Research Article

Evaluation of Neuroprotective Potential of Methanolic Extract of *Tamarindus indica* Bark against Experimentally Induced Parkinsonism

Dona Sarkar*, Dr. Ananya Bhattacharjee, Dr. Manodeep Chakraborty, Dr. Nihar Ranjan Bhuyan
Himalayan Pharmacy Institute, Majhitar, Rangpo, East Sikkim, India-737136.
***Corresponding author's E-mail:** sarkardona21@gmail.com

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ABSTRACT

The four cardinal signs of Parkinson disease are stiffness, resting tremor, slowness (bradykinesia), and loss in movement (hypokinesia). Several conventional treatments for parkinson were discovered in past decades but have side effects and sometimes toxicity. Plant products with active phytochemicals are employed for a variety of pharmaceutical purposes because they have fewer harmful effects. In this present study in vivo antiparkinson activity of the methanolic extract of *Tamarindus indica* (*T. indica)* bark has been evolved by using animal experimental models. Parkinson disease is induced by administration of Haloperidol (1 mg/kg/day, i.p) for 7 consecutive days. The symptoms of PD such as akinesia and rigidity were evaluated. The effect was evaluated by assessing various behavioral parameters (catalepsy, grip strength and locomotor activity), The methanolic extract of *T. indica* bark at the doses of 100 mg/kg and 200 mg/kg have shown significant ($p<0.001$) reduced in catalepsy. Daily administration of *T. indica* (200mg/kg) have shown significantly improved motor functions as compared to haloperidol treated groups. As a result, it was determined that plant products will become an important therapeutic option in the management of Parkinson's disease in the future. As a result, more research is needed to identify its active ingredients and molecular-level target mechanisms responsible for antiparkinson efficacy in humans.

Keywords: Antiparkinson activity, *T. indica*, Haloperidol, Levodopa-carbidopa combination, catalepsy test.

INTRODUCTION

The second-most prevalent neurodegenerative ailment, Parkinson's disease (PD) is clinically characterised by tremor, stiffness, bradykinesia, and postural instability.[1] The key hallmarks of the progression of Parkinson's disease have been shown to include oxidative stress, the buildup of misfolded protein, and the death of dopaminergic neurons in the substantia nigra pars compacta.[2] PD is caused by the suppression of mitochondrial complex-1 , various cell damage processes such as excitotoxicity, inflammatory disorders, apoptosis, and accumulation of proteins , and the interaction of hereditary and environmental variables.[3] The dopamine D_2 receptor antagonist haloperidol causes catalepsy and extrapyramidal Parkinson's symptoms.[4] Haloperidol can produce chronic movement problems, whose aetiology is connected to the effects of oxidative stress and neurotoxicity. PD affects 8 to 18 persons per 100,000 people per year. The average age at which a patient develops the condition is 60 years of age, and the average time it takes for a patient to pass away is fifteen years. Most epidemiological research show that males have prevalence and incidence rates that are twice as high as those of females.[5]

The present pharmacological therapies for Parkinson's disease (PD) are not only ineffective at slowing the death of dopaminergic neurons, but also have a variety of unfavourable side effects. In recent years, natural products with antiparkinson action have gained popularity due to their lower cost and lack of adverse effects. One of the most widely used medicinal herbs, *T. Indica*, is renowned for its powerful anti-inflammatory properties. *T. indica* includes phenolic components such catenin, procyanidin, epicatechin, pectin, arabinose, xylose, galactose, glucose, and triterpenes, according to the results of phytochemical analyses.[6] *T. indica's* pericarp and seed are primarily made up of phenolic antioxidant substances. All *T. indica* extracts showed strong antioxidant activity.[7] The presence of phytochemicals in the various plant components, such as flavonoids, alkaloids, tannins, phenols, triterpenoids, fatty acids, saponins, and steroids, is thought to be the cause of *T. indica's* therapeutic properties and use in traditional folk medicine.[8] *T. indica* bark has long been used to relieve pain, and the current study sought to confirm this scientifically by employing appropriate animal screening models. *T. indica* possesses a wide range of antibacterial activities. The antioxidant activity of *T. indica* methanolic bark extract was investigated.[9]

