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Anticonvulsive and antioxidant activity of aqueous root extract of *Moringa oleifera* in ferric chloride-induced epileptic rats

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Abstract

Moringa oleifera (MO), commonly known as drumstick tree in South Asian countries are consumed as food and have immense medicinal value. The consumption of the root of MO reduced neuronal hyper-excitability in psychiatric disorders. The present study has tested the efficacy of the aqueous root extract of MO in preventing epilepsy, a serious neurological disorder, from non-penetrating brain injury. Reducing power, polyphenol, flavonoid content of MO, and high performance liquid chromatographic identification of free radical scavenging compounds in the extract in the present study encourages its use for preventing free radical-induced epilepsy. Holtzman Strain adult rats, weighing 200-250 g, were assigned into four groups (n=6 in each group): normal control; sham operated; intracortically $FeCl_3$ injected (100mM; 8µl); $FeCl_3$ injected + MO pretreated (350mg/kg, orally) and $FeCl_3$ injected + diazepam (DZ) pretreated (20mg/kg, i.p). Lipid peroxidation (LPO), catalase (CAT), superoxide dismutase (SOD) activities were studied as indirect parameters of free radical-induced brain damage. Serotonin, dopamine and nor-epinephrine were also evaluated biochemically from different brain regions. A statistically significant reduction in lipid peroxidation, CAT and SOD activity was observed in MO pretreated group when compared to the untreated epileptic rats. Serotonin level was found to be elevated significantly in cerebral cortex whereas dopamine and nor-epinephrine levels declined in the caudate nucleus and in cerebellum of MO-pretreated rats in comparison to untreated epileptic group and synchronizes with the changes with anticonvulsant diazepam. MO effectively prevents the advent of $FeCl_3$ induced epilepsy by ameliorating free radical damage and by regulating protective neurotransmitters to restrain neuronal hyper excitability.

Keywords: *Moringa oleifera*, free radical, $FeCl_3$-induced epilepsy, neurotransmitter, brain damage, seizure.

Introduction

Non-penetrating brain injury has been postulated to release metabolites of hemoglobin from ruptured arteries or veins [1]. Release of ferric or ferrous ions from hemoglobin causes neuronal damage at the injured site by producing excessive free radicals and thus resulting in post-traumatic epileptogenesis. Iron induced brain damage resembles non-penitrating traumatic brain injury in humans who are prone to develop epilepsy in their latter part of life [2]. Post-traumatic epilepsy is a common neurological disorder associated with chronic and recurrent episodes of spontaneous seizures, neuronal hyper-excitability and is a result of severe brain injury [3]. Several anticonvulsant drugs are symptomatic in the sense as they inhibit seizures but could not cure epilepsy. Since, 20 to 30% of all epileptic patients are resistant to current anticonvulsant drugs [4], a drug that prevents or retards epileptogenesis will be appropriate for the treatment. Previously, controlled randomized trials in patients with a high risk of the development of "posttraumatic" epilepsy have shown that early prophylactic administration of the major anticonvulsant drugs phenytoin and carbamazepine did not yield any favorable difference to placebo in the proportion of patients developing seizures [5]. Thus a major concern has always remained to elucidate the proper and effective treatments for patients of hemorrhagic brain injury in order to prevent the development of epileptogenesis in the later period of their life. Recently, our laboratory has demonstrated an effective way for treating and preventing post-traumatic epilepsy by feeding aqueous extract from rhizome of *Acorus calamus* in ferric chloride-induced somatocortical damage and thus prevents post-traumatic epileptogenesis in rats [6].

Intracortical injection of ferrous or ferric chloride produces focal paroxysmal electroencephalographic discharges and behavioral convulsions in rats [6] and thus is of particular interest due to its similarity with post-traumatic epilepsy in humans. Willmore and Rubin, 1984 [2] showed the animal model for post traumatic epilepsy by injecting $FeCl_3$ in rat cerebral cortex and proposed that reactive oxygen species are involved for the post traumatic

epilepsy. Thus by preventing excessive production of free radicals could be an effective strategy to attenuate the convulsive seizures in iron-induced epilepsy.

Another prime factor known to be responsible for modulating epileptic seizures is neurotransmitter metabolism [7]. Excitatory neurotransmission in traumatized brain injury evokes the pathogenesis for epileptic seizures [8]. Moreover, neurotransmitter such as serotonin, dopamine and nor-epinephrine exhibit neuroprotective role in preventing seizure-induced neuronal damage in various parts of the brain in kianic acid-induced and temporal lobe epilepsy [9].

