

## Neuroprotective effect of *Zingiber officinale* in 3-np-induced huntington disease

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**Abstract**—Huntington's disease (HD) is an autosomal dominant inherited neurodegenerative disease that causes clinical manifestations such as progressive choreiform movements, cognitive abnormality and psychiatric deterioration. HD results from the destruction of neurons in the basal ganglia, and oxidative stress, Excitotoxicity and energy impairment has been implicated in its pathogenesis. 3-Nitropropionic acid (3-NP), a potent neurotoxin, has been reported to induce oxidative/ nitrosative stress and causes neurobehavioral and biochemical changes that mimic HD in humans. In the present work, we evaluated the effects of a well-known antioxidant on behavioral, biochemical, and mitochondrial dysfunction induced by 3-NP. The study was designed to investigate the neuroprotective effects of Ethanolic extract of *Zingiber officinale* against 3-NP-induced neurotoxicity. Intraperitoneal administration of 3-NP (20 mg/kg for 7 days) caused a decline in motor function (locomotor activity and impaired rotarod activity). Chronic treatment with EEZO root extracts (100 and 200 mg/kg) for 1 week dose-dependently improved 3-NP-induced behavioral, biochemical, and enzymatic changes ( $P < .05$ ). Biochemical analysis revealed that systemic 3-NP administration significantly increased lipid peroxidation and nitrite and acetylcholinesterase level, Chronic administration of EEZO (100 and 200 mg/kg) dose-dependently restored biochemical alterations induced by chronic 3-NP treatment ( $P < .05$ ). These findings suggest that neuroprotective actions of *Zingiber officinale* are mediated via its anticholinesterase activity. However, further studies are required to elucidate the molecular mechanisms involved in order to support the clinical use of the plant extract as a therapeutic agent for the treatment of HD.

**Keywords**—Huntington's disease, oxidative stress, *Zingiber officinale*, Neuroprotective, 3-nitropropionic acid.

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### I. INTRODUCTION

Huntington's disease (HD) is an autosomal dominantly inherited progressive neurodegenerative disorder, characterized by progressively worsening chorea, psychiatric disturbances, cognitive impairment and weight loss (1,2). First reported by George Huntington in 1872 the degenerative process primarily involves medium spiny striatal neurons (MSN) and to a lesser extent cortical neurons (3). Cognitive deficits are core neuropsychiatric problem among HD patients (4,5). HD patients often exhibit deficits in executive tasks requiring planning, problem solving, and cognitive flexibility with progress of the disease (6,7). HD involve the generation of excitotoxic events, the alteration in energy metabolism and mitochondrial dysfunction, the resulting oxidative stress, and the htt aggregation, all leading to neurotoxicity(8). 3-Nitropropionic acid (3-NP) is a well known fungal toxin causing neurotoxicity in both animals and humans (9,10). 3-NP systemic administration has been proposed to produce 3-NP treatment induces memory impairment in rodents as one of the symptoms of HD further related to an increase in oxidative stress specially glutathione levels (11). Although, the precise mechanism of neuronal degeneration is not yet perfectly understood however, experimental evidences demonstrate that 3-NP administration produce lesions in striatum and cortex areas of the brain.(12,13). Besides, oxidative damage has been reported to play an important role in 3-NP induced neurotoxicity. 3-NP depletes antioxidant defense enzymes and increases levels of reactive oxygen/nitrogen species in different areas of the brain (14,15) These observations have been further strengthened by successful use of several endogenous and exogenous antioxidants against 3-NP induced neuronal damage in rats .It has been further reported that oxidative damage occurs at early stage of 3-NP toxicity in addition to mitochondrial dysfunction (16, 17,18). *Zingiber officinale* is widely used around the world in foods as a spice. For centuries, it has been an important ingredient in Chinese, Ayurvedic and Tibbi-Unaniherbal medicines for the treatment of catarrh, rheumatism, nervous diseases, gingivitis, toothache, asthma, stroke, constipation an diabetes, hypertension ,antiplatelet ,nausea ,antiemetic , antihyperlipidemic (19-21). Several reviews have appeared in the literature about this plant, and this may reflect the popularity of the subject and its common use as a spice

and a medicinal plant (22,23). It has been demonstrated that the plant possesses a potent antioxidant activity (24). But no evidences are still present that indicate its importance in the management of HD. Hence the study has been designed to explore the neuroprotective effect of *Zingiber officinale* against 3-NP-induced Huntington's disease.

## II. MATERIALS AND METHODS

### 2.1. Animals

The experimental protocol used in the present study was approved by the Institutional Animal Ethical Committee. Age matched young Wistar rats weighing about 150–200 g were employed in the present study. They were fed on standard chow diet and water *ad libitum*. They were acclimatized in the institutional animal house and were exposed to normal cycle of day and night.