Numerous studies have been done to find out how important antioxidants are in controlling oxidative stress, which is the root cause of many illnesses, including "major parkinson disorder." Methanolic bark extract is high in active ingredients such as phenolics, ascorbic acid, carotenoids, and so on, giving it a high antioxidant potential. In order to explore its possible antiparkinson effects, this herbal resource was chosen for the present investigation. This research mainly aimed at investigating the neuroprotective activity of the methanolic extract of *T. indica* bark against experimentally induced Parkinsonism in rats.

MATERIALS AND METHODS

Requirements

Soxhlet apparatus, Round Bottom Flask (RBF), Condenser, Heating mantle, Rotary evaporator (HS-300), methanol (as a solvent for extraction), normal saline, distilled water, beakers, glass rod, containers, Whatman filter paper, Haloperidol, Levodopa-carbidopa (standard drug) and Oral feeder (for oral drug administration).

Animals

Male albino rats, were used for the study weighing 200-250gm and isolated in the experimental room at temperature not exceeding 26°C, controlled humidity conditions with alternative 12 hours day and night cycles and caged in proper housing. The rat were fed with food pellets and sufficient amounts of distilled water ad libitum. The animals were kept in animal house for 1week for acclimatization before starting the experiment. the studies were carried out in accordance with the ethical standards of CPCSEA guidelines.

Collection of plant materials, authentication and extraction of plant materials

The bark of *T. indica* (Family: Fabaceae) was collected from Majhitar region, East Sikkim in the month of October. The barks are of the local variety. After the collection, barks were kept for shade drying avoiding the direct sunlight for three weeks. The barks were then coarsely powdered to facilitate the process of extraction. A herbarium was prepared and was authenticated at Botanical Survey of India, Gangtok, Sikkim. It was identified and authenticated as *Tamarindus indica* belonging to family Fabaceae.

For the process of extraction, the Soxhlet extraction method was followed using ethanol as the solvent. This process is also known as continuous hot extraction. A dried, grounded and coarsely powdered plant material was placed inside thimble made up of filter paper and was tightly packed. Methanol was poured in the round bottom flask, while the thimble was placed in the extraction chamber. Methanol was then heated maintaining the temperature of 55-60°C, which evaporated and passed through the condenser. The condensed solvent then flowed down to the extraction chamber and came in contact with the plant material which facilitated the extraction. As the level of the solvent in the extraction chamber reached the top of the siphon, the solvent and the extracted plant material flowed back to the round bottom flask. The process was continuously repeated until the drug was completely extracted, until the solvent flowing from the extraction chamber did not leave any residue behind. Then the obtained extract was subjected to vacuum evaporation using rotary evaporator to facilitate solvent recovery and drying.

Acute toxicity study

The acute oral toxicity was studied in Wistar albino rat as per OECD guideline 423. The extract was administrated orally in an increasing dose of up to 2000 mg/kg. Vehicle (0.5% w/v) was administered to the control group. The general behaviour of the rat was continuously monitored for 1 h after dosing, periodically during the first 24 h with special attention given during the first 4 h, and daily thereafter, for a total of 14 days. Changes in the normal activity of rat and their body weights, food, and water intake were monitored and the time at which signs of toxicity or death appeared recorded. The acute toxicity studies showed that there were no toxic signs up to the dose level of 300 mg/kg but at dose level, 2000 mg/kg animals showed signs of toxicity.

Experimental design

The animals were divided into 5 groups containing 3 animals in each group

Group 1: Normal control group [Normal saline treated group]

Group II: Disease control group [treated with haloperidol 1mg/kg, 7 days treatment][10.]