Thus effective formulations of anti-epileptic drugs which can reduce free radical-induced oxidative damage and modulates the neurotransmitter function towards cessation of convulsive seizures are in great demand [10]. Researches on dietary antioxidants and herbal extracts have demonstrated that many of them are effective in attenuating the development of post-traumatic epilepsy and are also advantageous for their frequent dietary consumption and having no adverse effects [6]. MO, commonly known as drumstick tree is used as medicinal food in South Asian countries and in Africa [11]. Different parts of the plant have been found to exhibit anti-diabetic, wound healing, anti-ulcerative [12] and anti-inflammatory activity with rich contents of antioxidants [11]. Earlier our laboratory has provided evidence that, aqueous root extract of MO at a dose of 350 mg/kg body weight inhibited excitatory neurotransmission in penicillin-induced convulsions [13, 14]. Thus, in the present we are using the dose 350 mg/kg body weight. This study might possibly be the first to examine the effect of the aqueous root extract of MO on preventing iron-induced oxidative damage in brain and thereby preventing post-traumatic epilepsy. Another aim of the study is to investigate that whether MO extract has any beneficial effect on post-traumatic epilepsy by modulating the concentration of neuroprotective biogenic amines (such as, dopamine, serotonin and nor epinephrine) in different brain regions of rats.

Material and Methods

Collection of Plant material

The roots of MO were procured in bulk from United Chemical and Allied products (Kolkata) and authenticated by Botanical Survey of India (Howrah, India). The bulk amount was used throughout the experimental study.

Preparation of aqueous extract of MO

The bulk amount was used throughout the experimental study. The root bark was discarded because it has toxic action [15]. The woody portion of the roots (1 kg) was crushed, sun-dried, grinded and spread over tray with shifting of materials daily to avoid growth of fungus. The powder was soaked in water overnight and the extractive solution was filtered with Whatman No.1 filter paper and subjected to lyophilization [16]. The final yield was found to be 13% of dry matter [17].

Total reducing capacity of aqueous extract of MO

The reducing power of aqueous extract of MO was determined according to the method of Oyaizu, 1986 [18].

Measurement of Total antioxidant capacity in aqueous extract of MO by FRAP method

Total antioxidant activity was measured according to the method of Pe arrieta et al, 2008 [19].

Measurement of total phenolic compounds in aqueous extract of MO

The total phenolic compounds were measured according to the method of Pe arrieta et al, 2008 [19].

Measurement of total flavonoid content in aqueous extract of MO

Total flavonoid content was measured according to the method of Pe arrieta et al, 2008 [19].

High-performance liquid chromatography (HPLC) of aqueous extract of MO

In order to identify plant phenolic compounds such as gallic acid, catechin, epicatechin and quercetin by HPLC was done according to the method of Pe arrieta et al, 2008 [19] with slight modification. A volume of 20 µl of the filtered aqueous extract (0.45 µm syringe filter, Millipore, USA) was injected into a C8 column (250mm x 4.6mm I.D.; 3Å particle diameter; Waters, USA) from an autosampler (Waters 717, USA), using gradient flow of two solvents (Solvent A: 0.1 M sodium acetate pH 3.5: methanol: actonirile = 90:9:1; solvent B; methanol) by binary HPLC pumps (Waters 515, USA). The gradient was set as 5% solvent B from 0-10 mins, 40% of B for 10-40mins, 100% B for 40-60 mins, and followed by 5% of B upto 70mins. Aqueous solutions of gallic acid and methanolic solution of catechin, epicatechin, quercetin at different concentrations were injected as standards into the column under same conditions. The run time was set for 70 mins and peaks were identified in the sample by spiking with individual standards at 280 nm by a dual absorbance detector (Waters 2875, USA). Concentrations of the compounds in the extract were evaluated from the standard curves of gallic acid (10-1000 µg/ml), catechin (50-1000 µg/ml), epicatechin (100 µg-1500 µg), quercetin (40-500 µg/ml). Values were expressed in mg per gram of dry matter.

Superoxide anion radical scavenging activities of polyphenolic constituents identified in aqueous root extract

Measurement of superoxide anion scavenging activity of catechin, epicatechin, gallic acid, and quercetin was done according to the modified method of Nishimiki et al, 1972 [20].

Nitric oxide radical scavenging effect of polyphenolic constituents identified in aqueous root extract

Nitric oxide generated from sodium nitroprusside in aqueous solution at physiological pH interacts with oxygen to produce nitrite ions, which were measured by Griess reagent [21].

Animal and maintenance

Adult male Holtzman strain rats weighing 200-250g were used for the study. Animals were housed in cages (46 × 24 × 20 cm) with two animals per cage in a temperature (22º±1ºC), humidity (55-60%) and light-controlled room (lights on at 6:30 hrs, lights off at 18:30 hrs). Rats were provided commercial rodent diet (Lipton India, Delhi) and water *ad libitum*. Tail pinch and hand clapping tests were also performed to check any pre-existing epileptogenesis. All procedures and protocols used in the present study were approved by the Animal Care and Use Committee of the Institute and followed the guidelines documented in the National Institute of Health's Guide for the Care and Use of Laboratory Animals.