### 2.2. Drugs and treatment schedule

- 3-Nitropropionic acid (3- NP) ((Sigma USA) Ethanolic extract of *Zingiber officinale* solutions were made freshly at the beginning of each experiment. A solution of 20 mg/kg 3- NP was prepared in normal saline and administered in a volume of 1 ml/100 g body weight intraperitoneally (i.p.). EEZO was suspended in 0.5% sodium carboxymethylcellulose (CMC) and administered 1ml/100g body weight perorally (p.o.). Animals were randomly divided into five groups of 6 each: Group 1 (Normal group) received vehicle for EEZO (perorally) and also normal saline (intraperitoneally); Group 2 received 3-NP (20mg/kg, i.p.) for 7 days; Group 3 received EEZO (100 mg/kg) +3-NP (20 mg/kg, i.p.) for 7 days; Group 4 received EEZO (200 mg/kg) + 3-NP (20mg/kg, i.p.) for 7 days; and Group 5 received EEZO (200 mg/kg) alone for 7 days. In Groups 3 and 4, EEZO was given 1 hour prior to 3-NP administration, and the doses were selected on the basis of previous studies (24).

### 2.3 Behavior assessment

#### 2.3.1. Morris water maze

The acquisition and retention of memory was examined using Morris Water Maze test (25)

#### 2.3.2 Assessment of locomotor activity

The locomotor activity was monitored using Digital Actophotometer apparatus. Each interruption of a beam on the x- or y-axis generated an electric impulse, which was presented on a digital counter. The apparatus was placed in a darkened, light and sound attenuated and ventilated testing room. Each animal was observed over a period of 5 min and values expressed as counts per 5 min (26).

#### 2.3.3 Rota Rod Activity

All animals were evaluated for grip strength by using the rotarod. Each rat was given a prior training session before initialization of therapy to acclimatize them on a rotarod apparatus. Animal was placed on the rotating rod with a diameter of 7 cm (speed 25 rpm). Three separate trials were given to each rat at 5 min interval and cut off time (180) was maintained throughout the experiment. The average results were recorded as fall of time (27).

#### 2.3.4 Elevated plus maze

Another paradigm to assess memory dysfunction is elevated plus maze (28).

### 2.4 Biochemical study

#### 2.4.1. Preparation of homogenate

For the biochemical analysis, animals were sacrificed by decapitation immediately after behavioural assessment. The brains were removed. 10% (w/v) tissue homogenate was prepared in 0.1 M phosphate buffer (pH 7.4). The homogenate was centrifuged at 10,000 g for 15 min and clear supernatant was separated and used for biochemical estimations (29).

#### 2.4.2. Measurement of lipid peroxidation

Malondialdehyde (MDA) which is a measure of lipid peroxidation was measured as described by Ohkawa. Briefly, brain tissues were homogenized with 10 times (w/v) 0.1 sodium phosphate buffer (pH 7.4). The reagents acetic acid 1.5 ml (20%) pH 3.5, 1.5 ml thiobarbituric acid (0.8%) and 0.2 ml sodium dodecyl sulfate (8.1%) were added to 0.1 ml of processed tissue sample. The mixture was then heated at 100 °C for 60 mins. The mixture was cooled with tap water and 5 ml of n-butanol: pyridine (15:1 % v/v), and 1 ml of distilled water were added. The mixture was shaken vigorously. After centrifugation at 4000 rpm for 10 mins, the organic layer was withdrawn and absorbance was measured at 532 nm (30).

#### 2.4.3 Estimation of acetylcholinesterase (AChE) levels

The quantitative measurement of acetylcholinesterase levels in brain was performed according to the method of Ellman et al. The assay mixture contained 0.05 ml of supernatant, 3 ml of 0.01M sodium phosphate buffer (pH 8), 0.10 ml of acetylthiocholine iodide and 0.10 ml of 5,5'-dithio-bis(2-nitrobenzoic acid) (DTNB). The change in absorbance was measured immediately at 412 nm using spectrophotometer. Protein estimation was done by biuret method (31).

### **2.3.4 Estimation of nitrite**

The accumulation of nitrite in the supernatant, an indicator of the production of nitric oxide (NO) was determined using a colorimetric assay with the Griess reagent (0.1% N-(1-naphthyl) ethylenediamine dihydrochloride, 1% sulfanilamide and 2.5% phosphoric acid) as described by Green et al. Equal volumes of supernatant and the Griess reagent were mixed. Then, the mixture was incubated for 10 min at room temperature in the dark, and the absorbance was measured at 540 nm using a spectrophotometer (32).

### **2.3.5 Estimation of reduced glutathione (GSH)**

Reduced glutathione in the brain was estimated according to the method described by Ellman. One milliliter of supernatant was precipitated with 1 ml of 4% sulfosalicylic acid and cold digested at 4°C for 1 h. The samples were centrifuged at  $1,200 \times g$  for 15 min at 4°C. One milliliter of this supernatant was added to 2.7 ml of phosphate buffer (0.1 M, pH 8) and 0.2 ml of DTNB. A yellow color developed that was read immediately at 412 nm using a Perkin Elmer Lambda 20 spectrophotometer. Results were calculated using the molar extinction coefficient of chromophore ( $1.36 \times 10^4 \text{ M}^{-1}\text{cm}^{-1}$ ) and expressed as a percentage of control (33).

### **2.3.6 Protein estimation**

The protein content was measured by the biuret method using protein kit (34).