Group III: Standard control group [Levodopa + carbidopa (30mg/kg) treated for 7 days +haloperidol (1mg/kg), 7 days treatment][11.]

Group IV: Plant extracted treated group [Low dose *T. indica* bark extract (100mg/kg) for 7 days + haloperidol (1mg/kg), 7 days treatment].

Group 5: Plant extract treated group [High dose *T. indica* bark extract (200mg/kg) 7 days treatment].

The *Tamarindus indica* bark extract was given in two doses i.e., 100mg/kg as low dose and 200mg/kg as high dose which was given orally once a day through oral gavage for 7 days. Levodopa + carbidopa was given at a dose of 30mg/kg orally for 7 days for standard control group.[11] Haloperidol was given at a dose of 1mg/kg through intraperitoneal route for 7 days treatment for group II, III, 1V, V to induce parkinsonism.[10]

After 24 hours of last treatment the animal was subjected for behavioral parameters to study the motor symptoms.

Behavioral parameters

Akinesia- Holding the animal's tail while placing its front paws on a platform and allowing it to move were used to measure its akinesia. The number of steps the animal took with its forelimbs were recorded for three minutes.[11] (Figure 1)

Grip strength- Using a rota rod instrument, the latency to grip strength was assessed. Each animal was mounted on a 20rpm spinning rod, and various animal groups' latency to fall over was noted.[11] (Figure 2)

Hang test- The motor neuron integrity and muscular strength have both been evaluated using this exercise. The rats used the front limbs to suspend themselves 70 cm above a foam cushion to sustain their body weight on a wire that was hung between two 30 cm poles. Before the

rat fell, the duration (measured in seconds) was noted. There was a waiting period before scoring; if the rat dropped right away, a result of zero was given. For each rat, three trials were conducted.[10](Figure 3)

Bar test- The bar test was used to measure stiffness and catalepsy. For a moment, animals were perched on a bar. When the animal elevates one or both paws, the latency bar test time is recorded. The 180-second time restriction was taken into account.[12](Figure 4)

Catalepsy test- After the administration of drug and treatments, severity of catatonic response was observed.[13](Figure 5)

Figure 1: Akinesia test

Figure 2: Rotarod activity

Figure 3: Hang test

Figure 4: Bar test

Figure 5: Catalepsy test

Stage I Rat moves normally when placed on the table, score = 0

Stage II Rat moves only when touched or pushed, score = 0.5

Stage III Rat placed on the table with front paws set alternately on a 3cm high block fails to correct the posture in 10 seconds, score = 0.5 for each paw with a total of 1 for this stage.

Stage IV Rat fails to remove when the front paws are placed alternately on a 9cm block, score = 1 for each paw with a total score of 2 for this stage

Thus for a single rat, the maximum possible score would be 3.5 revealing total catatonia. The severity of catatonia at 30,60,90,120,150,180,210 minutes.

Statistical analysis

The results obtained were analysed using Graph-pad prism software. The data was compiled and expressed in Mean ± SEM by one-way ANOVA for analysis of variance followed by Tukey-Kramer Multiple comparisons test. *P<0.05* was considered as significant.

RESULTS

Effect on akinesia and grip strength against haloperidol induced Parkinsonism.

Effect on akinesia:

The toxic control group treated with haloperidol 1mg/kg demonstrated an extremely significant (P<0.001) increase in akinesia when compared with normal control.

Standard group treated with levodopa + carbidopa (30mg/kg + haloperidol), Low dose of *T. indica* (100mg/kg + haloperidol), and high dose of *T. indica* (200mg/kg + haloperidol) showed extremely significant (P<0.001) decrease in akinesia when compared with toxic control group (Table 1) (Fig. 6).

