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Anxiolytic interventions of extracts and pure compounds from *Adenanthera pavonina* and *Peltophorum pterocarpum* leaves to treat acute anxiety and depression symptoms in mice

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Abstract: Anxiolytic effect of ethanol, hexane extracts and pure compounds β- sito sterol glucoside and bergenin isolated from *Adenanthera pavonina* AP (*Fabaceae*) and *Peltophorum pterocarpum* PP (*Fabaceae*) leaves were monitored in this study. Mice were treated with dose of 125mg/kg body weight of ethanol and hexane leaves extracts of both tested plants while, 5mg/kg body weight of β-sito sterol glucoside and 25mg/kg body weight of bergenin. The effect was monitored by hole board test, forced swimming test, open field apparatus and stationary rod test. Results from neuropharmacological effects revealed that ethanol extract of AP leaves and hexane extract of PP leaves had significant anxiolytic (forced swimming test) exploratory (head dip and open field test) and neuro activator activity (stationary rod test) at tested dose. The greatest anti-depressant and anxiolytic effect was found in ethanol extract of AP leaves when compared to all treated drugs. A part from memory enhancing effects, diazepam treated mice also exhibited anxiolytic and antidepressant effects and found comparable with ethanol extract of AP. These findings may clarify the impact of ethanol, hexane extracts, and pure substances β-sitosterol glucoside and bergenin at tested concentrations, as well as their potential to treat the Parkinson's and related disorders as an alternative therapy.

Keywords: Anxiolytic, anti-depressant, ethanol and hexane leaves extracts, pure compounds, *Adenanthera pavonina* (AP), *Peltophorum pterocarpum* (PP).

INTRODUCTION

The prevalence of mental, neurological and behavioral disorders is incalculable in people and causes immense distress all over the world. Individuals suffering from these disorders often facing social isolation, a diminished standard of living and an increased risk of mortality. (Brand *et al*., 2022). To overcome this problem the evaluation of medicinal plants' interactions with living organs and systems has enabled the development of a scientific foundation for their therapeutic applications, as well as significantly broadening the range of treatments for a variety of disorders. Form the past 40 years, different synthetic anti-depressants like buspirone and β-blockers have been most frequently prescribed medication to treat various types of anxiety. However, they can lead to pharmacological dependency and have noticeable side effects like sedation, myorelaxation, ataxia, amnesia, lightheadedness, dizziness, headaches, dry mouth, constipation and diarrhea (Kenda *et al*., 2022).

Numerous biologically active phytochemicals from different plants generate therapeutic effect on the central nervous system, in that way demonstrating anxiolytic effects (Lundstrom *et al*., 2017; Savage *et al*., 2018). Most active phytochemicals work through the brain's neurotransmitters including the gamma-aminobutyric acid-ergic (GABAergic) system, dopaminergic (DA) system, serotonergic (5-hydroxytryptamine or 5-HT) system and noradrenergic (NA) system (Phootha *et al*., 2022). Those Phytochemicals are chemical compounds namely flavonoids, alkaloids, saponins, polyphenols, triterpenoids and fatty acids have shown promising anxiolytic effect. Previously the phytochemical screening of (AP) leaves extract showed the presence of alkaloids, carbohydrates, glycosides, flavonoids glucosides of β-sitosterol, saponin, steroids, stigma sterol and tannins. Whereas *Peltophorum pterocarpum* leaves extract showed the presences of flavonoids, alkaloids, steroids, saponin, stigma sterol and glycoside (Tasleem, 2016) These are the substances that might be useful in the future for treating isolation-related anxiety disorders.

Now a days, people are more open to trying traditional herbal remedies, especially those that have been clinically proven to be effective or that have been proven to work in controlled trials and in some cases have even been shown to have better galenic benefits than traditional medicines. The study of medicinal plants and the self-administration of herbal medications may lead to new therapeutic options for the treatment of anxiety disorders and it is one of the most widely used alternative therapy for treating anxiety problems, so it is a great deal of interest in the creation of novel anxiolytic therapies.

