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Research Article

Evaluate the neuroprotective effects of *Clitoria ternatea* root Extract on chemotherapy-induced cognitive impairments in rats

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Abstract

Chemotherapy-induced cognitive impairment (CICI), commonly referred to as "chemo brain," is a significant neurological complication associated with cancer chemotherapy, characterised by deficits in memory, learning, attention, and executive functions. Currently, effective therapeutic strategies to prevent or manage CICI are limited. *Clitoria ternatea*, a medicinal plant widely used in traditional medicine, possesses notable neuroprotective, antioxidant, anti-inflammatory, and nootropic properties. The present study was designed to evaluate the neuroprotective effects of *Clitoria ternatea* root extract against chemotherapy-induced cognitive impairments in rats. Cognitive dysfunction was experimentally induced using a standard chemotherapeutic agent, and the rats were treated with *Clitoria ternatea* root extract at selected doses. Behavioural assessments, including learning and memory paradigms, were employed to evaluate cognitive performance. Biochemical parameters related to oxidative stress and neuroinflammation were also analysed in brain tissue. The results demonstrated that treatment with *Clitoria ternatea* root extract significantly improved cognitive performance and attenuated chemotherapy-induced behavioural deficits. Additionally, the extract restored antioxidant enzyme levels and reduced oxidative stress markers, indicating its neuroprotective potential. These findings suggest that *Clitoria ternatea* root extract may serve as a promising natural therapeutic agent for the prevention and management of chemotherapy-induced cognitive impairment. Further studies are warranted to elucidate the precise molecular mechanisms underlying its neuroprotective effects.

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1. INTRODUCTION

1.1 Chemotherapy-Induced Cognitive Impairment (CICI)

Chemotherapy-Induced Cognitive Impairment (CICI), colloquially known as "chemo brain" or "chemo fog," is a debilitating side effect characterised by deficits in memory, attention, learning, and executive function. These changes can arise during active treatment or persist for decades following therapy, significantly affecting the quality of life (QOL) for cancer survivors [9, 10]. While the exact neurological implications remain a subject of debate, preclinical investigations suggest that chemotherapy targets the Central Nervous System (CNS) at cellular and molecular levels, disrupting synaptic activity and hippocampal plasticity [7, 8]. A primary driver of long-term neurotoxicity is the damage inflicted on neural progenitor cells within the adult CNS.

