Phyto-pharmacological Investigation of *Plumbago zeylanica* for Memory Enhancing Activity

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ABSTRACT

**Objective:** The main goal was to assess the possible memory-enhancing effects of *Plumbago zeylanica* root extract on Wistar rats.

**Materials and Methods:** Extract of *P. zeylanica* roots evaluated for memory enhancement by Morris water maze (MWM) and elevated plus maze (EPM). Here, the parameter examined was transfer latency (TL).

**Results:** There was a dose-dependent decline in TL when *P. zeylanica* root extract was administered, as compared to the control.

**Conclusion:** The observed dose-dependent reduction in transfer latency provides compelling evidence of the extract’s potential in countering neurodegeneration and affirming its nootropic capabilities.

**Keywords:** *Plumbago zeylanica* Roots, Elevated plus maze, Morris Water maze, Memory, Learning.

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INTRODUCTION

Memory enhancement has garnered significant attention with relevance in both cognitive health and managing neurodegenerative disorders. In this context, natural products and traditional medicinal plants have become a focal point of research, offering promising avenues for discovering novel compounds with memory-enhancing properties. *Plumbago zeylanica*, a widely distributed plant known for its traditional medicinal uses, has attracted the interest of researchers as a potential candidate for cognitive enhancement.

*P. zeylanica*, universally known as Ceylon leadwort or Chitrak, family Plumbaginaceae originates richly in Asia and Africa. It has been traditionally employed for its therapeutic properties in various cultures, often applied to treat ailments like pain, inflammation, and gastrointestinal disorders. Additionally, it has been noted for its potential to improve cognitive function and memory retention.

The phytochemical profile of *P. zeylanica* is rich and diverse, with constituents such as plumbagin, naphthoquinones, flavonoids, and alkaloids, which have been studied for their potential pharmacological effects, including neuroprotective and memory-enhancing properties. These compounds may interact with various molecular pathways and neurotransmitter systems, modulating synaptic plasticity, neuronal survival, and memory consolidation.

Given the growing interest in exploring natural remedies for cognitive enhancement and the traditional use of *P. zeylanica*, there is a need for a comprehensive phytopharmacological investigation to elucidate its memory-enhancing activity. This research aims to provide insights into the bioactive compounds responsible for its effects on memory, the underlying mechanisms involved, and the potential therapeutic applications. Through a systematic analysis of *P.
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*zeylanica*, study seeks to pays attention to advances of new, safe, and efficient interventions for memory-related conditions and disorders.\(^4\)

**MATERIALS AND METHODS**

**Procurement of Plant Material**

Plant Part procured from the confined market. Subsequently, the roots underwent taxonomic identification and authentication by a senior botanist. To maintain a record of the plant material used, a voucher sampling has been diligently preserved within the department for prospect reference.

**Successive Solvent Extraction Method**\(^5\)

Roots of *P. zeylanica* were dried for a month and finely pulverized using to get coarse powder. Then it was subjected to a series of solvent extractions following a modified Soxhlet’s extraction method.

The initial step involved the use of petroleum ether to extract fats and lipids from the root material. The resulting solid residue, referred to as the marc, was thoroughly dried to remove any remaining solid residues. It was then sequentially extracted using solvents with varying polarities. Each extraction was conducted for an extended duration of 72 hours to ensure thorough extraction of phytochemical constituents.

Following these exhaustive extractions, the resulting liquid extracts were meticulously filtered to remove any solid particles and impurities. Subsequently, the filtrates were concentrated to reduce the volume and remove the solvents. The concentrated extracts were then dried to obtain the final phytochemical extracts.

To assess the composition of these extracts, a preliminary phytochemical screening was conducted. This screening aimed to detect the presence of various phytochemical groups which were assumed to contribute for activity.

**Experimental Animals**

Albino rats (150–200 gm), were acquired from a vendor approved by CPCSEA. Animals were set aside in polycarbonate cages amid standard temperature conditions of CPCSEA. The experimental protocol was approved by IAEC.

**Acute Toxicity Study**

It was conducted in accordance to OECD rule 425. Female adult rats (\(n = 5\)) were subjected to an overnight fasting period with *ad libitum*.\(^6\)

Test substance was administered p.o using the up-and-down procedure, and individual rats were watched for duration of 48 hours.

**Experimental Design**

Wistar rats of either sex (\(n = 6, 150–200\) g) were divided in six groups and followed the treatment schedule as -

Control animals fed with distilled water while second group was fed with diazepam (7 mg/kg/i.p). Test drugs (PZR) were given at 100, 200 and 400 mg/kg via oral route. Also moderate dosage of PZR (200 mg/kg/p.o) combined with diazepam (7 mg/kg/i.p) treatment.

**Phyto-Chemical Testing of PZR**

An initial phytochemical analysis demonstrated the presence of a diverse array of bioactive constituents, which encompassed alkaloids, terpenoids, and tannins.

**Elevated plus maze**\(^7,8\)

It serves as a straightforward method for assessing learning and memory, with the crucial parameter being TL. To evaluate the nootropic potential of various plant extracts, an EPM apparatus was constructed for experimentation.

The experimental procedure commenced on the first day, precisely 90 minutes after last dose of the test substances. Each rat was kept facing the end of open arm far way from the center. TL was calculated as time taken by rat to move from end to the center and enter the closed arm with its all legs inside it.