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Different pharmacological assays have been employed for the analysis of therapeutic plants for neuro-pharmacological potential. The selection of test methods demonstrates effectiveness and, in some cases, provides information about the mechanism of the test drug.

Current study was established to evaluate the anxiolytic effects of ethanol, hexane extracts and pure compounds isolated from leaves of (AP) and (PP) using the head dip activity into holes, open field activity, force swimming test and stationary rod test. Furthermore, the effects of tested drugs and diazepam on these animal models were compared to determine whether the behavioral profile of *Adenanthera pavonina* and *Peltophorum pterocarpum* plants were helpful to established the anxiolytic drug.

MATERIALS AND METHODS

Animals

For this assessment, Swiss albino mice weighing 20-25g of either sex were employed. The animals were housed in an animal house with conventional environmental conditions, including light/dark cycles, 23°±4°C and 66% relative humidity. They were also given free access to standard laboratory feed and water. The extracts were examined for toxicity prior to the start of anxiolytic action. The Board of Advanced Studies and Research, University of Karachi, Karachi, approved the biological use and care of the animals in the current study against Resol. No.07 (09) File No.0265/Pharm.

Chemicals and drugs

Dimethyl sulfoxide (10%), normal saline (0.9%), hexane, ethanol, distilled water. Standard drug diazepam was used in the study.

Plant material

A. pavonina and *P. pterocarpum* aerial sections were gathered from the University of Karachi in Karachi, Pakistan. Dr. Muhammad Mohtasheemul Hassan of the Faculty of Pharmacy and Pharmaceutical Sciences carried out the identification. In accordance with Resolution No. 07 (09) File No.0265/Pharm, a voucher specimen was taxonomically recognized and placed in the herbarium of the Department of Pharmacognosy, Faculty of Pharmacy and Pharmaceutical Sciences, University of Karachi, Karachi, Pakistan.

Preparation of extracts

For fifteen days, 3 kg of washed and air-dried AP and PP leaves were soaked in hexane. Hexane extract was filtered and evaporated after a period of 15 days. For the next fifteen days, the residues were immersed in ethanol. Finally, after that time, the ethanol extract was filtered and concentrated under reduced pressure using a rotary evaporator at 45°C. The extracts were then kept in airtight glass containers for use in identification-related biological research (Tasleem, 2015).

Isolation of pure compounds

Method used, already documented in an earlier research publication (Mahmood *et al*., 2017; Tasleem *et al*., 2017).

Treatment schedule

An oral dose of ethanol, hexane extracts at 125mg/kg body weight and β-sitosterol glucoside at 5mg/kg body weight of (AP) leaves, was administered to experimental groups (N=5) of mice. While the control groups were given the normal saline (20ml/kg body weight). Diazepam 2mg/kg body weight was administered as a standard drug. Same treatment schedule to be repeated for ethanol, hexane extracts and bergenin at 25mg/kg body weight of (PP) leaves.

Hole-board test

The evaluation of anxiolytic and CNS depressant activity were conducted using the hole-board apparatus. The device is a rectangular transparent polyacrylic box with sixteen equal distance holes on a black sheet. Each mouse was given a test dose orally, put in the middle of the device, and given 10 minutes to explore it. The number of head dips was only recorded after the mouse's two eyes vanished into the hole for 0, 30, 60, 120 minutes after the drug had been administered. (Pisula *et al*., 2021)

Forced swimming test

FST is the most broadly used and verified method to determine the anti-depressant activity in animal module. The apparatus was a transparent Plexiglas cylinder, measuring 20cm by 12cm. It was filled with water and kept up with the temperature up to 24±1°C. Animals were treated with respective group treatments and after that, each animal was placed individually in the plexiglass cylinder for 5 minutes of swimming session. Period of immobility was recorded for 5 minutes, immediately after 30, 60 and 120 minutes of treatment. When the mouse did not try to escape, it was deemed immobile and it's the only movement when its head keep out of the water (Ráez *et al*., 2020).