Animal experimentation

Sixty rats were equally divided into five groups (n=12 per group) and assigned the following treatments: Group I the normal control; Group II sham control received intracortical injection of saline; Group III the $FeCl_3$ group received intracortical injection of freshly prepared $FeCl_3$ solution (100mM, 8µl); Group IV received MO (350mg/kg; orally) for 14 days prior to $FeCl_3$ administration and Group V received an antiepileptic drug diazepam (DZ) (20mg/kg i.p) 1 hr prior to $FeCl_3$ administration. Behavioral observations and EEG recordings were continuously monitored from 1 hr to 5 hr after administration of $FeCl_3$ solution. The convulsive effect was estimated using the number of wet dog shakes (WDS) as an indicator according to the method of Lanthorn and Isaacson (1978). After EEG recording rats were sacrificed by cervical dislocation and antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT) and lipid peroxidation (LPO) were estimated from 6 rats in each groups (i.e. 30 rats) and neurotransmitters serotonin (5HT), norepinephrine (NE) and dopamine (DA) level were estimated in cerebral cortex (CC), midbrain (MB), cerebellum (CB) and caudate nucleus (CN) from 6 rats in each group (i.e. 30 rats).

Induction of epilepsy by intra-cortical administration of ferric chloride ($FeCl_3$) stereotaxically

Iron-induced experimental epilepsy model was prepared by injecting 8 µl freshly prepared aqueous solution containing 100mM $FeCl_3$ over a period of 5 min into the sensorimotor cortex [6] by using stereotaxic apparatus (INCO, India Ltd).

EEG recording

For electrocortiocographic recordings, electrodes were implanted stereotaxically on the surface cortex and a reference electrode was implanted over the frontal bone [6].

Behavioral study on epileptic seizures by Wet Dog Shakes (WDS)

Behavioral observations [22] were noted for 4 h after administration of FeCl3 solution. Animals were placed in individual observation chambers (40 x 24 x 15 cm). The frequencies of WDS were recorded throughout a 1 hr period by observers with no knowledge of the treatment given to the rats. WDS was identified as a rapid rhythmic shaking of the head in a radial motion. The signs were counted and presented as number of events per hour [6].

Biochemical estimation of SOD, CAT, LPO

After completion of EEG recordings at day 5, rats(n=6 in each group) were killed by cervical dislocation and the brain was removed immediately and washed in ice-cold phosphate buffer saline to remove blood and homogenized (10% w/v) in ice-cold 150mM potassium chloride in a homogenizer (Polytron, Switzerland). The homogenate was then centrifuged at 3000 g for 10 min in a cooling centrifuge (REMI, India) and the supernatant was collected for analysis of superoxide dismutase [23], catalase [24] and lipid peroxidation [25].

Estimations of brain monoamines

5HT, DA and NE level wereestimated from different brain tissues (CC, MB, CN and CB) after homogenizing in acidified butanol [14].

Estimation of protein

Protein concentration in the tissue homogenate was estimated by the method of Lowry et al, 1951 [26] using bovine serum albumin as the reference standard.

Statistical analysis

The values of estimation of total reducing power, total antioxidant activities, total phenolic content, flavonoid content, concentrations of gallic acid, catechin, epicatechin, quercetin and their superoxide anion and nitric oxide scavenging activities were averaged and expressed as mean ± SEM. A one-way analysis of variance (ANOVA) was carried out to evaluate whether there was any significant effect of MO treatment on the serotonin, dopamine and nor-epinephrine levels in different brain regions in post-traumatic epilepsy and also to investigate its effect on superoxide dismutase, catalase activity and on lipid peroxidation. The level of significance was set at $P<0.05$. When significant was found, multiple – comparison *t* test was used as post hoc analysis of the data in between the MO treated and untreated groups.

Results

Antioxidant properties and radical scavenging of aqueous root extract of MO

The total polyphenolic content and flavonoid content in aqueous root extract of MO was estimated to be 662.33± 2.517µmol gallic acid/gm and 11.95±0.197 catechin µmol/gm of dry weight (Fig 1). It was found that the estimated total polyphenol content was higher than the reported values in the leaf, seeds and stem. The ferric reducing antioxidant power was found to be 607.83± 2.22 µmol trolox/gm of dry weight (Fig 1).

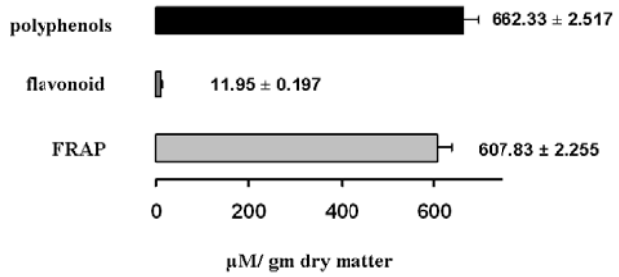


Figure 1 Polyphenol, Flavonoid content and ferric reducing antioxidant power (FRAP) of aqueous root extract of *Moringa oleifera*. Values expressed in Mean± SEM of triplicates.

Gallic acid, catechin, epicatechin and quercetin were identified from the HPLC profiling of the aqueous root extract (Fig 2) and a high content of gallic acid (18.51± 0.372 mg/ gm of dry weight) followed by quercetin (7.18 ± 0.074 mg/ gm of dry weight), epicatechin (2.91 ± 0.032 mg/ gm of dry weight) and caechin (1.08 ± 0.08 mg/ gm of dry weight) (Table 1).

