Article

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Anti-Cataleptic Activity, Evaluation of Graded Doses and Different Ratios of Polyherbal Formulation Triphala against Haloperidol-Induced Catalepsy in Swiss Albino Mice

Kuntal Das[1], Benson Mathai[2,*], Preeti Kaur[3], Manjeshwar Shrinath Baliga[4], Saad Alobid[5], Ali Ibrahim Almoteer[6], Moneer E. Almadani[7], Fuzail Ahmad[8], Syed Imam Rabbani[9], Syed Mohammed Basheeruddin Asdaq[10,*]

[1]Department of Pharmacognosy, Mallige College of Pharmacy, 560090 Bangalore, India
[2]Department of Pharmacology, Dr. Joseph Mar Thoma Institute of Pharmaceutical Sciences and Research, 690503 Kerala, India
[3]Department of Pharmacology, St. John's Pharmacy College, 560104 Vijayanagar, Bangalore, India
[4]Mangalore Institute of Oncology, Mahavir Circle, Pumpwell, 575002 Mangalore, India
[5]Department of Pharmacology and Toxicology, College of Pharmacy, King Saud University, 11451 Riyadh, Saudi Arabia
[6]Department of Pharmacy, King Saud University Medical City, 12372 Riyadh, Saudi Arabia
[7]Department of Clinical Medicine, College of Medicine, AlMaarefa University, 13713 Riyadh, Saudi Arabia
[8]Department of Respiratory Therapy, College of Applied Sciences, AlMareefa University, 13713 Riyadh, Saudi Arabia
[9]Department of Pharmacology and Toxicology, College of Pharmacy, Qassim University, 51452 Buraydah, Saudi Arabia
[10]Department of Pharmacy Practice, College of Pharmacy, AlMaarefa University, 13713 Riyadh, Saudi Arabia
*Correspondence: bensonpharma74@gmail.com (Benson Mathai); sasdag@um.edu.sa; sasdaq@gmail.com (Syed Mohammed Basheeruddin Asdaq)
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Background: Extrapyramidal side effects are a considerable risk associated with most drugs used to treat psychotic illnesses, and they can negatively impact treatment outcomes. Consequently, there is an ongoing requirement to investigate alternative medicines for managing the side effects. Triphala consists of *Terminalia chebula* Retz. (Haritaki), *Terminalia bellirica* (Gaertn.) Roxb. (Bibhitaki) and *Emblica officinalis* Gaertn. (Amlaki) in equal ratio (1:1:1) is one of the most important polyherbal formulations used in the Indian system of medicine. The objective of the present study was to investigate the preventive efficacy of graded doses of different ratios of Triphala (formulations) and antioxidant effects against haloperidol in Swiss albino mice.

Methods: Graded doses (2.5, 6.25, 12.5 mg/kg) of Triphala in the ratio of 1:1:1, 1:2:3, and 1:2:4 proportions of the three myrobalans, or scopolamine (1.0 mg/kg) or ondansetron (0.5 mg/kg) were pretreated daily (per oral) for 7 days, before administration of haloperidol (5.0 mg/kg, i.p, on 7th day) to induce catalepsy in Swiss albino mice. The behavioral parameters were recorded using the standard bar test, akinesia, and rotarod test at various time points. The animals were euthanized, and the brain was used to study the levels of superoxide dismutase and lipid peroxidation. The data was subjected to a one-way analysis of variance (ANOVA) followed by the Dunnett test and a p-value of <0.05 was considered significant.

Results: The findings show that Triphala enhanced superoxide dismutase and decreased lipid peroxidation, and it significantly ($p < 0.001$) decreased the cataleptic, rotarod, and akinesia scores. The results were comparable with the observations recorded for standard drugs. Besides, lower (2.5 mg/kg) and higher (12.5 mg/kg) doses of Triphala formulations exhibited enhanced efficacy ($p < 0.001$) than standards at the initial periods after haloperidol exposure. Further, the improvement in antioxidant status was also found to be better than standards.

Conclusion: The data suggested that the Triphala formulations might possess better efficacy than scopolamine and ondansetron in preventing haloperidol-induced complications. More research in this direction might identify an alternative safe herbal medicine for preventing as well as treating the extrapyramidal side effects of antipsychotics.

Keywords: drug screening; herbal formulation; extrapyramidal side effects; antioxidant; scopolamine; ondansetron

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Introduction

Psychotic illnesses are mostly neurosis and psychosis, and the conventional treatment for psychosis is principally with drugs and behavioral therapies [1]. However, extended use of antipsychotics, like haloperidol, which is used to treat schizophrenia and other affective disorders, is linked to a high rate of extrapyramidal side effects (EPS), which can impair quality of life and necessitate additional medication to lessen drug-induced toxicity. Examples of EPS associated with antipsychotics include catalepsy, acute dystonias, akathisia, tardive dyskinesia, tachycardia, hypotension, impotence, lethargy, seizures, intense dreams or nightmares, and hyperprolactinemia [2,3].

Among different EPS, catalepsy is considered as one of the important conditions characterized mainly by the rigidity of extremities, decreased sensitivity to pain, and an animal behavioral state in which they fail to correct the externally imposed posture [3]. In rodents, the administration of neuroleptic drugs like haloperidol is reported to induce catalepsy-associated behaviors [4]. These neuroleptic drug-induced catalepsies have long been used as an animal model of EPS and to screen potential antiparkinsonian activity [5].

Schizophrenia medication users are severely burdened by EPS, and taking anticholinergic medications regularly to prevent EPS increases side effects and regimen costs. For instance, antipsychotic-induced EPS can be effectively prevented by anticholinergic medications like scopolamine [6]. However, the main shortcomings of these drugs are their numerous adverse effects, including dry mouth, blurred vision, constipation, urinary retention, and tachycardia, as well as a plethora of central nervous system (CNS) disturbances, encompassing decreased concentration, confusion, disorientation, attention deficits, and memory impairment [7]. Similarly, the use of ondansetron (5-HT_3 antagonist) for the management of EPS is reported to contribute headaches, constipation, tiredness, and drowsiness [8]. Such adverse effects of conventional drugs used to prevent EPS make it necessary to find new pharmacotherapy options that are safe and effective [9].

Triphala is one of the most important traditional polyherbal medicinal preparations and is composed of three myrobalans, namely *Terminalia chebula* Retz. (Haritaki; TC), *Terminalia bellirica* (Gaertn.) Roxb. (Bibhitaki; TB), and *Emblica officinalis* Gaertn, *Amalaki* or the Indian gooseberry (EO) [10]. Several scientific studies have shown the effectiveness of *Triphala* and its constituents in the treatment of catalepsy [11], memory loss [12], epilepsy [13], Alzheimer's disease [14], neuropathy [15], paralysis [16], and depression [17].

