCHEMICAL STUDIES OF COMBINATION IN THREE HERBAL DRUGS IN 95% ABSOLUTE ETHANOLIC EXTRACT

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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* and rhizome of *Curcuma longa.* The mature green leaves of *Annona squamosa, Azadiracta indica* and rhizome of *Curcuma longa* 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 49.3% and 61.73% 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].*Curcuma longa* (Family: Zingiberaceae) is a rhizomatous herbaceous perennial plant of the ginger native to tropical South Asia. It is used as cough, amenorrhea, toothache, chest pain, blood urine, hemorrhage, skin disorders, diabetes, arthritis and wounds. The main active constituents are Curcuminoids, Curcumin, Demethoxy-curcumin and Bisdemethoxy-curcumin [[1-2,5]. A literature survey reveals that no systematic approach has been made to study the anti-inflammatory activity of polyherbal formulation of these plants. In the present work, we have investigated the we have investigated of polyherbal formulation against Indomethacin, Anxiolytics, Anti-Inflammatory activity, Analgesic activity, and Antidepressant activity.

2. Experimental results. Discussion

The leaves of *Azadiracta Indica*, *Annona squamosa* and rhizome of *Curcuma longa* were collected from Guna (M.P). The plant authenticated by comparing with the herbarium voucher specimen. The material was air dried under shade, pulverised 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 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 1. 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	0.86 ± 0.02	--	0.96 ± 0.02	--
01	0.73 ± 0.04**	15.11	0.86 ± 0.02**	10.41
02	0.46 ± 0.02*	46.51	0.63 ± 0.02*	34.37
03	0.66 ± 0.03*	23.25	0.80 ± 0.03*	16.66
04	0.53 ± 0.02*	38.37	0.66 ± 0.02*	31.25
-05	0.63 ± 0.02*	26.74	0.76 ± 0.02*	20.83
Indomethacin	.20 ± 0.03*	76.74	0.40 ± 0.03*	58.33
One way F ANOVA df P	53.52 16,85 <0.001		46.13 16,85 <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.

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 2. Analgesic Activity.

Compound	Pre drug reaction time in sec (Mean ± SEM)	Post drug reaction time in sec. (Mean ± SEM			
		30 Min. (Mean±SEM)	60 Min. (Mean±SEM)	90 Min. (Mean±SEM)	180 Min. (Mean±SEM)
Control	4.36 ± 0.13	4.35 ± 0.12	4.35 ± 0.11	4.29 ± 0.10	4.33 ± 0.09
01	3.46 ± 0.30*	4.20 ± 0.20	6.03 ± 0.27*	5.73 ± 0.36*	4.86 ± 0.36**
02	4.44 ± 0.04	4.91 ± 0.02**	6.51 ± 0.08*	6.56 ± 0.20*	6.01 ± 0.01*
03	4.04 ± 0.05	4.05 ± 0.06	7.13 ± 0.05*	6.82 ± 0.34*	6.04 ± 0.04*
04	4.77 ± 0.06**	4.80 ± 0.07**	5.04 ± 0.08*	5.29 ± 0.15*	5.45 ± 0.11*
05	4.79 ± 0.07**	4.91 ± 0.04**	5.73 ± 0.10*	5.99 ± 0.02*	5.91 ± 0.06*
Tramadol HCl	4.22 ± 0.18	6.16 ± 0.05*	7.97 ± 0.15*	9.44 ± 0.06*	9.39 ± 0.06*
One –way F ANOVA df P	8.75 16,85 P<0.01	16.16 16,85 P<0.001	75.89 16,85 P<0.001	81.21 16,85 P<0.001	183.30 16,85 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					

3. Statistical 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. Conclusions

Polyherbal formulation possesses potent anti-inflammatory 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 signification anti inflammatory activity and analgesic activity . The polyherbal formulation reduced the inflammation induced by carrageenan by 49.3% and 61.73% 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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