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Effect of Spinach (*Spinacea oleracea*) on DNA fragmentation in pentylenetetrazole induced experimental epileptic rat model

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Abstract

Background

Epilepsy is a restrained neurological disorder, with a constant neuronal damage, ranging from severe, life-threatening and disabling situations. It leads to oxidative brain damages through DNA fragmentation. Pentylenetetrazole (PTZ) is a convulsant used to produce experimental epileptic animals. Investigation proved; antioxidant enriched *Spinacea oleracea* (SO) or spinach, a commonly available herb, has a modulatory role on the damaging effects of free radicals.

Methods

The study was conducted with twenty-four adult male Holtzman strain albino rats (200-250gm). These rats were divided into groups of Control, SO treated control, PTZ induced experimental epileptic group and SO pretreated PTZ induced experimental epileptic group. The epileptic model was prepared by intraperitoneal administration of PTZ at a dose of 40 mg/kg body weight. Aqueous leaf extract of SO was orally given at a dose of 400 mg /kg body weight, for fourteen consecutive days. After the behavioral study serum and brain tissue samples were collected for the estimation of nitric oxide (NO), DNA fragmentation and antioxidants level.

Results

Pretreatment with SO leaf extract showed significant decrease in the seizure score, ictal phase, serum NO level, LPO levels and rate of DNA fragmentation. The interictal phase, SOD, CAT, GSH activity of different parts of the brain were significantly increased in SO pretreated PTZ induced group.

Conclusion

SO is found to play a vital role to provide protection against the oxidative damage of epileptic brain by amending the levels of antioxidants and serum NO level.

Key words

Epilepsy, DNA fragmentation, nitric oxide, Pentylenetetrazole, seizure, spinach,

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Background

Epilepsy is a most common chronic and a non communicable neurological disorder affecting over 50 million people worldwide [1]. More than 3,500 years ago Hippocrates described this oldest medical condition; Epilepsy. It is also recognized by the Ayurvedic system of medicine in India for over twenty centuries. According to WHO approximately 15 million people that comprises to about 1% of the South-East Asian population are suffering with epilepsy [2]. Based on a study in India, in the year 2001, it was found that 5.5 million people are epileptic with an incidence of about half a million new cases each year. Research works stated that Indian rural population constitutes of about 74% of the total and among them approximately three fourths remain untreated [3]. A study of West Bengal state of India showed that about 38 cases were detected in a population of 20,966 [4].

Epilepsy is a restrained neurological disorder, characterized with constant neurological damage, ranging from severe, life threatening and disabling situations. It is termed as seizure disorder. Research proved that seizures can set off long-term inflammatory reactions. Brain injuries like head trauma, stroke, seizure, infection may lead to brain inflammation [5]. Oxidative injury has been studied to be an initiator and a cause behind the progression of epilepsy. Functional or structural brain injuries are observed with an increase in the levels of extracellular glutamate, intracellular calcium ions, proteases and free radicals [6]. NO, hydroxyl radicals, and superoxide ions are known to contribute to nucleic acid damage. Peroxynitrite which is formed from the combination of reactive NO and superoxide ion is identified to be cause behind oxidative damage of nucleic material either directly or indirectly [7, 8]. Brain inflammation is studied to be related to pro-inflammatory cytokine gene expression and DNA damage [9].

Oxidative stress is considered to be the major factor behind the etiology of seizure-induced neuronal death [10]. Studies on human brains showed epileptic condition lead to damage of the brain due to constant exposure to oxidative stress [11]. Free radicals act as the causative agents for the degradation of proteins, lipids, and nucleic acids [12]. Within minutes of initiation of oxidative stress DNAs damage takes place, which is due to the activation of endonucleases or by the attack of free radicals; thereby leading to senescence during brain oxidative stress [13]. Nitric oxide synthase is evidenced to play an important role in DNA fragmentation [14].

Following a line of investigation it is found that epileptics are low in many antioxidants, including glutathione, superoxide dismutase as intrinsic and vitamin E, vitamin C, vitamin A as extrinsic antioxidants [15]. A handful of studies done on epileptic patients regarding the use of antioxidants was not enough to support the fact that brain injury in such cases are protected. However abundant data on animal models that are available showed an improvement of the oxidative brain damage with the use of antioxidant [16]. The research data available on human population suggest that vitamins A, C, and E are vital for brain functioning [17]. A combination of vitamin E and vitamin C proved to safeguard the nerve cell membranes against the oxidation damages in people with posttraumatic seizures [18].

