Int J Pharm Bio Sci 2014 July; 5(3): (P) 501 - 507

Research Article **Pharmacology**

International Journal of Pharma and Bio Sciences **ISSN 0975-6299**

ANXIOLYTIC AND ANTI-DEPRESSANT LIKE EFFECTS OF LEAVES OF *ANDROGRAPHYS PANICULATA*

RAVINDRA PATIL[*1], VIMAL SINGH CHAUHAN[2] AND VENKAT R. P[1].

[1]Department of Pharmaceutical Sciences, Allana College of Pharmacy, Pune, Maharashtra, India, 411038.
[2]Department of Pharmacology, Allana College of Pharmacy, Pune, Maharashtra, India, 411038.

ABSTRACT

The current study was undertaken to explore the effect of ethanolic extract (ext.), ethyl acetate (EA) fractions and precipitate fraction (ppt.) of total ethanolic extract of *Andrographis paniculata* on depression in rats. In the present study, the antidepressant effect of *Andrographis paniculata* was examined using two behavioral models, the forced swimming test (FST) in rats and the tail suspension test (TST) in rats. In both the screening effects, the immobility time was reduced by 10 and 15 seconds when the ethanolic extract was administered at an acute dose of 50 mg/kg of the body weight compared to the control immobility time of 50 and 60 seconds. In both the tests, the immobility time was reduced by 10 and 15 seconds showing the best activity. The results showed potent activity considering the tested drugs after the standard, imipramine HCl given at 30 mg/kg. Ethanolic extract when administered at an acute dose of 50 mg/kg of body weight ($P<0.01$) reduced the immobility time by 10 and 15 seconds as compared to the immobility time of control in both the screening models. Similarly EA reduced latter by 30 and 35 secs. The ppt. fraction showed the best activity, reducing the immobility time by 50 and 60 secs. in both the tests. These results showed that after standard i.e. Imipramine HCl (30 mg/kg), the ppt. fraction is potent amongst all the studied drugs. The present study clearly demonstrated that *Andrographis paniculata* exerts an antidepressant effect in these two behavioral models. It could be due to presence of labdane diterpenoids and flavonoids.

KEY WORDS: ***Andrographis paniculata*, Labdane diterpenoids, Flavonoids, tail suspension test (TST), forced swimming test (FST).**

RAVINDRA PATIL
Department of Pharmaceutical Sciences, Allana College of Pharmacy, Pune, Maharashtra, India, 411038.

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 of the extracts of *A. paniculata* and its related chemical constituents, such as anti-inflammatory, hepatoprotective, antimalarial, antibacterial, antithrombotic, immune stimulant, antidepressive, antiallergic, central nervous system disorders, anti HIV, and anticancer [2-18]. Diterpenoids and flavonoids are the primary constituents found in leaves of *A. paniculata*, in particular, andrographolide is the major metabolite[1]. 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 nitric oxide 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.[19-27] The various medicated formulas for depression contain *Andrographis* species. But till now there is no scientific work has been reported on its anti-depressant activity. Therefore the current study was aimed to explore this indigenous plant for anti-depressant activity.

MATERIALS AND METHODS

Plant Identification

Whole plant of *Andrographis paniculata* was collected from botanical herbarium (Herbarium no. Bot/117A); Orissa, India in the months of March 2013.

Preparation of extracts and fractions

Leaves of *Andrographis paniculata* were shade dried at room temperature. The shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether in a soxhlet apparatus. The extraction was sustained until the material was defatted. The defatted material was then subjected to ethanolic extraction for about 6-7 days. The obtained ethanolic extract was then dissolved in distilled water and left overnight which was later filtered. The supernatant liquid has been fractionated with ethyl acetate and then dried at 40°C in a rotavapor to get a semisolid, that was later used for antidepressant activity. The extraction was continued till the defatting of the material had taken place. The defatted marc of the drug was subjected to ethanolic extraction for a period of 6-7 days. The ethanolic extract obtained was dissolved in dist. water and kept overnight so as to settle down the undissolved matter, which was filtered off later. The supernatant was fractionated with ethyl acetate (400 ml) in separating funnel (250 ml) both fractions were dried at 40^0C in rotatory evaporator up to a semisolid consistency and were utilized for the antidepressant activity.