## **III. RESULTS**

### **3.1 Effect of EEZO on locomotor activity**

3-NP treatment caused a significant decrease in locomotor activity as compared to vehicle treated group ( $P < 0.05$ ). Further, chronic treatment with Z.O (100 and 200 mg/kg, p.o.) significantly improved locomotor activity in 3-NP treated rats (Fig. 1). However lower dose of EEZO (100 mg/kg) did not show any significant effect on locomotor activity as compared to 3-NP treated rats. Z.O (200 mg/kg, p.o.) per se treatment did not show any significant change as compared to vehicle treated rats (Fig 1).

### **3.2 Effect of EEZO on rotarod activity in 3-NP-treated rats**

3-NP (20 mg/kg) treatment decreased muscle grip strength as assessed by the rotarod test on day 1<sup>st</sup>, 3<sup>rd</sup>, 5<sup>th</sup>, 7<sup>th</sup> day compared to the vehical group, which did not show any change in the activity of the animal throughout the experiment. EEZO (200mg/kg, p.o.) alone did not alter grip strength of the animals compared to control animals ( $P < .05$ ). Further, EEZO (100 and 200 mg/kg, p.o.) treatment significantly improved muscle grip strength of 3-NP (20 mg/kg)-treated rats ( $P < .05$ ) (Fig 2).

### **3.3 Effect of EEZO on memory performance in elevated plus maze paradigm**

In elevated plus task the 3-NP treatment significantly delayed retention transfer latency as compared to vehicle treated group. In contrast, chronic administration of EEZO (100,200mg/kg, p.o.) significantly shortened mean retention latencies in 3-NP treated groups (Fig. 3). However, EEZO (200 mg/kg, p. o.) per se treatment did not show any significance on transfer latency as compared to vehicle treated group.

### **3.4 Effect of EEZO on spatial navigation task in 3-NP treated rats**

In Morris water maze test, the mean escape latency of trained rats gradually decreased during training session from day 1st to 3rd in 3-NP treated animals. However, escape latency was significantly increased on the 5<sup>th</sup> and 7th day in Morris water maze as compare do the vehicle treated group ( $P < 0.05$ ). Chronic EEZO (200 mg/kg, p. o.) treatment showed a significant improvement in memory performance on the 5th and 7th day as compared to the 3-NP treated group ( $P < 0.05$ ) (Fig 4).

## **IV. BIOCHEMICAL ASSESSMENTS**

### **4.1. Effect of EEZO on acetyl cholinesterase levels in 3-NP treated rats**

Chronic 3-NP treatment significantly increased acetylcholinesterase enzyme level in rat brain as compared to the normal control ( $P < 0.05$ ). EEZO (100 and 200 mg/kg, p.o.) treatment significantly attenuated acetylcholinesterase enzyme activity as compared to 3-NP treated rat (Fig. 5).EEZO(100 and 200 mg/k.) per se treatment did not produce any significant effect on acetylcholinesterase enzyme activity as compared to 3-NP treated rats.

### **4.2 Effect of EEZO on brain lipid peroxidation level**

Administration of 3-NP showed significant increase in brain MDA level in disease groups compared to normal control ( $P < 0.001$ ). Pretreatment with EEZO. at a dose of 100 and 200 mg/kg respectively showed significant decrease in the brain LPO level as compared with disease group ( $P < 0.05$ ) It shows that 200mg/kg dose produce maximum significant as compared to 200mg/kg. (Fig. 6)

### **4.3 Effect on brain reduced glutathione (GSH)**

GSH level in the brain homogenate of 3-NP administered rats showed significant decrease as compared with Normal Group ( $P < 0.01$ ). In 3-NP induced Huntington's disease in rats, pre treatment with the ethanolic extract of Z.O.(EEZO)(100 and 200 mg/kg) showed significant increase in brain GSH level as compared to

disease group ( $P < 0.01$ ). It shows that 200mg/kg dose produce maximum significant as compared to 100mg/kg (Fig. 7).

#### 4.4. Effect of EEZO on brain nitrite levels in 3- nitropropionic acid-treated rats

Systemic 3-NP administration caused a significant increase in brain nitrite levels, which was significantly reversed by pretreatment with ZO (100 mg/kg, 200 mg/kg orally) and 200mg/kg shows maximum effect .No significant effect of EEZO (200mg/kg, orally) alone was observed on brain nitrite levels as compared with normal (Fig 8)

#### 4.5. Histological examination

A significant histopathological changes were seen in the brain of rats administered 3-NP as compared to normal rats. However, rats treated with EEZO (100mg/kg and 200mg/kg) significantly protected brain of rat indicated by reduction in inflammation, necrosis and glosis (Fig 9)

