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Research Article

ANXIOLYTIC AND ANTIULCER ACTIVITY OF LEAVES OF *ANDROGRAPHYS 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).

Keywords: *Andrographis paniculata*, methanolic extract, pylorus ligation, famotidine, diazepam.

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, 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), antiallergic (Gupta *et al*., 1998), central nervous system disorders (White *et al*., 2014, Fajemiroye *et al.,* 2014, Polepally *et al*., 2013, Prabhakar *et al.,* 2014, Zjawiony *et al*., 2011), anti HIV (Li *et al*., 2007, Raju *et al*., 2008), 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.

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.

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 Durenta repens 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 10 mg/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 (35×5 cm^2) crossed with two closed arms ($35\times5\times20$ cm^3). The arms were connected together with a central square (5×5 cm^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 5 minutes. 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 2 mg/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 gastroprotective 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.

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

treatment	Volume of Gastric juice	pH	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

A. paniculata exert its property by one or more of this proposed mechanism however it should be pointed out that *A. paniculata* contains 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.

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

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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REFERENCES

Anne, M.; Dominic, S. Philip, S. Robert, S.; Kenneth, P. David, V. Curtis, H. John, L. Paul, C. Anne, V. Marcia, G. Hugh, C. Joseph, M. Michael, B. (1991). Feasibility of a High-Flux Anticancer Drug Screen Using a Diverse Panel of Cultured Human Tumor Cell Lines. J. Nat. Cancer Inst. 83, 757.

Chakravarti, R. N. Chakravarti, D. (1951). Andrographolide, The Active Constituent of *Andrographis paniculata* Nees A Preliminary Communication. Ind. Med. Gaz. 86, 96.

Gupta, P. P. Tandon, J. S. Patnaik, G. K. (1998). Antiallergic Activity of Andrographolides Isolated from *Andrographis paniculata* (Burm. F) Wall. Pharm. Biol. 36, 72.

Gupta, S. Choudary, M. A. Yadava, J. N. S. Srivastava, V. Tandon, J. S. (1990). Antidiarrhoeal Activity of Diterpenes of *Andrographis paniculata* (Kal-Megh) against *Escherichia coli* Enterotoxin in *in vivo* Models. Pharm. Biol. 28, 273.

Handa, S. S. Sharma, A. (1990). Hepatoprotective Activity of Andrographolide from *Andrographis paniculata* Against Carbontetrachloride. Indian J. Med. Res. 92, 284.

He, X. J. Li, J. K. Gao, H. Qiu, F. Hu, K. Cui, X. M. Yao, X. S. (2003). Four New Andrographolide Metabolites in Rats. Tetrahedron Lett. 59, 6603.

Husen, R.; Pihie, A. H. L. Nallappan, M. (2004). Screening for Antihyperglycaemic Activity in Several Local Herbs of Malaysia. J. Ethanopharmacol. 95, 205.

Kumar, R. A. Sridevi, K. Kumar, N. V. Nanduri, S. Srinivas, N. Rajagopal, S. J. (2004). Anticancer and Immunostimulatory Compounds from Andrographis paniculata. J. Ethnopharmacol. 92, 291.

Li, Z.; Huang, W. Zhang, H. Wang, X. Zhou, H. (2007). Synthesis of Andrographolide Derivatives and their TNF-α and IL-6 Expression Inhibitory Activities. Bioorg. Med. Chem. Lett. 17, 6891.

Madav, S. Tandan, S. K. Lal, J. (1996). Tripathi, H. C. Anti-inflammatory Activity of Andrographolide. Fitoterapia 67, 452.

Najib, N. A. R. N. Furuta, T. Kojima, S.Takane, K. Ali, M. M. (1999). Antimalarial Activity of Extracts of Malaysian Medicinal Plants. J. Ethanopharmacol. 64, 249.

Nanduri, S. Nyavanandi, V. K. Thunuguntla, S.S.R. Kasu, S.; Pallerla, M.K.; Ram, P.S. Rajagopal, S.; Kumar, R.A.; Ramanujam, R. Babu, M.; Vyas, K. Devi, A.S. Reddy, G.O. Akella, V. (2004). Synthesis and Structure-Activity Relationships of Andrographolide Analogues as Novel Cytotoxic Agents. Bioorg. Med. Chem. Lett. 14**,** 4711.

Nanduri, S. Nyavanandi, V. K. Thunuguntla, S. S. R. Velisoju, M. Kasu, S. Rajagopal S. Kumar, A. R. Rajagopalan R.; Iqbal J. (2004). Novel Routes for the Generation of Structurally Diverse Labdane Diterpenes from Andrographolide. Tetrahedron Lett**.** 45, 4883.

Newman, D. J. Cragg G. M. (2012). Natural Products as Sources of New Drugs over the 30 Years from 1981 to 2010. J. Nat. Prod. *75*, 311.