Administration of the extracts and fractions

Suspensions of ethanolic extract, ethyl acetate and ppt. fractions were prepared in distilled water using Tween-80 (0.2% v/v) as the suspending agent. The extract and fractions were administered in a dose of 2000 mg/kg to rats by oral route, 45 min before the test procedures for pre-pharmacological screening as per OECD guidelines. Control groups were given only the vehicle (0.2% v/v Tween-80 solution) in volume equivalent to that of the plant extracts and fractions.

Acute Toxicity Studies

Ethanolic extract and fractions in a dose of 2000 mg/kg were given orally for the assessment of acute toxicological studies. All the parameters were thoroughly checked and dose for the further studies was calculated as per OECD. After the conduct of acute toxicological studies

the dose of each extract and the two fractions were decided i.e. 50 mg/kg, 10 mg/ kg. oral route was selected for the administration of drugs.

Forced swimming test (FST)

Rats of either sex were individually forced to swim in an open cylindrical container (diameter 10 cm, height 25 cm), containing 19 cm of water at 25±1 °C. All the rats of either sex were divided into five different groups. The first group assigned as control received only vehicle (NaCl 5ml/kg). The other three groups received an acute dose of ext., EA and ppt. fraction (50, 10, 10 mg/kg). The fifth group received standard drug Imipramine (30 mg/kg). The total duration of immobility was recorded during the last 6 min of the 10-min period. Each mouse was judged to be immobile when it ceased struggling and remained floating motionless in the water, making only those movements necessary to keep its head above water. A decrease in the duration of immobility is indicative of an antidepressant like effect.

Tail suspension test (TST)

All the rats of either sex were divided in five different groups. The first group assigned as control receiving only vehicle (NaCl 5ml/kg). The other three groups received an acute dose of ext., EA and ppt. fraction (50, 10, 10 mg/kg). The fifth group received standard drug Imipramine (30 mg/kg).Briefly, mice both acoustically and visually isolated were suspended 50 cm above the floor by adhesive tape placed approximately 1 cm from the tip of the tail. Immobility time was recorded during a 6-min period. Mice were considered immobile only when they hung passively and were motionless.

Statistical analysis

The immobility time in the tail suspension test and the forced swimming test was analyzed with ANOVA, further comparisons between vehicle and drug-treatment groups were performed using the Dunnett's t-test. Results are expressed as the means ± SEM. Analyses were performed using the software SPSS version 13 for windows. The level of statistical significance adopted was **P<0.01, when compared with the control group.

RESULTS

The behavioral despair model was performed in order to investigate the ability of this herbal drug in the elevation of suppressed mood, which is quite common in today's scenario. The results obtained from FST and TST clearly reveals the fact that this drug is potentially quite useful in cases of depression.

The present findings suggested that ethanolic extract when administered at an acute dose of 50 mg/kg of body weight (P<0.01) reduced the immobility time by 15 seconds as compared to the immobility time of control i.e. 190 sec. the time shown by animals treated with extract was found to be **170 sec. Ethyl acetate showed a reduction in immobilization time at an acute dose of 10 mg/kg (P<0.01) the mean immobility time of EA treated animals was **165 sec. The precipitate fraction of the drug shown the best results when it was compared with control and standard. The decrease in the immobility time was quite close to that of the standard. The time of mobility was increased by ppt. fraction at a dose of 10 mg/ml, shown the immobility time **140 sec. (P<0.01) to that of standard **135 sec. (P<0.01). These results shows that after standard i.e. Imipramine HCl (100 mg/ml) the ppt. fraction is most potent amongst all the treated groups. (Table 1).

Table 1
Effects of *A. paniculata on immobility time in FST*

Group no.	Drug treatment	Dose (mg/kg)	Immobility period, mean±SEM (n=6)
I	control	Nacl (5 mg/kg)	180 sec
II	Ehanolic extract	50	**160 sec
III	Ethylacetate extract	10	**145 sec
IV	Ppt, fraction	10	**130 sec
V	Standard drug	30	**125 sec

Values were mean±S.E.M. for (n=6 rats) expressed as the time in seconds of 6 animals in each group; Date analysis was performed using Dunnet's test; **p<0.01 vs. control.