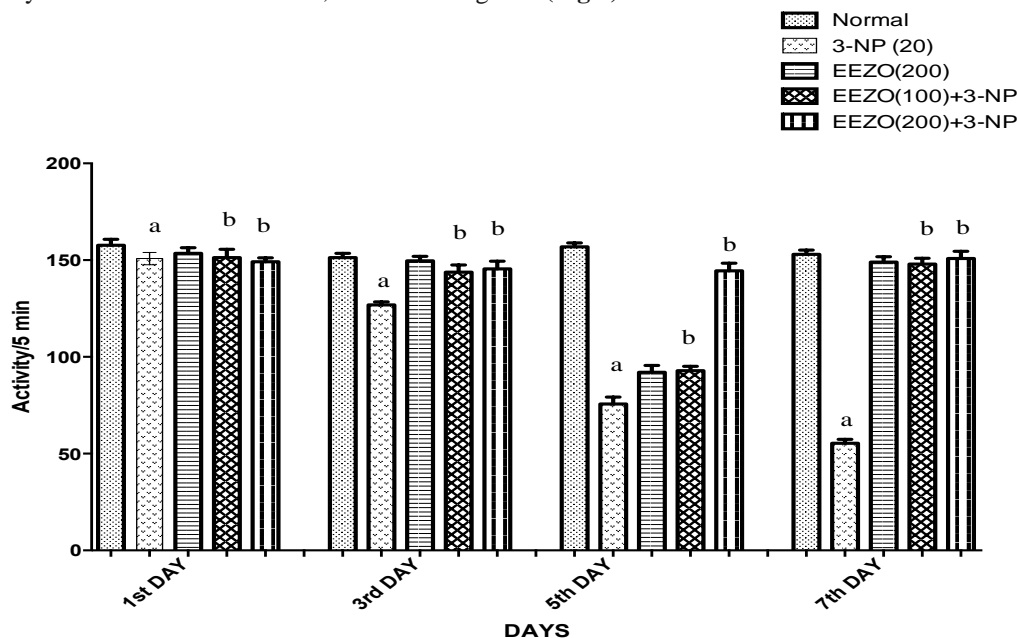


Fig 1 Effect of EEZO (100 and 200 mg/kg) on locomotor activity in 3-NP administered rats. Data are mean SEM values (n=6 in each group). a=  $p < 0.05$  vs. compared to vehicle-treated normal group; b=  $p < 0.05$  compared to 3-NP-injected group (one-way ANOVA followed by Tukey test)

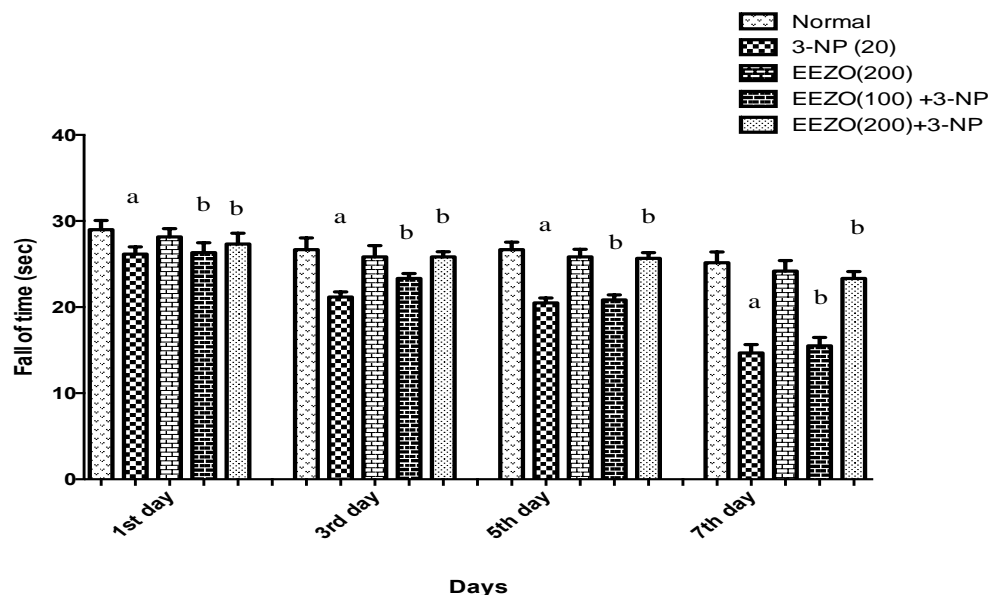


Fig 2 Effect of EEZO (100 and 200 mg/kg) on Rotarod activity in 3-NP administered rats. Data are mean  $\pm$ SEM values (n=6 in each group). a=  $p < 0.05$  compared to vehicle-treated normal group; b=  $p < 0.05$  compared to 3-NP-injected group (one-way ANOVA followed by Tukey test)

