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Research Article

Anti-inflammatory activity of ethanolic extract of *Pueraria tuberosa* tubers

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ABSTRACT:
Depression is one of the leading health problems worldwide and is commonly connected with oxidative stress and monoaminergic dysfunction. The present investigation was designed to evaluate the antidepressant-like efficacy of ethanolic extract of *Pueraria tuberosa* tubers (EEPT) in validated behavioural models in mice. The extract yield was 25.79% w/w and phytochemical screening showed the presence of alkaloids, flavonoids, phenols, tannins and triterpenoids. The quantitative analysis showed the high presence of phenolics (66.62 ± 0.453 mg GAE/g) and flavonoids (62.03 ± 0.588 mg QE/g). The Forced Swim Test (FST) and Tail Suspension Test (TST) were used to evaluate antidepressant activity. EEPT significantly enhanced the swimming frequency in FST (22.16 ± 2.483 at 200 mg/kg; 31.16 ± 3.061 at 400 mg/kg) compared to control (17.66 ± 1.366) and was close to the impact of fluoxetine (35.83 ± 3.371). In TST, the immobility period was dramatically decreased at 400 mg/kg (25.17 ± 1.352 sec) than control (43.00 ± 1.211 sec) with similar findings to fluoxetine (10.17 ± 0.667 sec). The present results demonstrate that EEPT shows dose-dependent antidepressant-like effects probably mediated by its phenolic and flavonoid contents, thereby indicating its potential as a natural treatment agent for depression

Key Words: *Pueraria tuberosa*, antidepressant, forced swim test, tail suspension test, phenolics, flavonoids

Introduction:

Depression is a complex mental illness marked by persistent poor mood, loss of interest and reduced cognitive performance.[1] Current pharmaceutical treatments, such as selective serotonin reuptake inhibitors (SSRIs) are successful, however often have a delay in the beginning of action and unpleasant effects, so that the search for safer alternatives is necessary.[2] Natural products, especially those high in phenolics and flavonoids, have been studied for their antioxidant, neuroprotective and monoaminergic modulatory activities that could contribute to antidepressant-like effects.[3,4]

The tubers of *Pueraria tuberosa* or Indian Kudzu are used for their revitalizing and healing effects in ayurvedic and folk medicine.[5-8] Phytochemical analysis revealed the presence of alkaloids, flavonoids, tannins and triterpenoids in the plant, which are known to affect central nervous system activity.[9] Antidepressant potential of *Pueraria tuberosa* is not adequately established by scientific experimentation, yet it is traditionally used.

Hence, the present study was designed to investigate the antidepressant like effect of ethanolic extract of *Pueraria tuberosa* tubers (EEPT) by employing forced swim test (FST) and tail suspension test (TST) in mice.

Material and Method

The tubers of *Pueraria tuberosa* were purchased from the local road side vegetable sellers of Gwalior, Madhya Pradesh in the month of January. The authentication of the specimen was done by botanist at RB Science, Bhopal. The collected plant material, after authentication was washed with distilled water and dried under shade. The completely dried tubers were converted to coarse powdered form using a blender at low speed.

Extraction of plant material

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The powdered material prepared using the above procedure were used for extraction process. Hot continuous extraction was performed for extracting out the phytochemicals from the powder. Briefly, 100 g of the tuber powder was evenly packed in the extractor of the soxhlet apparatus and extracted using ethanol as the solvent after defatting with petroleum ether (500 mL). The extraction process was carried out for about 8 h for each solvent, till a clear solution was visible in the siphon tube of the extractor. The extracts (solvents) were filtered while hot to remove any un-dissolved material (debris or impurities). The extracts were concentrated by evaporation using rotary vacuum evaporator and transferred to 100 mL beaker and the remaining solvents were evaporated on thermostatically heated water bath. The oleo-resinous extracts were collected and placed in desiccators to remove the excessive moisture.[10]