Findings of the tail suspension test were quite comparable to the previous FS test. As shown in the observation table and bar graph, it is quite evident that none of the drug treated animals showed excellent results compared to the standard.[28] The immobility of Imipramine HCL (P<0.01) 100 mg/ ml was came out to be **135 sec. In this test the time of animals treated with ethanolic extract was found to be **170 sec. (P<0.01) when it was compared to the control group of animals which was 195 sec. The immobility time of ethyl acetate and ppt. fractions when given an acute dose of 10 mg/kg each of body weight significantly reduced the time of immobility by **165 sec. (P<0.01) and **140 sec. The results clearly reveals the fact that standard treated animals showed better response as compared to the plant extract and fraction treated groups but even though the ppt. fraction treated group showed better response as compared to the extract and EA fraction treated group of animals. (Table 2)

Table 2
Effects of *A. paniculata on immobility time in TST*

Group no.	Drug treatment	Dose (mg/kg)	Immobility period, mean±SEM (n=6)
I	control	Nacl (5 mg/kg)	180 sec
II	Ehanolic extract	50	**160 sec
III	Ethylacetate extract	10	**145 sec
IV	Ppt, fraction	10	**130 sec
V	Standard drug	30	**125 sec

Values were mean±S.E.M. for (n=6 rats) expressed as the time in seconds of 6 animals in each group; Date analysis was performed using Dunnet's test; **p<0.01 vs. control

DISCUSSION

For the purpose of investigation of antidepressant activity of this plant, we used two animal models, the forced swimming test (FST) in rats and the tail suspension test (TST) in mice. When the rodents were subjected to unavoidable stress as seen with the forced swimming test, the immobility displayed reflects the lowered or despair moods which are similar to the depressive disorders in humans. Also, when antidepressant drugs were used, the immobility time has been reduced. A significant correlation was seen between the clinical effectiveness of the antidepressant drugs and their potency. The immobility displayed by rodents when subjected to unavoidable stress such as forced swimming is thought to reflect a state of despair or lowered mood, which are thought to reflect depressive disorders in humans. In addition, the immobility time has been shown to be reduced by treatment with antidepressant drugs. Moreover, a significant correlation was found between the clinical efficacy of antidepressant drugs and their potency in both models. It has been recently shown that the regulation of α-2-adrenergic receptor may be the major mechanism of this

model. The results indicate that *A. paniculata* may have an antidepressant-like effect. However, further experiments evaluating the levels of noradrenaline and serotonin in different brain regions are necessary to confirm this hypothesis. This behavioral model for the screening of new antidepressant compounds, concluded that the immobility time observed in the test reflected a state of lowered mood or hopelessness in animals, thus, this animal model is the most widely used tool for preclinical screening of putative antidepressant agents. The FST shows a strong sensitivity to monoamine alterations and is a very specific cluster of stress-induced behaviors that are not related to depression symptoms in humans, but which are nonetheless exquisitely sensitive to monoaminergic manipulations. It also provides a useful model to study neurobiological and genetic mechanisms underlying stress and antidepressant responses. In both these studies, ppt. fraction significantly reduced the immobility time 145 and 140 sec at a dose of 10 mg/kg, which was more than the extract and EA fractions that reduced time by 160, 170 and 145 and 165 sec. at a dose of 50 and 10 mg/kg. Ethanolic extract is a complex product prepared from the green leaves of the *A. paniculata* plant. Major ingredients are terpenoids, especially flavonoids including quercetrin, quercetrin and kaempferol. Recently, several studies have suggested the antidepressant effect of quercetin glycosides such as hyperoside, isoquercitrin and rutin using the positive results of FST. Flavonoid glycosides are mostly hydrolyzed into their aglycons by mucosal and bacterial enzymes in the intestines, and then converted to conjugated metabolites during the absorption process. This perhaps indicates that the active form of the antidepressant effect of quercetin glycosides is the conjugated form, and not the glycoside form. Additionally, the plant contains kaempferol and Rutin. These flavonoid glycosides seem to appear as conjugated forms in the blood stream as with quercetin glycosides. Transportation of these metabolites into the brain tissues via the blood brain barrier and their effect on the CNS system have been recently argued.[29] Moreover, quercetin metabolites were previously found in the brain tissues of rodents after oral administration. Therefore, one of the antidepressant mechanisms of *A. paniculata* is thought to involve falvonoid glycosides, which reach the brain tissues through to involve flavonoid glycosides, which reach the brain tissues through the metabolizing process, protecting brain function from CNS disturbance, and consequently exerting an antidepressant effect. Our results confirm the traditional use of the plant as an antidepressant.

