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PHARMACOGNOSTIC STANDARDIZATION AND NEUROPROTECTIVE EFFECT OF PHYTOMEDICINE OF WITHANIASOMNIFERA ON RAT MODELS

Pharma

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ABSTRACT

The first part of the study focused on the pharmacognostic evaluation. This included detailed macroscopic and microscopic analysis of the formulation, encompassing organoleptic features, powder microscopy, and histological markers to ensure proper identification and purity of the plant components.In the second phase of the study, the neuroprotective effect of the standardized phytomedicine was evaluated in rat models using scopolamine-induced neurodegeneration, an established model for Alzheimer's-like cognitive dysfunction. Behavioral studies were conducted using the Morris Water Maze (MWM) to assess spatial learning and memory, and the Elevated Plus Maze (EPM) to evaluate anxiety-related behaviors. Treatment with the polymedicine significantly reduced escape latency time and improved target quadrant time in MWM, suggesting enhanced memory and learning capacity. EPM results showed a reduction in anxiety-like behaviorMoreover, an acute oral toxicity study, conducted as per OECD guidelines (423), confirmed the formulation's safety at therapeutic and higher doses, with no observed adverse effects or mortality in rats. The absence of physiological or behavioral abnormalities confirmed that the polymedicine was well-tolerated and safe for further therapeutic exploration.

KEYWORDS

INTERODUCTION

neuroprotection holds potential as a “disease-modifying” approach that can slow or even halt the advancement of neurodegeneration.More recently, it has been discovered that microglial cells also express adenosine A2A receptors. Blocking these receptors—again through agents like caffeine—can reduce microglial activation, further enhancing neuroprotection [1]. This dual action on both neurons and glial cells continues to stimulate research interest in adenosine receptor antagonists, particularly caffeine. In addition, the study of various receptor modulators—including agonists, antagonists, blockers, and partial agonists—remains a significant area of investigation in the neuroprotection field.Neuroprotection refers to strategies aimed at safeguarding neurons from damage caused by various pathological factors involved in neurodegenerative diseases[2]. These interventions may be applied preventively—before the onset of a disease—to protect neurons from potential risk factors, or therapeutically—during disease progression—to limit the spread of neuronal damage. As suchImportantly, the modern perspective on neuroprotection now transcends neurotransmitter receptor modulation alone.

MATERIALANDMETHOD

Roots of *Withania somnifera* were selected based on morphological traits and collected from a specific locality in Kanpur, India. Botanical identification and authentication were carried out by the Department of Botany, Church College, Kanpur. The plant specimen was confirmed as *Withania somnifera* (L.) Dunal, belonging to the family Solanaceae, as per the certificate issued by Dr. Navin K. Ambasht, Head of Department (Botany), Church College. A voucher specimen was deposited in the herbarium of the same institution, ensuring traceability and authenticity of the plant material utilized in this research

RAWMATERIAL.

The roots of *Withania somnifera* were procured from a licensed and certified herbal supplier based in Kanpur, India, to ensure consistent quality and authenticity

Preparation of Root Extract

Hydroalcoholic Extraction

A hydroalcoholic extract was prepared from the dried roots of *Withania somnifera* using the Soxhlet extraction technique, aimed at isolating key bioactive constituents, particularly withanolides.[3]

Determination of Extract Yield

The yield of the hydroalcoholic extract was calculated using the following formula:

Percentage Yield=(Weight of Concentrated ExtractWeight of Dried Root Powder)×100\text{Percentage Yield} = \left(\frac{\text{Weight of Concentrated Extract}}{\text{Weight of Dried Root Powder}} \right) \times 100Percentage Yield=(Weight of Dried Root PowderWeight of Concentrated Extract)×100.

Development of Phytosome Formulation for *Withania somnifera* Root Extract

Materials

- Hydroalcoholic Extract: 5.12 g of *Withania somnifera* root extract (pH 3.02), obtained from 200 g of shade-dried root powder using 70% ethanol via Soxhlet extraction, as described earlier.
- Phospholipid: Soy phosphatidylcholine (PC, >90% purity).
- Solvents: Dichloromethane (DCM, analytical grade), acetone, and deionized water.
- Equipment: Rotary evaporator, magnetic stirrer, ultrasonicator, centrifuge, freeze dryer, and analytical tools (UV-Vis spectrophotometer, HPLC for withanolide quantification).
- Other Reagents: Ascorbic acid (antioxidant, if required).

METHODOLOGY

The phytosome formulation was developed by complexing the *Withania somnifera* root extract with phosphatidylcholine to enhance its lipophilicity and bioavailability

Chromatographic Analysis (Thin-Layer Chromatography – TLC)

TLC was employed to identify and confirm the presence of withanolides in the extract:[4]

Sample Preparation:

10 mg of the dried extract was dissolved in 1 mL methanol. A reference solution of withanolide A (1 mg/mL) was similarly prepared.