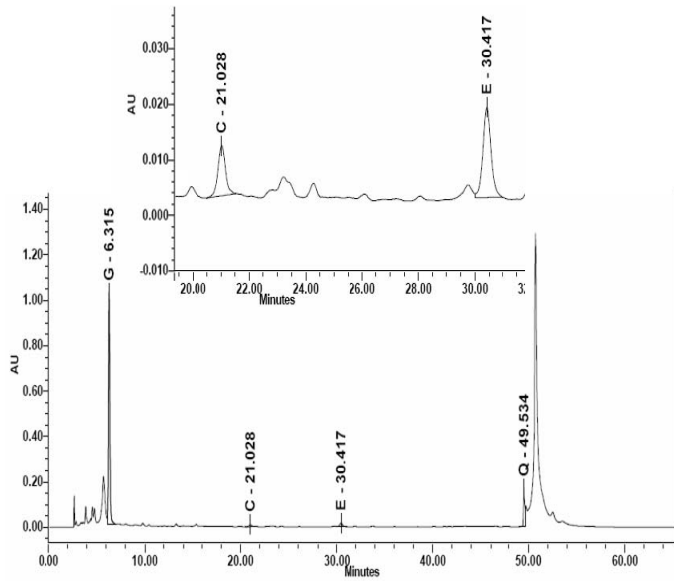


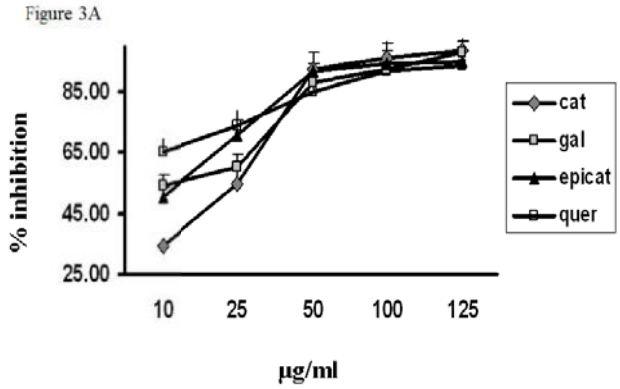
Figure 2 Identification of plant polyphenols by high performance liquid chromatography of aqueous root extract of *Moringa oleifera*. G= gallic acid; C= catechin; E= epicatechin; Q= quercetin.

Table 1: Quantification of some plant polyphenols in queous root extract of *Moringa oleifera* by HPLC

	Run 1	Run 2	Run 3		mean SEM
Gallic acid	18.94	18.26	18.34	18.51	0.037
Catechin	1.00	1.16	1.08	1.08	0.08
Epicatechin	2.94	2.87	2.91	2.91	0.032
Quercetin	7.27	7.14	7.15	7.18	0.074

Values are Mean± SEM of triplicate run. µg/100g of extract

The superoxide scavenging (Fig 3A) and nitric oxide scavenging (Fig 3B) effects of the identified polyphenol and flavonoids were found in concentrations between 10- 125 µg and 500-2500 µg. Superoxide scavenging activities of gallic acid, catechin, epicatechin and quercetin were found to be higher in lower concentrations (10- 125 µg) than their nitric oxide scavenging effects in relatively higher concentrations (500-2500 µg).



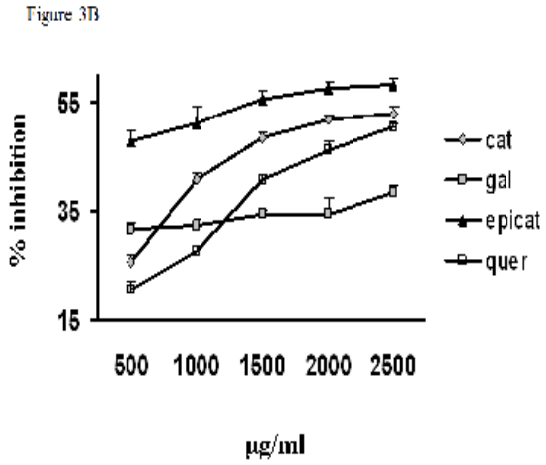


Figure 3 Free radical scavenging activities of plant polyphenols identified in the aqueous root extract of *Moringa oleifera*. A) Superoxide scavenging activity; B) nitric oxide scavenging activity. Results were expressed as Mean± SEM of triplicates of polyphenolic compounds.

Behavioral manifestations in $FeCl_3$-induced epilepsy

Administration of FeCl3 resulted in development of WDS in three groups of animals ($FeCl_3$, $FeCl_3$+MO, $FeCl_3$+DZ) whereas WDS was never observed after intracortical injection of saline in sham control group of animals. Significant reduction in WDS counts was observed in MO pretreated - $FeCl_3$ injected group ($P<0.01$) and in DZ pretreated -$FeCl_3$ injected group ($P<0.01$) in comparison to untreated-$FeCl_3$ injected group of animals (Fig 4). None of the animals have shown behavioral evidences of seizures.