CONCLUSION

In summary, we explore the effect of ethanolic extract (ext.), ethyl acetate (EA) fractions and precipitate fraction (ppt.) of total ethanolic extract of *Andrographis paniculata* on depression in rats. In this study, the antidepressant effect of *Andrographis paniculata* was examined using two behavioral models, the forced swimming test (FST) in rats and the tail suspension test (TST) in rats. Ethanolic extract when administered at an acute dose of 50 mg/kg of body weight ($P<0.01$) reduced the immobility time by 10 and 15 seconds as compared to the immobility time of control in both the screening models.

ACKNOWLEDGEMENT

The authors are thankful to Head of the department of Pharmaceutical sciences, and Pharmacology Allana College of Pharmacy. We also thankful to Invocan Pharmaceuticals, Aurangabad, Maharashtra for providing chemicals and standard drugs for biological activity studies.

REFERENCES

1. Chakravarti RN, Chakravarti D, Andrographolide, The Active Constituent of *Andrographis paniculata* Nees; A Preliminary Communication, Ind. Med. Gaz. 86:96-97, (1951).
2. Shen YC, Chen CF, Chiou WF, Andrographolide Prevents Oxygen Radical Production by Human Neutrophils: Possible Mechanism(s) Involved in its Anti-Inflammatory Effect, Br. J. Pharmacol, 135: 399-406, (2002).
3. Najib Nik ARN, Furuta T, Kojima S, Takane K, Ali MM, Antimalarial Activity of Extracts of Malaysian Medicinal Plants, J. Ethanopharmacol., 64: 249-254, (1999).
4. Gupta S, Choudary MA, Yadava JNS, Srivastava V, Tandon JS, Antidiarrhoeal Activity of Diterpenes of *Andrographis paniculata* (Kal-Megh) against *Escherichia coli* Enterotoxin in *in vivo* Models, Pharmaceutical Biology, 28: 273-283, (1990).
5. Gupta PP, Tandon JS, Patnaik GK, Antiallergic Activity of Andrographolides Isolated from *Andrographis paniculata* (Burm. F) Wall, Pharmaceutical Biology, 1998, 36: 72-74, (1998).
6. Madav S, Tanda SK, Lal J, Tripatthi HC, Anti-inflammatory Activity of Andrographolide. Fitoterapia, 67: 452-458, (1996).
7. White KL, Scopton AP, Rives ML, Bikulatov RV, Polepally PR, Brown PJ, Kenakin T, Javitch JA, Zjawiony JK, Roth BL, Identification of Novel Functionally Selective κ-Opioid Receptor Scaffolds. *Mol. Pharmacol.* 85: 83-90, (2014).
8. Husen R, Pihie AHL, Nallappan M, Screening for Antihyperglycaemic Activity in Several Local Herbs of Malaysia, J. Ethnopharmacol. 95: 205-208, (2004).
9. Polepally PR, White K, Vardy E, Roth BL, Ferreira D, Zjawiony JK, Kappa-Opioid Receptor-Selective Dicarboxylic Ester-Derived Salvinorin A Ligands. Bioorg. Med. Chem. Lett., 23: 2860-2862, (2013).
10. Handa SS, Sharma A, Hepatoprotective Activity of Andrographolide from *Andrographis paniculata* Against Galactosamine and Paracetamol-induced toxicity Indian J. Med. Res. 92: 284-292, (1990).
11. Shen YC, Chen CF, Chiou WF, Suppression of Rat Neutrophil Reactive Oxygen Species Production and Adhesion by the Diterpenoid Lactone Andrographolide, Planta Med. 66: 314-317, (2000).
12. Li Z, Huang W, Zhang H, Wang X, Zhou H, Synthesis of Andrographolide Derivatives and their TNF-α and IL-6 Expression Inhibitory Activities, Bioorg. Med.Chem. Lett. 17: 6891-6894, (2007).
13. Zjawiony JK, Polepally PR, Roth BL, Setola V, Vardy E, Design and Synthesis of Natural-Product Based Ligands with High Affinity to the Kappa-Opioid Receptor, Planta Med. 77(12): SL4, (2011).
14. Kumar RA, Sridevi K, Kumar NV, Nanduri S, Srinivas N, Rajagopal SJ, Anticancer and Immunostimulatory Compounds from *Andrographis paniculata*., J. Ethnopharmacol. 92: 291-295, (2004).
15. Polepally PR, Setola V, Vardy E, Roth BL, Mosier PD, Zjawiony JK, New Salvinorin A-Derived Ligands to Opioid Receptors. Planta Med. 78: PI238, (2012).
16. Reddy PP, Raju BC, Rao JM, A Facile One-Pot Friedlander Synthesis of Quinoline Derivatives, J. Chem. Res. 12: 679-682, (2008).
17. Polepally PR, Setola V, Vardy E, Roth BL, Zjawiony JK, Convenient Synthesis And *In Vitro* Pharmacological Activity of Thioesters of Salvinorin B, Planta Med. *79*(05), P43, (2013).
18. Raju BC, Pradeep DVS, Reddy PP, Rao JM, CBr_4 Catalyzed Synthesis of Aryl-14H-dibenzo [a,j] Xanthenes Under Solvent-Free Conditions, Lett. in Org. Chem. 5: 450-454, (2008).
19. Polepally PR, Setola V, Vardy E, Roth BL, Zjawiony JK, New Michael Acceptor-Type of Salvinorin A Ligands to Kappa-Opioid Receptor, Planta Med. 79(05): P41, (2013).