Spinach or *Spinacia oleracea* (SO); known as an important dietary vegetable often associated with beneficial health effects. Fresh spinach consists of flavonoids and phenolic compounds. The hydroxyl groups of these compounds exert an antioxidants function due to the free radical (such as superoxide, OH anion radical and singlet oxygen) scavenging properties [19]. Water soluble extract of spinach leaves is a natural antioxidant mixture (NAO) [20]. This mixture is considered to exhibit antiproliferative [21] and anti-inflammatory properties [22]. Glucurinated flavonoid *i.e*; 6-(3, 4-dihydroxy-phenyl)-9-hydroxy-7-methoxy-dioxolo (4,5-) chromen-8-one 4'-ß-glucuronid [23, 24], is isolated and purified from NAO. This component is, highly stable at high temperature and has a long shelf life.

Considering the above mentioned antiproliferative, anti-inflammatory and immunomodulatory role of SO leaves extract through its high antioxidant contents; this study was performed to investigate in experimental epileptic animals by analysis of their behavioral, seizure patterns, alteration immune system and to elucidate the role of antioxidants in immunomodulation of epilepsy using SO.

Material and Methods

Study Period

The present study was carried in S.N. Pradhan Centre for Neurosciences, University of Calcutta for a period of 12 months (2008 Sep. -2009 Sep.) including animal habituation, their treatment, behavioral observation followed by the biochemical estimations.

Sample size calculation

In a pilot study done prior to this research showed Standard deviation of serum NO in group I (SO+PTZ) = 0.08
Standard deviation of serum NO in group II (Control) = 0.09
Mean difference = 0.123
Effect size = 1.45
Alpha Error (%) = 5
Power (%) = 70
sided = 2
Required sample size per group = 6 [25, 26].

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Study design and the collection of data

Twenty four adult male Holtzman strain albino rats were used for this experiment weighs between 200-250 gm. Standard laboratory condition was maintained (room temperature 27±1°C, humidity 60% and 12 hr light/dark cycle) along with standard laboratory diet, which supplemented the necessary proteins, carbohydrates and minerals. Drinking water was supplied ad libitum. Throughout the experimental period the body weight of the rats were recorded and maintained on a daily basis and maintained in the. Regularly between 12:00 and 14:00 hr all the behavioral procedure was carried out.

Collection and preparation of water extract from SO leaves

The SO leaves were procured from the local markets. The identity of these leaves was authenticated by the Botanical Survey of India, Howrah (Voucher specimen no – CHN/I-I/[239]/2008/Tech.II/278). These leaves were shade dried and grinded by an electrical grinder to obtain a free flowing powder and a water extract (1:3) at room temperature for 48 hours was obtained which was subjected to filtration through Whatman filter paper. This extract was vacuum dried at 40° - 50° C. This dry powder was dissolved in double distilled water for further use.

Treatment

With the help of orogastric cannula the SO leaf extract was orally administered at the dose of 400 mg/kg b.w. This treatment was carried on for fourteen consecutive days (between 10:00 and 11:00 hrs). This dose was standardized earlier [27]. The behavior parameters such as seizure score, ictal phase and interictal phase were measured during these periods (between 12:00 and 1:00 hrs).

Grouping of Animal

The rats were divided into 4 groups, viz; (1) Control (2) Only SO treated control (3) PTZ induced experimental epileptic model (4) SO pretreated PTZ induced experimental epileptic model. Each day, these experimental groups were given aqueous leaf extract of SO orally at a dose of 400 mg/kg body weight [27], for fourteen consecutive days by using an orogastric cannula.

Preparation of experimental Epileptic rat model by Pentylenetetrazol

Intraperitoneal injections of PTZ were given to the animals at a dose of 40 mg/kg body weight to obtain generalized epileptic models. The animals were kept under strict supervision and seizure score, ictal phase and interictal phase were observed for next two hours [28].

Behavioral analysis

PTZ induced epileptic rats:

The PTZ induced experimental epileptic animals were observed for the progression of seizures every 15 minutes for 2 hours according to a modified version of Patel *et al* [29].

