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Research article

# The therapeutic potential of Berberine chloride hydrate against harmaline-induced motor impairments in a rat model of tremor

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## HIGHLIGHTS

- Harmaline significantly increased step width and tremor scores.
- Muscle strength and time on rod decreased in harmaline group.
- BBR partially reversed the effects of harmaline on motor and balance function.
- Berberine in a dose dependent manner improved harmaline – induced neurotoxicity.
- High dose of berberine had the same tremor score as compared to harmaline.

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## ABSTRACT

Essential tremor (ET) is a progressive neurological disorder with motor and non-motor symptoms. It has conclusively been shown that modulation of glutamate receptors could ameliorate ET. Recent studies have suggested that Berberine (BBR) has an inhibitory effect on glutamate receptors. Therefore, BBR may have therapeutic effects on ET. In this study, male Wistar rats ($n = 10$ in each group) weighing 40–60 g were divided into control, harmaline (30 mg/kg, i.p.) and berberine (10, 20 or 50 mg/kg, i.p, 15 min before harmaline injection) groups. Open field, rotarod, wire grip and foot print tests were used to evaluate motor performance. The results indicated that the administration of BBR (10 and 20 mg/kg) attenuated harmaline-induced tremor in rats, but the beneficial effects of BBR could not be identified at dose 50 mg/kg. In addition, BBR ameliorated gait disturbance in doses of 10 and 20 mg/kg. The high dose of BBR not only failed to recover step width but also showed an adverse effect on left and right step length. The results indicate that BBR only in dose of 20 mg/kg recovers mobility duration. The current study found a dose-dependent manner for the therapeutic effects of BBR in ET. Our study provides the initial evidence for the effects of BBR on motor function. Since BBR exerts its effects mainly through regulation of neurotransmitter release or blocke of NMDA receptors, thus, it is predicted that BBR ameliorate harmaline effect through blockade of NMDA receptors or glutamate release. This is an important issue for future research to evaluate the possible mechanisms involved.



## 1. Introduction

Tremor is defined as the involuntary, rhythmic and sinusoidal oscillation involving one or more body parts [1] seen in Essential tremor (ET) and Parkinson's disease (PD) [2]. ET as a progressive neurological disorder has negative effects on the quality of life in patients. By focusing on the concept of disability, its social and economical burden is estimated to be high [3,4]. The clinical manifestations consist of motor and non motor aspects, including tremor, ataxia and cognitive impairment [5]. In addition, post-mortem studies have revealed that ET is a neurodegenerative disorder [6]. The pathophysiological mechanisms underlying ET are not clearly defined yet; however, the components that decreased neuronal excitability play an important role in the treatment of ET [7]. Therefore, there is an unambiguous relationship between ET and neuronal excitability.

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Experimental studies using animal models of harmaline, a tremorgenic alkaloid that resembles centrally induced tremors in rodents and primates, have provided considerable insight into the pathogenesis and pharmacotherapy of ET [8]. Harmaline induces a nonspecific tremor by increasing glutamate discharge in climbing fibers, resulting in marked purkinje cell destruction and changes in the olivocerebellar pathway in the laboratory animals [9–11]. Considerable research has additionally been performed to investigate a large range of neuroprotective and neurotransmitter modulator agents using animal models which mimic such disorders [12,13]. Administration of harmaline causes excitotoxic damage of purkinje cells resulted by excessive release of glutamate from climbing fibers [14]. Hence, the modulation of glutamatergic system could preserve the normal levels of glutamate in the neuronal synapses, which positively affect motor disease. Previous studies have reported that activation of GABA by Gabapentin and inhibition of glutamatergic transmission by ethanol may be effective in ET [15,16], but questions have been raised about the safety of prolonged use of these drugs and medications. Thus, it is important to find new pharmacotherapies for inhibition of glutamatergic system and neuronal excitability as a therapeutic strategy for ET treatment.

Berberine (BBR), an isoquinoline alkaloid is found in many plants such as *Berberis*, Coptidis rhizoma and Berberis integerrima or Zereshk and is widely used in traditional medicine [17,18]. BBR has been shown to exert neuroprotective, anti apoptotic, anti inflammatory and anti-oxidative properties in various animal models of CNS-related disorders such as Alzheimer's disease, Parkinson·s disease, forebrain ischemia, mental depression and anxiety [17,20,21].