20. Nanduri S, Nyavanandi VK, Thunuguntla SSR, Kasu S, Pallerla MK. *et al* Synthesis and Structure-Activity Relationships of Andrographolide Analogues as Novel Cytotoxic Agents, Bioorg. Med. Chem. Lett. 14: 4711-4717, (2004).
21. Polepally PR, Roth BL, White K, Ferriera D, Zjawiony JK, Synthesis and *In vitro* Biological Evaluation of New Dicarboxylic Ester-Type Salvinorin A Analogs, Planta Med. 79(05): P42, (2013).
22. He XJ, Li JK, Gao H, Qiu F, Hu K, Cui XM, Yao XS, Four New Andrographolide Metabolites in Rats, Tetrahedron Lett. 59: 6603-6607, (2003).
23. Polepally PR, Roth BL, White K, Zjawiony JK, Synthesis and Biological Evaluation of New Salvinorin B-Sulfonate Ester Ligands to Opioid Receptors. Planta Med.79 (05): P44, (2013).
24. Polepally PR, White K, Roth BL, Zjawiony JK, Synthesis and *In Vitro* Pharmacological Activity of C-2 Modified New Salvinorin A Analogues. Planta Med. 79(05): P45, (2013).
25. Li Z, Huang W, Zhang H, Wang X, Zhou H, Synthesis of Andrographolide Derivatives and their TNF-α and IL-6 Expression Inhibitory Activities, Bioorg. Med. Chem. Lett. 16, 6891-6894, (2007).
26. Fajemiroye OJ, Galdino PM, Florentino IF, DaRocha FF, Ghedini PC, Polepally PR, Zjawiony JK, Costa EA, Oleanolic acid: Plurality of anxiety and depression alteration mechanism by oleanolic acid, Journal of Psychopharmacology, 2014, doi:10.1177/0269881114536786.
27. Salaga M, Prabhakar RP, Sobczak DG, Sibaev A, storr M, Dorego JC, Jordan KZ, Jakub JF, (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. Therapeutics, 350: 69-78, (2014)..
28. Lucki I, Dalvi A, Mayorga A, Sensitivity to the effects of pharmacologically selective antidepressants in different strains of mice. J. Psychopharmacology, 155: 315–322, (2001).
29. Kumar, Amitabh A., Padma Sravani, Vishnu Priya, "Anxiolytic Profile of 5-HT3 Antagonist Ondansetron and its Clinical Correlation, *International Journal of Pharma & Bio Sciences* 5(2): 1-5, (2014).