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TheComplementaryStudyofAntidepressant and Anxiolytic Potential of Acorus Calamus Rhizome Extract in Rats

JyotiYadav[1], RaniYadav[1], DeepuYadav[2], NeelamYadav[2], Sunita Arya[1], Arvind Prakash[3], Shalini Jain[4], Mukesh Yadav[3*]

[1]Department of PharmacyP.K. UniversityThanara DinaraShivpuri-473665, Madhya Pradesh, India
[2]Department of Pharmacy Advance College of Pharmacy Datia-475661, Madhya Pradesh, India
[3]Department of Chemistry PG College Datia-475661, MadhyaPradesh, India
[4]MusBResearch, NewPort Richey, FL, USA

Abstracts
In present study *Acorus calamus* (AC) rhizome extract within two solvents (Methanol and Water) alone and combination with standard drug imipramine were tested for anxiolytic and antidepressant activity in rats. The anxiolytic effect of Methanol extract and Water extract were tested in rats using the elevated plus-maze (EPM) test, the hole-board test (HBT), and the light-dark box (LDB) test. Its antidepressant effectwasevaluatedbythetailsuspension(TST)andtheforced swim(FST)tests.Thetotal alkaloidand flavonoid content were measured using standard colorimetric assays. The Methanol extract exhibited highest anxiolytic and antidepressant activity in rats among two extracts while Water extract exhibited moderate activity respectively. Methanol extract was further studied to dose dependent [250, 150.50 mg/kgbodyweight(b.wt,)]anxiolyticandantidepressanteffectaloneandincombinationwithimipramine (2,1.5, 1 mg/kg b.wt.), the combination of Methanol extract (250 mg/kg b. wt. and lower dose of imipramine 5 mg/kg b,wt.) has shown safer and potent anxiolytic as well as antidepressant while higher dose (250 mg/kg b. wt.of Methanol extract and 1.5 and 2 mg/kg b, wt.) of imipramine generated lethal effect in control animals. The result of present study shows that Methanol extract of AC rhizome has significantly anxiolytic antidepressant activity may use as a complementary medicine to treat anxiolytic (EPM,HBTandLDB)andantidepressant(TSTandFST)bysignificantlyreducingdoseofstandarddrugs.

KeywordsAcoruscalamus,Phytochemicals,HerbalBehaviouraltestandImipramine

Introduction

Human survival in the world depends upon a variability of biotic and abiotic considerations. The basic needs from the ancient time continuously have been delivered by the nature to encourage, progress and modify in all circumstances. Our ancestries were continuously dependent on herbs, shrubs and trees for their expanded demands that mostly involved nutrition and treatment. The continued existence of wild-life significantly and conspicuously involves the presence of flora for their favourable properties as they provide us provisions, attire, accommodation, and remedy.
Anxietyanddepressivedisordersarehighlyprevalentintheelderly,oftenappearascomorbiddisorders

and both haveadverseconsequences such as reduced quality oflifeand excess mortality (deBeurset al.,1999,Charney et al., 2003). In addition, subthreshold symptoms of anxiety and depression are common and serious, causing significant disruption in daily living. Although late-life anxiety and depression are treatable conditions (Wetherell, 1998,McCusker_et al., 1998), they are often underrecognized and undertreated in primary care (Mulsant and Ganguli, 1999,Olafsdottir et al., 2001,Volkers et al., 2004). ACiscommonlyknownasthe“sweetflag”andisindigenoustoIndia,Myanmar,Japan,Mongolia,China andothers(Gongetal.,2019).ACisagrass-like,perpetualherbaceousplant.Itspreadsatanelevationof 20–2,600m,usuallyinthesoilsbetweenthewaterandthefoothillorbetweenthewaterandnettleinthe ditch. AC is notable for its pharmaceutical consequence in Asia. It is one of the important apparatuses of the usual methods of prescription in China and India (Zhang et al., 2015). The perfumed rhizomes and leaves have long been exploited as medicine. Remaining to the consequence in medicine and pharmacology, the plant is the matter of seriously research (Ganjewala et al.,2011). Roots and rhizomes have recently been recognized to be antibacterial agents opposed to fish pathogen (Bahukhandi et al., 2021).A. calamus, a significant herb, is used as a "rejuvenator" for the nerve system and brain in ayurvedic medicine (Sharma et al.,2014). A. calamus rhizome was administered with hot extraction (by using soxhlet apparatus) and cold extraction using the solvents like ethyl acetate, chloroform, methanol, water and n-hexane, ethyl-acetate, chloroform, methanol and water correspondingly. Each extract was made from developing the extractions of mixed semisolid material (Lokapur et al., 2022). The primary goalofphytochemicalscreeningistodeterminesecondarymetabolites,whicharecomprisedintheplant material (AC rhizome). The existing secondary chemicals' facilitates pharmacological research on the plant. AC contains glucoside, alkaloids, and essential oils that contain sesquiterpenes, calamen, asarone, acorine,eugenol,pinene,andcampheneisalsopresent.Asaronewasidentifiedasasignificantbioactive component (46.78%) (Gyawali et al.,2009). AC rizhome extracts encompasses different secondary metabolites like alkaloids, terpenoids, flavonoid, Tannins, saponins, proteins amino acids, Saponins and glycosides etc (Elhikh et al.,2022). This study is directed to conclude the significant pharmacognostical, physicochemical, and phytochemical guidelines for AC rhizome to perfectly recognize it for showing various investigation.