Open field test

An open field test was conducted to assess the impact of test drugs on locomotion of mice. The experiment was carried out in a quiet and dimly lit area. The open field's floor was partitioned into a grid of squares, each of which was alternately painted in black and white. Each mouse was given treatment with control, diazepam, and test drugs before being placed at the center of the field. After that, the numbers of squares traveled were counted at 0, 30, 60 and 120 minutes of treatment for 10 minutes (La-Vu *et al*., 2020).

Stationary rod test

This technique is used to study the balance and memory of animals. The stationary rod apparatus consists of elevated and fixed steel rod with platforms at the ends.

Before the start of study all of the treated mice were trained to cross a rod and reach to platform. To conduct the observations, each mouse was placed on rod's midpoint and forced to walk across the rod to reach the platform. Each mouse was treated with test drugs and after 0, 30, 60 and 120 minutes record the time required by each mouse to reach the platform from the midpoint was recorded (Ahmed *et al*., 2017).

STATISTICAL ANALYSIS

The results were expressed as mean ± standard error of the mean (SEM) and analyzed by Student's t-test using SPSS 21.0 analysis software. P value <0.05 and **P<0.01 were considered statistically significant.

RESULTS

Being the primary sources of secondary metabolites, herbal medicines are essential in the prevention and treatment of many disorders. The objective of this study was to evaluate the anxiolytic intervention of ethanol, hexane extracts and isolated pure compounds β-sito sterol glucoside and bergenin from *Adenanthera pavonina* and *Peltophorum pterocarpum* leaves respectively to cure depression and anxiety using animal behavioral models. In this case study four different assays were applied on mice for assessment of anti-depressant activity. Experimental models for the assessment of anxiolytic properties of substances are head dip activity into holes used to demonstrate exploratory behaviors. Open field activity has been used to inspect various behavioral features like anxiety related response. Force swimming test could be helpful to explore the alleviating anxiety feelings and depression symptoms in rodents (Wahid *et al*., 2023).

DISCUSSION

The exploratory effect of ethanol, hexane extracts and β-sito sterol glucoside and bergenin from (AP) and (PP) leaves at concentrations of 5 and 125mg/kg body weight as well as 25 and 125mg/kg body weight respectively were determined by the hole board test to observe how many times mice dip their heads. Placement of each mouse in hole board apparatus is a tense situation, upon compression of both plant ethanol extract at a dose of 5 mg/kg body weight of (AP) leaves indicated the maximum decrease of heads dipping as compared to other treated drugs and standard drug diazepam. Results will suggest that anxiety was relieved and there was a reduction of fear and temper.

Open field test is a general motor function test and results in mice tested by ethanol, hexane extracts and β- sito sterol glucoside and bergenin from (AP) and (PP) leaves at 5 and 125mg/kg body weight as well as 25 and 125 mg/kg body weight accordingly presented time dependent reduction in striving period this distress reflects the anxiolytic characteristic of tested samples. After comparison of both plants activity it was found that ethanol extract at 5mg/kg body weight of *Adenanthera pavonina* leaves presented the lowest number of squares in crossing during whole analysis period of time and that was also found significant and more than standard drug diazepam. Furthermore, anxiolytic medications are known to increase the time required to cross a given number of squares.

Force swimming test relating to behavioral distress and presented the outcome on floundering and immovability duration. This test is widely used to assess how effective anti-depressants medications and how well they respond to different behavioral and neurological tests in fundamental and pre-clinical research. It has been characterized as creating a situation in which the animal experiences a state of "behavioral despair"; i.e., the animal no longer has any hope of escaping the stressful situation (Molendijka and Kloe., 2019). On the basis of this evaluation mice treatment with ethanol, hexane extracts and β-sito sterol glucoside and bergenin from (AP) and (PP) leaves respectively at doses of 5 and 125 mg/kg body weight as well as 25 and 125mg/kg body weight accordingly showed short immobility time throughout the analysis. However, ethanol extract of (AP) leaves presented least number of heads out of water as compared to other treated groups but this increase time in immobility found less than diazepam.