Triphala has been reported to be a rich source of vitamin C, ellagic acid, gallic acid, chebulinic acid, bellericanin, β-sitosterol, ascorbic acid, and flavonoids. Other constituents identified in the fruit include lipids, sitosterol, saponins, cardiac glycosides, and various carbohydrates [17]. Triphala also has antioxidant and nitric oxide-scavenging activities [16].

The Triphala formulation generally consists of equal proportions of the dried pericarps of TC, TB, and EO [18]. However, modified formulations of Triphala are also prevalent, and the two most important modifications are where TC, TB, and EO are in a 1:2:3 ratio and in a 1:2:4 ratio [18]. The standard combination of Triphala with TC, TB, and EO in a 1:1:1 ratio has been reported to possess anti-cataleptic activity [11]. However, other known formulations such TC, TB and EO in 1:2:3 and 1:2:4 ratios are not well studied for anti-psychotic medications-induced EPS in the literature. Hence, for the first time, the current study is planned to test the anti-cataleptic activity of the remaining two Triphala formulations (1:2:3 and 1:2:4) using a haloperidol-induced catalepsy mouse model. The current analysis was designed to find the best Triphala formulation among the three and compare its preventive efficacy with available standard drugs (such as scopolamine and ondansetron) for treating extrapyramidal side effects induced by haloperidol.

Materials and Methods

Drug and Reagents

Triphala powder was procured from Natural Remedies Ltd., Bangalore, Gum acacia from Nice Chemicals, India (B.N. 2125), scopolamine from German Remedies Ltd., Mumbai, India (Lot # 3118) Sodium carbonate and Bicarbonate, ethylenediaminetetraacetic acid (EDTA) from Nice chemicals, Cochin, India (B.N. 295 and B.N. 3668), Trichloroacetic acid by Bangalore fine Chemicals, Bangalore, India (Batch # 4821), Thiobarbituric acid from Spectrochem India Pvt. Ltd., Mumbai, India (Lot No. 2214), Ondansetron from Cipla Ltd., Mumbai, India (Batch No. CP-4521) and Haloperidol from RPG Life Sciences, Ltd., Mumbai, India (Batch # R-010799).

Preparation of Different Triphala Formulations

The powders of the individual constituents of Triphala, *T. bellerica*, *T. chebula*, and *E. officinalis* were obtained from Natural Remedies Ltd., Bangalore, India (batch no. TB-1102; TC-1106 and EO-1115 respectively). For the Triphala 1:1:1 ratio formulation (F1) equal quantities of *T. chebula*, *T. bellerica*, and *E. officinalis* powder were in a blender for five minutes to attain uniform mixing. For the Triphala in a 1:2:3 ratio (F2) one part of *T. chebula*, two parts of *T. bellerica*, and three parts of *E. officinalis* were accurately weighed and mixed; while for the Triphala in a 1:2:4 ratio (F3) one part of *T. chebula*, two parts of *T. bellerica* and four parts of *E. officinalis* were accurately weighed and mixed to attain uniform mixing. The aqueous extract of Triphala powder was prepared as described earlier [19]. Briefly, 100 grams of the powder [F1, F2, or F3 ratio] was boiled in 1000 mL of double distilled water till

the volume was reduced to one-fourth of the original (250 mL). After that, the extract was cooled and filtered through two layers of filter paper in addition to cotton cloth at first. By letting the filter's liquid contents evaporate in a water bath within an evaporating china dish, the filtrate was gathered and concentrated. The extract obtained was scraped off from the china dish into a clean plastic bottle. An approximate 22.5% yield of the extract was obtained for F1, 22.8% for F2, and 28.7% yield for F3. The required amount of Triphala extract was freshly prepared every day taking into consideration the dose to be used (2.5, 6.25, 12.5 mg/kg) and the formulations (F1 or F2 or F3) carefully.

Drug Treatments

Haloperidol (RPG Life Sciences Ltd., Mumbai, India, Batch No. R-010799) was dissolved in distilled water and administered at a 5.0 mg/kg concentration. Scopolamine (anti-cholinergic drug) in 1.0 mg/kg concentration [20] (German Remedies Ltd., Mumbai, India, Lot # 3118) was dissolved in 1% gum acacia (Nice chemicals, Cochin, India, B.N. 2125) solution and administered orally. Ondansetron (5-HT_3 antagonist) (Cipla Ltd., Mumbai, India, Batch No. CP-4521) was dissolved in 1% gum acacia solution and administered orally in 0.5 mg/kg doses [21]. For seven days, all drugs were administered orally daily. On the seventh day, after 1 hour of the last drug dosing, haloperidol was administered intraperitoneally, and various parameters were recorded at different times post-haloperidol exposure.

Experimental Animals and Maintenance

Swiss albino mice were used and maintained as per the animal ethical guidelines given by the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA), and the experimental designs were approved by the Institutional Animal Ethics Committee (IAEC) (Ref. no. SJPC/IAEC/M-28/2010). About 6–8 weeks aged weighing up to about 20–25 g mice were procured from Central animal facility, St. John's Pharmacy College, Bangalore, Karnataka, and were aboded in polypropylene cages and provided standard pellet diet (Agro Corporation Pvt. Ltd., Mumbai, India, Batch No. 145/22) and excess of filter water. All experiments including handling and maintenance were performed humanely according to the guidelines of CPCSEA.

Experimental Design

The animals in the present study were allocated randomly and divided into 12 groups as described in Table 1.

Behavioral Parameters

Standard Bar Test

Catalepsy was determined by the standard bar test (Implex Scientific Inst Ltd., Ambala, India, Model No. 6621), and the degree of catalepsy was measured at 30, 60, 90, and 120 min after 30 minutes of Haloperidol administration and was scored by the method described previously [22]. The cataleptic scoring pattern is as follows; if the animal maintained the imposed posture for at least 20 seconds when placed on a standard bar, it was considered cataleptic and was given one point. For every additional 20 seconds for which the cataleptic posture was maintained, one extra point was given.

Akinesia

Akinesia was measured using a elevated wooden block (30 cm) platform (40 cm × 40 cm) (SYNAX Instruments Ltd., Ambala, India, Model No. 9227). The procedure includes placing the experimental animals on the wooden platform and recording the latency period. Each animal was acclimatized for 5 minutes on wooden platform before starting the test. Akinesia scores were recorded by noting the latency in seconds (s) in moving all four limbs. Using a stopwatch, the time taken by the animal to move all four limbs was recorded. Further, the test was terminated if the latency period exceeds 120 seconds [23].