No behavioral change – '0'; Facial movements, ear twitching and tail raising – '1'; myoclonic jerks of the whole body – '2'; clonic convulsion with rearing – '3'; forelimb clonus – '4'; clonic convulsion with falling down and loss of body control – '5'

Estimations for NO release

Sample preparation: Blood was collected from tail vein on the fourteenth day immediately after behavioral study and serum samples were separated from blood cells.

Serum NO level: The cells in the serum were suspended in Phosphate buffer solution-bovine serum albumin and were stimulated with lipopolysaccharide (100 µg/ml).These was centrifuged at 10,000 rpm for 15 min, afterwards the cell free supernatant was collected and NO released was measured using the Griess reaction [30].

DNA fragmentation assay

Intact brain tissue was resuspended in hypotonic lysis buffer till it was homogenized. From this homogenized tissue a tissue sample (106 cells/ 200µl) was resuspended in hypotonic lysis buffer (0.2%), TritonX-100, 10 mM Tris, 1 mM EDTA (pH 8.0) and centrifuged for 15 minutes at 14,000 X g. The supernatant containing small DNA fragments were collected and the cell pellet containing large pieces of DNA and cell debris were subjected to electrophoresis [31]. Estimation of antioxidant enzymes and lipid peroxidation:

Tissue preparation

On the 14th day, immediately after behavior study, rats were sacrificed by cervical dislocation. The Cerebral cortex (CC), Cerebellum (CB), Caudate nucleus (CN), Pons and Medulla (PM) and Midbrain (MB) were dissected out. The tissues were weighed and homogenized in ice-cold phosphate buffer and prepared for biochemical estimation.

Catalase (CAT) estimation

Brain tissue samples were homogenized with 5 ml of ice-cold 0.1 M phosphate buffer (pH-7.4). Then at a speed of 3000 rpm for 10 min the homogenates was centrifuged. The precipitate was then stirred with the addition of 15 ml of ice-cold 0.1 M phosphate buffer and allows standing in cold condition with occasional shaking. The shaking procedure was repeated for thrice. 1 ml of sample was added with 9 ml of H_2O_2. The rate of decomposition of H_2O_2 was measured spectrophotometrically from the changes in absorbance at 350 nm. The activity of Catalase was expressed as % inhibition unit [32].

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Superoxide dismutase (SOD) estimation

Superoxide Dismutase (SOD) was estimated by the method of ray *et al* [32]. Brain tissue samples were homogenized with 5 ml of ice-cold 0.1 M phosphate buffer (pH-7.4). At a speed of 3000 rpm for 10 min the homogenates was centrifuged. Then 0.8 ml of triethanolamine diethanolamine HCl buffer (TDB) was mixed to 0.1 ml of sample. Reaction started by the addition of 4 µl of NADH. Then 25 µl of EDTA-$MnCl_2$ mixture was added and the first spectrophotometric readings were taken at 340 nm. The second was taken at 340 nm after 0.1 ml of Mercaptoethanol was added to it.

Reduced glutathione (GSH) estimation

For Glutathione estimation equal quantity of homogenate was mixed with 10% trichloroacetic acid (TCA). This was centrifuged to separate the proteins. 2 ml of phosphate buffer [pH 8.4], 0.5 ml of 5, 5-dithiobis [2-nitrobenzoic acid] and 0.4 ml of double distilled water were added to 0.01 ml of this supernatant. The mixture was vortexed and the absorbance was read at 412 nm within 15 min. The concentration of reduced glutathione was expressed as µg/g of tissue [33].

Lipid peroxidation (LPO) estimation

Lipid peroxidation was measured according to the method of Roy *et al.* [32]. Brain tissue samples were homogenized with 5 ml of ice-cold 0.1 M phosphate buffer (pH-7.4). The homogenates was then centrifuged at 3000 rpm for 10 min. 0.5 ml of sample was mixed with 1 ml of TDB and then the mixture was incubated at 37°C for 1 hour. 0.5 ml of trichloroacetic acid (TCA) was added to it. It was vortexed and at an absorbance of 350 nm the spectrophotometric reading was obtained. A second reading was taken after the addition of 500 µl Mercaptoethanol to 1 ml sample.