Preliminary phytochemical screening of extracts

The ethanolic extract was subjected to qualitative phytochemical testing procedures for identifying the presence or absence of usual plant secondary metabolites. The test was performed for alkaloids, triterpenes/steroids, glycosides, tannins, flavonoids, saponins, and phenolic acids. The color, intensity of color or the precipitate formation was used as observational responses to the reactions occurring in these tests.[11,12]

Total Phenolic Content Determination

A 50 mg/mL solution of the extract was prepared in methanol for phenolic content estimation. 200 µL of the extract solution was mixed with 1.4 mL purified water and 100 µL of Folin-Ciocalteu reagent. After 3 min, 300 µL of 20%Na2CO3 aqueous solution was added and the mixture was allowed to stand for 2 h.[13,14] The absorbance was measured at 765 nm using a UV-Vis spectrophotometer. Standard solutions of gallic acid (10-100 ppm) were similarly treated to plot the calibration curve. The control solution contained 200 µL of methanol and suitable reagents, and it was prepared and incubated under the same conditions as the rest of the samples. Results were expressed as milligrams of gallic acid equivalent (GAE) per 100 g of the dry sample.

Total flavonoid content determination

Total flavonoid content (TFC) was determined by using aluminium chloride method.[15] Briefly, the extract was prepared by dissolving 5 mg of extract in 5 mL of distilled water. Then, 100 µL of extract solution was mixed with 250 µL of distilled water and 15 µL of NaNO3 (5% w/v in distilled water). The mixture was incubated for 5 min at room temperature. Next, 30 µL of AlCl3 (10% w/v) solution was added into the mixture and left at room temperature for 6 min and to it were added 100 µL of NaOH (1 M) and 55 µL of distilled water. The mixture was incubated in the dark for 20 min. The absorbance was measured at 510 nm using UV visible spectrophotometer. The TFC was expressed as µg quercetin equivalents (QE) per mg extract. Standard solutions of quercetin (10-100 ppm) were similarly prepared to obtain a calibration curve.

Antidepressant Action

The in vivo antidepressant action of the ethanolic extract of *Pueraria tuberosa* (EEPT) was carried out in male albino mice weighing between 25–30 g by forced swim test (FST) method. Animal were divided into 5 groups of 6 animals each for conducting the study. Group I was administered with normal saline and served as control, group II & III were administered 200 mg/kg (i.p) and 400 mg/kg of the EEPT respectively, whereas group IV served as positive control and was administered with fluoxetine, 10 mg/kg (i.p).

Forced Swim Test

The extract (EEPT) and fluoxetine were dissolved in DMSO and injected intraperitoneally in a standard volume of 0.05 mL per 20 g body weight, to each mouse 30 minutes prior to the test. To determine the effect of the test compound mice were individually placed in a glass cylinder (25 cm height, 10 cm diameter) filled with water (22-25°C) up to 10 cm height. Each mouse was allowed to swim for 6 minutes during the test, and the duration of immobility was observed and noted during the final 4 minutes of the test. The time spent by the mouse floating in the water without struggling and making only those movements necessary to keep its head above water was regarded as the immobility period. The animals were dried using tower and returned back to their housing conditions.[16-18]

Tail Suspension Test

The extract (EEPT) and fluoxetine were dissolved in DMSO and injected intraperitoneally in a standard volume of 0.05 mL per 20 g body weight, to each mouse 30 minutes prior to the test. To determine the effect of the test compound mice were individually suspended by tail using clamp (2 cm from the tip of the tail) in a box (25 × 25 × 30 cm) with the head 5 cm from the bottom. Minimal background noise was maintained and the testing was carried out in dark room. All animals were suspended for total 6 minutes, and the duration of immobility was observed and noted during the final 4 minutes of the test. Mice were considered immobile only when they hung passively and completely motionless. The animals were used only once for this test.[16-18]

Statistical Analysis

The results of pharmacological studies were expressed as mean ± S.D. The total variations present in data were evaluated by using Graph Pad Prism 5 project software one way ANOVA (analysis of variance) followed by Dunnett's multiple comparison Test. The results were considered statistically significant when P- value less than 0.05 ($P<0.05$) vs control.