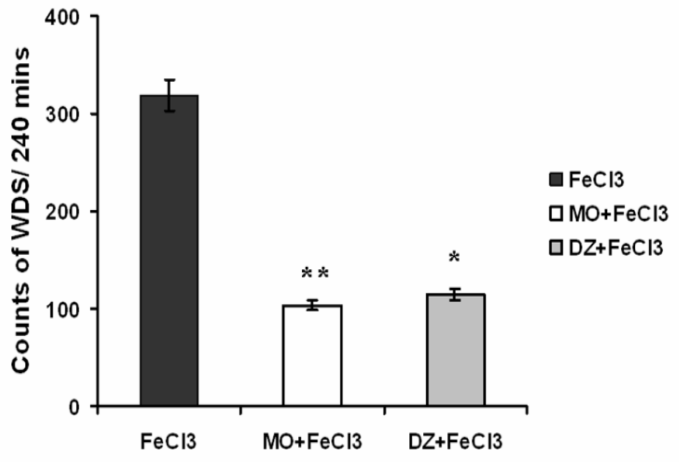


Figure 4. Effect of pretreatment of aqueous root extract of *Moringa oleifera* (MO) and diazepam (DZ) on $FeCl_3$-induced Wet Dog Scores (WDS). MO (350 mg/kg) and DZ (20 mg/kg) reduced WDS counts. Values are expressed as Mean± SEM (n=6). * $P< 0.05$; ** $P<0.01$.

Effect of aqueous root extract of MO on cortical waveform of $FeCl_3$-induced epilepsy

The continuous EEG recordings showed progressive development of high-voltage fast activity with spiking, burst of polyspiking, and high-voltage sharp waves in untreated-$FeCl_3$ injected group (Fig 5b). The sham control group showed no evidences of high-voltage waveform and spiking (Fig 5a). Rats pretreated with MO extract did not exhibit high voltage waves and sparse spiking was observed (Fig 5c). The DZ-pretreated group also did not show any high voltage wave and polyspiking (Fig 5d), indicating that pretreatment of MO extract prevented epileptiform activity in $FeCl_3$-induced epilepsy.

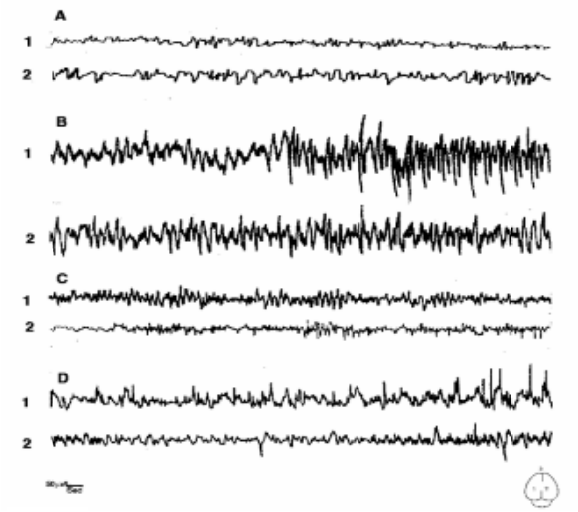


Figure 5. Representative electroencelographic (EEG) recordings of Moringa oleifera (MO) and diazepam (DZ) pretreated rats on seizure activity after FeCl3 administration. Numbers 1-3 indicate the position of the recording electrodes: 1 and 2- surface cortex; 3- frontal lobe. A) EEG from sham control rats injected with saline at sensorimotor cortex; B) epileptiform seizures of FeCl3 injected cerebral cortex; C) Appearance of single positive waves in MO pretreated FeCl3 injected rats; D) Low voltage waves with single spikes in DZ pretreated FeCl3 injected rats.

Effect of aqueous root extract of MO on superoxide dismutase (SOD) and catalase (CAT) activities and on LPO in $FeCl_3$-induced epilepsy

SOD activity was significantly higher ($P<0.01$) in untreated $FeCl_3$-induced group (28.09±1.25 U/mg of protein) of animals in comparison to sham control group (14.81±1.02 U/mg of protein, Fig 6A). CAT activity was also significantly higher ($P<0.001$) in untreated $FeCl_3$-induced group of animals (20.15 ± 0.92 U/mg) of protein in comparison to control groups (15.11±0.96 U/mg of protein, Fig 6B). Whereas, in animals pretreated with MO and DZ, a significant reduction in SOD (MO+ $FeCl_3$: 20.87± 0.76 U/mg; $P<0.01$; DZ+$FeCl_3$: 18.41± 0.08 U/mg; $P<0.01$) were observed in comparison to their untreated $FeCl_3$-induced group (Fig 6A). Although CAT activity (MO+ $FeCl_3$: 17.03± 0.63 U/mg; $P<0.01$; DZ+$FeCl_3$: 16.21± 0.75 U/mg; $P<0.01$) decreased in these groups but were not significant in comparison to $FeCl_3$-induced group (Fig 6B).

