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Evaluation of *Andrographis paniculata* leaves extract for analgesic activity

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ABSTRACT

Objective: Aqueous extract of *Andrographis paniculata* leaves was subjected to analgesic activity in animal models. **Materials and Methods:** The study was carried out in male swiss albino mice weighing 25-30 gm.Analgesic activity of the aqueous extract of *Andrographis paniculata* leaves at doses 50,100,500mg/kg p.o was evaluated using two animal models *viz.*acetic acid induced writhing and Eddy's hot plate model. **Results:** Aqueous extract of Andrographis paniculata (AP) at doses 100mg and 500mg/Kg p.o. significantly reduced (*P<0.01*) acetic acid induced writhing in both acute and chronic study. The test drug at doses 100, 500mg/kg was better than the standard drug aspirin 150mg/kg in acute study.The percent reduction of abdominal constrictions was 20%, 56% and 57% at doses 50, 100 and 500mg/kg p.o. respectively compared to control in acute study. In chronic study the percent reduction of abdominal constriction was 15%, 69.4% and 70.4% at doses 50, 100 and 500mg/kg respectively. The extract increased the reaction time significantly at doses 50, 100 and 500mg/kg p.o in acute study at 15min, 30min and 60 min by Eddy's hot plate model and maximum increase (*P<0.01*) was observed at dose 500mg/kg. In chronic study the analgesic activity of the extract was observed at all doses at 15 min and the mean reaction time increased significantly (P<0.001) at dose 500mg/kg at 30 min and diminished later. **Conclusion:** The results confirm the analgesic activity of *Andrographis paniculata* by both peripheral and central actions which were comparable to standard drug. The maximum effect was observed at dose 500mg/kg in both the models.

Key words: Analgesic, acetic acid, *Andrographis paniculata*, Eddy's hotplate, writhing

INTRODUCTION

Andrographis paniculata known in the Indian subcontinant as Kalmegh is commonly used plants in the traditional systems of Unani and Ayurvedic medicines. *Andrographis paniculata* (AP) has been reported to have antibacterial, antifungal, antiviral, choleretic, hypoglycemic, hypocholesterolemic activity.[1] Studies have shown AP improve liver histology in CCl_4 induced liver damage,[2] has antiviral activity,[3] reduces BP,[4] restrict infarct size in animals,[5] have antioxidant,[6] antiinflammatory,[7] antihyperglycemic activities.[8] Clinical studies have shown its use in relieving symtoms of common cold, URTI.[9,10] There is very limited data available on its analgesic activity. Hence this study is carried to evaluate the analgesic activity of AP.

MATERIALS AND METHODS

Preparation of the extract:

The fresh plant collected locally, identified and authenticated at the department of pharmacognosy, Manipal College of Pharmaceutical Sciences, Manipal. Leaves were separated from the plant, cleaned and dried undershade and coarsely powdered. Preparation of aqueous extract was done by the method of Pirie NM (1979)[11] and Khosla P.et,al, (2000).[12] 50gm of powdered leaves were extracted using Soxhlet apparatus with 500 ml of distilled water till the eluent was colorless.The dark green color liquid was obtained as extract and evaporated, shade-dried, scraped out and weighed and used for the study.Phytochemical studies indicated the presence of diterpins, Lactones and Flavonoids.Diterpins include two bitter principles andrographolide and a compound Kalmeghin.

Animals and treatment regimen:

Swiss male albino mice weighing 20-30gm were used for the study.The animals were housed in animal room in a group of six with alternate light dark cycle of 12 hr each at constant temperature of 25±1^{0}C, had free access to food and water. They were deprived of food but not water for 12hr before the experiment.The animals were acclimatized to the laboratory conditions for atleast 7 days prior to experiment.

Acute oral toxicity study:

An acute oral toxicity study was done for the aqueous extract of AP for the determination of LD_{50} in mice by administering different doses according to the dose described by Ghosh.[13] It was observed that dose upto 2 gm/kg was nontoxic and produced no behavioral changes or mortality.Therefore LD_{50} was considered greater than 2 gm/kg body weight.