Results and Discussion

The yield of the extract obtained using ethanol as solvent was found to be 25.79%w/w indicating the presence of significant amount of polar and semi-polar secondary metabolites. The extract was dark yellow in color and the qualitative phytochemical screening revealed the presence of alkaloids, flavonoids, triterpenoids, phenols and tannins in the extract (Table 1).

Table 1. Qualitative phytochemical screening of *P. tuberosa* ethanolic extract

Chemical Tests	Observation checked for	Inference
Alkaloids		
Mayer's reagent	cream colour precipitate	+
Hager's reagent	yellow colour precipitate	+
Wagner's reagent	reddish brown precipitate	+
Dragendorff's reagent	reddish brown precipitate	+
Glycosides		
Froth test	Frothing is seen	-
Kedde's Test	No color	-
Bontrager's Test	Rose pink or red color in the ammonical layer not found	-
Keller-Kiliani	No color in acetic acid layer	-
Phenols/Tannins		
Ferric chloride	Blue green color	+
Gelatin Solution	White precipitate	+
Alkaline reagent test	Yellow to red precipitate	+
Vanillin HCl test	Purplish red color	+
Flavonoids		
Shinoda test	red color	+
Alkaline reagent test	Yellow color that turns red on acidification	+
Zinc HCl reductino test	red color	+

Proteins		
Millon's Test	white precipitate, turns red on heating	-
Ninhydrin Test	Voilet color	-
Sterols/triterpenoids		
Liberman-Burchard Test	Brown ring at junction Upper layer turns green	+
Salkowski Test	Yellow color in lower layer	-

+ indicates positive observation; - indicates negative observation

Total Phenolic and flavonoid content

The standard curve of gallic acid was constructed using the absorption data. Using the standard curve data, the total phenolic content of the extract of *Pueraria tuberosa* was calculated and found to be 66.62 ± 0.453 GAE mg/g. The total flavonoid content in the extracts was determined using colorimetric method and are reported as quercetin equivalent (QE). Quercetin was used as the standard flavonoid and the absorbance of various concentration of quercetin standard was plotted to obtain the calibration curve. The total flavonoid content was calculated from the quercetin calibration curve and was found to be 62.03 ± 0.588 QE mg/g.

Antidepressant action

The antidepressant action of the extract (EEPT) was tested using forced swim test and tail suspension test at two dose levels (200 and 400 mg/kg). The immobility time and the swimming frequency was recorded and statistically analyzed using one way ANOVA followed by Dunnett's multiple comparison test.

The results of the study revealed that the extract, was able to exhibit dose dependent improvement in swimming frequency in mice. Forced swim test is used as a stress induced depression model of assessing the effectiveness of antidepressant drugs. The effect of extract treatment was found to be significant at both the tested doses ($p<0.05$). At 400 mg/kg dose, the extract was able to improve the swimming frequency almost equal to the standard drug fluoxetine ($p<0.001$) (Figure 1).

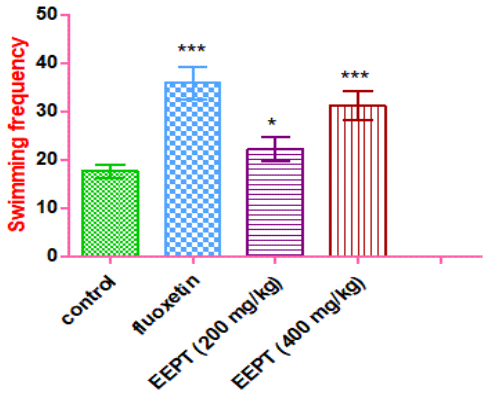

Figure 1. Effect of treatment of swimming frequency. ***p<0.001 compared to control, Values are represented as mean ± SEM, (n = 6)