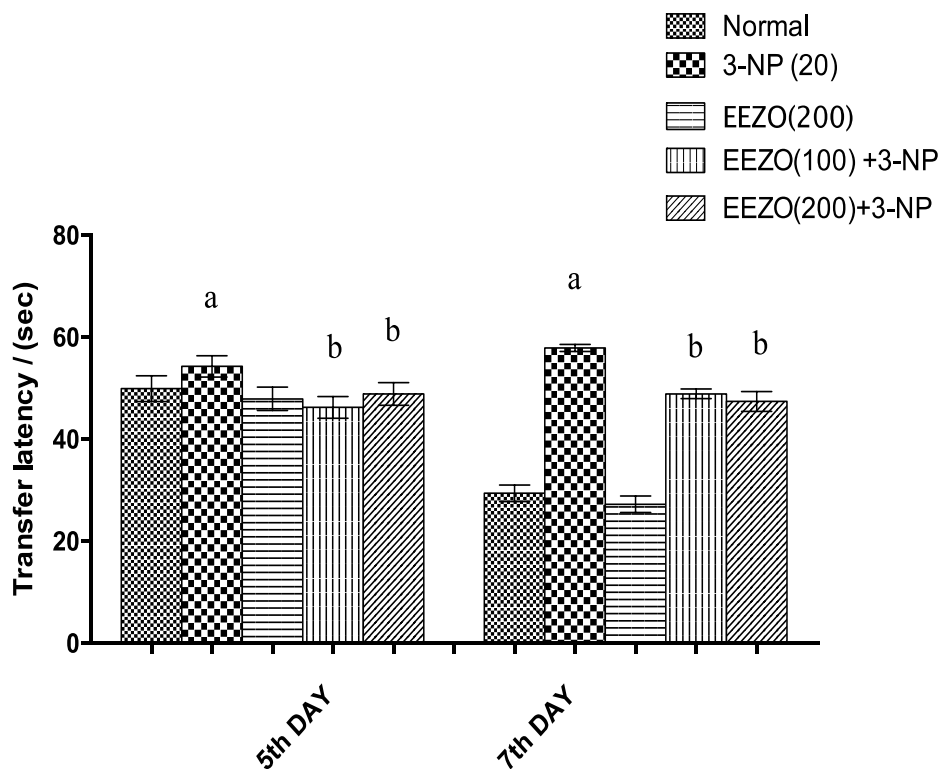


Fig 3 Effect of EEZO (100 and 200 mg/kg) on elevated plus maze activity in 3-NP administered rats. Data are mean  $\pm$  SEM values (n =6 in each group). a= $p < 0.05$  compared to vehicle-treated normal group; b= $p < 0.05$  compared to 3-NP injected group (one-way ANOVA followed by Tukey test)

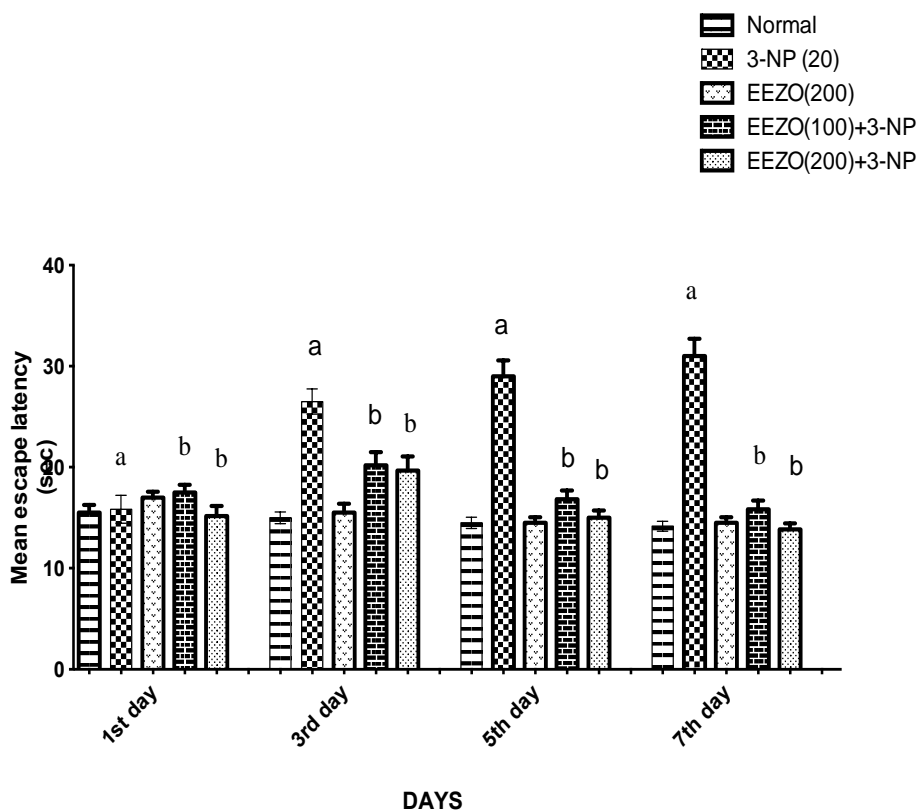


Fig 4 The effect of the administration of EEZO on time spent by rat to reach submerged quadrant or( we check for )transfer latency in MWM. All values are represented as mean  $\pm$  S.E.M. a= $p < 0.001$  v/s Normal; b=  $p < 0.001$  (one-way ANOVA followed by Tukey test)