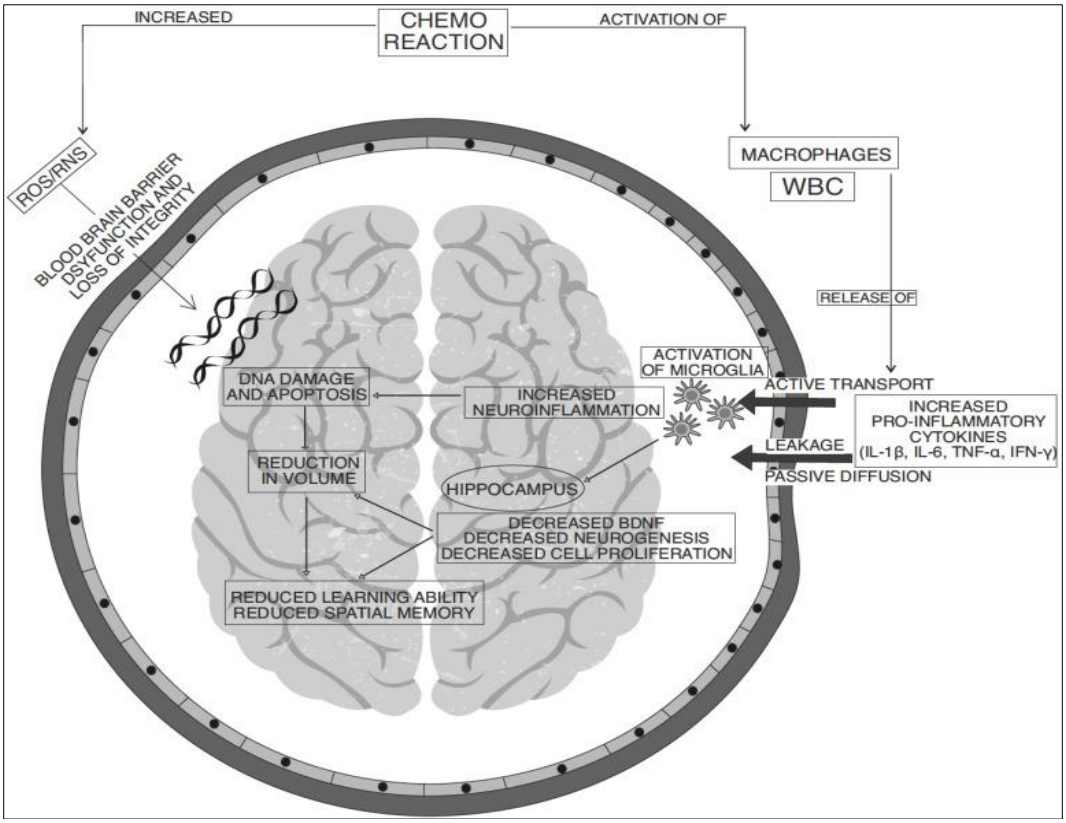

Fig 1: Schematic representation of "Chemo Brain" and its impact on CNS integrity.

1.2 Incidence and Epidemiology

Clinical data indicate that approximately 35–75% of cancer patients experience cognitive impairment during treatment [11]. Meta-analyses show that CRCI (Cancer-Related Cognitive Impairment) remains prevalent at rates of 23% within one year and 31% beyond one year post-treatment [13]. In some subsets, these deficits, including slowed processing speed and impaired verbal memory, persist for up to 20 years [17].

In the Indian context, the burden is particularly significant. With approximately 1.4 million new cancer cases annually (ICMR, 2022), the population at risk for CICI is expanding rapidly. However, the condition remains underdiagnosed in Indian clinical settings due to a lack of culturally validated screening tools and limited awareness [21].

1.3 Aetiology and Principal Sites of Damage

The aetiology of CICI is multifactorial, involving direct neurotoxicity where drugs cross the blood-brain barrier (BBB) to cause white-matter injury and synaptic dysfunction [27]. Neuroimaging has identified the prefrontal cortex and the hippocampus as the primary sites of damage [19, 20]. These regions are essential for learning and memory; thus, any depletion in grey or white matter density directly manifests as behavioural and cognitive decline [36].