Rotarod Test

The Rotarod test was carried out by placing each animal in 5-compartment rotarod equipment (Dolphin Labs, Mumbai, India, Model # 2213), and the time was noted for 5 min time intervals. The speed of rotation was kept appropriate (20–25 rpm). Each animal was initially acclimatized to the equipment. The "fall off time" when the mouse falls from the rotating rod was noted. A cut-off time of 300 s (5 min) was applied. The scoring pattern is recorded by observing the number of limbs the animal grips on the rotating rod when it is moved at an acceleration of 20–25 rpm/min [24].

Antioxidant Enzyme Estimation

The experimental mice were euthanized under the supervision of a veterinarian who is also a member of the Institutional Animal Ethics Committee. Humane care and precautions were followed while performing the euthanasia on the experimental animals. As indicated in the literature, the procedure was conducted by utilizing anesthetic agents [25]. An injection of 5% ketamine/xylazine (NexGen Pharmaceutical Ltd., Mumbai, India, Lot No. 01328) diluted in normal saline (2 mL) was administered to animals by intraperitoneal route [25]. After confirming the death of animals, the brain was collected and homogenized. The homogenate solution was used for biomarker estimation such as lipid peroxidation (LPO) (HiMedia Laboratories Pvt Ltd., Mumbai, India, Lot # 3416) [26] levels, and superoxide dismutase (SOD) (HiMedia Laboratories Pvt Ltd., Mumbai, India, Lot # 74431) [27] enzyme activity was calculated and reported.

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Table 1. Experimental protocol for pretreating the animals with Triphala.

Study	Treatment	Dosing
Group 1	Vehicle (control)	10 mL/kg
Group 2	Scopolamine	1.0 mg/kg
Group 3	Ondansetron	0.5 mg/kg
Group 4		2.5 mg/kg
Group 5	F1 = 1:1:1 of *Terminalia chebula*, *Terminalia bellirica* and *Emblica officinalis*	6.25 mg/kg
Group 6		12.5 mg/kg
Group 7		2.5 mg/kg
Group 8	F2 = 1:2:3 of *Terminalia chebula*, *Terminalia bellirica* and *Emblica officinalis*	6.25 mg/kg
Group 9		12.5 mg/kg
Group 10		2.5 mg/kg
Group 11	F3 = 1:2:4 of *Terminalia chebula*, *Terminalia bellirica* and *Emblica officinalis*	6.25 mg/kg
Group 12		12.5 mg/kg

Haloperidol (5 mg/kg); Number of animals for each group = 6; Std.1 = Scopolamine; Std.2 = Ondansetron. Ratios of Triphala: F1 = 1:1:1; F2 = 1:2:3; F3 = 1:2:4.

Lipid Peroxidation in the Brain

The assay method was followed as per the protocol described previously [26]. In brief, two milliliters of suspension medium were taken from 10% of tissue homogenate. To this, 2 mL of 30% of trichloroacetic acid (TCA; HiMedia Laboratories Pvt Ltd., Mumbai, India, Lot # 0015) was added, followed by 2 mL of 0.8% thiobarbituric acid (TBA; HiMedia Laboratories Pvt Ltd., Mumbai, India, Lot # 1124) reagent. The tubes were covered with aluminum foil and kept in a shaking water bath for half an hour at 80 °C after half an hour; the tubes were taken out and kept in ice-cold water for half an hour. They were then centrifuged at 3000 rpm for 15 minutes. The absorbance of the supernatant was read at 535 nm at room temperature against the appropriate blank. Blank consists of 2 mL distilled water, 2 mL of 30% TCA and 2 mL of 0.8% TBA. The content of malondialdehyde (MDA), expressed as moles formed per milligram of protein in the tissue, was calculated using the formula, concentration = A × (V/E) × P where A is the volume of solution, E is extinction coefficient (1.56×10^5 M^{-1} cm^{-1}), and P is the protein content of tissue calculated as milligram of protein per gram of tissue.

Superoxide Dismutase (SOD) Assay

The assay method was followed as reported earlier [27]. In brief, the brain was dissected out and homogenized with ice-cold saline and centrifuged at 3000 rpm for 10 min at 4 °C. Then, 0.1 mL of homogenate sample was mixed with 0.1 mL EDTA (1×10^{-4} M) (HiMedia Laboratories Pvt Ltd., Mumbai, India, Lot # 1016), 0.5 mL of carbonate buffer (pH 9.7), and 1.0 mL of epinephrine (3×10^{-3} M) (Laborate Pharma P Ltd., Haryana, India, Batch # 0151) in the test tube. The optical density of the formed adrenochrome was read to 480 nm for 3 min at an interval of 30 sec, and results were expressed as U/gm of tissue.

Statistical Analysis

The results were expressed as mean ± standard error (SEM) (n = 6). The statistical analysis was performed using a one-way analysis of variance (ANOVA) followed by the Dunnett test. A p-value at <0.05 was considered statistically significant. Data was processed with GraphPad Prism version 5.00 software (Dotmatrics,Scientific Ltd., La Jolla, CA, USA).

Results

Cataleptic Activity

The observation recorded with different treatments on the cataleptic scores after haloperidol administration is represented in Fig. 1. The study indicated that haloperidol administration has shown a sequential increase in catalepsy scores at various time durations. When compared to the vehicle control, both scopolamine and ondansetron significantly ($p < 0.01$) reduced cataleptic ratings at all examined periods (30, 60, 90, 120, and 240 minutes). It was also shown that administering the Triphala formulations (F1, F2, and F3) markedly ($p < 0.01$) lowered the cataleptic scores brought on by haloperidol.

The data from the study suggested that F1 formulation at high dose (12.5 mg) and F2 Triphala at low (2.5 mg) and F3 (2.5 mg) doses provided better protection ($p < 0.001$) than scopolamine against haloperidol-induced catalepsy at 30 min intervals. Further, when the test was conducted at 90, 120, and 240 min, all the doses of Triphala formulations (F1, F2, and F3) exhibited equipotent protection against haloperidol-induced cataleptic scores, and the effect was found to match with the efficacy ($p < 0.001$) of scopolamine. Additionally, the results recorded with Triphala match with the efficacy of ondansetron as well, in reducing the cataleptic scores induced by haloperidol.