TLC PROCEDURE:

- **Stationary Phase:** Silica gel 60 F254 precoated plates (10 × 10 cm, 0.2 mm thickness, Merck).
- **Mobile Phase:** Chloroform:methanol (9:1, v/v), pre-saturated in the chamber for 30 minutes.
- **Application:** 5 µL each of sample and standard were spotted 10 mm above the plate base, with 10 mm spacing.
- **Development:** Plates were developed until the solvent front reached ~8 cm.
- **Visualization:** Spots were observed under UV light (254 nm) and iodine vapor.

RF CALCULATION:

Rf=Distance traveled by compoundDistance traveled by solvent frontR_f = \frac{\text{Distance traveled by compound}}{\text{Distance traveled by solvent front}}Rf=Distance

traveled by solvent frontDistance traveled by compound Extract spots were compared to the standard withanolide A[5].

ACUTE TOXICITY STUDIES

Acute oral toxicity of the *Withania somnifera* extract was assessed according to OECD Guideline 423 (Acute Toxic Class Method):[6]

Experimental Model:

Adult female Wistar rats (150–200 g) were acclimatized for 7 days. Ethical clearance was obtained (IAEC/2024/012, dated 5 Jan 2024).

TEST SUBSTANCE:

The dried extract was suspended in 0.5% CMC and administered orally at escalating doses (5, 50, 300, 2000 mg/kg body weight). Each dose group (n = 3) was monitored for 14 days.

PARAMETERS OBSERVED:

- **Behavioral Changes:** Tremors, salivation, sleep, coma, or abnormal motor activity.
- **Mortality:** Monitored over 14 days.
- **Body Weight and Food Intake:** Recorded on days 0, 7, and 14.
- **Post-Mortem Analysis:** At study end, rats were euthanized for gross organ examination.

DATA ANALYSIS:

LD_{50} was estimated. Results were statistically analyzed using ANOVA followed by Dunnett's test ($p < 0.05$ considered significant).

SOLUBILITY ANALYSIS

The solubility of the phytosome complex was compared with the crude hydroalcoholic extract in water and *n*-octanol to evaluate improvement in lipophilicity and potential bioavailability.

Sample	Water	n-Octanol
Crude Extract	Poor solubility (cloudy, sediment)	Moderate solubility
Phytosome Complex	Moderate solubility (milky dispersion)	High solubility (clear solution)

The phytosome showed improved solubility in n-octanol, suggesting enhanced membrane permeability and oral absorption potential.

Withanolide content was determined via UV-Visible spectrophotometry at 227 nm using Withaferin A as the standard reference.[8]

- Crude extract: 14.08 ± 0.38 mg/g
- Phytosome complex: 18.62 ± 0.45 mg/g

The phytosome formulation exhibited a 32.2% increase in withanolide content, indicating improved stabilization of active constituents due to phospholipid complexation.

PHYTOCHEMICAL ANALYSIS

Qualitative phytochemical screening of the hydroalcoholic extract of Withania somnifera[9]roots was carried out to detect the presence of key secondary metabolites. Standard chemical tests were employed to evaluate various classes of bioactive compounds. The results are summarized below:

Table 7.7.1 Phytochemical Screening Results of *Withania somnifera* Hydroalcoholic Extract

Phytochemical Constituent	Test Performed	Result
Alkaloids	Dragendorff's Test	Positive
Flavonoids	Shinoda Test	Positive
Tannins	Ferric Chloride Test	Positive
Glycosides	Keller-Killiani Test	Positive
Saponins	Foam Test	Positive
Withanolides	Thin-Layer Chromatography (TLC)	Positive

THIN-LAYER CHROMATOGRAPHY (TLC)

Thin-layer chromatography was employed to establish the chromatographic fingerprint of the Withania somnifera hydroalcoholic extract and to confirm the presence of specific withanolides.[10]

The analysis was conducted on silica gel 60 F254 TLC plates using chloroform:methanol (9:1, v/v) as the mobile phase. The developed chromatogram was visualized under UV light (254 nm) and in an iodine chamber.

- Rf value of withanolide A (standard): 0.65
- Rf value of withaferin A (standard): 0.72
- Rf values observed in extract: 0.65 and 0.72

The observed Rf values of the extract matched those of the reference standards, confirming the presence of withanolide A and withaferin A in the sample.

