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# Evaluation of nootropic and safety profiles of berberine and ferulic acid in wistar rats

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**Abstract**

The present study evaluates the acute oral toxicity and cognitive-enhancing potential of berberine and ferulic acid, isolated from *Tinospora cordifolia* and *Centella asiatica*, respectively. Acute toxicity studies performed according to OECD 423 guidelines revealed no mortality or severe behavioral abnormalities up to 2000 mg/kg, confirming the safety of the polyherbal suspension (PHS). Cognitive performance was assessed using scopolamine-induced amnesia models in Wistar rats via T-maze, Morris Water Maze, and Novel Object Recognition tests. Results demonstrated significant improvements in transfer latency, escape latency, probe trial retention, and recognition index in treatment groups compared to scopolamine controls. Combination therapy exhibited the most pronounced effect, surpassing the efficacy of individual compounds. These findings validate the synergistic nootropic potential of berberine and ferulic acid and provide a scientific rationale for their use in polyherbal formulations targeting cognitive impairment.

**Keywords:** Berberine, Ferulic acid, Wistar rats, Nootropic activity, Acute oral toxicity, Morris Water Maze, Novel Object Recognition, Scopolamine

## Introduction

Cognitive decline associated with neurodegenerative disorders such as Alzheimer's disease is a major global health challenge. Current therapies offer limited efficacy and are often accompanied by adverse effects, prompting interest in natural bioactive compounds with neuroprotective properties (Zhu *et al*., 2018) [5]. *Tinospora cordifolia* (Guduchi) is a well-established medicinal plant recognized for its immunomodulatory, antioxidant, and neuroprotective properties. Its stem contains bioactive alkaloids, flavonoids, and phenolics, which contribute to memory enhancement and cholinesterase inhibition (Saha & Ghosh, 2012) [2]. Conversely, *Centella asiatica* (Gotu kola), widely used in Ayurvedic medicine, is renowned for its cognitive enhancement, wound healing, and neuroprotective actions, attributed to triterpenoid saponins such as asiaticoside and madecassoside (James & Dubery, 2013; Matthews *et al*., 2021) [3, 4].

While traditional uses highlight their potential in improving cognitive health, comparative experimental studies combining these two bioactives remain limited. The present investigation was designed to evaluate the safety profile and nootropic activity of berberine and ferulic acid, individually and in combination, in scopolamine-induced amnesia models in Wistar rats.

## Materials and methods

### Experimental animals

Male Wistar rats (180–200 g) were housed under controlled conditions (25 ± 2 °C, 12 h light/dark cycle) with free access to food and water. Protocols were approved by the institutional ethics committee.

### Acute oral toxicity study

Toxicity was evaluated as per OECD 423 guidelines. Rats received polyherbal suspension (PHS: berberine + ferulic acid) at doses of 5, 50, 300, and 2000 mg/kg orally and were monitored for 14 days for behavioral, physiological, and mortality parameters.

### Induction of amnesia

Amnesia was induced by intraperitoneal injection of scopolamine (1 mg/kg), a muscarinic receptor antagonist widely used to model memory impairment in rodents (Srinivasan *et al*., 2019) [6].

### Drug administration

- **Group I**: Normal control (vehicle)
- **Group II**: Scopolamine (inducer)
- **Group III**: Berberine (10 mg/kg, p.o.) + Scopolamine
- **Group IV**: Ferulic acid (10 mg/kg, p.o.) + Scopolamine
- **Group V**: Combination (1:1, berberine + ferulic acid, 1 mg/mL, p.o.) + Scopolamine

### Behavioral Studies

**a) T-maze test:** Transfer latency (TL) measured spatial

working memory.

**b) Morris Water Maze (MWM):** Escape latency (learning) and probe trial (retention).

**c) Novel Object Recognition (NOR):** Recognition Index (RI) determined memory performance. (Ennaceur & Delacour, 1988).

**Statistical analysis**

Data were expressed as mean ± SD. One-way ANOVA followed by Bonferroni's post-hoc test was applied.

## Results and discussion

### Acute oral toxicity

No mortality was observed up to 2000 mg/kg. Mild reversible effects (hair loss, nasal discharge) occurred at higher doses. Body weight remained stable across groups.

**Table 1:** Effect of Polyherbal Suspension (PHS) on body weight and mortality in wistar rats

| Group | Dose (mg/kg) | Mortality | Body Weight (Day 0, g) | Body Weight (Day 7, g) | Body Weight (Day 14, g) |
|---|---|---|---|---|---|
| A | 5 | 0/3 | 193–199 | 184–189 | Stable |
| B | 50 | 0/3 | 186–190 | 185–191 | Stable |
| C | 300 | 0/3 | 198–200 | 190–198 | Stable |
| D | 2000 | 0/3 | 180–185 | 176–181 | Stable |

This absence of toxicity supports the safety of PHS for further pharmacological testing, consistent with previous reports that berberine and ferulic acid are well-tolerated at high doses (Zhu *et al*., 2018; Srinivasan *et al*., 2019) [5, 6].