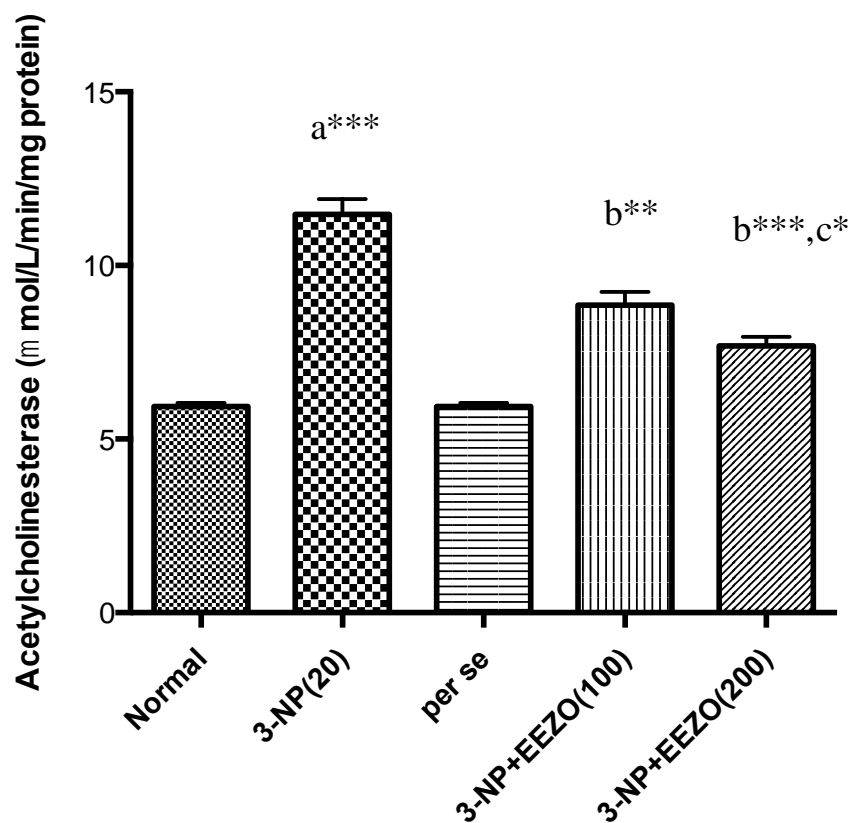


Fig 5 The effect of administered EEZO on AChE level. Data are mean  $\pm$ SEM values (n =6 in each group). a=p< 0.05 compared to vehicle-treated normal group; b=p<0.05 compared to 3-NP injected group; c= p< 0.05 compared to (3-NP+ 100 mg/kg) group (one-way ANOVA followed by Tukey test)

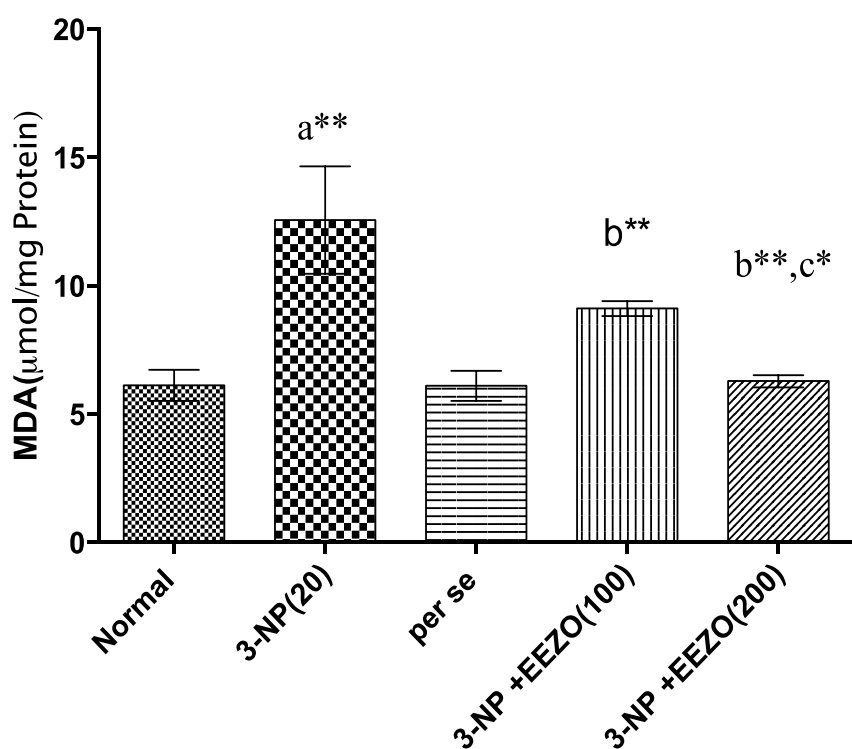


Fig 6 The effect of administered EEZO on Lipid Peroxide level. Data are mean  $\pm$ SEM values (n =6 in each group). a=p< 0.05 compared to vehicle-treated normal group; b=p<0.05 compared to 3-NP injected group; c= p< 0.05 compared to (3-NP+ 100 mg/kg) group (one-way ANOVA followed by Tukey test)