$FeCl_3$ –induced damage was increased as evident from significantly higher ($P<0.001$) level of LPO in untreated group of animals (8.92 ±0.47 nmole/gm tissue) as compared to sham control (3.00±0.34 nmole/gm tissue). Peroxidation of lipids was found to be significantly reduced in MO treated animals (4.29±0.79 nmole/gm tissue; $p<0.01$) and in DZ treated group (3.88±0.61 nmole/g tissue; $p<0.01$) as comparison to control (Fig 6C).

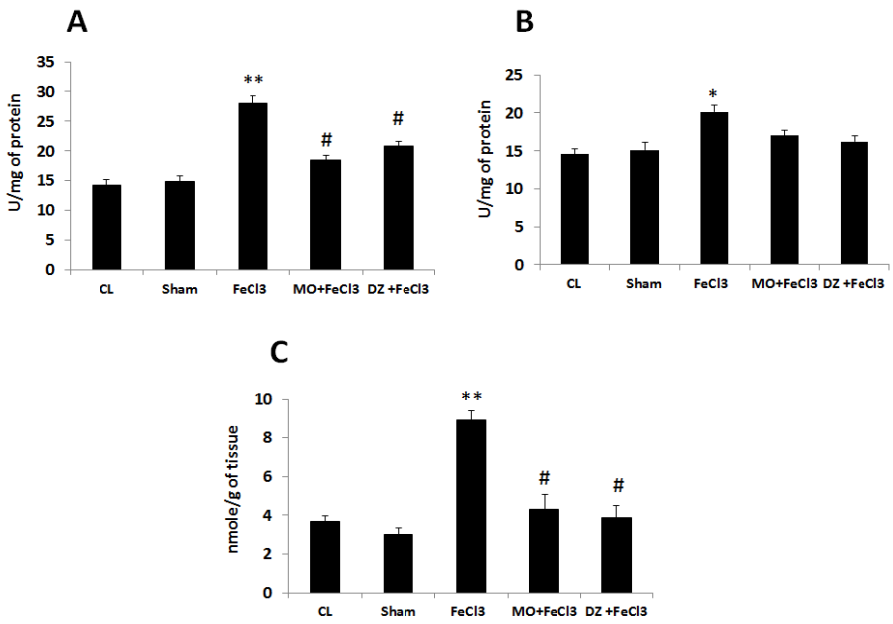


Figure 6 Changes of antioxidant enzymes like superoxide dismutase (SOD) [A], catalase (CAT) [B] and Lipid peroxidation (LPO) [C] level in control (cl), sham control (sham), ferric chloride ($FeCl_3$) induced epileptic and *Moringa oleifera* (MO) and diazepam (DZ) pretreated epileptic rats brain. A significant reduction in SOD, CAT and LPO was observed in MO pretreated group than $FeCl_3$ induced epileptic rats. The results are expressed as mean ± S.E.M. *p<0.01, **p<0.001 when compared with sham control group, #p<0.01 when compared with FeCl3 induced epileptic group.

Effect of aqueous root extracts of MO on 5HT, DA and NE contents in different brain regions of $FeCl_3$-induced epileptic rats

A significant decrease in the 5HT level in CC (0.051± 0.005 µg/100g wet tissue; P<0.01) and in MB (0.181± 0.024µg/100g wet tissue; P<0.02) of untreated $FeCl_3$-induced group was found in comparison to sham control group of CC (0.085± 0.008µg/100g wet tissue) and MB (0.292± 0.019µg/100g wet tissue) (Fig 7A). 5HT levels were found to be increased significantly in CC of MO (0.113± 0.009µg/100g wet tissue; P<0.001) and DZ (0.097± 0.007µg/100g wet tissue; P<0.001) pretreated rats in comparison to the untreated $FeCl_3$-induced group. No significant change was observed in CB and CN between the groups (Fig 7). Significant increase in DA level in CB (1.029±0.047µg/100g wet tissue; P<0.001) and CN (3.676±0.117µg/100g wet tissue; P<0.01) was found in $FeCl_3$-induced group in comparison to their respective sham control (CB: 0.786± 0.018µg/100g wet tissue; CN: 2.788± 0.164µg/100g wet tissue) group (Fig 7B). When pretreated with MO (CB: 0.512 ± 0.59µg/100g wet tissue; CN: 1.701 ± 0.248µg/100g wet tissue) and DZ (CB: 0.625 ± 0.064µg/100g wet tissue; CN: 1.954 ± 0.156 µg/100g wet tissue), the level was found to be significantly decreased (p<0.001) in comparison to the $FeCl_3$-induced untreated group (Fig 7B). No significant changes were observed in DA levels in CC and MB in between groups.

Cerebral NE was significantly increased in $FeCl_3$-injected untreated group (0.019 ± 0.003 µg/100g wet tissue; P<0.02) when compared with sham control group, whereas the level increased significantly in MO treated (0.036 ± 0.005 µg/100g wet tissue; P<0.01) and DZ (0.047 ± 0.002 µg/100g wet tissue; P<0.001) treated $FeCl_3$-injected animals (Fig 7C). Similar changes were also found in the mid brain region of the animals between sham control, untreated, MO treated and DZ treated $FeCl_3$-injected groups.