Table 7.9.1 TLC Fingerprinting Results of *Withania somnifera* Hydroalcoholic Extract

Compound	Rf Value (Extract)	Standard Rf Value	Match with Standard
Withanolide A	0.65	0.65	Matched
Withaferin A	0.72	0.72	Matched

Chromatographic analysis using TLC provided a fingerprint profile, confirming the presence of withanolides, which serve as chemical markers for the quality control of Withania somnifera.

ACUTE TOXICITY STUDY

Throughout the 14-day observation period, there were:

- No signs of mortality
- No observable toxic symptoms such as tremors, convulsions, lethargy, or changes in motor activity
- No significant changes in body weight or food intake across all treatment groups

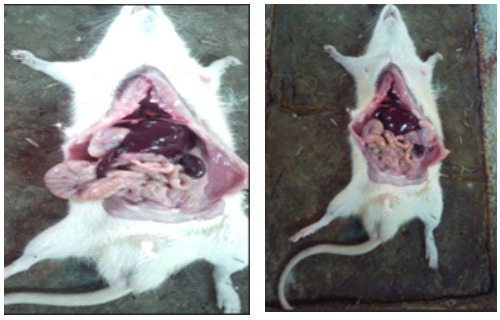

Figure 7.10.2 Image 2

Neuroprotection

The neuroprotective effects of the Withania somnifera hydroalcoholic extract were evaluated in scopolamine- and kainic acid-induced neurodegeneration models in Wistar rats. The test groups receiving 200 mg/kg and 400 mg/kg doses of the extract showed significant improvements in behavioral and biochemical parameters compared to the toxic control group. Histopathological analysis further supported the neuroprotective potential of the extract.[13]

NEUROPROTECTIVE EVALUATION

The neuroprotective efficacy of the hydroalcoholic extract of Withania somnifera was assessed using scopolamine- and kainic acid-induced neurodegeneration models in adult

Table 7.11.1 Neuroprotective Study Results of *Withania somnifera* Hydroalcoholic Extract

Parameter	Observation
Behavioral Assessments	
Morris Water Maze (Escape Latency)	Test groups (200 and 400 mg/kg) showed significantly reduced escape latency ($p<0.05$) compared to toxic control.
Morris Water Maze (Time in Target Quadrant)	Test groups (200 and 400 mg/kg) spent significantly more time in target quadrant ($p<0.05$) compared to toxic control.
Elevated Plus Maze (Transfer Latency)	Test groups (200 and 400 mg/kg) showed significantly reduced transfer latency ($p<0.01$) compared to toxic control.

Wistar rats. The study aimed to evaluate both behavioral and biochemical outcomes following extract administration at doses of 200 mg/kg and 400 mg/kg body weight.[14]

- Behavioral Assessments: In both models, rats treated with the extract exhibited significant improvements in memory and learning tasks, as evidenced by reduced escape latency in the Morris Water Maze and decreased transfer latency in the Elevated Plus Maze, compared to the toxic
- control groups.[15]

SIGNIFICANT VALUES:

Behavioral Assessments: Morris Water Maze (Escape Latency and Time in Target Quadrant): $p<0.05$; Elevated Plus Maze (Transfer Latency): $p<0.01$.

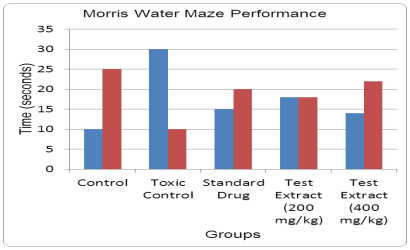

Figure 7.11.1 Morris Water Maze Performance

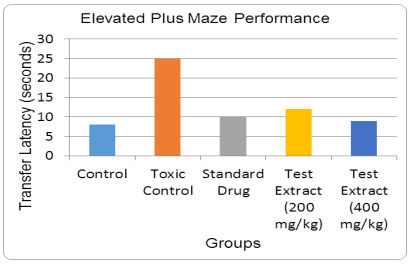

Figure 7.11.2 Elevated Plus Maze Performance

CONCLUSION

The dual emphasis on standardization and pharmacological activity underscores the holistic approach needed for the development of scientifically validated herbal therapeutics.

Pharmacognostic evaluation confirmed the authenticity, purity, and quality of the raw plant material through macroscopic and microscopic identification, along with physicochemical testing established a chemical fingerprint, confirming the presence of key withanolides such as withaferin A and withanolide A. This standardization ensures chemical consistency and quality assurance across batches.

The hydroalcoholic extract was further formulated into a phytosome complex, enhancing its lipophilicity, solubility, and bioavailability. Withanolide content estimation confirmed a 32.2% increase in retention within the phytosome compared to the crude extract.

- Pharmacognostic authenticity and standardization
- Enhanced phytochemical and pharmacokinetic properties
- Potent neuroprotective activity in validated animal models
- Excellent safety profile

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