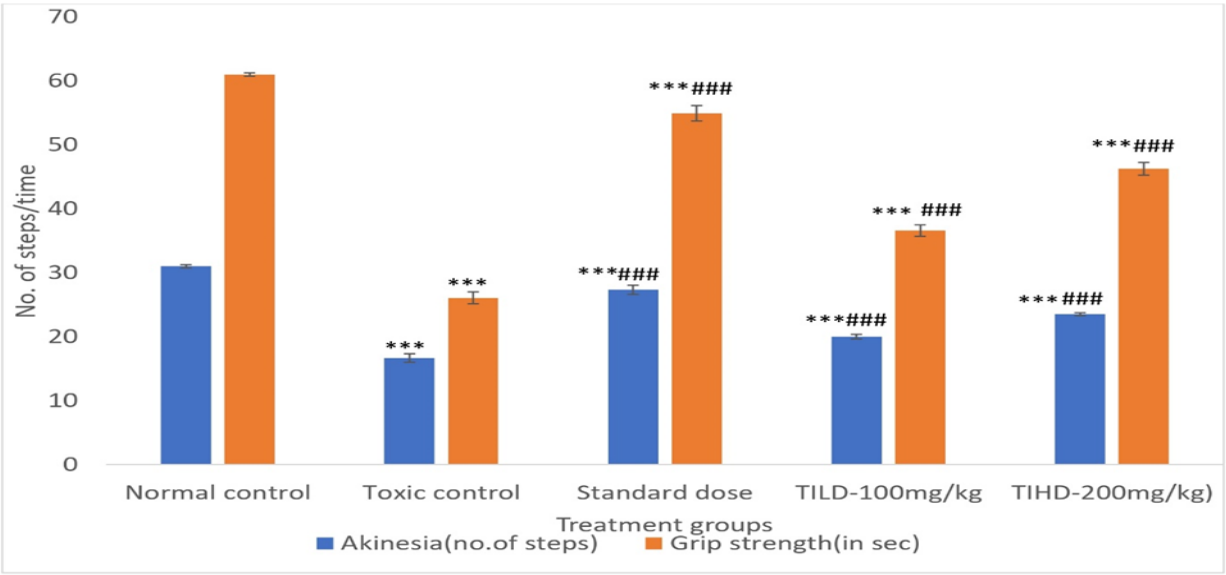
Effect on grip strength:

The toxic control group treated with haloperidol 1mg/kg demonstrated an extremely significant (P<0.001) increase in grip strength when compared with normal control. Standard group treated with levodopa + carbidopa (30mg/kg + haloperidol), low dose of *T. indica* (100mg/kg + haloperidol), and high dose of *T. indica* (200mg/kg + haloperidol) showed extremely significant (P<0.001) decrease in grip strength when compared with toxic control group (Table 1) (Fig. 6).

Table 1: Effect on akinesia, grip strength against haloperidol induced Parkinsonism.

Treatment groups	Akinesia (no. of steps taken with forelimbs)	Grip strength (latency to fall in seconds)
Normal control	31±0.25	61±0.26
Toxic control (Haloperidol 1mg/kg)	16.66±0.66***	26.08±0.93***
Standard dose (Levodopa+carbidopa 30 mg/kg)	27.33±0.71***###	54.94±1.20***###
Low dose of *T. indica* (100mg/kg+ haloperidol)	20±0.36***###	36.59±0.89***###
High dose of *T. indica* (200mg/kg+ haloperidol)	23.5±0.22***###	46.25±0.99***###

Values are expressed as mean ±SEM, n=6, ***P<0.001, when compared to normal group; ###P<0.001, when compared to toxic group (Haloperidol 1mg/kg).



Values are expressed as mean ±SEM, n=6, ***P<0.001, when compared to normal group; ###P<0.001, when compared to toxic group (Haloperidol 1mg/kg).

Figure 6: Effect on akinesia and grip strength against haloperidol induced parkinsonism.

Effect on hang test and bar test against haloperidol induced Parkinsonism.