Inclusion criteria

Adult male Holtzman strain albino rats weighing between 200-250 gm were used for the experiment.

Exclusion criteria

Female rats were excluded as there is hormonal level change during estrous cycle. Rats weighing more than 250 gm and less than 200 gms were also excluded as the dose of SO was standardized on rats weighing between 200-250 gm.

Ethical committee approval

Prior to the experiment, animal ethical committee approval was obtained. This research was conducted according to the declaration of Helsinki (Latest version).

Outcome variable

The outcome variables are NO, SOD, CAT, LPO, GSH and DNA fragmentation.

Explanatory variables

The explanatory variables of this study were SO, PTZ, Control, SO+PTZ

Data management and statistical analysis

The data were expressed as mean±SEM and were analyzed statistically using one way analysis of variance [one way ANOVA], followed by multiple comparison't' test, was used for statistical evaluation of the data. In addition to this, two-tailed't' test was performed to determine the level of significance between the means. P value less than 0.01 was considered statistically significant.

Results

The seizure score and ictal phase were significantly increased ($p<0.001$) and the interictal phase was significantly decreased ($p<0.001$) in PTZ induced experimental epileptic group as compared to the control. Pretreatment with SO leaf extract for 14 consecutive days showed significant improvements in the behavioral analysis of the experimental epileptic group. The seizure score and ictal phase significantly increased the interictal phase when compared to PTZ induced experimental epileptic group. The seizure score and ictal phase were significantly decreased and the interictal phase was significantly increased in SO pretreated control group compared to that of control group (table – 1).

Table - 1 Changes in duration of seizure score, ictal phase and interictal phase of SO pretreated PTZ induced experimental epileptic rat model (mean±SEM)

Group	Seizure score	Ictal phase (sec.)	Interictal phase (sec.)	Percentile protection (%)
PTZ	96.00±0.92	82.00±0.21	125.00±0.38	0
SO+PTZ	42.00±0.41	30.00±0.23	365.00±0.42	71. 52

The serum NO level was significantly increased in PTZ induced experimental epileptic group as compared to the control group. Pretreatment with SO leaf extract for 14 consecutive days showed significant decline serum NO level in experimental epileptic group when compared to PTZ induced experimental epileptic group. The serum NO level was significantly decreased in SO pretreated control group rather than control group (table – 2).

Table - 2 Effect of SO on serum NO activity in PTZ induced experimental epileptic rat model (mean±SEM)

GROUP	NO [µg/ml of serum]	P value
Control	0.235±0.09	0.000†
PTZ	0.589±0.11	0.000†
SO	0.248±0.07	0.000†
SO+PTZ	0.358±0.08	0.000†

†P<0.01 statistically significant

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There was a sharp decline in SOD activity in CC, CB, CN, MB and PM in the PTZ induced experimental epileptic group as compared to the control group. The SOD activity was significantly increased in SO pretreated control group rather than control group in CC, CB, CN, MB and PM. SO significantly increased SOD activity in SO pretreated PTZ induced group in comparison with PTZ induced experimental epileptic group in all mentioned parts of the brain (table-3).

There was a significant rise in lipid peroxidation levels in the PTZ induced experimental epileptic group as compared to the control group in CC, CB, CN, MB and PM. The LPO level was significantly decreased in CC, CB, CN, MB and PM in SO pretreated control group rather than control group. SO significantly decreased LPO levels in SO pretreated PTZ induced group rather than PTZ induced experimental epileptic group in all mentioned parts of the brain (table-4).

There was a sharp decrease ($p<0.001$) in CAT activity in CC, CB, CN, MB and PM in the PTZ induced experimental epileptic group as compared to the control group. The CAT activity was significantly increased in SO pretreated control group rather than control group in CC, CB, CN, MB and PM. SO significantly increased CAT activity in SO pretreated PTZ induced group rather than PTZ induced experimental epileptic group in CC, CB, CN, MB and PM (table-5).

There was a sharp decrease in GSH level in the PTZ induced experimental epileptic group as compared to the control group in CC, CB, CN, MB and PM. The GSH level was significantly increased in SO pretreated control group rather than control group in CC, CB, CN, MB and PM. SO significantly increased GSH level in SO pretreated PTZ induced group rather than PTZ induced experimental epileptic group in different brain regions (table-6).