Previous studies have shown that BBR has an inhibitory effect on glutamate receptors and can alleviate neuronal injury by reducing glutamate, serotonin and norepinephrine levels [17]. Moreover, it has conclusively been shown that modulation of glutamate receptors could ameliorates ET and Parkinson disease (PD) [7,22]. More recently, Ji and Shen suggested that BBR can interact readily to three key enzymes AChE, butyrylcholinesterase (BChE), and monoamine oxidase (MAO) [23]. These findings have provided new insights for interaction of BBR with neurological disorders such as ET.

In view of all that has been mentioned so far, one may suppose that BBR may have beneficial effects on ET. This study, therefore, set out to assess the neuroprotective effect of BBR on harmaline-induced termor and ataxia in rats.

## 2. Methods and materials

### 2.1. Drugs and chemicals

Harmaline 51330 and Berberine chloride hydrate were purchased from Sigma–Aldrich, India and dissolved in normal saline on the day of experiment.

### 2.2. Animals

Male Wistar rats (40–60 g) were used for the current study. All the procedures in the current experiment were carried out according to the Kerman medical university guidelines for reporting research on animals (Ethics code: KNRC/93/46). Animals were kept under standard conditions (12/12 light-dark cycle) with access to food and water ad libitum.

### 2.3. Drug preparation and administration

On the day of experiments, animals were brought to the testing room and left for 1 h to acclimate. They were randomly assigned to five experimental groups: control group animals that received no treatment, harmaline (30 mg/kg, i.p), harmaline + Berberine (10, 20, 50 mg/kg, i.p). Berberine was administered 15 min before harmaline injection [17,20,24].

All behavioral assays were performed 30 min after BBR injection with suitable interval among each assay in the following order: observation, open field test, rotarod, wire grip test and footprint.

### 2.4. Observation

The occurrence of tremors was rated by an observer who was blinded to the treatment groups. Fifteen minutes after harmaline administration, data was acquired at the same time of open field test. Balance disturbances were scored as the following scale: 0 = lack of disturbances, 1 = unsteady gait appearing occasionally, 2 = staggering while changing the body position and strongly unsteady gait, 3 = frequent episodes of losing a natural body posture and falling down and maintenance of lying position [25].

### 2.5. Open field test (OFT)

The open field test was used to evaluate the possible effects of BBR on locomotion and anxiety-like behaviors. The apparatus consisted of an arena made of opaque Plexiglas ($90 \times 90 \times 45$ [H] cm). The arena was divided into 16 small squares, so that the rats spent time in either central or peripheral squares. Rats were placed in the middle of the arena and their behavior was recorded and analyzed by an automated video tracking system (Ethovision, Noldus Technology, Netherlands) during a 5 min interval. Total time spent in the center or periphery, total distance moved (TDM), speed, and the number of grooming and rearing were recorded for each rat [26].

### 2.6. Rotarod

The accelerating rotarod was used in the current study. The rotarod experiment started at a speed of 10 revolutions per min (RPM) to the maximum speed of 60 RPM. Each rat underwent three trials (inter-trial interval = 5 min), with each trial lasting for a maximum of 300 s. Total duration that each rat spent on the rod maintaining its balance was recorded as a measure of balance [26].

### 2.7. Wire grip test

The wire grip test assays muscle strength and balance of the animals. Each rat was suspended on a horizontal steel wire hanging on both forepaws (80 cm long, 7 mm diameter). While the rat's forepaws were put in contact with the steel wire, the rat was placed in a vertical posture and released whenever it grasped the wire. Latency to fall was recorded for each animal using a stop watch. Each rat underwent three trials with five minutes inter-trial interval [26].

### 2.8. Footprint

Footprint test was used to assess the walking pattern and gait kinematics of rats. Hindpaws of the animals were painted with non-toxic inks, and the rats were allowed to spontaneously transverse a clear plexiglass tunnel (100 cm × 10 cm × 10 cm) ending in a darkened cage. A sheet of white absorbent paper (100 cm × 10 cm) was placed at the bottom of the track. The resulting tracks provide the spatial relationship of consecutive footfalls from which the stride length and width were measured [27].

### 2.9. Statistical analysis

SPSS (V.16, IBM, USA) was used for the analysis of data. Standard error of the mean (Mean ± S.E.M) was used to describe the level of