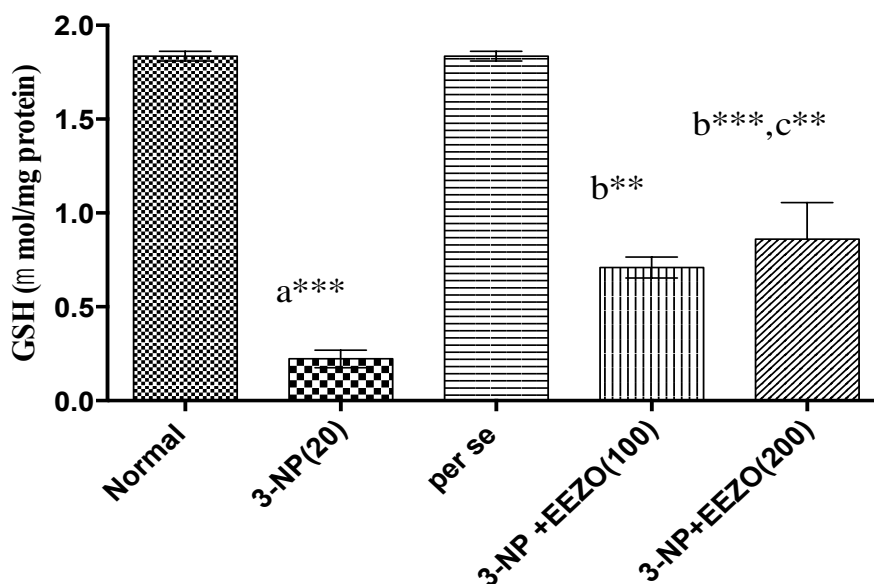


Fig 7 The effect of administered EEZO on GSH level. Data are mean  $\pm$ SEM values (n =6 in each group). a= $p < 0.05$  compared to vehicle-treated normal group; b= $p < 0.05$  compared to 3-NP injected group; c=  $p < 0.05$  compared to (3-NP+ 100 mg/kg) group (one-way ANOVA followed by Tukey test)

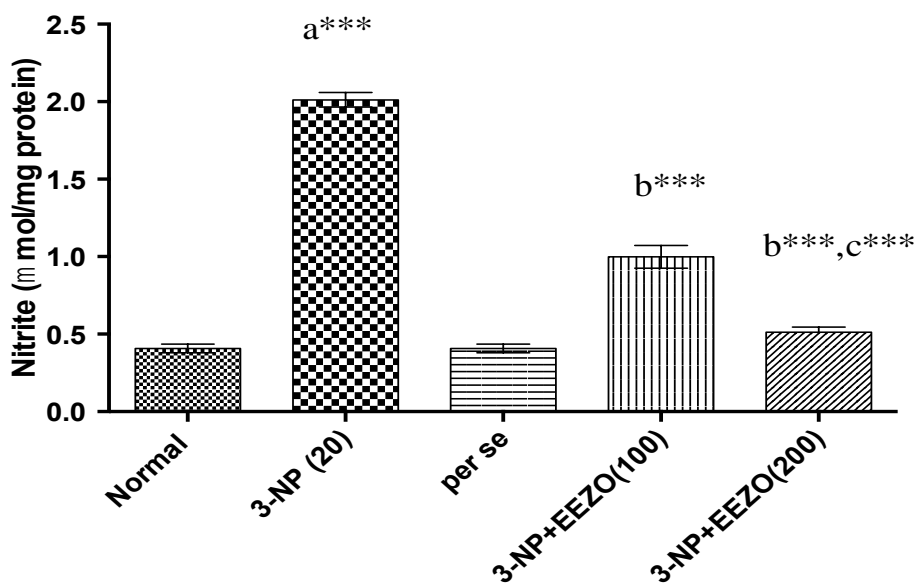
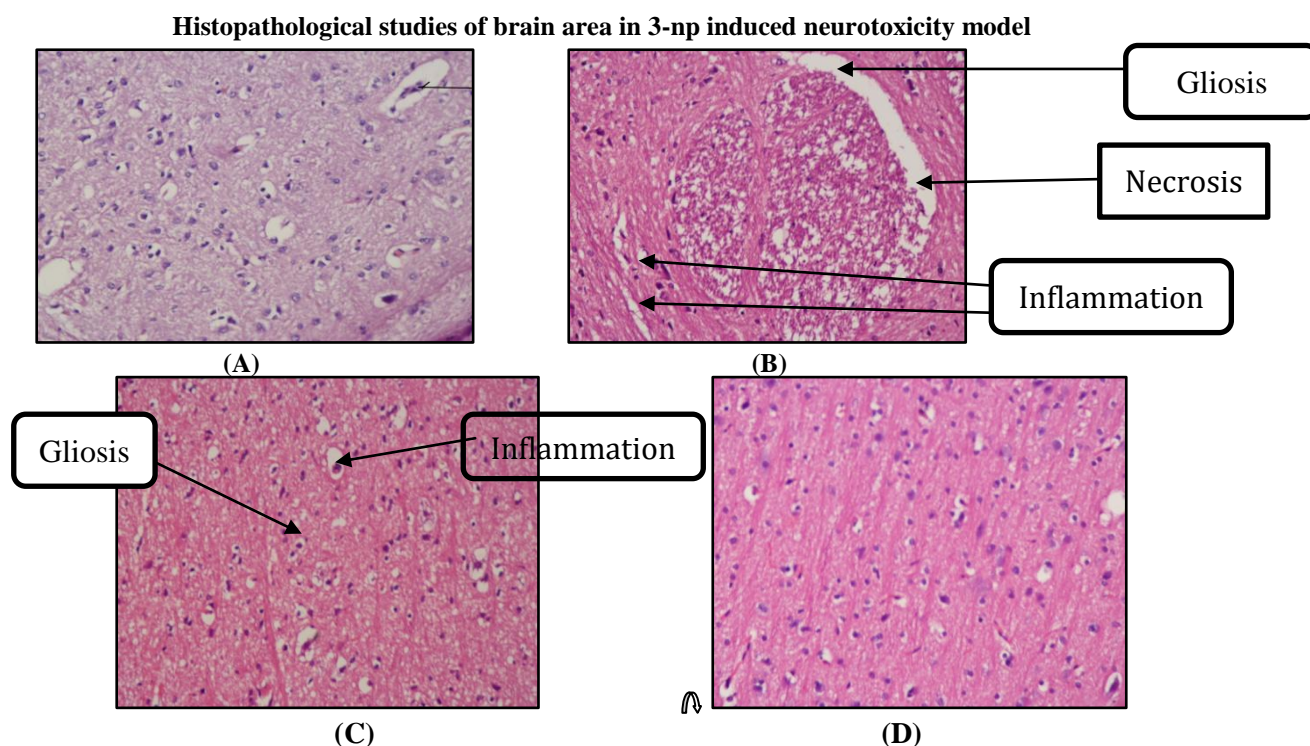