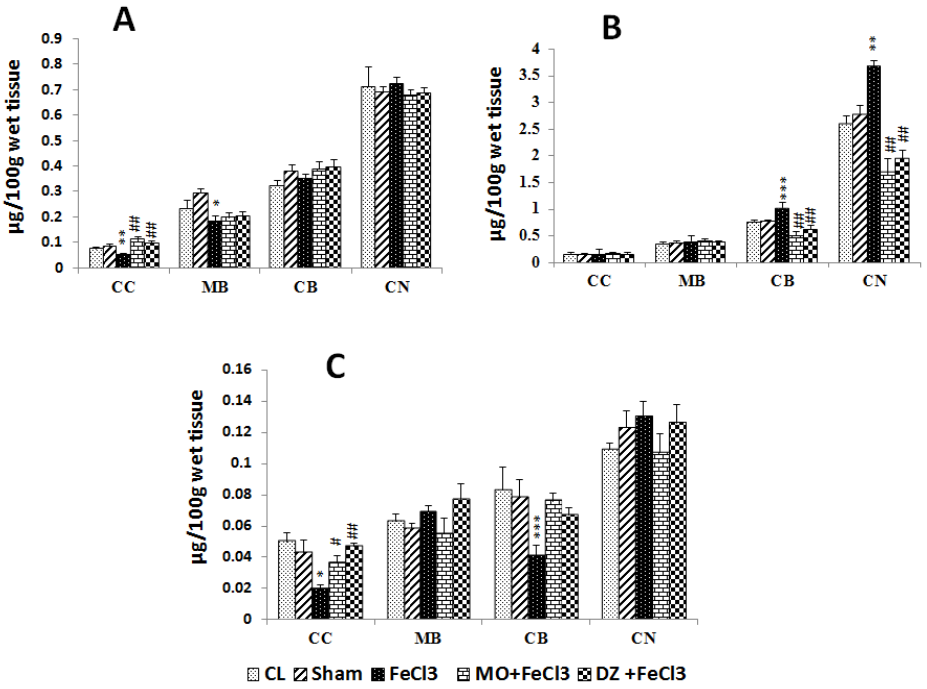


Figure 7. Changes of neurotransmitters like serotonin (5HT) [A], dopamine (DA) [B] and norepinephrine (NE) [C] level in cerebral cortex (CC), midbrain (MB), cerebellum (CB) and caudate nucleus (CN) of control (CL), sham control (sham), ferric chloride ($FeCl_3$) induced epileptic and *Moringa oleifera* (MO) and diazepam (DZ) pretreated epileptic rats brain. The results are expressed as mean ± S.E.M. *p<0.02, **p<0.01, ***p<0.001 when compared with sham control group, #p<0.01, ##p<0.001 when compared with $FeCl_3$ induced epileptic group.

Discussion

The present study demonstrated that pretreatment with the aqueous extract of MO significantly reduced ferric chloride-induced free radical injury in brain on rats and modulated the neurotransmitter homeostasis to prevent epilepsy from oxidative damage. The data showed that aqueous root extract of MO exhibited prominent antioxidant activity and constitutes effective free radical scavengers. Recently Kasolo et al, 2011 [27] reported about phytochemicals and acute toxicity of *Moringa oleifera* roots in mice. They have isolated "saponin" from water extract of Moringa root and it is established that saponin has anticonvulsant activity [28] and antioxidant activity [29]. Thus, saponin might be the active compound which is responsible for anticonvulsant and antioxidant activity of *Moringa oleifera* aqueous root extract.

Several lines of evidence suggested that excessive free radicals such as reactive oxygen and nitrogen species are the cause of oxidative stress and free iron catalyses the formation of some of these reactive species which damages the membrane-lipid integrity of brain structures [30]. Moreover, excitatory neurotransmitter glutamate and N-methyl-D-aspartate in epileptic seizures can produce reactive oxygen and nitrogen species [30]. The free radical induced brain damage may evoke hyper-excitability in neuronal transmission as is manifested in the form of epileptic seizures [31]. In the present study, $FeCl_3$-induced epileptic animals exhibited significant rise in peroxidation of membrane lipids. The increase in enzymatic activity of SOD and catalase demonstrated the activation of antioxidative defense system in the brain which is indicative of free radical–induced brain damage after intracortical administration of $FeCl_3$.