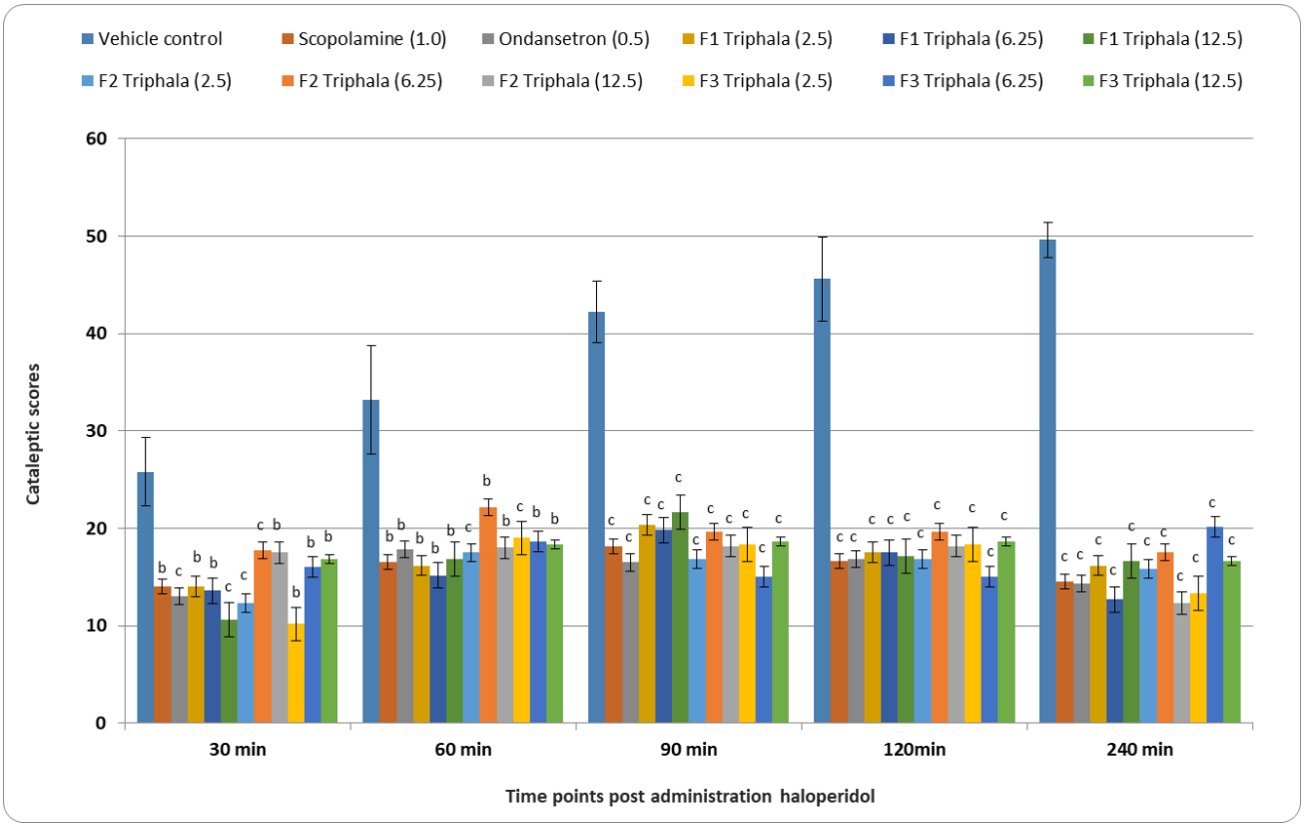

Fig. 1. Preventive effect of different formulations of Triphala on the cataleptic scores at various time intervals after haloperidol administration. Note: All values are expressed as Mean ± standard error (SEM) (n = 6 in each group). p values: b < 0.01, c < 0.001 as compared with vehicle control (1.0% gum acacia) by one-way analysis of variance (ANOVA) followed by Dunnett test.

Rotarod Test

Fig. 2 summarizes the influence of various treatments on the rotarod scores after haloperidol administration. The vehicle control group that did not receive any drug treatment showed a progressive increase in the rotarod scores after the administration of haloperidol. When compared to vehicle control, the two common medications used to lessen haloperidol complications—scopolamine and ondansetron—were found to significantly ($p < 0.01$) lower the rotarod scores at various test durations. Different formulations of Triphala (F1, F2, and F3) at various doses were found to reduce significantly ($p < 0.01$) the rotarod scores after haloperidol exposure.

The important findings of the study suggested that lower (2.5 mg) and higher (12.5 mg) doses of F2 Triphala produced better protection compared to the standard drugs (scopolamine and ondansetron) in reducing the haloperidol-induced rotarod scores before 90 min. Moreover, all the tested doses of F1, F2, and F3 formulations beyond 90-minute intervals after haloperidol exposure were found to have similar efficacy in reducing the rotarod scores like those of standard drugs.

Akinesia Test

The findings of the study revealed that, when compared to the vehicle control, the conventional medication scopolamine significantly ($p < 0.01$) reduced the akinesia scores following haloperidol at various time intervals (Fig. 3). Similarly, when compared to the control, ondansetron, another standard agent, was found to be preventive ($p < 0.01$) in decreasing akinesia scores at different time intervals. Triphala was tested in three formulations (F1, F2, and F3), and the results showed that different doses of Triphala formulation were effective ($p < 0.01$) in reducing akinesia scores after haloperidol administration.

Analysis of the data from the findings suggested that all the formulations of Triphala have shown potency in reducing the akinesia scores after haloperidol administration. The efficacy of the polyherbal formulations appears to match with the standard drugs such as scopolamine and ondansetron. In addition, the F1 formulation of Triphala was observed to be better than standards in preventing the haloperidol-induced akinesia at all intervals of exposure (Fig. 3).