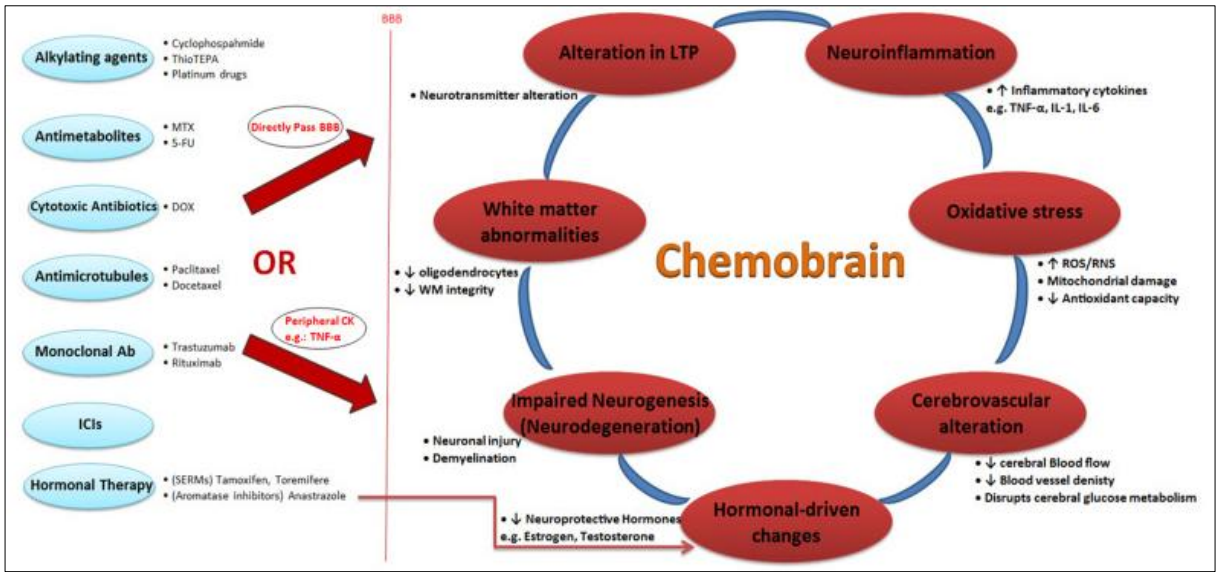

Fig 2: Etiology and Principal Anatomical Sites of CICI.

1.4 Pathophysiology and Molecular Mechanisms

The biological underpinnings of CICI involve several interrelated pathways:

- **Oxidative Stress:** Enhanced production of ROS/RNS leads to lipid peroxidation and mitochondrial energy failure.
- **Neuroinflammation:** Activation of microglia and astrocytes results in the release of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6 [27].
- **Neurotransmitter Dysregulation:** Decreased levels of acetylcholine, dopamine, and serotonin contribute to attention and memory deficits.
- **Impaired Neurogenesis:** Suppression of Brain-Derived Neurotrophic Factor (BDNF) inhibits the survival and maturation of neural progenitor cells in the dentate gyrus.

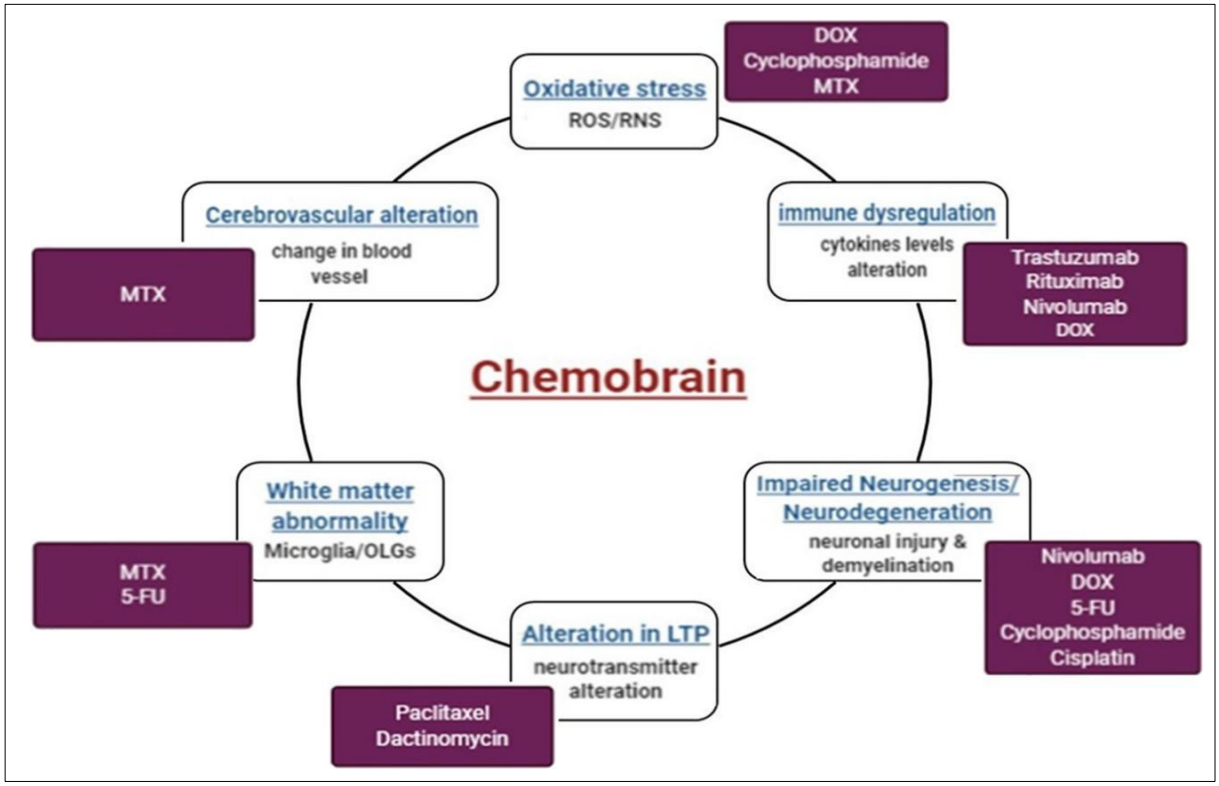

Fig 3: Pathophysiology of CICI and the cascade of molecular neurotoxicity.