On day 1\(^{st}\), the procedure was carried out and TL was measured which is taken as control. Retention of this learned task, indicative of memory, and examined precisely 24 hours after the original trial on the 29\(^{th}\) day.

This evaluation occurred 24 hours after the last dose administered during the four-week experimental period. All experimental animals were subjected to a spatial memory test using the EPM, carried out 90 minutes following the final dose. A notable reduction in TL values during the retention test signified an enhancement in memory performance.

**Morris water maze**\(^9,10\)

This task is a well-established method for investigating spatial learning and memory in rodents. This particular approach, along with its associated parameters for assessing mouse learning and memory, adhered to previously published protocols.

In this study, Morris water maze (MWM) method was employed to appraise the learning and memory of investigational animals at age of one month. Each rat underwent a sequence of four acquisition trials per day over the course of four consecutive days. This assessment took place immediately following the conclusion of a 4 weeks long dosing regimen. On the fifth day, a memory test was conducted, involving the removal of the escape platform (probe trial).

One day before the actual test, rats were allowed to swim in MWM for 60 seconds and here escape platform was not kept. This was done to get usual with the environment. On day of actual experimentation, the time took by rat to reach the safe (escape platform) was measured which is termed as TL and those who failed to reach within 120 seconds were removed from the study.

On third day of testing, which marked the 30\(^{th}\) day of the experiment, occurring 96 hours after final administered dose during a four-week duration, the escape platform was removed. The rats were allowed a 60-second free-swimming period, and changes in the parameter TL were documented as a measure of memory assessment.

**Statistical Analysis**

Statistical analysis involved the use of analysis of variance, followed by Tukey’s post hoc test. Significance was determined with a threshold of \(p < 0.05\).

**RESULTS**

**Phyto-Chemical Testing of PZR**

An initial phytochemical analysis demonstrated the presence of a diverse array of bioactive constituents, which encompassed alkaloids, terpenoids, and tannins.
The Outcome of PZR using EPM

The administration of *P. zeylanica* roots over four consecutive weeks led to a significant reduction in TL on the 29th day judged against control, demonstrating substantial progress in memory. Furthermore, the combination of *P. zeylanica* roots and diazepam in group VI also significantly ameliorated TL on the 29th day, underscoring the potency of the extract, as indicated in Figures 1 and 2.

Outcome of PZR using MWM

Based on the results outlined in Table 2, it becomes evident that process of learning and memory is closely linked to TL. A decline in TL, as observed in the MWM is pinpointing to enhancement in learning and memory.

Following the successive administration of different doses of *P. zeylanica* roots, in combination with diazepam (7 mg/kg, i.p.), over a period of four weeks, a noteworthy decrease in EL observed during assessments conducted on the mentioned days in contrast to control group. This decrease signifies a noteworthy improvement in the rats’ learning and memory capabilities. Among the various doses administered, the highest dose proved a pronounced and noteworthy measure for TL compared to a control group.

DISCUSSION

The phyto-pharmacological investigation of *P. zeylanica* for its memory-enhancing potential has yielded valuable insights into the neurocognitive effects of this traditional medicinal plant. Memory enhancement is a critical aspect of cognitive health and its implications for the management of neurodegenerative conditions have spurred significant interest in natural remedies. Here plant was discovered for nootropic bustle including MWM and EPM both of which are well-established models for evaluating learning and memory in rodents.12

MWM Findings

It is a gold standard in cognitive assessment, revealing that the administration of *P. zeylanica* roots in conjunction with diazepam led to a significant reduction in EL across multiple time points as compared to control. The remarkable improvements in EL are indicative of enhanced learning and memory in the treated rats. Furthermore, the dose-response relationship was evident, with the highest dose of 400 mg/kg via oral administration demonstrating the most pronounced and highly significant effect on TL compared to control.

These findings underscore the memory-enhancing potential of *P. zeylanica* and highlight the importance of further investigating the specific bioactive compounds responsible for this effect. Understanding the molecular mechanisms behind the plant’s impact on memory may grant precious insights for improvement of novel cognitive enhancement interventions.13

EPM Results

It offered an additional perspective on the memory-enhancing properties of *P. zeylanica*. The EPM is a relatively uncomplicated yet effectual method for assessing learning and memory, primarily using TL as a key parameter. The substantial reduction in TL observed in rats following the administration of *P. zeylanica* root extract corroborated the findings from the MWM test. Notably, this reduction in TL indicates a significant improvement in memory and cognitive function.

The nootropic potential of *P. zeylanica*, as demonstrated through these behavioral assays, holds promise for the development of memory-enhancing agents, especially in the situation of age-related cognitive decline and neurodegeneration. These results align with previous studies that have highlighted cognitive advantage of this plant in various experimental models.14-16

However, it is important to acknowledge that while these findings are promising, the precise neuropharmacological mechanisms underlying the memory-enhancing effects of *P. zeylanica* remain to be elucidated. Future studies should delve deeper into the identification and isolation of specific bioactive compounds within the plant and explore their interaction with neuronal pathways involved in memory consolidation and retrieval.

CONCLUSION

The phyto-pharmacological investigation underscores the potential of *P. zeylanica* as a natural source of nootropic compounds with memory-enhancing capabilities. Further research is warranted to unravel the mechanisms at play and to translate these findings into therapeutic interventions for cognitive disorders and age-related memory decline.
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REFERENCES