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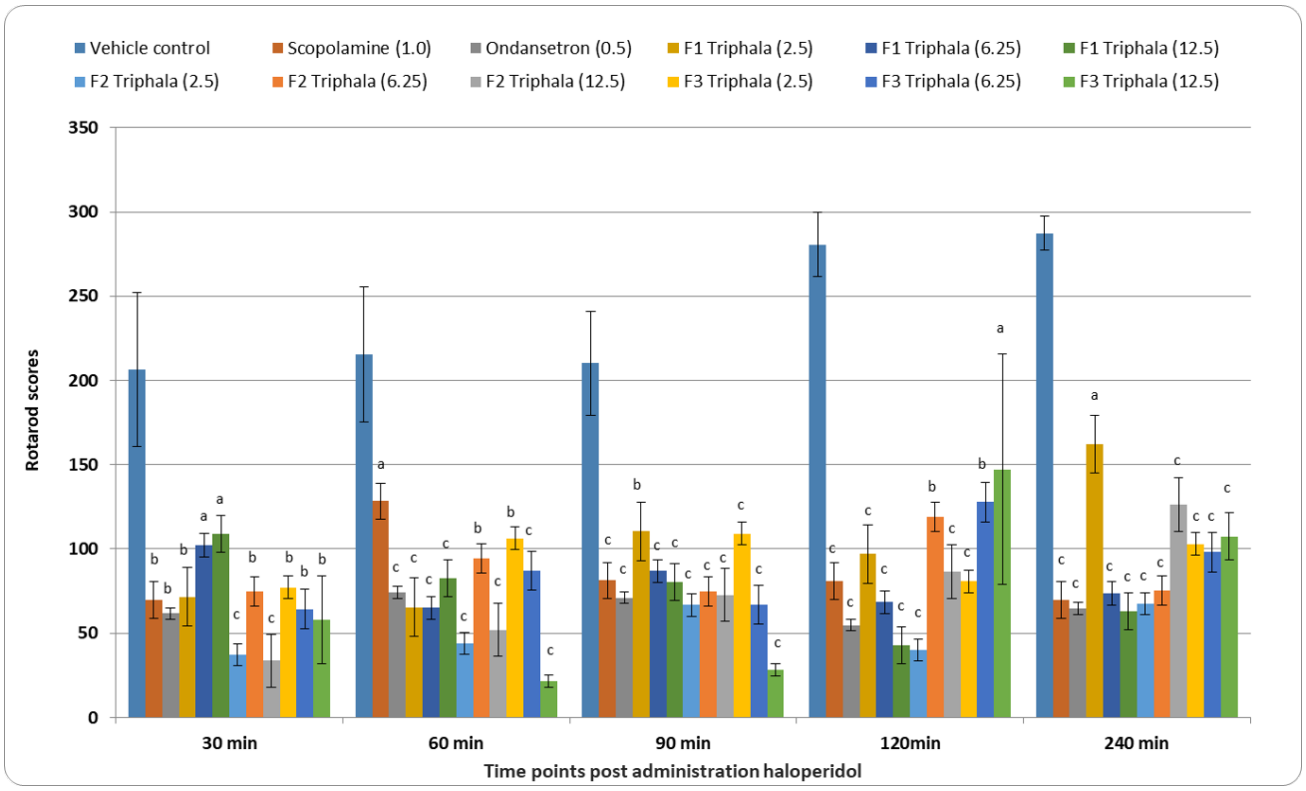

Fig. 2. Preventive effect of different formulations of Triphala on the rotarod scores at various time intervals after haloperidol administration. Note: All values are expressed as Mean ± SEM (n = 6 in each group). *p* values: a < 0.05, b < 0.01, c < 0.001 as compared with vehicle control (1.0% gum acacia) by one-way ANOVA followed by the Dunnett test.

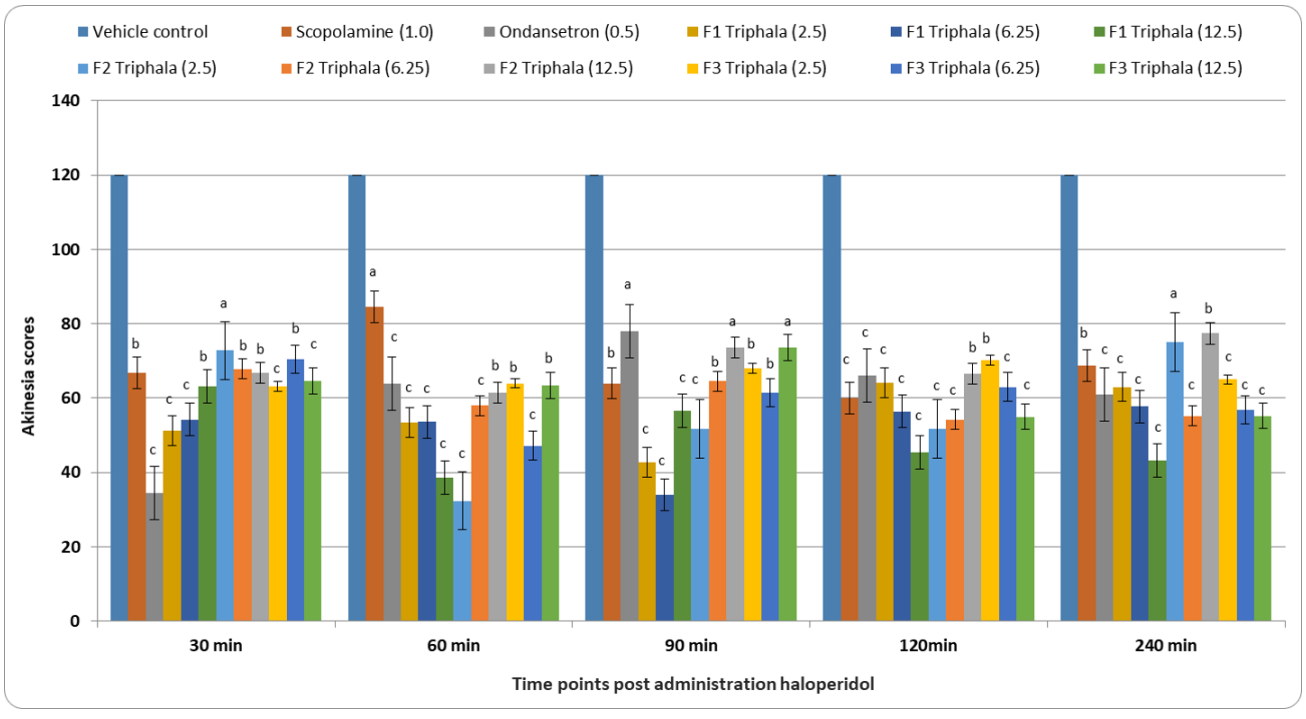

Fig. 3. Preventive effect of different formulations of Triphala on the akinesia scores at various time intervals after haloperidol administration. Note: All values are expressed as Mean ± SEM (n = 6 in each group). *p* values: a < 0.05, b < 0.01, c < 0.001 as compared with vehicle control (1.0% gum acacia) by one-way ANOVA followed by the Dunnett test.