The use of synthetic antidepressants and anxiolytics medications can have negative impact on memory. So, the execution of learning ability and memory enhancing ability along with general motor activity of animal model were evaluated in this analysis. According to this study, administration of ethanol and hexane extracts as well as pure compounds β-sito sterol glucoside and bergenin from two different plants (AP) and (PP) respectively belongs to same family- *Fabaceae* found effective to waned the anxiety state. While ethanol extract at 5mg/kg body weight of *Adenanthera pavonina* leaves presented most potent and comparable activity against depression and relieve anxiety symptoms among all tested drugs. This might be brought on by higher dopamine and noradrenaline concentrations as well as the slower noradrenaline metabolism. The reason may be due to presences of reported CNS active metabolites (Matraszek *et al*., 2019). that are responsible for anxiolytic and sedative effects. The presence of flavonoids in ethanol extract of AP leaves can be hypothesized for its antianxiety potential. Furthermore, the presence of flavonoids, tannin, phenols and saponin alkaloids was also confirmed by phytochemical analysis (Tasleem, 2015) and the results suggest that, ethanol extract of AP leaves had substantial anxiolytic interference in this model

Table 1: Effect of β-sitosterol glucoside, hexane and ethanol extracts of *Adenanthera pavonina* in head dip test

Compound and Extracts	Dose mg/kg	Number of head dips counted in 10 minutes				
		0 min ♦	30 mins ♦	60 mins ♦	120 mins ♦	Mean
β-sitosterol glucoside	5	25.330±0.003	29.000±0.570	30.000±0.003**	31.330±0.003**	28.915±0.144
Adenanthera pavonina (Hexane Extract)	125	21.666±0.014	25.666±0.002**	25.666±0.010*	32.660±0.007**	26.410±0.008**
Adenanthera pavonina (Ethanol Extract)	125	18.500±0.002	15.500±0.001**	11.167±0.001**	4.500±0.006**	12.415±0.002**
Standard Drug (Diazepam)	2	21.877±0.007	11.600±0.003	12.400±0.001	13.400±0.007	14.819±0.004
Control	-	21.200±0.001	20.400±0.002	22.000±0.001	22.000±0.001	21.400±0.001

♦ showing observation intervals of reaction time
Results were presented as Mean ± SEM, n=5,*P<0.05 and **P<0.01 significant as compared to control

Table 2: Effect of β-sitosterol glucoside,hexane and ethanol extracts of *Adenanthera pavonina* inopen field test

Compound and Extracts	Dose mg/kg	Number of squares crossed in 10 minutes				
		0 min ♦	30 mins ♦	60 mins ♦	120 mins ♦	Mean
β-sitosterol glucoside	5	109.500±0.001	93.200±0.001**	75.000±0.008**	56.660±0.005**	83.590±0.004**
Adenanthera pavonina (Hexane Extract)	125	74.400±0.002	70.400±0.002**	64.000±0.001**	48.000±0.001**	64.200±0.002**
Adenanthera pavonina (Ethanol Extract)	125	52.000±0.003	40.330±0.009	24.500±0.003*	13.330±0.003**	32.55±0.004**
Standard Drug (Diazepam)	2	105.087±0.006	11.000±0.002	14.000±0.003	12.000±0.001	35.521±0.003
Control	-	105.000±0.020	105.660±0.020	107.666±0.023	108.660±0.022	106.580±0.021

Table 3: Effect of β-sitosterol glucoside, hexane and ethanol extracts of *Adenanthera pavonina* in forced swimming test

Compound and Extracts	Dose mg/kg	Struggling time in seconds				
		0 min ♦	30 mins ♦	60 mins ♦	120 mins ♦	Mean
β-sitosterol glucoside	5	53.660±0.001	48.000±0.001**	39.500±0.002**	24.000±0.002**	41.290±0.002**
Adenanthera pavonina (Hexane Extract)	125	58.830±0.001	66.830±0.001**	48.330±0.001**	34.000±0.002**	51.997±0.001**
Adenanthera pavonina (Ethanol Extract)	125	48.000±0.002	40.330±0.002**	23.500±0.006**	12.500±0.001**	31.082±0.003**
Standard Drug (Diazepam)	2	63.283±0.001	1.600±0.060	1.300±0.051	1.030±0.063	16.803±0.043
Control	-	64.133±0.002	62.000±0.005	64.330±0.004	57.330±0.003	61.947±0.003