Animals were divided in to five groups of six animals each. The first group served as a control Group I received the vehicle (1% Gum acacia solution, 10ml/kg). Aspirin [*I.P. grade*, Vikas, Pharma, Mumbai] was suspended in 1% Gum acacia solution and administered at 150mg /kg to Group II.Groups III, IV and V received 50, 100 and 500mg/kg of AP extract respectively.The vehicle, standard drug and the test compound were administered orally. The research was conducted in accordance to ethical rules of the institute.

Acetic acid induced writhing model in mice[14]:

Freshly prepared 0.6% acetic acid solution in the volume of 10ml /kg was administered intraperitoneally to each animal of all the groups. In acute study, vehicle/aspirin/ the test drugs were administered orally 60 minute prior to the administration of acetic acid. While in chronic study they were given once a day for 10 days and the last dose was given 60 minute prior to acetic acid administration. Onset and the number of writhes were counted for 15min. Percentage of reduction in writhing syndrome was calculated and compared with control group. Treatment with doses received by each group is mentioned in Figure 1. Percent reduction indicates the percentage protection against abdominal constriction which was taken as an index of analgesia.

{(Wc-Wt) X 100}/Wc

where Wc= No of writhing of the control group

Wt= No of writhing of the treated group

Eddy's Hot plate model in mice[15]:

In this model, animals having basal reaction time not exceeding 15 seconds were included in the study.They were divided into 5 group for acute and chronic study separately. Number of animals and treatment received by each group is given in Table 2 and Table 3. Animals were pretreated with vehicle/ drugs orally 60 min before the experiment in acute study and in chronic study, animals were treated once a day for 10 days and the last dose was given 60 minutes before the experiment. Animals were placed individually on Eddy's hot plate (Techno instruments, India) maintained at 55±1^{0}C and the reaction was noted either by licking the paw or jumping or rising the limb whichever was observed first taken as the end point. Observations were made at 0, 15, 30, 60, 90 and 120 min following the administration of drugs. A cutoff time of 20 seconds was considered.

Statistical Analysis:.

Results were expressed as mean ± SEM. Statistical analysis of the data was done using One-way analysis of variance followed by Dunnet test by using spss software version 14. P<0.05 was considered significant.

RESULTS

Acetic acid induced writhing model in mice:

AP produced significant reduction in number of abdominal constrictions in

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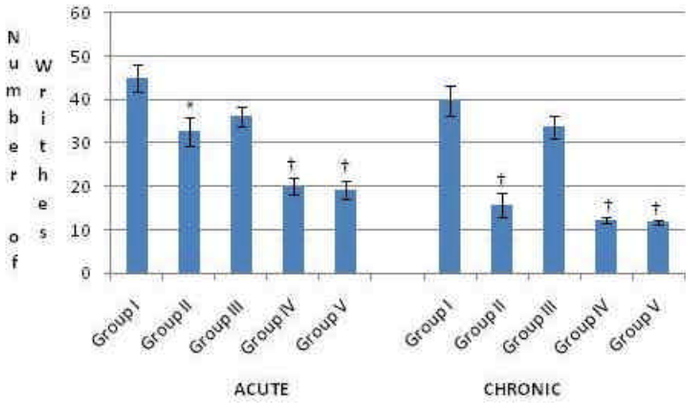
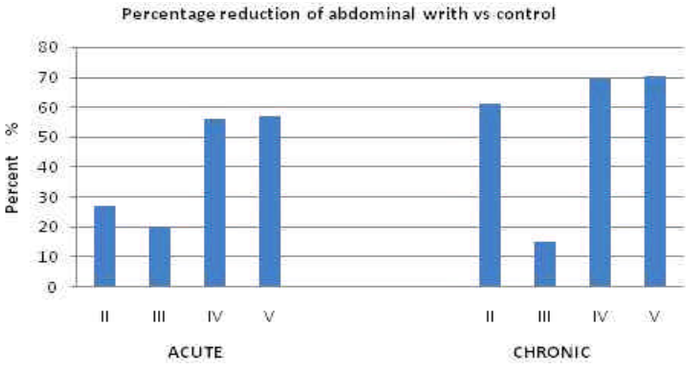
mice in dose dependent manner. Aqueous extract of AP significantly reduced the number of writhing in mice both in acute and chronic study [Figure 1].