### T-maze performance

Scopolamine significantly increased TL (51.3 ± 3.6 sec). Berberine and ferulic acid reduced TL, while combination treatment showed the greatest reduction.

**Table 2:** Effect of PHS and isolated compounds on transfer latency in T-maze test

| Group | Treatment | Transfer Latency (sec) |
|---|---|---|
| I | Normal Control | 32.3 ± 2.8 |
| II | Scopolamine | 51.3 ± 3.6 |
| III | Berberine + Scopolamine | 29.7 ± 2.4* |
| IV | Ferulic Acid + Scopolamine | 27.4 ± 2.1* |
| V | Combination + Scopolamine | 21.2 ± 1.9** |

(*$p < 0.05$, **$p < 0.001$ vs. Scopolamine group)

### Morris Water Maze (MWM)

Escape latency was significantly reduced in treated groups, with combination treatment showing the best performance.

**Table 3:** Effect of PHS and isolated compounds on escape latency (acquisition phase)

| Group | Treatment | Day 1 (sec) | Day 2 (sec) | Day 3 (sec) | Day 4 (sec) |
|---|---|---|---|---|---|
| I | Normal Control | 48.2 ± 3.1 | 36.5 ± 2.8 | 27.1 ± 2.6 | 18.6 ± 2.4 |
| II | Scopolamine | 62.7 ± 4.3 | 57.3 ± 4.0 | 52.1 ± 3.8 | 46.5 ± 3.9 |
| III | Berberine + Scopolamine | 50.4 ± 3.2 | 38.7 ± 2.9 | 30.2 ± 2.7 | 25.3 ± 2.5* |
| IV | Ferulic Acid + Scopolamine | 49.1 ± 3.3 | 36.9 ± 3.0 | 29.6 ± 2.5 | 23.6 ± 2.3* |
| V | Combination + Scopolamine | 44.6 ± 3.1 | 30.8 ± 2.7 | 21.5 ± 2.2 | 15.4 ± 1.8** |

(*$p < 0.05$, **$p < 0.001$ vs. Scopolamine group)

**Table 4:** Effect of PHS and isolated compounds on probe trial performance (memory retention)

| Group | Treatment | Time in Target Quadrant (sec) |
|---|---|---|
| I | Normal Control | 38.5 ± 2.9 |
| II | Scopolamine | 15.6 ± 2.1 |
| III | Berberine + Scopolamine | 29.2 ± 2.4* |
| IV | Ferulic Acid + Scopolamine | 31.4 ± 2.3* |
| V | Combination + Scopolamine | 42.8 ± 2.6** |

(*$p < 0.05$, **$p < 0.001$ vs. Scopolamine group)

The combination's superior effect supports a complementary mechanism: berberine enhancing cholinergic signaling (Zhu *et al*., 2018) [5] and ferulic acid providing antioxidant and neuroprotective benefits (Srinivasan *et al*., 2019) [6].

### Novel Object Recognition (NOR)

Recognition index was lowest in scopolamine-treated rats. Combination therapy significantly improved RI beyond normal control values.

**Table 5:** Effect of PHS and isolated compounds on recognition index in NOR test

| Group | Treatment | Recognition Index (%) |
|---|---|---|
| I | Normal Control | 68.4 ± 3.1 |
| II | Scopolamine | 41.2 ± 2.8 |
| III | Berberine + Scopolamine | 59.7 ± 2.6* |
| IV | Ferulic Acid + Scopolamine | 61.3 ± 2.7* |
| V | Combination + Scopolamine | 74.5 ± 2.5** |

(*$p < 0.05$, **$p < 0.001$ vs. Scopolamine group)

## Conclusion

A study in Wistar rats demonstrates that berberine and ferulic acid are safe and exhibit significant neuroprotective effects. When combined, they provide superior protection against scopolamine-induced memory impairment compared to individual treatments. These results provide scientific validation for the traditional use of polyherbal formulations containing *Tinospora cordifolia* and *Centella asiatica* in supporting cognitive health. The enhanced efficacy of the combination highlights its potential as a therapeutic strategy for preventing or managing neurodegenerative disorders. This study bridges traditional herbal knowledge with modern neuroscience, suggesting promising avenues for natural interventions in cognitive decline.

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