Effect on hang test:

The toxic control group treated with haloperidol 1mg/kg demonstrated an extremely significant (P<0.001) increase in hanging time when compared with normal control. Standard group treated with levodopa + carbidopa (30mg/kg + haloperidol), low dose of *T. indica* (100mg/kg + haloperidol), and high dose of *T. indica* (200mg/kg + haloperidol) showed extremely significant (P<0.001) decrease in hanging time when compared with toxic control group (Table 2) (Fig 7).

Effect on bar test:

The toxic control group treated with haloperidol 1mg/kg demonstrated an extremely significant (P<0.001) increase in bar test when compared with normal control. Standard group treated with levodopa + carbidopa (30mg/kg + haloperidol), low dose of *T. indica* (100mg/kg + haloperidol), and high dose of *T. indica* (200mg/kg + haloperidol) showed extremely significant (P<0.001)

decrease in hang time when compared with toxic control group (Table 2) (Fig 7).

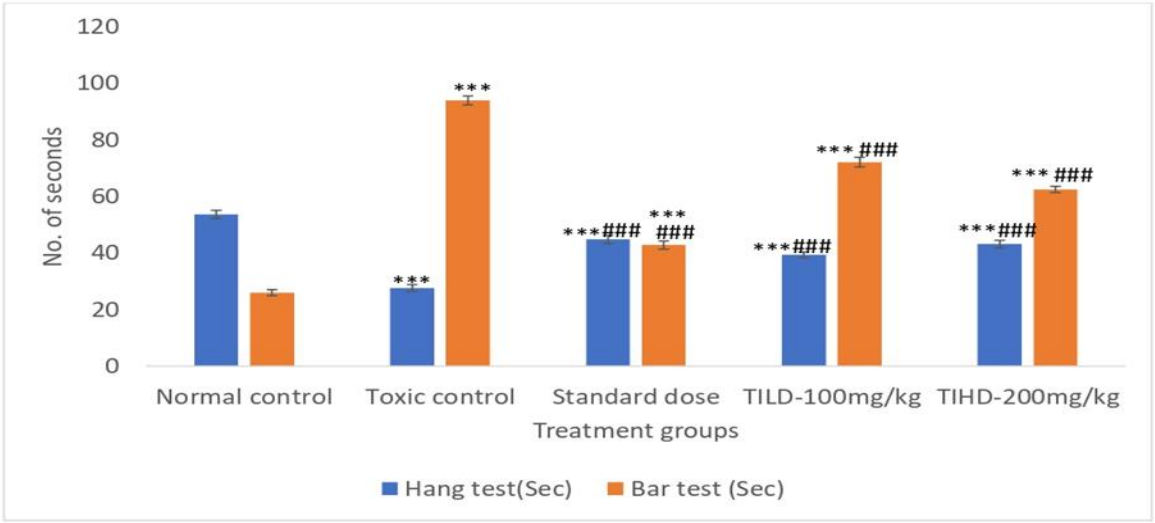
Effect on catalepsy against haloperidol induced parkinsonism:

The toxic control group treated with haloperidol 1mg/kg demonstrated an extremely significant (P<0.001) increase in catalepsy in different time intervals when compared with normal control.

Standard group treated with levodopa + carbidopa (30mg/kg + haloperidol), low dose of *T. indica* (100mg/kg + haloperidol), and high dose of *T. indica* (200mg/kg + haloperidol) showed significant decrease in catalepsy when compared with toxic control group (Table 3) (Fig 8).