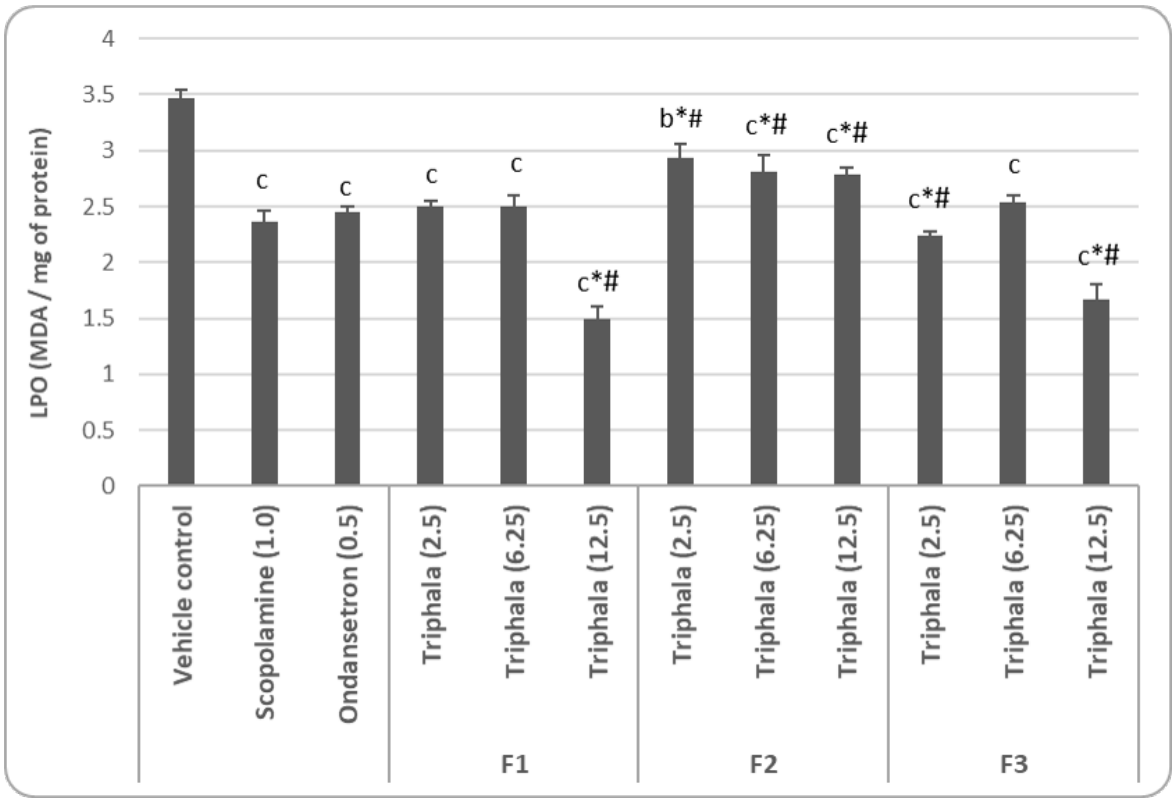

Fig. 4. Preventive effect of different formulations of Triphala on lipid peroxidation after haloperidol administration. Note: All values are expressed as Mean ± SEM (n = 6 in each group). *p* values: b < 0.01, c < 0.001 as compared with vehicle control (1.0% gum acacia); * <0.05 compared to scopolamine; # <0.05 compared to ondansetron by using one-way ANOVA followed by the Dunnett test.

Antioxidant Status

Lipid Peroxidation Test

The analysis of data for the effect of different treatments on lipid peroxidation (LPO) is represented in Fig. 4. The observations suggested that the standard drugs such as scopolamine and ondansetron significantly ($p < 0.001$) reduced the LPO levels in brain homogenate compared to vehicle control. All the tested formulations of Triphala (F1, F2, and F3) in different doses also produced significant ($p < 0.001$) reductions in the level of LPO, compared to vehicle control.

The comparative evaluation revealed that the LPO activity of various doses of Triphala formulations, including F1, F2, and F3, showed a similar level of decrease ($p < 0.001$) to that of the standard medications. Moreover, higher doses (12.5 mg) of F1 and F3 Triphala formulations appear to have lowered the LPO levels beyond the values observed with standard drugs (scopolamine and ondansetron). Further, the group comparison indicated that higher doses of F1 and F3 as well as low doses of F3 significantly ($p < 0.05$) reduced the LPO levels compared to both the tested standards (scopolamine and ondansetron). On the other hand, all the tested doses of F2 produced significant ($p < 0.05$) elevation of LPO activity compared to ondansetron and scopolamine.

Superoxide Dismutase Activity

The data for estimating the level of superoxide dismutase (SOD) in the brain suggested that scopolamine significantly ($p < 0.001$) increased the level compared to vehicle control. Similarly, ondansetron also exhibited significant ($p < 0.001$) elevation in SOD levels when comparison was done with vehicle control. The Triphala formulations (F1, F2, and F3) tested in graded doses showed improvement ($p < 0.001$) in SOD levels upon comparison with vehicle control.

The data analysis also revealed that all three doses of the Triphala formulations (F1, F2, and F3) increased SOD levels with comparable efficacy to standards (scopolamine and ondansetron). However, F3 Triphala at lower (2.5 mg) and higher (12.5 mg) doses produced more elevation in SOD levels of the brain than the standard drugs such as scopolamine and ondansetron. Furthermore, the group comparison indicated that high dose of F2 and all tested doses of F3 significantly ($p < 0.05$) elevated the SOD levels compared with scopolamine (Fig. 5).

Discussion

The present study investigated the anti-cataleptic activity of the three ratios of Triphala against haloperidol in mice. At various intervals following haloperidol treatment,

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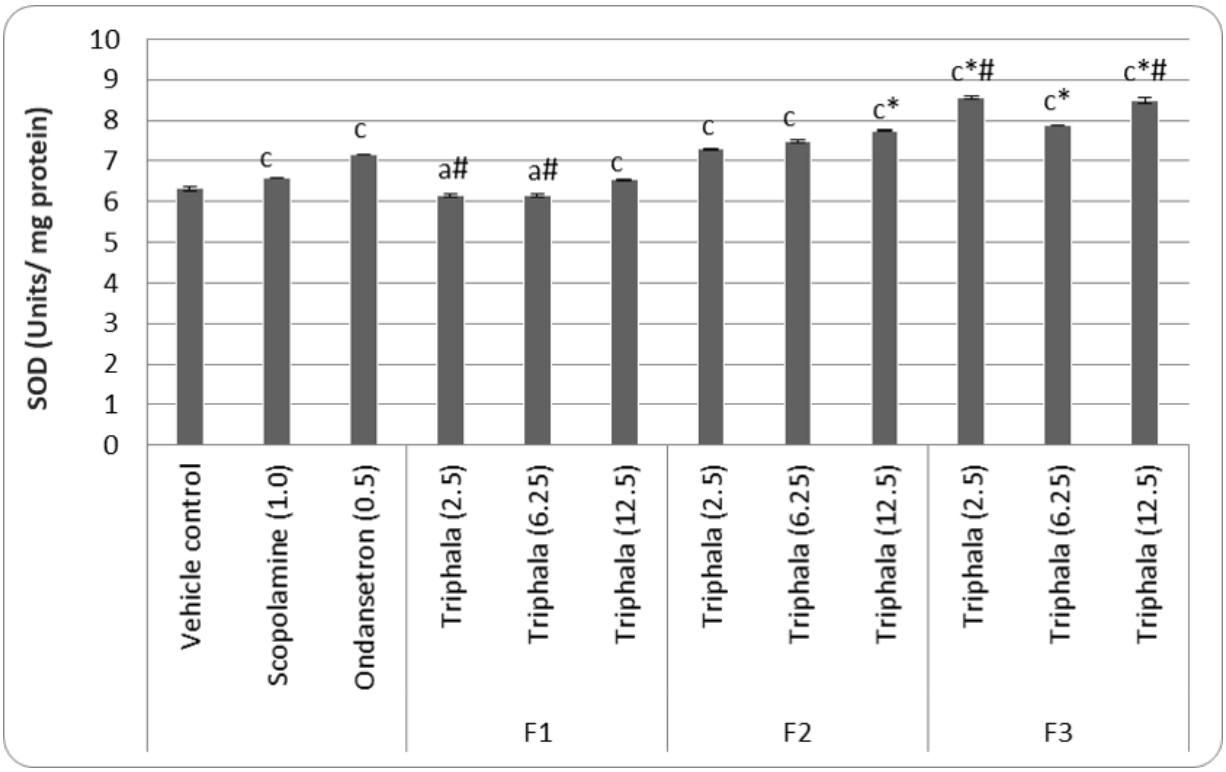