♦ showing observation intervals of reaction time
Results were presented as Mean ± SEM, n=5,*P<0.05 and **P<0.01 significant as compared to control

Table 4: Effect of β-sitosterol glucoside, hexane and ethanol extracts of *Adenanthera pavonina* in stationary rod test

Compound and Extracts	Dose mg/kg	Time taken to reached at end point of rod in seconds				
		0 min ♦	30 mins ♦	60 mins ♦	120 mins ♦	Mean
β-sitosterol glucoside	5	25.166±0.001	18.333±0.001**	16.166±0.001**	11.000±0.001**	17.664±0.001**
Adenanthera pavonina (Hexane Extract)	125	22.800±0.003	21.400±0.001**	17.400±0.002**	13.200±0.004**	18.700±0.009
Adenanthera pavonina (Ethanol Extract)	125	18.000±0.001	17.000±0.005**	13.000±0.002**	8.833±0.002**	14.207±0.003**
Standard Drug (Diazepam)	2	24.054±0.001	11.600±0.003	7.400±0.001	6.400±0.007	11.819±0.004
Control	-	11.633±0.011	11.433±0.019	11.200±0.018	11.366±0.017	11.405±0.016

♦ showing observation intervals of reaction time
Results were presented as Mean ± SEM, n=5,*P<0.05 and **P<0.01 significant as compared to control

Table 5: Effect of Bergenin, hexane and ethanol extracts of *Peltophorum pterocarpum* in head dip test

Compound and Extracts	Dose mg/kg	Number of head dips counted in 10 minutes				
		0 min ♦	30 mins ♦	60 mins ♦	120 mins ♦	Mean
Bergenin	25	27.000±0.001	22.833±0.001**	16.660±0.001**	12.667±0.001**	19.787±0.001**
Peltophorum pterocarpum (Hexane Extract)	125	18.330±0.001	25.166±0.006**	17.666±0.002**	12.000±0.001**	18.287±0.002**
Peltophorum pterocarpum (Ethanol Extract)	125	25.833±0.002	23.66±0.003**	19.500±0.003**	14.000±0.003**	20.747±0.003**
Standard Drug (Diazepam)	2	21.877±0.007	11.600±0.003	12.400±0.001	13.400±0.007	14.819±0.004
Control	-	21.200±0.001	20.400±0.002	22.000±0.001	22.000±0.001	21.400±0.001

♦ showing observation intervals of reaction time
Results were presented as Mean ± SEM, n=5,*P<0.05 and **P<0.01 significant as compared to control

Table 6: Effect of Bergenin, hexane and ethanol extracts of *Peltophorum pterocarpum* inopen field test

Compound and Extracts	Dose mg/kg	Number of squares crossed in 10 minutes				
		0 min ♦	30 mins ♦	60 mins ♦	120 mins ♦	Mean
Bergenin	25	105.000±0.001	113.000±0.002**	.330±0.001**	67.000±0.002**	91.330±0.002**
Peltophorum pterom (Hexane Extract)	125	90.000±0.001	71.000±0.001**	43.000±0.002**	15.500±0.001**	43.900±0.001**
Peltophorum pterocarpum (Ethanol Extract)	125	120.333±0.001	111.333±0.001**	87.666±0.002**	71.666±0.002**	97.745±0.002**
Standard Drug (Diazepam)	2	105.087±0.006	11.000±0.002	14.000±0.003	12.000±0.001	35.521±0.003
Control	-	105.000±0.020	105.660±0.020	107.666±0.023	108.660±0.022	106.580±0.021

♦ showing observation intervals of reaction time
Results were presented as Mean ± SEM, n=5,*P<0.05 and **P<0.01 significant as compared to control

Table 7: Effect of Bergenin, hexane and ethanol extracts of *Peltophorum pterocarpum* in forced swimming test

