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FORMULATION AND EVALUATION OF ANALGESIC ACTIVITY, ANTI-INFLAMMATORY AND ANTI-ANXIETY ACTIVITY OF USING PLANT EXTRACTS

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The aim of the present work is to evaluate the anti-anxiety activity of ethanolic extract of *Datura stramonium (leaves), Terminalia arjuna (bark), Withania somnifera (root)* in mice. Elevated plus-maze apparatus, light/dark apparatus were used for finding anti-anxiety activity of the ethanolic extract of *Datura stramonium (leaves), Terminalia arjuna (bark), Withania somnifera (root)* (100, 200 mg/kg i.p.) and diazepam (1 mg/kg i.p.) were administered half an hour before the administration. The result showed that the ethanolic extract of polyherbal formulation (100and 200 mg/kg) and diazepam (1 mg/kg) induced significantly ($P<0.01$) increase in the occupancy in the open arm and showed a decrease preference for the closed arm entries. Polyherbal formulation of the extracts of bark of *Terminalia Arjuna* leaves of *Datura stramonium* and roots of *Withania somnifera* were developed and compared with control saline sample and treated Diazepam sample. We concluded that Polyherbal formulation was endowed with significant antianxiety activity.

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Keywords: Datura stramonium, Terminalia Arjuna, Withania somnifera, Antianxiety activity, Analgesic activity, Anti-inflammatory activity.

1. Introduction

Datura stramonium **(**Family: Solanaceae**)** is consist of dried leaves contain 0.25 % of alkaloids of stramonium. It is indigenous to Caspian region and in United States, South America, France, Germany and Hungary. The main active constituents of plant are atropine, hyoscyamine and scopolamine. It is used as a aphrodisiac, medicinal, psychotropic, sacred and antispasmodic [1-4]. *Terminalia arjuna* **(**Family:Combretaceae**)** is a dried stem bark. The tree is common in Indian peninsula it is grown by the side of stems and very common in Chotta-nagpur region. The pieces of various-sizes, about 15×10×1cm.According to ayurevedic text it is used as cardiotonic, styptic, febrifugal and antidysentric and diuretic. The main active constituents of the plant are 15% tannins, arjuonic acid, arjunogenin, arjunetine, arjunolone [1-4]. *Withania somnifera* **(**Family: Solanaceae**)** is consists of dried roots. The main active constituents are steroidal lactones, somniferine, somnine, somniferinine, tropane and anahydrine. It is used as sedative and hypnotic, hypotensive, as respiratory stimulant along with bradycardia. Literature survey reveals that no systematic approach has been made to study the anti-anxiety activity of polyherbal formulation of these plants. In the present work, we have investigated the anti-anxiety activity of polyherbal formulation against Diazepam [1-4].

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2. Experimental

Datura stramonium (leaves), Terminalia Arjuna (bark), Withania somnifera (root) were collected from SAGAR, M.P. The plant authenticated by comparing with the herbarium voucher specimen. The material was air dried under shade, powdered mechanically and stored in airtight containers. About 30 g of the powdered material was subjected individually for cold maceration with 95% ethanol for 7 days. This ethanolic extract was concentrated to dryness under reduced pressure and controlled temperature (50-60 ^{o}C) to yield solid masses.

Table 1. Anti-anxiety activity

Compounds	Group I (Mean± SEM)	Group II (Mean ± SEM)	Group III (Mean ± SEM)	Group IV 5hours (Mean)
Control	0.61 ± 0.05	0.76 ± 0.05	------	0.36 ± 0.02
01	0.33 ± 0.07**	12.11	0.66 ± 0.02**	13.41
02	0.66 ± 0.01*	36.51	0.43 ± 0.02*	24.31
03	0.40 ± 0.01*	33.25	0.60 ± 0.01*	18.33
04	0.56 ± 0.02*	24.37	0.41 ± 0.02*	21.25
05	0.63 ± 0.02*	36.21	0.76 ± 0.02*	26.83
Diazepam	10 ± 0.03*	56.74	0.40 ± 0.03*	18.33
One way r ANOVA df P	43.12 36.81 <0.001		46.13 11,51 <0.001	22.13 16.21 <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-anxiety activity

Healthy inbred Wister albino rats of either sex, (22-35 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. In both the model [5], Group-I rats were treated with simple saline (control). Group-II rats were treated with a reference standard (Diazepam 1 mg/kg), Group-III and Group-IV rats were treated with 100 mg/kg and 200 mg/kg ethanolic extract, respectively.

Table 2. 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	0.75 ± 0.02	--	0.82 ± 0.02	--
01	0.71 ± 0.04**	12.11	0.76 ± 0.02**	13.43
02	0.56 ± 0.02*	33.17	0.80 ± 0.02*	41.53
03	0.69 ± 0.03*	13.19	0.72 ± 0.03*	22.40
04	0.63 ± 0.02*	48.55	0.76 ± 0.02*	29.81
-05	0.73 ± 0.02*	55.74	0.62 ± 0.02*	21.39
Indomethacin	.20 ± 0.03*	81.22	0.52 ± 0.03*	61.91
One way f ANOVA df p	43.12 11,81 <0.001		56.32 34.15 <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 3. 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	0.45 ± 0.02	--	0.71 ± 0.02	--	0.45 ± 0.02
01	0.38 ± 0.04**	17.11	0.66 ± 0.02**	12.43	0.38 ± 0.04**
02	0.36 ± 0.02*	23.17	0.70 ± 0.02*	31.53	0.36 ± 0.02*
03	0.41 ± 0.03*	33.19	0.63 ± 0.03*	12.40	0.41 ± 0.03*
04	0.43 ± 0.02*	38.55	0.56 ± 0.02*	34.81	0.43 ± 0.02*
05	0.40 ± 0.02*	45.74	0.62 ± 0.02*	21.39	0.40 ± 0.02*
Tramadol HCl	.40 ± 0.03*	65.22	0.60 ± 0.03*	61.91	.40 ± 0.03*
One –way F ANOVA df P	33.19 16,31 <0.001		46.26 41,52 <0.001	-----	33.19 16,31 <0.001
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).

3. Statistical analysis

The data were analyzed by one-way ANOVA. According to this test, there was a significant difference between the drug treated groups and control at the level of P<0.05. Results, expressed as Mean ± SEM were evaluated using the t-test. Values of P < 0.05 were considered statistically significant.

4. Conclusions

Antianxiety is used to reduce the stress condition. The Polyherbal formulation possesses potent anti anxiety activity on elevated plus maze model and light and dark model which was comparable to that of standard drug diazepam. Based on the results of the present study, it can be concluded that polyherbal formulation showed signification antianxiety activity. 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 .

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