Fig. 5. Preventive effect of different formulations of Triphala on the SOD activity after haloperidol administration. Note: All values are expressed as Mean ± SEM (n = 6 in each group). *p* values: a < 0.05, c < 0.001 as compared with vehicle control (1.0% gum acacia); * <0.05 compared with scopolamine; # <0.05 compared with ondansetron by using one-way ANOVA followed by the Dunnett test. SOD, superoxide dismutase.

the cataleptic, rotarod, and akinesia scores were significantly modified for the three Triphala formulations (F1, F2, and F3). Besides, the formulations significantly reduced lipid peroxidation and elevated superoxide dismutase levels. The Triphala formulation appears to exhibit equipotent action similar to standard drugs such as scopolamine and ondansetron. The lower and higher doses of Triphala formulations were found to be better than standards, especially during the initial periods after haloperidol administration (Figs. 1,2,3,4,5).

Typical neuroleptic agents like chlorpromazine, haloperidol, and reserpine induce a cataleptic state in rodents, and it is being used as a model to test the extrapyramidal side effects (EPS). Neuroleptic-induced catalepsy has been linked to a blockade of the postsynaptic striatal dopamine D1, and D2 receptors [28,29]. Despite this evidence, several other neurotransmitters such as acetylcholine, serotonin, angiotensin, adenosine, and opioids have also been implicated [30]. In addition to the implications of various neurotransmitters in catalepsy, many pre-clinical and clinical studies have proposed reactive oxygen species in haloperidol-induced toxicity [31]. Evidence indicates that drugs that potentiate or attenuate neuroleptic-induced catalepsy in rodents might aggravate or reduce extrapyramidal signs, respectively in human beings [32]. Research conducted in the past suggested that several experimental models can be used to study the cataleptic activity influenced by an agent. Instruments such as standard bar test, wooden block for akinesia and rotarod test are some of the routinely employed models for screening the cataleptic activity [33].

In the present study, the most prominent activity was seen with F1 Triphala (high dose), F2 Triphala (low and medium dose), and F3 Triphala (low dose) compared to the standards for protecting against haloperidol-induced catalepsy (HIC) starting from 30 min of haloperidol administration and up to 240 min of exposure. The animals in the control group exhibited more cataleptic behavior, a higher catalepsy score, and prolonged periods with their paws on the bar. Furthermore, cataleptic scores decreased with treatment with different medications, including standards. Analysis of the present study data indicated that low doses of Triphala formulations (F1, F2, and F3) are better at protecting the catalepsy at initial intervals after haloperidol administration. Further, as the duration of exposure progressed, all the doses of Triphala formulations showed similarity in protection comparable with standard drugs (scopolamine and ondansetron). Hence, the data from our study suggested that all three Triphala ratios might possess enhanced efficacy compared to standard agents. Furthermore, the mice appeared to recover completely from catalepsy at 240 minutes after haloperidol administration with the treat-

ment of different test compounds. Similar observations were reported when the extract of *Tagetes lucida* was tested against haloperidol-induced catalepsy [34].

The observations show the presence of anticataleptic compounds in multiple formulations of Triphala indicating that they might be preventive in suppressing HIC. In addition, the aqueous extracts of Triphala decreased HIC, which is comparable to that of the standard drugs, scopolamine and ondansetron. The preventive effect of Triphala against HIC was consistent with the previous studies on the anticataleptic effect of a polyherbal product, NR-ANX-C [3] and BR-16A (Mentat®) [4] in which Triphala is one of the components.

Catalepsy patients show increasing motor impairment, gait, cognitive dysfunction, and postural difficulties. The Rotarod test was carried out to assess animals' muscle strength and motor impairments following haloperidol administration and the effects after Triphala administration [3,4]. The observations of the study suggested that the formulations of Triphala (F1, F2, and F3) showed efficacy similar to the standard agents. Furthermore, F2 Triphala dosages of 2.5 mg/kg and 12.5 mg/kg seem to work better than normal medications in lowering the raised rotarod scores caused by haloperidol during the first 30 and 60 minutes of exposure. Additionally, it was seen that the rats receiving standard medications displayed neuromuscular incoordination and rapidly lost balance on the rod. The drug-treated animals could maintain themselves on the rotating rod for a sufficient quota of the cut-off time (300 min). The findings are based on the previous research reported for vinpocetine (a synthetic derivative of the vinca alkaloid vincamine), where the plant-derived substance was found to reduce rotarod scores in mice [35].

The Akinesia test has also been extensively used to study motor impairments in animal models [36]. Our observations suggest that all formulations of Triphala have efficacy like standard compounds in reducing akinesia scores. The F1 Triphala was found to be better than standard agents such as scopolamine and ondansetron in reducing the scores at different intervals after haloperidol exposure. The control mice in the study exhibited an inability to move after 30 min of haloperidol administration itself and achieved a maximum latency of 120 s [37]. The observations are suggestive of the protective role of Triphala in the akinesia induced by haloperidol [38].