Compound and Extracts	Dose mg/kg	Struggling time in Seconds				
		0 min ♦	30 mins ♦	60 mins ♦	120 mins ♦	Mean
Bergenin	25	64.166±0.001	57.833±0.001**	38.666±0.001**	20.000±0.001**	45.162±0.001**
Peltophorum pterocarpum (Hexane Extract)	125	59.833±0.001	56.666±0.001**	32.500±0.003**	18.166±0.002**	41.787±0.002**
Peltophorum pterocarpum (Ethanol Extract)	125	65.000±0.001	54.833±0.001**	38.000±0.003**	20.833±0.003**	42.165±0.002**
Standard Drug (Diazepam)	2	63.283±0.001	1.600±0.060	1.300±0.051	1.030±0.063	16.803±0.043
Control	-	64.133±0.002	62.000±0.005	64.330±0.004	57.330±0.003	61.947±0.003

♦ showing observation intervals of reaction time
Results were presented as Mean ± SEM, n=5,*P<0.05 and **P<0.01 significant as compared to control

Table 8: Effect of bergenin, hexane and ethanol extracts of *Peltophorum pterocarpum* in stationary rod test

Compound and Extracts	Dose mg/kg	Time taken to reached at end point of rod in seconds				
		0 min ♦	30 mins ♦	60 mins ♦	120 mins ♦	Mean
Bergenin	25	24.000±0.001	20.833±0.004**	17.666±0.001**	11.166±0.001**	18.412±0.002**
Peltophorum pterocarpum (Hexane Extract)	125	23.833±0.001	18.666±0.001**	13.833±0.002**	6.000±0.002**	15.580±0.002**
Peltophorum pterocarpum (Ethanol Extract)	125	24.333±0.001	18.166±0.001**	13.333±0.001**	6.500±0.001**	15.580±0.001**
Standard Drug (Diazepam)	2	24.054±0.001	11.600±0.003	7.400±0.001	6.400±0.007	11.819±0.004
Control	-	21.200±0.001	20.433±0.002	22.000±0.001	22.000±0.001	21.405±0.001

♦ showing observation intervals of reaction time
Results were presented as Mean ± SEM, n=5,*P<0.05 and **P<0.01 significant as compared to control

study to treat anxiety and depression in mice. Therefore, further studies are planned so establish the exact mechanism of CNS depressant and anxiolytic activities of by using agonists and antagonists.

Stationary rod test is an accurate pharmacological technique for evaluating the grip strength to sustain the balance on rod. This evaluation showed the improves learning and memory ability in mice. Stationary rod test results revealed that ethanol, hexane extracts and β-sito sterol glucoside and bergenin from (AP) and (PP) leaves at concentration of 5 and 125mg/kg body weight as well as 25 and 125mg/kg body weight respectively showed the time dependent reduction in time to reach the plate form. Although, the greatest activity in learning and memory improvement, presented by ethanol extract of (AP) leaves through better muscles relaxation, but lesser than standard drug diazepam.

Individuals' emotions, behavior and mental health are impacted by depression. Moreover, there are also signs of altered neurotransmitters in the central nervous system. Specifically, norepinephrine, serotonin (5-hydroxy-tryptamine 5-HT) and dopamine (Yohn *et al*., 2017). Depression's underlying neurobiological origins are still unclear. It is assumed that the onset of depression is caused by a decrease in the concentration of monoamine transmitters in the central nervous system, such as 5-HT, NA and DA. Depression and a "foggy brain" can be caused by a lack of dopamine in the central nervous system. Decreased dopamine levels can lead to depression. On the other hand, manic symptoms may arise from elevated dopamine levels.

CONCLUSION

It is possible to conclude from the analysis that the pure isolated compounds, ethanol and hexane extracts from AP and PP leaves are promising source of therapeutically active phytoconstituents having neuropharmacological activity to relieve anxiety and depression. So tested plants may be a better option when treating anxiety disorders in a traditional manner. In order to highlight the development of more effective treatments with no side effects, it may therefore recommend additional research to reveal the underlying causes of anxiolytic actions.

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