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RESEARCH ARTICLE

ANXIOLYTIC ACTIVITY OF LEAVES OF *ANDROGRAPHIS PANICULATA* IN EXPERIMENTAL MODEL

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ABSTRACT

The Petroleum ether and Chloroform extract of *Andrographis paniculata* leaves was investigated for its potential to protect gastric mucosa against pylorus ligation induced ulcer and to find out the anxiolytic action in elevated plus maze model. Chloroform extract at the dose of 200mg/kg protected the gastric mucosa in the pylorus ligation ulcer induction significantly ($p<0.001$) when compared with that of the standard drug famotidine (10mg/kg) and acts as a potent antiulcer effect. Elevated plus maze results were significant in alleviating the anxiety in the animals' results in increased time spent and entries into the open arm compared with the standard drug diazepam (1mg/kg).

INTRODUCTION

Andrographis paniculata (Acanthaceae) is an Indian herbal medicine used as an anti-inflammatory and antipyretic drug for the treatment of fever, cold, laryngitis, diarrhea, and rheumatoid arthritis[1]. Experimental studies have revealed numerous pharmacological activities by extracts of *A. paniculata* and its related chemical constituents, such as antimalarial (Najib *et al*., 1999, Li *et al*., 2007), antibacterial (Li *et al*., 2007), anti-inflammatory (Shen *et al*., 2002, Madav *et al*., 1995, Shen *et al*., 2000, Reddy *et al*., 2008, 2009, 2010; Suresh *et al.,* 2010; Rao *et al.,* 2009, 2012; Salaga *et al.,* 2014), hepatoprotective (Handa *et al.,* 1990), antithrombotic (Li *et al*., 2007), immune stimulant (Kumar *et al*., 2004), antidepressive (White *et al*., 2014, 2015, Salaga *et al.,* 2014), antiallergic (Gupta *et al*., 1998), central nervous system disorders (White *et al*., 2014, Fajemiroye *et al.,* 2014, Polepally *et al*., 2012, 2013, 2014; Zjawiony *et al*., 2011), anti HIV and anticancer (Kumar *et al*., 2004, Nanduri *et al*., 2004). Diterpenoids and flavonoids are the primary constituents found in leaves of *A. paniculata*, in particular, andrographolide is the major metabolite (He *et* al., 2003, Li *et al*., 2006, Nanduri *et al*., 2004). Recent reports revealed that andrographolide may be beneficial in the treatment of endotoxic shock by suppressing the production of nitric oxide (NO) and expression of inducible nitricoxide synthase, reactive oxygen species (ROS), hydrogen peroxide (H_2O_2) and superoxide anion (O_2^-), are important toxic metabolites involved in the intracellular killing of microorganisms and tissue damage by phagocytes during inflammation. Moreover, stimulated neutrophils are more likely to adhere to extracellular matrix protein, where they become "activated" to release hydrolytic enzymes and large amounts of ROS that results in tissue damage. The other species of the same genera are being used as an antidepressant, anti-ulcer, memory and learning enhancers, etc. ROS have been implicated in the aetiology and pathophysiology of gastrointestinal inflammation and gastric ulcers, and antioxidant actions have been reported to be effective in the cytoprotection and/or healing in the experimentally induced peptic ulcers. However, until now there is no scientific works reported on its anti-ulcer and antioxidant. Therefore, the present study aimed to explore this indigenous plant for antiulcer and antioxidant activity.

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Animals

Normal healthy male wistar albino rats and mice (180-240g) were housed under standard environmental conditions at temperature (25±2º C) and light and dark (12: 12 h). They were fed with standard pellet diet and water ad libitum.

Phytochemical Test

Phytochemical tests on the extract and fractions were performed using standard procedures.

Table 1. Antiulcer effect of leaves extracts of *Andrographis paniculata*

treatment	Volume of Gastric juice	p^H	Total acidity (mEq/L)	Free acidity (mEq/L)
control	1.6±0.08	1.2±0.04	93±5.8	73±4.1
Chloroform extract (2 mg/kg)	0.54±0.04	4.51±0.14*	29±2.8*	18±1.4*
Pet ether extract (2 mg/kg)	1.4±0.02**	1.3±0.03**	90±0.12**	68±1.2**
Famotidine (10 mg/kg)	0.59±0.03	4.3±0.07*	30±1.6*	18±1.3*