Material and Methods

Preparationofextracts

TherhizomeoftheACwascollectedfromWildlifeandayurvediccommercialsupplier,Gwalior,Madhya PradeshIndia,andwasauthenticatedandconfirmedbyDr.VivekGupta,Professorandhead,department of pharmacy, P.K. University Dinara, Shivpuri Madhya Pradesh. The Rhizome of AC were air dried and powderedwithgrinderthepowderedmaterialwasextractedwithwaterandmethanolofextractsbyusing soxhlation.

PhytochemicalAssessment

The chemical composition Anthraquinone, phlabatannin, alkaloids, flavonoids, glycosides, phenols, saponins, steroids, terpenoids and tannins of rhizome extracts of Acorus calamus in methanol was qualitatively examined for the described by using standard protocols. (Edeoga et al., 2005 and Rahmi Nurdiani, et al., 2012)

Test for Alkaloids 10 ml of the crude extracts was stirred with 20 ml of 1% aqueous HCl in water bath and then filtered.

Mayer'stest:To2mlfiltratefewdropsofMayer'sreagent(solutionofpotassiummercuriciodide) were added appear buff coloured precipitate was taken as existence of alkaloids.
Inbrieftanninswererecordedbyreactingwith0.1%ferricchloridesolution,saponinsweremeasuredby mixing with olive oil and flavonoids were estimated with 1% aluminium solutions.

Experimentalanimals

MaleWistarratsof4-6weeksoldweighingbetween121-129gbodyweightwerehousedinpolypropylene cages at 22±3˚C ambient temperature and 55±5 humidity in 12/12 light and dark cycle and were given standard feed and water ad libitum.

Experimentalapplication

This study was divided in four groups all experimental groups encompassed six animals with trueefforts taken to limit the number and application of animals and to take a best probable way to maintain their comfort. This study is completed in two phases: Phase.1 Each animal was tested only once for each experiment. Group- I was administrated the vehicle (1% Tween 80 in distilled water, bw). Group-II received the standard drug Imipramine (1 mg/kg, i. p.) in the elevated plus-maze (EPM) test, the hole-board test (HBT), the light-dark box (LDB) test and the standard drug imipramine (1 mg/kg, i. p.) - a tricyclic antidepressant - in thetail suspension test (TST)and the forced swim test (FST). Theremaining groups III, IV, were given AC methanol and Water extract (300 mg/kg, bw), respectively. These doses were selected based on the acute oral toxicity results and were like those reported in previous studies examining the anxiolytic and antidepressant of plant extracts (Bures et al., 1983, Nandi et al., 2021 and Raietal.,2023).Phase2.Methanolwasfoundtothemostactiveanxiolyticandantidepressantotherthan Water extract three different doses of Methanol extract (50,150 and 250 mg/kg b.wt.) and imipramine (2,1.5 and 1mg/kg b.wt) were tested for anxiolytic and antidepressant potential in normal animals.