The effect of EEPT in TST was recorded as the immobility time of test animal on hanging the animal by its tail. A higher immobility suggests depression whereas the lower immobility suggests antidepressant action. As it can be seen from Figure 2 that the immobility time for the compounds EEPT reduced significantly when compared to the control group. The immobility time for EEPT (400 mg/kg) was found to be 18.17 ± 1.994 sec and was comparable to that of fluoxetine at a dose on 10mg/kg (10.17 ± 0.667 sec) ($p<0.001$).

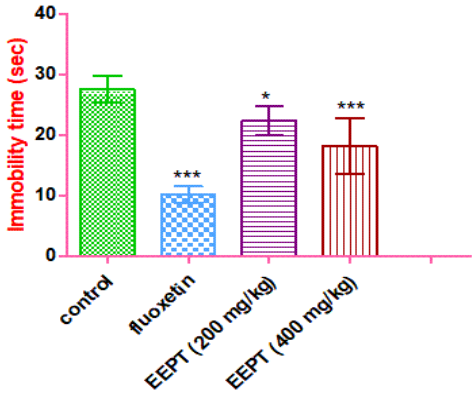

Figure 2. Effect of treatment of immobility time. ***p<0.001 compared to control, Values are represented as mean ± SEM, (n = 6)

In the FST, the swimming frequency of the control group was 17.66 ± 1.366. Treatment with EEPT at 200 mg/kg significantly increased swimming frequency to 22.16 ± 2.483 ($p < 0.05$), while the higher dose of 400 mg/kg produced a more pronounced effect (31.16 ± 3.061, $p < 0.001$). The standard drug fluoxetine (10 mg/kg) showed the highest activity (35.83 ± 3.371, $p < 0.001$). These findings demonstrate a clear dose-dependent improvement in swimming behavior, suggesting that EEPT enhances active coping responses and reduces immobility, a hallmark of antidepressant activity.

In the TST, immobility time was significantly reduced in EEPT-treated groups compared to saline controls (17.33 ± 0.494). At 200 mg/kg, immobility decreased to 43.00 ± 1.211 ($p < 0.001$), while at 400 mg/kg, immobility was further reduced to 25.17 ± 1.352 ($p < 0.001$). Fluoxetine produced the most pronounced reduction (10.17 ± 0.667 sec). Notably, the higher dose of EEPT (400 mg/kg) showed immobility times approaching those of fluoxetine, indicating strong antidepressant-like efficacy.

The phytochemical analysis of EEPT revealed the presence of alkaloids, flavonoids, phenols, tannins, and triterpenoids, along with high levels of phenolic (66.62 ± 0.453 mg GAE/g) and flavonoid content (62.03 ± 0.588 mg QE/g). These bioactive compounds are well known for their antioxidant and neuroprotective properties, which may underlie the observed behavioral improvements. Flavonoids, in particular, are reported to modulate monoaminergic systems and reduce oxidative stress, mechanisms that are consistent with antidepressant activity.

Conclusions

The ethanolic extract of *Pueraria tuberosa* tubers showed considerable antidepressant-like effect in mice as evident from increase in swimming frequency in FST and decrease in immobility duration in TST. The effects were dose-dependent and the higher dose (400 mg/kg) produced results comparable to fluoxetine, a common antidepressant. The phytochemical analysis revealed the presence of alkaloids, flavonoids, phenols and tannins. The high phenolic and flavonoid content is probably responsible for the reported activity via antioxidant and neuroprotective mechanisms. These results not only support the traditional usage of *Pueraria tuberosa* but also reveal its potential as a natural treatment agent for depression. Further investigations should be carried out to isolate the active elements, elucidate the molecular mechanisms and undertake clinical tests to establish its efficacy and safety in humans.

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