Data expressed as mean±S.E.M (n=6), *p<0.01; **p<0.05

Table 2. Anxiolytic effect of leaves extracts of *Andrographis paniculata*

treatment	Dose (mg/kg, p.o.)	Number of entries in open arms	Time spent in open arms (seconds)
control	Vehicle (1 ml/kg)	4.80±0.62*	3.06±0.63*
Chloroform extract (2 mg/kg)	200	5.54±0.68*	14.53±0.71*
Pet ether extract (2 mg/kg)	200	2.83±2.45**	9.20±0.10**
Diazam	2	9.90±1.66*	16.20±0.58*

Data expressed as mean±S.E.M (n=6), *p<0.01; **p<0.05

Acute toxicity studies

The acute toxicity studies were performed to study the acute toxic effects and to determine the minimum lethal dose of the drug extracts as per the guideline OECD 423. Swiss albino mice of either sex weighing between 18-25gm were used for the study. The pet ether and chloroform extracts of Durentarepens were administered orally to different groups of overnight fasted mice at the dose 30, 100, 300, 1000 and 2000mg/kg body weight. After the administration of the extracts, animals were observed continuously for the first 8hrs for any toxic manifestation. Thereafter observations were made at regular intervals for 24hrs. Further the animals were under investigation upto a period of one week.

Pharmacological screening

Antiulcer activity by pylorus ligation method

Adult albino rats of either sex weighing between 100-130 gm were divided into 3 groups of 6 animals. The animals were deprived of food for 24 hours before the commencement of experiment but water was allowed ad libitum. The drugs were given orally 2 hours prior to pylorus ligation, which was carried out according to the technique reported. Group I received acacia suspension 1ml/kg, Group II and Group II received the chloroform and pet ether extract 200mg/kg and Group IV received anitidine 10mg/kg respectively. The animals were sacrificed six hours after pyloric ligation to observe gastric lesion. The gastric juice was collected, centrifuged and its pH was determined. Free and total acidity were estimated titrimetrically using 0.01NaoH solution.

The data concerning the pH, acid secretion and ulcer analysed by one way followed by Tukey multiple comparison test. Anxiolytic activity by elevated plus maze model The plus maze apparatus consists of two open arms ($35\times5cm^2$) crossed with two closed arms ($35\times5\times20$ cm^3). The arms were connected together with a central square ($5\times5cm^2$). The apparatus was elevated to the height of 25 cm in a dimly illuminated room. Animals were divided into 3 groups of 5 animals each, Group I control received distilled water (1ml/kg, p.o), Group II received Diazepam (1mg/kg, p.o) and Group III and IV received pet ether and chloroform extract (200mg/kg, p.o). After 30 minutes they were placed individually in the center of the apparatus, facing the closed arm. The time spent in both the open and closed arms was recorded for 5minutes. The numbers of entries into open and closed arms were also counted during the test. An entry was defined as having all four paws within the arm.

RESULTS AND DISCUSSION

The extractive values for pet ether and chloroform extract was 20% and 25% which shows the solubility of the phytoconstituents in the particular solvent used. The phytochemical studies revealed the presence of carbohydrates, alkaloids, glycosides, reducing sugar, resins, flavonoids and terpenoids and the absence of tannins, saponins and acidic compounds. The toxicity study reveals that 2mg/kg as the therapeutic dose and up to 2mg/kg both the extracts were safe and not produced any toxicity symptoms. Pretreatment with pet ether and chloroform extracts reduced the incidence of ulcers in rats. There was no ulcer lesion in oral administration of acacia suspension and pretreatment groups. Gastric ulcer is believed to be due to an imbalance between acid and pepsin, and the weakness of mucosal barrier. Several mechanisms have been suggested for the effect of gastro protective principles, including increasing hexosamine level and enhancing the strength of gastric barrier either physically or by blocking the H+, K+ ATPase pump, stimulation of membrane stabilization by interference with Ca2+ influx, scavenging oxygen generated free radical and inhibition of biological membranes.

A. paniculata exert its property by one or more of this proposed mechanisms However it should be pointed out that *A. paniculata* contain tannins and flavonoids to which the gastro protective effect could be attributed. Further studies are required to isolate the active protective properties of *A. paniculata*. In elevated plus maze the animals spend greater time in the closed arms when placed in maze comprising of open and closed arms. Avoidance of the open arm portrays a manifestation of fear and anxiety. The results obtained showed chloroform had anxiolytic property by increasing the cumulative time spent in the open arm. This effect was mainly due to modulation of GABAA–chloride channel receptor complexes. This may also exert pharmacological action by increase in GABA content in the cerebral hemisphere.

Conclusion

From the above study it was confirmed that *Andrographis paniculata* can be safely used in the treatment of ulcer and

anxiety disorders. Further studies were needed to confirm the exact molecular action and the specified pharmacological mechanism. Also the active phytoingredient to be isolated for the further studies.

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