Determinationofanxiolyticbehaviour

a. Elevatedplus-maze(EPM)test

TheEPMapparatusconsistedoffourarms,comprisingtwoopen(5×16cm)andtwoclosed(5×16×12cm) arms, joint together with acentral platform(5 ×5cm).Themaze was elevated 30 cm above ground level. TheanimalsfromgroupsI to IVweretreated30 minbeforethetestwas startedby employing individual animalsonthecentralplatform.Thetimespentintheopenarmsandthenumberofentrancesintheopen arms were recorded over a period of 5 min (Pellow et al., 1986).

b. Hole-boardtest (HBT)

Thehole-board(HB)testtackleisawoodencubicle(40×40×25cm)with16holeseach3cmindiameter. The animals from groups I to IV were treated 30 min before being placed individualistically on the HB apparatus. The number of head-dips was counted over a 5 min period of observation.

c. Light-Darkbox(LDB)test

The light dark test utilizes a box (45 cmx30cmx27cm) where one third of the box is a covered dark chamber and the other two thirds is an open light chamber. Both the chambers relate to an opening of (7.5cmx7.5cm)which allowsthemicetomove between thelightand darkchamber.Theanimals from groupsIto IVweretreated60minbeforebeingplacedindividually inthelightchamberoftheapparatus and allowed to move around. The time that the animals spent in the light and the dark chambers was recorded for a period of 5 min (Shaha et al., 2022).

Determinationofanti-depressantbehaviour

a. Tailsuspensiontest(TST)

ThetailsuspensiontestwasbasedonthemethodofSteruwithlittlemodifications(Setal., 2005) animals was separately suspended on the edge of A, 50 cm above the floor with the help of paste tapeplacedaround1cmfromthetipofthetail.Testingwascarriedoutinanisolatedroomwithminimal backgroundnoise.Themotionlessnesswasobservedduring6min.periodoftotalduration.Animalswere considered immobile only when they hung passively and completely motionless.

b. Forceswimtest(FST)

Animalwasseparatelyforcedtoswiminaglassjar(25×12×25cm3)containingfreshwateruptoaheight of 15 cm at (23±1˚C) for 6 min. The duration of motionlessness was measured during the final 4 min of the total test duration of 6 minutes. Motionlessness period was regarded as the time spent by mouse floating without moving in the water and ceased struggling, making only those movements necessary to keep its head above water (S et al., 2005).

Statistical Analysis

Data were represented as mean ± SD of 6 animals in each group and analysis of variance was performed byusingSPSS(SPSSincChicago).Thesignificantdifferenceamonggroupswereanalysedwiththehelp of student's test. The value with $P<0.05$ were considered statistically significant.

PhytoconstituentsinRhizomeofAcorusCalamus (AC)

Alkaloids and flavonoids contents were significantly higher in Methanol extracts than those of water extracts. However, tannins and saponins were significantly higher in Water extracts than other, while no significant difference were observed in phenol contents among all the extract (Table 1).

Results

Anxiolyticandantidepressantpotentialwith2extractsofrhizomeofAC

The results of table 2 indicate that the oral administration of Methanol extract of rhizome of AC shows 38%anxiolyticand36%antidepressanteffectinnormalrats.Afterexperimentswaterextractofrhizome of AC also shows 25% anxiolytic and 21% antidepressant after experiments in the normals.

1. EvaluationoftheAnxiolyticactivity

A. EffectofMethanolextractandWaterextractofACrhizomein theEPMtest

TheeffectofMethanolextractandWaterextractonthetimespentthenumberofentriesintheopenarms in the EPM test are illustrated in figure 1. Administration of Methanol extract and Water extract showed anxiolytic activity by increasing both the time and number of entries in the open arms. The Methanol extract the time spent and the number of entries in the open arms were significantly increased in groups treated with Methanol extract of AC rhizome. While Water extract showed a moderate anxiolytic effect in both time and the number of entries in the open arms.

B. EffectofMethanolextractandWaterextractinthe(hole-boardtest) HBT

The effect of Methanol extract and Water extract of AC rhizome on the head dip counts in the HBT are illustrated in figure 2. Methanol extract evaluated significantly increased the number of head dips. No significant effect was recorded with Water extract of AC rhizome.

C. EffectofMethanolextractandWaterextractinthelight-darkbox(LDB)test

TheeffectofMethanolextract and Waterextractonthetimespentin thelightand darkcompartmentsin

theLDBtestareillustratedinfigure3.Methanolextractsignificantlyincreasedthetimespentinthelight box as compared with the group of animals treated.

AlthoughWaterextractdidnot manifestasignificantincreased inthetimespent inthelight-dark box.