O. J. Fajemiroye, P.M. Galdino, I.F. Florentino, F. F. Da Rocha, P.C. Ghedini, P. R Polepally, J.K. Zjawiony., E.A. Costa. (2014). Oleanolic acid: Plurality of anxiety and depression alteration mechanism by oleanolic acid. Journal of Psychopharmacology, *i*n press

Polepally, P. R. White, K. Vardy, E. Roth, B. L.; Ferreira, D. Zjawiony, J. K. (2013). Kappa-Opioid Receptor-Selective Dicarboxylic Ester-Derived Salvinorin A Ligands. Bioorg. Med. Chem. Lett. 23**,** 2860.

Polepally, P. R. Setola, V. Vardy, E. Roth, B. L.; Zjawiony, J. K. (2013). New Michael Acceptor-Type of Salvinorin A Ligands to Kappa-Opioid Receptor. Planta Med. 79 (05), P41.

Polepally, P. R. White, K. Roth, B. L. Zjawiony, J. K. (2013). Convenient Synthesis and *In Vitro* Pharmacological Activity of Thioesters of Salvinorin B. Planta Med. 79 (05), P43.

Polepally, P. R.; Roth, B. L.; White, K. Zjawiony, J. K. (2013). Synthesis and Biological Evaluation of New Salvinorin B-Sulfonate Ester Ligands to Opioid Receptors. Planta Med. 79(05), P44.

Polepally, P. R. Roth, B. L. White, K. Ferriera, D. Zjawiony, J. K. (2013). Synthesis and *In Vitro* Biological Evaluation of New Dicarboxylic Ester-Type Salvinorin A Analogs. Planta Med. 79 (05), P42.

Polepally, P. R. White, K. Roth, B. L. Zjawiony, J. K. (2013). Synthesis and *In Vitro* Pharmacological Activity of C-2 Modified New Salvinorin A Analogues. Planta Med.79 (05), P45.

Polepally, P. R. Setola, V. Vardy, E. Roth, B. L. Mosier, P. D.; Zjawiony, J. K. 2012. New Salvinorin A-Derived Ligands to Opioid Receptors. Planta Med. 78, PI238.

Prabhakar R. Polepally, Vincent Setola, Eyal Vardy, Bryan L. Roth, Jordan K. Zjawiony. 2014. Michael acceptor approach to the design of new Salvinorin A-based high affinity ligands to the kappa-opiod receptor. European Journal of Medicinal Chemistry, in press

Raju, B. C. Pradeep, D. V. S. Reddy, P. P. Rao, J. M. (2008). CBr_4 Catalyzed Synthesis of Aryl-14H-dibenzo [a,j] Xanthenes Under Solvent-Free Conditions. Lett. in Org. Chem. 5, 450.

Reddy, P. P. Raju, B. C. Rao, J. M. (2008). A Facile One-Pot Friedlander Synthesis of Quinoline Derivatives. J. Chem. Res. 12, 679.

Salaga Maciej, Prabhakar R. Polepally, M. Sobczak, D Grzywacz, A Sibaev, M storr, J C Dorego, Jordan K. Zjawiony**,** Jakub J. Fichna. (2014). Novel orally available salvinorin A Analog PR-38 inhibits gastrointestinal motility and reduces abdominal pain in mouse Models mimicking irritable bowel syndrome. J. Pharmaceutical. Exper. Theraupetics, in press.

Shen, Y. C. Chen, C. F. Chiou, W. F. (2002). Andrographolide Prevents Oxygen Radical Production by Human Neutrophils: Possible Mechanism(s) Involved in its Anti- Inflammatory Effect. Br. J. Pharmacol. 135, 399.

Shen, Y. C.; Chen, C. F. Chiou, W. F. (2000). Suppression of Rat Neutrophil Reactive Oxygen Species Production and Adhesion by the Diterpenoid Lactone Andrographolide. Planta Med. 66, 314.

Wender, P. A. Baryza, J. L. Bennett, C. E. Bi, F. C. Brenner, S. E.; Clarke. M. O.; Horan, J.

C.; Kan, C. Lacote, E. Lippa, B. Nell, P. G. Turner, T. M. (2002). The Practical Synthesis of a Novel and Highly Potent Analogue of Bryostatin. J. Am. Chem. Soc. 124, 13648.

White, K. L. Scopton, A. P. Rives, M. L. Bikulatov, R. V. Polepally, P. R. Brown, P. J.Kenakin, T. Javitch, J. A. Zjawiony, J. K. Roth, B. L. (2014). Identification of Novel Functionally Selective κ-Opioid Receptor Scaffolds. Mol. Pharmacol. 85, 83.

Zjawiony, J. K. Polepally, P. R.; Roth, B. L. Setola, V. Vardy, E. (2011). Design and Synthesis of Natural-Product Based Ligands with High Affinity to the Kappa-Opioid Receptor. Planta Med. 77(12), SL4.