Thus, developing drugs or introducing traditional medicinal herbs in order to prevent oxidative stress in brain could effectively attenuate the seizure development from traumatic brain damage [10]. Thus need of medicines with consistent benefits and devoid of any adverse effects has implicated to test the efficacy of MO root extract, as traditional dietary supplement. Our hypothesis was supported with the rich presence of some plant polyphenolic compounds in roots and also exhibited potent antioxidant activities and free radical scavenging activities. Pretreatment with aqueous root extract on $FeCl_3$-administered rats has significantly reduced the behavioral abnormalities in comparison to untreated animals and were also accompanied with reduced magnitude and intensity of neuronal after-discharge. The improvement in behavioral manifestations and electrophysiological recordings were further investigated at the cellular and biochemical level. And a significant reduction in lipid peroxidation with decrease in anti-oxidative defense might indicate the reduction of free radical induced membrane damage and in maintaining membrane integrity as antioxidant compounds can exert neuroprotective function during acute phase of seizures, thereby decreasing the severity of

hippocampal lesion [32]. The decrease in oxidative parameters might be the results of the free radical scavenging activities of the compounds identified in the extract in present study.

Extensive researches based on neurotransmitter involvement in generating or abolishing epileptic seizure has displayed that an intricate mechanism of interactions exists between neurotransmitters and epileptic seizures [9]. A wealth of literature has provided an inverse relationship of serotonin concentration with epileptic seizures. Enhancement of 5HT transmission by administering 5HT agonists in epileptic foci of substantia niagra has been found to reduce convulsions [33]. The reduction of seizures in these studies can better be explained as an agonist-induced preferential activation of 5HT receptor subtypes in the epileptic foci [9]. This was confirmed in a study where mice having mutation in a 5HT receptor subtype was found to be susceptible to sound-induced epileptic seizures [34]. In the present study, we investigated serotonin concentration in different brain areas and found a significant rise in 5HT concentration in CC of MO pretreated-epileptic rats. Thus it can be speculated that MO-induced neuro-protection by 5HT may either be due to attenuation of free radical induced damage in the site of $FeCl_3$ injection or may be due to the direct central inhibitory effect of the extract.

DA is commonly known to exert neuro-protection against hippocampal cell death [35]. A study by Bozzi et al, 2000 [35] revealed that hippocampal cell death could not be averted in kianic acid-induced seizures in homozygous DA D2 receptor deficient mice and postulated that inhibitory control of dopamine on glutamate excitotoxicity is due to DA and D2 receptor interaction. A reciprocal relationship of glutamate and DA exits in neural networks in almost all parts of the brain. Paroxysmal activity in the cortex of epileptic patients leads to increased activity of glutamatergic fibers, thereby increasing tonic release of DA and down regulating DA receptors [36]. The down regulation of receptor reduces the phasic response of DA and thus could not prevent seizures. The mechanism pointed out the need for introducing D2 DA receptor agonists in treating epilepsy. In the present study different brain areas were used to analyse the DA levels in MO-pretreated and untreated $FeCl_3$ injected rats and a significant increase in DA concentration was observed in CN and CB in untreated epileptic animals. DA level was reduced significantly in MO pretreated epileptic group in the same parts of the brain in comparison to the untreated epileptic rats The CN, located within the basal ganglia of brain is highly innervated by dopaminergic neurons. These neurons originate mainly from the ventral tegmental area and the substantia nigra pars compacta, and electrical stimulation of the latter is known to suppress hippocampal epilepsy by activating dopaminergic projections [37]. CB plays an important role in the integration of sensory perception, coordination and motor control. From the data obtained in the present study we can postulate that MO might be responsible in modulating the receptor dependent phasic response in treating post traumatic seizures. This interesting observation may also lead the way to future investigation for further elucidation of the structures of biochemical compounds in the extract and their any direct role on DA receptor function.

Another neurotransmitter included in the present study is NE since the nor-adrenergic pathway by some extent is able to suppress the epileptic seizures generated by electric shock or certain chemical compounds [38]. Alteration in NE level in cerebellum of MO untreated epileptic rats was significant and was lower than the normal animals. It is known that noradrenergic deficits singly or with 5HT may contribute to some form of epilepsies and the degree of susceptibility to the seizures is modulated by noradrenergic pathway. Barry et al, 1987 [39] in a classical experiment in mice showed that grafting of NE-rich cell suspension effectively reduced the kindling-induced epileptic seizure. In the present model of post traumatic injury, the significant rise in NE concentration in MO-treated $FeCl_3$ injected animals with parallel reduction in seizure activity might suggest that root extract of MO potentiates the favorable balance between neurotransmitter function to reduce neuronal hyper excitability.

Conclusion

Based on the results of the present study, it may be concluded that the antiepileptic action of the aqueous root extract of MO in post-traumatic injury might be the result of attenuating the free radical generation, as evident from decreased brain tissue damage. Moreover, MO attributed to a favorable balance on neurotransmitter level for suppressing seizure activity. The present study also encourages further investigations for existence of any compounds modulating the neurotransmitter-receptor interaction and more importantly as the use of the plant product as food for reducing the chances of occurrence of epilepsy from post-traumatic brain injury.

Authors' contributions

Koushik Ray carried out the electrophysiological study and wrote the manuscript. Arkodeb Dutta managed the literature search and undertook the statistical analysis. Rimi Hazra carried out the neurochemical and antioxidant study. Debjani Guha designed the study and wrote the protocol and helped to draft the manuscript. All authors contributed to and have approved the final manuscript.

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