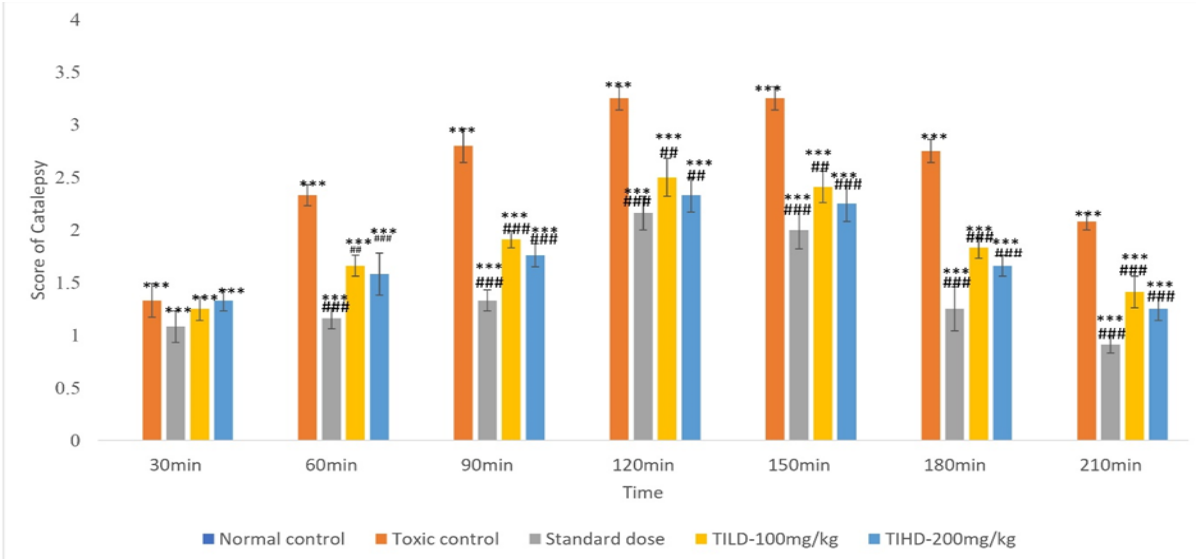
Table 2: Effect on hang test and bar test against haloperidol induced Parkinsonism

Treatment groups	Hang test	Bar test
Normal control	53.66±1.45	26±1.06
Toxic control (Haloperidol1mg/kg)	27.66±1.20***	94±1.57***
Standard dose (Levodopa+carbidopa 30 mg/kg)	44.83±1.44***###	42.83±1.44***###
Low dose of *T. indica* (100mg/kg+ haloperidol)	39.33±1.05***###	72.16±1.73***###
High dose of *T. indica* (200mg/kg+haloperidol)	43.16±1.35***###	62.5±1.11***###



Values are expressed as mean ±SEM, n=6, ***P<0.001, when compared to normal group; ###P<0.001when compared to toxic group (Haloperidol 1mg/kg).

Figure 7: Effect on hang test and bar test against haloperidol induced parkinsonism.



Values are expressed as mean ±SEM, n=6, ***P<0.001 when compared to normal group; ###P<0.001,## P<0.01, when compared to toxic group (Haloperidol 1mg/kg).

Figure 8: Effect on catalepsy against haloperidol induced parkinsonism.

Table 3: Effect on catalepsy against haloperidol induced Parkinsonism

Treatment groups	30min	60min	90min	120min	150min	180min	210min
Normal group	0.00±0.00	0.08±0.08	0.10±0.10	0.00±0.00	0.08±0.08	0.08±0.08	0.00±0.00
Toxic group	1.33±0.16***	2.33±0.10***	2.8±0.16***	3.25±0.11***	3.25±0.11***	2.75±0.11***	2.08±0.08***
Standard dose of levodopa+carbidopa (30mg/kg)	1.08±0.15***	1.16±0.10***###	1.33±0.10***###	2.16±0.16***###	2±0.18***###	1.28±0.21***###	0.91±0.08***###
Low dose of *T. indica* (100mg/kg+ Haloperidol)	1.23±0.11***	1.66±0.10***##	1.91±0.08***###	2.5±0.18***##	2.41±0.15***##	1.83±0.10***###	1.41±0.15***###
High dose of *T. indica* (200mg/kg+ Haloperidol)	1.33±0.10***	1.58±0.20***###	1.76±0.11***###	2.33±0.16***##	2.25±0.17***###	1.66±0.10***###	1.25±0.11***###

Values are expressed as mean ±SEM, n=6, ***P<0.001 when compared to normal group; ###P<0.001, ## P<0.01, when compared to toxic group (Haloperidol 1mg/kg).