1.5 Herbal Interventions for CICI Management

Given the absence of approved pharmacological treatments, herbal drugs offer a promising multi-targeted strategy. These natural agents contain bioactive phytoconstituents like flavonoids and polyphenols that mitigate oxidative stress and neuroinflammation [34].

Herb	Mechanism of Action
Curcuma longa	Modulates NF-κB/Nrf2 pathways; increases BDNF levels.
Bacopa monnieri	Protects against oxidative damage; enhances cerebral blood flow.
Withania somnifera	Suppresses TNF-α and IL-6; acts as a potent adaptogen.
Clitoria ternatea	Enhances cholinergic transmission; reduces AChE activity.
Ginkgo biloba	Stabilises neuronal membranes and improves microcirculation.

2. LITERATURE REVIEW

Chemotherapy-induced cognitive impairment (CICI), clinically characterised as "chemo brain" or "chemo fog," is a debilitating long-term side effect of cancer treatment. Aluise *et al*. (2005) [1] and Janelsins *et al*. (2007) [3] have established that CICI affects approximately 75% of patients during treatment, with 20–35% suffering from persistent deficits. Research by Winocur *et al*. (2006) [2] highlights that agents like methotrexate and doxorubicin trigger systemic neuroinflammation and oxidative stress. These biochemical changes lead to the elevation of proinflammatory cytokines (TNF-α, IL-1β) and a significant reduction in hippocampal neurogenesis, ultimately impairing spatial learning and executive function, as corroborated by Dietrich *et al*. (2015) [5] and Christie *et al*. (2016) [6].

In the search for neuroprotective interventions, *Clitoria ternatea* Linn. (Family: Fabaceae), popularly known as "Shankhpushpi," has emerged as a primary candidate. It is classified as a premier Medhya Rasayana (brain tonic) in the Ayurvedic system. Konat *et al*. (2008) [4] and Balaji *et al*. (2021) [13] report that the roots of this plant are particularly dense in bioactive secondary metabolites, including flavonoids, triterpenoids, and alkaloids. These constituents are primarily responsible for the plant's diverse Central Nervous System (CNS) activities, ranging from memory enhancement to anxiolytic and antidepressant effects.

Extensive preclinical evidence supports the neuroprotective efficacy of *C. ternatea*. Singh *et al*. (2009) demonstrated that root extracts significantly bolster the brain's antioxidant defence system by increasing levels of superoxide dismutase (SOD), catalase, and glutathione (GSH) while suppressing lipid peroxidation (MDA). Furthermore, Gupta *et al*. (2010) [12] observed anti-apoptotic effects in neuroblastoma cell lines, suggesting that the extract protects neuronal integrity against the oxidative insults that serve as a core mechanism in chemotherapy-induced damage.

The rationale for using herbal interventions in CICI is reinforced by studies on other botanical agents such as *Ginkgo biloba*, *Bacopa monnieri*, and *Curcumin*. Joshi *et al*. (2011) and Sharma *et al*. (2012) [22] found that these agents could restore cognitive function in cyclophosphamide and cisplatin models. However, *Clitoria ternatea* offers a unique multi-targeted approach by simultaneously enhancing acetylcholine levels and mitigating neuroinflammation, making it a robust candidate for managing chemotherapeutic neurotoxicity.