*Group I=Control, Group II=Aspirin 150mg/kg p.o. Groups III, IV, V=Andrographis paniculata at 50,100,500 mg/kg p.o respectively. Values are expressed as mean±SEM, n=6. Symbols * and † indicates significant values at P<0.05 and P<0.01 respectively vs control; ANOVA followed by Dunnet's test*

Figure 1: Effect of Andrographis paniculata leaves extract on acetic acid induced abdominal constriction in mice

In both the studies AP reduced writhing at 100 and 500mg/kg dose compared to control. However, at 50mg dose it did not show reduction. The percent reduction of abdominal constrictions in acute study was 20%, 56% and 57% at doses 50, 100 and 500mg/kg respectively compared to control. The percent reduction of abdominal constriction in chronic study was 15%, 69.4% and 70.4% at doses 50, 100 and 500mg/kg respectively [Figure 2].



Group II=Aspirin at 150mg/kg p.o, Groups III, IV, V=Andrographis paniculata at 50,100,500mg/kg p.o. respectively.Values are expressed as percentage reduction vs control, n=6

Figure 2: Percentage reduction of abdominal writh in mice

There was significant reduction in the number of abdominal constrictions in acute study with AP at doses 100mg/kg and 500mg/kg compared to standard drug aspirin. On contrary there was significant increase in number of abdominal constriction in chronic study at dose 50mg/kg. The percent reduction of abdominal constriction was 38% and 41% at doses 100 and 500 mg/kg respectively in acute study. In chronic study the percent reduction was 22% and 25% at doses 100 and 500 mg/kg respectively. An increase of 115% in abdominal constriction was observed at dose 50mg/kg of AP compared to standard drug in chronic study [Table 1].

Table 1: Comparison of anlagesic activity of aqueous extract of *Andrographis paniculata* leaves with the standard drug aspirin in acute and chronic study by acetic acid induced writhing model in mice.

Group	Dose (mg/kg) p.o	Mean no of writhing		% change in abd.writh Vs Aspirin	
		Acute	Chronic	Acute	Chronic
II	150	32.7±3.3	15.7±2.9	0	0
III	50	36.2±2.3	33.7±2.7‡	11	115
IV	100	20±1.9*	12.2±0.7 ns	38	22
V	500	19.3±2*	11.8±0.5 ns	41	25

*Group II=Aspirin, Group III, IV, V= Andrographis paniculata at different doses. Values are expressed as mean ± SEM, n=6.Symbols *and ‡ indicate significance at P<0.05and P<0.01 respectively vs standard; ANOVA followed by Dunnet's test.ns=Nothing significant*

Eddy's Hotplate model in mice:

In this model mean basal reaction time increased significantly in the AP treated group compared to control group from 15 min onwards in both acute and chronic study.In acute study the increase in mean basal reaction time was observed at intervals 15min, 30min and 60min at all three doses of AP [Table 2].In chronic study increase in reaction time was observed at all doses of AP at 15min interval but only at the high dose of the extract 500mg/kg the reaction time rised at 30min interval [Table 3].

Table 2: Effect of aqueous extract of *Andrographis paniculata* leaves on mean reaction time in acute study by hotplate method in mice

Group (N=6)	Dose (per kg p.o)	Reaction Time in Seconds 0 min	15 min	30 min	60 min	90 min	120 min
I	10ml	2.5±0.2	2.4±0.3	2±0.1	2.2±0.2	2.5±0.2	2±0.2
II	150 mg	2.5±0.1	4.2±0.3†	3.6±0.3*	3.6±0.4†	2.9±0.3	2.5±0.3
III	50 mg	2.8±0.2	4.1±0.1*	3.8±0.7*	3.5±0.2*	2.5±0.4	2.4±0.2
IV	100 mg	2.6±0.1	4.3±0.5†	3.9±0.2†	3.5±0.2*	2.5±0.2	2.9±0.4
V	500 mg	2.5±0.1	4.3±0.5†	3.4±0.3†	3.8±0.3†	2.7±0.2	2.5±0.1