2. EvaluationoftheAntidepressantactivity

A. EffectofMethanolextractandWaterextractrhizomeofACrhizomein TSTandFST

The observation of this study indicated that there was no death in Water extract had no beneficial effect onimmobilityperiodofratsinboththemodelsofdepressioni.e.FST&TST.Thedecreaseinimmobility period in both the models was observed starting from Methanol extract. But the suggesting the ceiling effect of Methanol extract showed antidepressant effect which is comparable to that of group of treated animals respectively. Shown in figure 4.

ComplementaryAnxiolyticandAntidepressantactivityofMethanolextractswith Imipramine

TheresultsintableshowsthatAnxiolyticandAntidepressantpotentialofMethanolextractsofrhizome of AC were dose dependent and decreased with the dose. Similarly dose dependent Anxiolytic and antidepressanteffectofimipramine alsonoted.Thereby250mg/kgbwt.ofmethanolextractwasselected for further complementary activity with different doses of Imipramine.

Oraladministrationof(250mg/kgb.wt,)alongwith2and1.5mg/kgb.wtofimipraminedevelopedchronic anxiolytic after experiments and animals felt uncomfortable and shocked. However, the animal's anxiolytic was significantly decreased showed in animals ingested with 250 mg/kg b.wt.of methanol extracts and 1 mg/kg b.wt. of Imipramine but not developed chronic effects.

It may also show in table 2 that oral administration of 250+2 mg/kg b.wt of imipramine fully restricted. The dose of 1.5 mg/kg b.wt imipramine with Methanol extracts also not allowed to significantly during screening period.

Slightly observed in animals with the dose of 250mg Methanol extract and 1mg/kg b. wt. of imipramine after experiments which was significantly lower than those of control animals.

Table.1 PhytoconstituentspresentinMethanolandwaterextractofACrhizome.

Phytoconstituents	Methanolextract	Waterextract
Alkaloid	5.06±0.32	0.91±0.06
Flavonoid	4.6±0.20	3.46±0.23
Tannin	5.58±0.16	10.26±0.16
Phenol	1.92±0.01	2.02±0.01
Saponin	1.8±0.01	6.63±0.10
Glycoside	2.28±0.14	1.56±0.05
Steroid	0.01±0.005	0.02±0.01
Terpenoid	0.72±0.15	0.50±0.01

Values are means ±SD of three independent measurements of each extract. Values with different are significantly different at the level of $p<0.05$.

Table2.ComplementarypotentialofMethanolextractofACrhizomeonnormalrats.

Methanol extract (mg/kgb. wt.)	EPM		TSTandFST		HBD	LDB	
	TSOA	NEOA	DM	DI	HDC	TSD	TSL
250	77.60±0.43	10.56±0.35	190.53±0.35	83.2±0.20	40.20±0.95	175.80±0.26	191.26±0.50
150	84.53±0.35	25.66±0.26	179.50±0.36	86.9±0.10	37.3±0.45	172.93±0.45	185.45±0.51
50	95.56±0.29	50.63±0.21	160.66±0.32	93.9±0.10	35.16±0.40	171.16±0.25	177.20±0.3
Imipramine							
2	71.50±.16	9.63±0.31	194.66±0.49	80.4±3.59	40.26±0.97	173.36±0.41	192.03±0.61
1.5	77.60±0.30	10.53±0.40	167.70±0.15	82.7±0.28	37.7±0.55	168.3±0.52	188.86±0.90
1	89.70±0.20	16.66±0.32	160.70±0.20	85.7±0.32	36.5±0.36	164.26±0.47	187.60±0.96
Methanol extract + imipramine							
250+2	50.46±0.25	3.53±0.31	199.0±0.20	50.7±0.26	48.66±0.41	180.33±0.51	193.70±0.47
250+1.5	60.63±031.	6.46±0.20	195.96±0.20	57.7±0.32	45.0±0.17	173.30±0.45	187.06±0.15
250+1	68.66±0.15	8.63±0.25	190.70±0.43	80.6±0.25	41.76±0.57	168.26±0.40	186.96±0.20

Values are means ±SD of six animals in each group. Values with different in row and column are significantly different at the level of $p<0.05$ (dose dependent for a particular group).