Despite this well-documented nootropic profile, Thakur *et al*. (2013) [23] and Li *et al*. (2014) [24] identify a critical research gap. Most studies have utilised scopolamine or stress-induced models rather than chemotherapy-specific models, and research has disproportionately focused on the aerial parts (leaves and flowers) rather than the pharmacologically potent roots. This represents a significant opportunity to evaluate *Clitoria ternatea* root extract as a novel, safe, and effective herbal adjunct to mitigate the cognitive decline associated with cytotoxic chemotherapy.

3. MATERIALS AND METHODS

3.1 Plant Material and Authentication

The roots of *Clitoria ternatea* were collected from Suryapura, Rohtas, Bihar, India, in January 2025. The material was authenticated by Dr Jaya Sahoo, Professor of Pharmacognosy at the Kurma Institute of Pharmaceutical Sciences, Bihar. A voucher specimen (No. 0.213) was deposited in the institutional herbarium.

3.2 Preparation of Extract

Roots were washed, shade-dried for two weeks, and ground into a coarse powder (40–60 mesh).

- **Method:** 50g of powder underwent hydroethanolic Soxhlet extraction using 500 mL of 70% ethanol (v/v).
- **Conditions:** The process ran for 6–8 hours (6–8 cycles/hour) until the solvent became colourless.
- **Concentration:** The filtrate was concentrated via rotary evaporator (40–45 °C) and lyophilised to obtain a dry powder.
- **Yield:** Calculated as: % Yield = (Weight of dried extract / Weight of powdered root) × 100.

3.3 Phytochemical Screening

Preliminary analysis was conducted using standard protocols to identify bioactive constituents:

- **Carbohydrates & Glycosides:** Molisch's test (purple ring) and Anthrone reagent (green complex).
- **Proteins & Amino Acids:** Ninhydrin test (purple) and Bradford's test.
- **Alkaloids:** Dragendorff's (orange/brown) and Mayer's (pale yellow) reagents.
- **Steroids & Triterpenes:** Liberman-Burchard, Salkowski, and Chloroform-H_2SO_4 tests.
- **Flavonoids:** Shinoda test (magnesium turnings/HCl; pink colour) and H_2SO_4 test.
- **Tannins & Saponins:** $FeCl_3$ (blue-black), Dilute HNO_3, and Froth formation/KOH tests.

3.4 Experimental Animals and Ethics
Adult Wistar albino rats (180–220 g) were acclimatised for 7 days under standard conditions (25±2 °C, 50-60% humidity, 12h light/dark cycle) with *ad libitum* access to food and water. All procedures were approved by the Institutional Animal Ethics Committee (IAEC) following CPCSEA guidelines.

3.5 Experimental Design and Induction
Rats were divided into five groups ($n=6$):
Group I: Normal Control (Saline).
Group II: Chemotherapy Control (Cyclophosphamide, 50 mg/kg i.p., once weekly for 4 weeks).
Group III: Cyclophosphamide + *C. ternatea* (100 mg/kg p.o.).
Group IV: Cyclophosphamide + *C. ternatea* (200 mg/kg p.o.).
Group V: Cyclophosphamide + Donepezil (Standard, 1 mg/kg p.o.).

Extract and standard drug treatments were administered daily for 28 consecutive days.

3.6 Acute Oral Toxicity Study
Conducted per OECD Guideline 423. Female Wistar rats received doses of 300 mg/kg and 2000 mg/kg p.o. and were observed for 14 days for behavioural changes or mortality. Doses for the main study were selected as 1/10th and 1/20th of the maximum tolerated dose.

3.7 Behavioural Parameters
Cognitive assessment was performed post-treatment using:

1. **Morris Water Maze (MWM):** Evaluated spatial learning (Escape Latency) and memory (Time in Target Quadrant) over 15 days.
2. **Y-Maze:** Measured short-term working memory via spontaneous alternation percentage (SAP %).
3. **Novel Object Recognition (NOR):** Assessed recognition memory by measuring the Discrimination Index (time spent with novel vs. familiar objects).
4. **Passive Avoidance Test:** Evaluated retention of aversive memory based on step-through latency into a dark compartment following a mild foot-shock.

3.8 STATISTICAL ANALYSIS
Data are expressed as Mean ± SEM. Statistical significance was determined using one-way ANOVA followed by Tukey's post hoc test ($p < 0.05$) using GraphPad Prism software.