Oxidative stress plays an important role in the pathogenesis of various diseases. Oxidative stress is initiated by reactive oxygen species (ROS), such as superoxide anion (O-2), per hydroxy radical (HOO-), and hydroxyl radical (HO•). These radicals are formed by a one-electron reduction process of molecular oxygen (O2). Thus, antioxidant defense systems have coevolved with aerobic metabolism to counteract oxidative damage from ROS. Most living things have effective defensive mechanisms to keep themselves safe from oxidative stress brought on by ROS [31]. ROS can easily initiate the lipid peroxidation of the membrane lipids [unsaturated fatty acids oxidatively degraded into a variety of products, including malondialdehyde (MDA)], causing damage to the cell membrane phospholipids and lipoproteins by propagating a chain reaction cycle. The accumulation of lipid peroxides is reported to introduce hydrophilic moieties into the hydrophobic phase and thus alter membrane permeability and its functions [39]. In our present study, all the formulations of Triphala at various doses exhibited similarity in reducing the LPO levels to the standard drugs. However, the higher dose (12.5 mg/kg) of F1 and F3 Triphala was found to be more potent than standards in decreasing the LPO activity in haloperidol-treated animals. These results showed that brain LPO levels were significantly elevated by haloperidol in the control mice and significantly reduced by the standards and test medications. Up to a point, the standards may lessen the LPO in the brain. The Triphala formulations, due to the presence of active phytoconstituents, showed better antioxidant activity than even the standards. According to the literature, compounds reducing LPO activity were reported to possess antioxidant potential. Such agents were found to reduce oxidative stress-mediated defects in living tissues [40].

Moreover, haloperidol-treated mice showed a reduction in SOD activity, which was significantly reversed by the test compound Triphala. The lower (2.5 mg/kg) and higher (12.5 mg/kg) doses of the F3 formulation have shown more elevation in SOD levels than the standard drugs after haloperidol induction. The findings suggest that Triphala has exhibited antioxidant potential [16], which could have overcome the oxidative stress induced by haloperidol. The possible mechanism of action induced by Triphala formulations against haloperidol-mediated catalepsy is represented in Fig. 6.

Despite the wide array of drugs, the control of psychosis is refractory to antipsychotic therapy, and consistent control is not always achieved. Although there are many different antipsychotics accessible in modern medicine, effective long-term control is still difficult [12]. The antipsychotics belonging to the first or second generation have their own set of adverse effects that preclude their effective usage or are insufficient in dosage, which compromises the effectiveness of the therapy [7]. Earlier studies have suggested that agents possessing antioxidant potential have reduced the complications associated with antipsychotic medications [41].

Triphala is a polyherbal formulation with multiple health benefits. It is composed of a myriad of components that have multisystem effects. When used concurrently with antipsychotics, it could help in free radical scavenging and exert antioxidant and anti-inflammatory action [17,19]. These preventive effects could culminate in considerably lowering the various extrapyramidal side effects of antipsychotics, thus making it possible to use them at their desired

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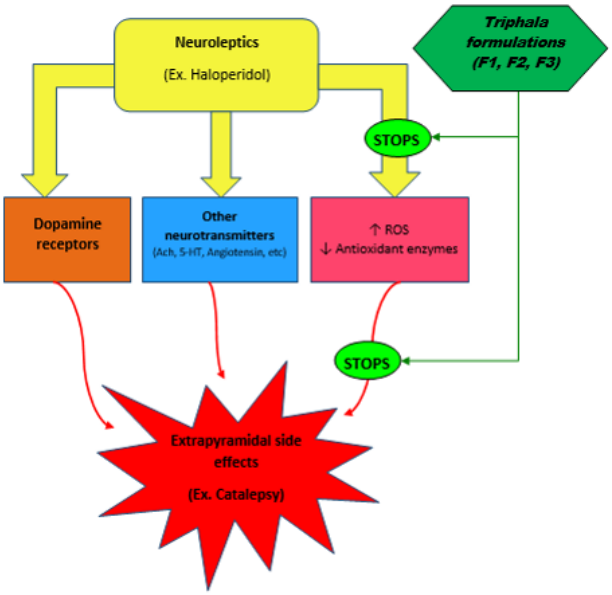

Fig. 6. Possible mechanism of Triphala in haloperidol-induced catalepsy. Ach, Acetylcholine; 5-HT, 5-hydroxytrypatamine; F1: Triphala formulation 1; F2: Triphala formulation 2; F3: Triphala formulation 3; ROS, reactive oxygen species.

dose for a longer period and allowing better control of psychosis [42]. Several important phytoconstituents, such as chebulic acid, gallic acid, gallotonic acid, and vitamin C, have been reported to be present in Triphala [43]. These active constituents (chebulic acid, gallic acid, and gallotonic acid) belonging to important classes of phytochemicals such as polyphenols are known to possess antioxidant potential [44]. The compounds exert their action through multiple pathways, such as scavenging free radicals, increasing antioxidant status, and promoting repair mechanisms in damaged cells [45].

The experimental studies show the preventive effectiveness of Triphala and a reduction in extrapyramidal side effects and catalepsy. Thus, Triphala, a well-known drug consumed by the population of the Asian subcontinent, could be an add-on drug with antipsychotic therapy that could prevent the frequency of extrapyramidal side effects such as catalepsy. Future studies need to consider this aspect with conventional drugs to mitigate the deleterious effects including the therapeutic efficacy of Triphala. Although, the objectives of this research is accomplished, there are some limitations of the study that should be considered while conducting future studies. The study was done on single species. Data from multiple animal species are required before a clinical evaluation could be conducted. Also, number of animals for each group should be improved to provide higher power to the findings, Lastly, additional experimental methods should be applied with the inclusion of evaluating neurotransmitter changes to validate the pharmacological outcomes.

Conclusion

The current study demonstrated the potential preventive benefit of several triphala formulations that exhibited anti-cataleptic activity in HIC at different dosages. Triphala formulations significantly reduced standard bar test scores, akinesia scores, and brain LPO, while increasing brain SOD and rotarod scores in all groups. These findings imply that Triphala has a more promising preventive effect than the other studied drugs. Due to its natural origins and widespread clinical usage, the formulation may have the ability to reduce the risk of extrapyramidal problems. However, more research into the use of polyherbal formulations is needed to demonstrate and ensure complete safety and efficacy, particularly when co-administered with different antipsychotic agents.

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding authors on reasonable request.

Author Contributions

KD, BM, PK, and MSB designed the research study. KD, SA, and AIA carried out the experimental part of the research. SMBA, MEA, FA, and SIR provided help and advice on research methodology and analysis of data. BM and SIR analyzed the data. BM wrote the manuscript. SMBA edited and reviewed the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

The experiment was conducted as per the guidelines of CPCSEA and after approval from the Institutional Animal Ethics Committee of St. John's Pharmacy College, Bangalore, India (SJPC/IAEC/M-28/2010).

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Conflict of Interest

The authors declare no conflict of interest.

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