Fig 8 The effect of administered EEZO on Nitrite level. Data are mean  $\pm$ SEM values (n =6 in each group). a= $p < 0.05$  compared to vehicle-treated normal group; b= $p < 0.05$  compared to 3-NP injected group; c=  $p < 0.05$  compared to (3-NP+ 100 mg/kg) group (one-way ANOVA followed by Tukey test)





**Fig. 9.. Effect of EEZO on 3-nitropropionic acid induced histopathological changes in rat brain striatum. Histological sections of rat striatum stained with H & E: (A) —Normal group with no pathological changes (B) 3-NP(20mg/kg), showed gliosis ,inflammation and Necrosis (C) EEZO(100mg/kg)+3-NP,shows mild changes (D) EEZO(200mg/kg) +3-NP,showed no pathological changes .**

## V. DISSCUSSION

HD is an autosomal dominant inherited disorder characterized by progressive decline in motor and cognitive functions (35). HD is characterized by destruction of cholinergic receptors leading to decreased level of acetylcholine (36). The exact pathogenesis of the disease is not yet known, but evidence suggest that impairment in energy metabolism and oxidative stress play a major role in the pathogenesis of the Huntington's disease (37). However, the events which prompt or intervene the loss of Ach neurons remain unclear. Plants pose an important and a safer alternative for the management of neurodegenerative disorders (,38). Even, World Health Organization (WHO) has also documented the importance of folk medicine and has created strategies, guidelines and standards for botanical medicines. Thus, the present study examined the therapeutic value of antioxidants in the prevention of 3-NP neurotoxicity. Our results demonstrated that 3-NP administration increases the oxidative stress through lipid peroxidation and subsequently decreasing level of endogenous antioxidants. Ginger, a potent antioxidant, can be protective in this model of neurotoxicity depicted by significantly improved cognitive defects, prevented oxidative stress induced by systemic 3-NP treatment.

3-NP is a neurotoxin that irreversibly inhibits succinate dehydrogenase (SDH), a relevant enzyme constituting the complex II of the respiratory chain during mitochondrial electron transport (39). 3-NP is known to produce oxidative/nitrosative stress and evokes an experimental model of Huntington's disease (40,41,42,43). Indeed, a growing body of evidence suggests oxidative stress as a culprit in mediating behavioral impairment and memory deficits in age-related neurodegenerative disorders (44). This is too supported in our study that 3NP administered rats showed a significant decrease in transfer latency with subsequent impairment in motor coordination and muscle grip strength. Further, 3-NP treated rats there is marked increase in the level of TBARS, marker of lipid peroxidation, nitric oxide (NO) with attenuation in the level of reduced glutathione. This is supported by earlier reports that 3-NP administration generated oxidative/ nitrosative stress (46). Further, it is well reported that 3-NP produces lesions in hippocampal CA1 and CA3 pyramidal neurons, the area of brain that is associated with cognitive performance (45,46,47) which is too revealed in our study by performing histopathology of brain. 3-NP administration to rats results in increased activity of acetylcholinesterase enzyme that reduces the level of Ach in the stratitum, that is the basic reason of HD (48).

Our results demonstrated that treatment with *Z.officinale* a neuroprotectant(49) , dose-dependently enhances the memory with improvement in the locomotor and muscle grip strength in 3-NP-administered rats.



Accumulating evidences indicates that ginger showed marked protection in various neurological disease models (50).

Recently, it has been reported that ginger prevents oxidative stress and improves impaired cognitive performance in animals. This is too supported in our study that treatment with *Zingiber officinale* increased the level of GSH and attenuated TBARS with NO, marker of oxidative and nitrosative stress. Further, Zinger officinale treatment had a significantly ( $p < 0.001$ ) increased the level of acetylcholine by inhibiting AChE.

Thus, it can be concluded that being a very potent antioxidant, *Z. officinale* too possess anticholinesterase potency, that is responsible for improving memory or cognitive functions. Hence, the neuroprotective role of *Zingiber officinale* may be due to its anticholinesterase activity.

## VI. CONCLUSION

*Zingiber officinale* was found to possess a therapeutic effect against Huntington's disease in 3-NP induced animal HD models. Thus being a very potent antioxidant zinger too possesses anticholinesterase potency, that is responsible for improving memory or cognitive functions. Further studies with different extracts and their fractions are encouraged to identify the chemical constituents responsible for neuroprotection in Huntington's disease.

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