Discussion

In present Study the Methanol extract of AC rhizome exhibited highest anxiolytic and antidepressant potential among the two extract and shown good complementary activity with imipramine. Anxiety is a chronic state with needs a deeper attention to control, because chronic anxiolytic and depressant are causative factor several complications (Walf et al., 2007). In this study, the effects of AC rhizome on anxiety-correlated performance were measured in mice using the EPM, HBT, and LDB tests; three behavioural models widely used to examine the anxiolytic potential of drugs, including plant-based ones (Zhouetal.,2021).TheEPMtestisoneofthefurthermostusedanimalmodelsfortestinganxiety-related behaviour.Itisbecauseelevatedandopensectionsofthemazeinitiationfearandanxietyinrodentswhich

in scare look after to escape concerning time in these places and like better safer (closed arm) sections. Treatment with an anxiolytic agent supports empirical activities and increases the time spent and the numberofentriesintheopenarmsofthemaze.Inthepresentstudy,ACrhizomeMethanolextractshowed significant anxiolytic activity by increasing both the time spent and the number of entries of treated animalsintheopenarms.IntheHBDtest,thedegreeofanxietyinanimalsisassessedbyobservinghead-dippingbehaviour,withanxiolyticdrugstriggeringanincreaseinthehead-dipcounts(Brownetal.,2008). In this study, AC rhizome (Methanol extract and Water extract) significantly increased the number of head-dipscomparedtothecontrolgroup,andMethanolextractshowedahead-dipcountand%head-dips increasesuperiortothatoftheWaterextract.TheanxiolyticeffectofACrhizomewasfurtherinvestigated using the LDB test. The LDB apparatus comprises of a dark (safe) compartment and a bright (aversive) compartment.TheLDBtesttrustsonthecharacteristicaversionofrodentstobrightareas,withanxiolytic drugsincreasingthetimespentbyanimalsinthelightcompartmentratherthanthedarkone(Blochetal., 2023). AC rhizome of Methanol extract and Water extract significantly increased the time spent in the light box and significantly decreased the time spent in the dark box. The effects observed for Methanol extract was comparable to those obtained after administration of Water extract. Methanol extract of AC rhizome only showed significant anxiolytic activity compared to untreated animals in all three tests. The antidepressant activity of AC rhizome was evaluated in rats using the well-established TST and FST behavioural models. When animals are placed under stressful conditions (i.e. inevitable positions), they tend to stay immobile for a long duration. This state of immobility echoes an inability to adjust to the stressful situation, despair orloss of hopeto be able to escape. Such behaviour closely resembles what is observedindepression.(NataliaLopez-GonzalezdelRey2023andCarvahloetal.,2021).Treatmentwith anantidepressantindicatestoareduceinthedurationofimmobility.Inthepresentstudy,MethanolExtract significantly reduced the duration of immobility compared to untreated animals in the FST model.

It has been found that various studies reported various combination/extract of AC rhizome in different CNS model, but it has not been clear which is the best and safer to be used for human consumption. Therebypresentstudywasconductedtofindoutthesaferandbestanxiolyticandanti-depressantpotential of extract of AC rhizome. Methanol extract was found best for its anxiolytic and anti-depressant among oftwo extracts (Methanol and Water extract)ofAC rhizome which is safer thanotherextract and can be useddirectlyforhumanconsumption.Theexactreasonbehindthehighestanxiolyticandanti-depressant activity of Methanol extract is not known, but because of results of present study it may speculated that this activity was might bedue higher contents of alkaloids and flavonoids in Methanol extract than those ofwaterextract(Table1.).Alkaloidsandflavonoidsaremethanolsolublecomponentshavebeenreported foranxiolyticandAntidepressantpotential(Gandaguletal.,2018andVanTanetal.,2018).Moreover,in present study the combination of Methanol extract with standard drug; imipramine was also investigated to find out how much dose of imipramine can be reduced by combination of Methanol extract of AC rhizome.ThispartofthisstudywasveryinterestedthatthecombinationofMethanolextractsignificantly reducedthedoseofimipramine(15to5mg/kgb.wt.)inanimalsanditwassaferincontrolanimals.Based on these results, it may be speculated that Methanol extract of AC rhizome had similar activity as imipramine in control animals. Synergistic effect of Methanol extract with imipramine supports it as an anxiolytic and anti-depressant activity.

In conclusion, it may suggest that the combination of Methanol extract of AC rhizome may play an importantroletosustainchronicCNSconditions,buthigherdosemaycauseCNSshockincontrolstate.

Moreover, further study is required to isolation, purification and characterisation of active components from the Methanol extract which may pave a good independent/or complementary regimen for the treatment of anxiety and depression.

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