DISCUSSION

Parkinson's disease is a long-term neurodegenerative condition marked by the death of SNpc dopaminergic neurons. A-synuclein buildup, mitochondrial malfunction, apoptosis, and neuronal excitotoxicity are a few examples. One of the most important pathogenic mechanisms underlying PD is oxidative stress. Due to the higher amount of dopamine it contains, SNpc is more susceptible to reactive oxygen species.[3]

In the haloperidol induced catalepsy, it was evident by a significant increase in the time spent on the block as compared to vehicle treated rats.[3] Treatment with the standard drug combination of levodopa + carbidopa significantly reduced it in haloperidol treated rats. The *T. indica* at doses of 100 and 200 mg/kg showed protective effect in a dose dependent manner against haloperidol induced catalepsy which indicated that *T. indica* methanolic bark extract may have an ability to protect dopaminergic neurotransmission in striatum.

The grip strength performed by Rotarod apparatus was used to assess the motor coordination and grip strength of muscles in rats.[14] In the both models, Haloperidol significantly decreased the grip strength compared to the normal animals, levodopa + carbidopa showed increased endurance time on the rotarod. Interestingly the high dose of *T. indica* (200mg/kg) also increased the endurance time on the rotarod which can be correlated with the increase in the dopamine level, due to haloperidol treatment.

Similarly, in hang test the haloperidol treated group exhibited difficulty to remain in the wire, where as in the standard group the neuromuscular strength was increased significantly.[10] In the similar manner, *T.indica* (100 and 200 mg/ kg) dose-dependently improved it which is an indication of neuroprotective effect of the drug, which was comparable to that of levodopa treatment group.

Haloperidol treatment induced akinesia probably by depletion of central catecholamine storage. L-dopa and carbidopa produced a significant reduction in these symptoms in rats. *T. indica* higher dose also exhibited significant reduction in these symptoms in rats.[11]

Haloperidol induces catalepsy by increase in time spent on horizontal bar compared to vehicle treated animals.[12] Treatment with levodopa + carbidopa significantly reduced the catalepsy in haloperidol treated rats. In the similar manner, *T. indica* also reduced the catalepsy significantly in a dose dependent manner, which indicated that it may be due to the ability of the extract to protect dopaminergic neurotransmission in striatum.

The above behavioral parameters results suggest that *T. indica* may have the ability to improve symptoms of Parkinsonism, probably by restoring the level of dopamine, and by the regulation of the antioxidant system. Thus neuroprotective activities may be responsible for Anti-Parkinson's effect. Hence *T. indica* may be useful as a neuroprotective agent in the treatment of PD. The above observed beneficial effects of *T. indica* may be attributed to diverse chemical components namely flavonoids, alkaloids, saponins and tannins. Observations of the present findings can be further investigated to establish this fact clinically.

CONCLUSION

In this current study, low dose (100mg/kg) and high dose of *T. indica* (200mg/kg) treatments was given orally to haloperidol induced parkinsonism for seven days. The particular doses of test drug showed significant neuroprotective effect towards the Parkinson's like symptoms produced by toxic control group. The doses of test drug showed significant effect on the behavioral parameters whereas symptoms like muscular rigidity, and catalepsy were reduced in outstanding manner. As from the phytochemical study, it was found that the bark is rich in flavonoids as well as phenols, and both of these constituents are reportedly responsible to exert anti-parkinsonian activity in experimentally induced parkinsonism, so from this study, it can be concluded that *T. indica* could be used as an alternative and/or adjuvant drug to prevent and treat extrapyramidal side effects of antipsychotic agents in clinical practice.

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