*Group I=Control 1%gum acacia,Group II=Aspirin,Groups III, IV, V= Andrographis paniculata at different doses.Values expressed as mean ±SEM,at 0,15,30,60,90 and 120min, n=6.Symbol * and † indicates significant values at P<0.05 and P<0.01 respectively vs control; ANOVA followed by Dunnet's test*

Table 3: Effect of aqueous extract of *Andrographis paniculata* leaves on mean reaction time in chronic study by hotplate method in mice

Group	Dose (per kg.p.o)	Reaction Time in Seconds 0 min	15 min	30 min	60 min	90 min	120 min
I	10ml	2.5±0.2	2.4±0.3	2±0.2	2.2±0.2	2.5±0.2	2±0.2
II	150 mg	2.6±0.1	3.9±0.2†	3.9±0.3‡	2.5±0.4	2.2±0.4	2.2±0.3
III	50 mg	2.6±0.1	3.8±0.2†	2.0±0.1	2±0.2	1.3±0.1	1.8±0.2
IV	100 mg	2.5±0.2	3.9±0.2†	2.6±0.1	1.8±0.1	1.6±0.1	1.7±0.1
V	500 mg	2.6±0.1	3.7±0.2†	3.7±0.2‡	1.3±0.1	1.4±0.1	1.4±0.1

Group I=Control 1%gum acacia,Group II=Aspirin, Groups III, IV, V= Andrographis paniculata at different doses. Values expressed as mean ±SEM, at 0,15,30,60,90 and 120 min, n=6. Symbols † and‡ indicates significant at P<0.01 and P<0.001 vs control; ANOVA followed by Dunnet's test

DISCUSSION AND CONCLUSION

The results of the present study confirm the analgesic activity of aqueous extract of AP leaves. Previous studies have shown the similar results.[16] The acetic acid-induced abdominal constriction method is widely used for the evaluation of peripheral antinociceptive activity [17] because it is very sensitive and able to detect antinociceptive effects of compounds at dose levels that may appear inactive in other methods.[18, 19] Local peritoneal receptors are postulated to be partly involved in the abdominal constriction response.[20] The method has also been associated with direct stimulation of nociceptive afferent fibers due to pH reduction [21] and generation of prostanoids in general, for example, increased levels of PGE2 and PGF2α in peritoneal fluids [22] as well as lipoxygenase products.[23] NSAIDs inhibit cyclooxygenase enzyme in the peripheral tissues and therefore interfere with the mechanism of transduction of primary afferent nociceptors.[24]Mechanism of action of AP could be due to inhibition of synthesis of endogenous substance that excites pain nerve endings similar to NSAIDs. Previous studies have shown that it reduces PGE_2 synthesis from macrophages [25] and its oral administration to mice suppresses acetic acid induced vascular permeability.[26] The reduction in the number of writhing indicates AP might exert antinociceptive activity by inhibition of prostaglandin synthesis or by interfering direct stimulation of nociceptive afferents in the periphery. This peripheral analgesic activity was dose dependent and was observed both in acute and chronic study with AP. The results also indicate that the peripheral analgesic activity of the A.Paniculata at doses 100 and 500mg/kg was better than the standard drug Aspirin at 150mg/kg in acute study but this was not significant in chronic study [Table 1]. Nevertheless, the reduction in writhing was comparable to standard drug.

Hotplate method is to evaluate a supraspinal mechanism of thermal nociceptors and it reflects activity in thermally sensitive afferent fibers (Ad and C fibers).[27]AP both in acute and chronic study was found to increase the latency of reaction time [Table 2 and Table 3]. However, it was not clear the exact pathway that is important for its analgesic activity.Studies have shown the possibility of nonopioid mechanisms for its actions.[28] The cause for short duration of analgesic activity in chronic study with AP is not known [Table 3] .Only with higher dose of AP the analgesic activity was observed at 30 min. There was no activity at doses 50mg and 100mg/Kg of AP after 15 min in chronic study. One possible reason may be due to development of tolerance. However, long term studies and studies on both pharmacokinetics and pharmacodynamics are required to conclude the above presumption.

The study confirms AP possesses analgesic activity by acting peripherally and by central actions. However, further studies on isolation and fractionation of the active components of the leaves of AP throw more light on the exact mechanism of action.

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