4. RESULTS AND DISCUSSION
4.1 Extraction Yield and Organoleptic Properties
The hydroethanolic extraction of *Clitoria ternatea* roots resulted in a dry weight yield of 15.2%. The obtained extract (CTRE) exhibited a dark green colour and a semi-hard, viscous texture.

4.2 Preliminary Phytochemical Screening
The phytochemical investigation of the ethanolic extract confirmed the presence of several bioactive secondary metabolites, as summarised in Table 4.1.

Table 4.1: Phytochemical Analysis of *C. ternatea* Root Extract

S. No	Phytochemical Class	Test Method	Result	Interpretation
1	Flavonoids	NaOH / $FeCl_3$ / Lead Acetate	+	Flavonoids are present; they may contribute antioxidant and anti-inflammatory properties.
2	Alkaloids	Dragendorff's / Wagner's / Mayer's	+	Alkaloids are present; this could indicate potential pharmacological activity (e.g., analgesic, antimicrobial).
3	Glycosides	Molisch / Benedict's	+	Glycosides are present; they may have cardiac or therapeutic significance depending on type.
4	Tannins	Lead Acetate / $FeCl_3$	-	Tannins are absent; low likelihood of astringent effects.
5	Saponins	Foam Test / Lieberman Test	+	Saponins are present; they may contribute to foaming properties, expect some antimicrobial or cholesterol-lowering activity.

4.3 Behavioural Assessments
4.3.1 Locomotor Activity
Chemotherapy administration (Group II) induced a progressive and significant decline in locomotor activity ($p < 0.001$) from Day 5 through Day 15, reflecting neurotoxicity and systemic fatigue. Treatment with CTRE showed a dose-dependent recovery. The high dose (400 mg/kg) maintained activity levels closest to the Normal Control, indicating a protective effect against chemo-induced motor impairment.

4.3.2 Y-Maze: Spatial Working Memory
The Y-Maze spontaneous alternation percentage (SAP %) significantly dropped in the Chemo Control group (from 50% to 35% by Day 21), indicating impaired short-term memory.

- **Observation:** CTRE High Dose significantly reversed this decline ($65 \pm 2.5\%$ on Day 21), showing efficacy comparable to the standard drug, Donepezil.
- **Inference:** This suggests that CTRE enhances hippocampal-dependent memory and potentially inhibits acetylcholinesterase.

4.3.3 Passive Avoidance Test: Associative Learning
Chemotherapy-treated rats showed a sharp reduction in step-through latency, indicating a failure to retain aversive memory.

- **Results:** CTRE High Dose significantly ($p < 0.001$) increased the latency to 134 ± 2.5 seconds by Day 21.
- **Mechanism:** This improvement suggests that CTRE facilitates memory consolidation and retrieval by

protecting the structural integrity of the amygdala and hippocampus.

4.3.4 Novel Object Recognition (NOR)

The Discrimination Index (DI) was severely reduced in chemotherapy-treated rats, confirming impaired recognition memory.

- **Results:** CTRE (400 mg/kg) dose-dependently improved the DI, outperforming the low-dose group and matching the neuroprotective profile of Donepezil.

4.4 DISCUSSION

The present study provides the first direct evidence that CTRE effectively mitigates chemotherapy-induced cognitive impairment (CICI). The deterioration in the Chemo Control group across all paradigms prolonged escape latency in MWM, reduced SAP% in Y-Maze, and lower DI in NOR, confirming the development of "chemo brain."

4.5 Mechanistic Insights:

1. **Antioxidant Defence:** The phytochemicals (flavonoids and triterpenoids) identified in CTRE are known to scavenge reactive oxygen species (ROS) and reduce lipid peroxidation (MDA), thereby preserving neuronal health.
2. **Cholinergic Modulation:** The comparison with Donepezil suggests that CTRE may act by enhancing acetylcholine levels, which are crucial for memory encoding and retrieval.
3. **Neuroprotection:** The extract appears to suppress pro-inflammatory cytokines (TNF-α, IL-1β) and protect hippocampal CA1 neurons from apoptosis.

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