There was an increase in the rate of DNA fragmentation in PTZ induced experimental epileptic group [Fig 1.1 – D,E,F,G; Fig 1.3 – E, F, G; Fig 1. 4 – D,E,F] as compared to the control group [Fig1.1 – B; Fig 1. 2 – C; Fig 1.3 – B ; Fig 1.4 – B]. Pretreatment with SO leaf extract showed significant decrease in DNA fragmentation in SO pretreated PTZ induced experimental epileptic group[Fig 1.1 – C; Fig 1.2 – D, E] when compared to PTZ induced experimental epileptic group. DNA fragmentation decreased in SO pretreated control group [Fig1.2 – B; Fig 1.3 – C, D] rather than control group. The result is shown in the following figures.

Discussion

The present study explains the immunomodulatory role of aqueous leaf extract of SO on DNA fragmentation in PTZ induced experimental epileptic rat model. From the behavioral study it was clear that SO leaf extract at the dose of 400 mg/kg b.w. significantly decreased the seizure activity, ictal phases and increased the interical phase considerably.

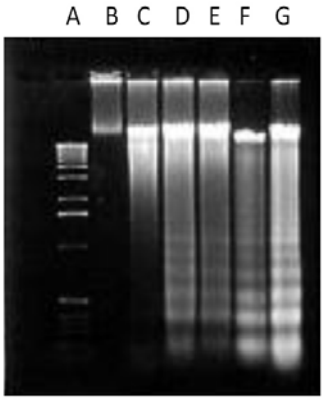

Figure 1.1
A – Marker, B – Control, C – SO + PTZ, D – PTZ, E – PTZ, F – PTZ, G – PTZ

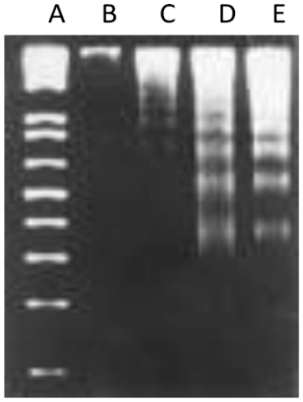

Figure 1.2
A – Marker, B – SO, C – Control, D – SO + PTZ, E – SO + PTZ

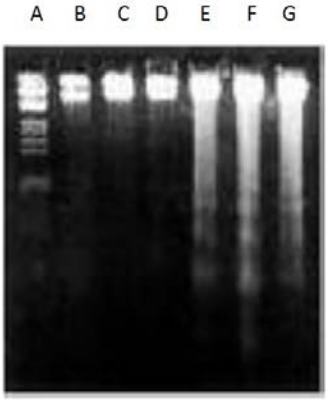

Figure 1.3
A – Marker, B – Control, C – SO, D – SO, E – PTZ, F – PTZ, G – PTZ

Figure 1.4
A – Marker, B – Control, C – PTZ, D – PTZ, E – PTZ, F – PTZ

Table - 3 Effect of SO on SOD activity on PTZ induced seizure (mean ± SEM)

Group	SOD [% inhibition unit]									
	CC	P value	CB	P value	CN	P value	MB	P value	PM	P value
Control	13.30±0.41	0.000†	11.28±0.32	0.000†	11.31±0.28	0.000†	11.12±0.14	0.000	12.47±0.19	0.000†
PTZ	20.66±0.41	0.000†	19.11±0.55	0.000†	19.60±0.67	0.000†	18.58±0.42	0.000	20.85±0.41	0.000†
SO	10.31±0.22	0.000†	9.82±0.18	0.000†	9.96±0.11	0.000†	9.79±0.21	0.000	10.27±0.26	0.000†
SO + PTZ	15.38±0.27	0.000†	14.19±0.27	0.000†	14.31±0.36	0.000†	14.46±0.37	0.000	14.43±0.39	0.000†

†P<0.01 statistically significant

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Table - 4 Effect of SO on LPO level on PTZ induced seizure (mean±SEM)

Group	LPO [nmol of TBARS / gm mol of tissue]									
	CC	P value	CB	P value	CN	P value	MB	P value	PM	P value
Control	4.03±0.10	0.000†	3.99±0.46	0.000†	3.46±0.07	0.000†	3.89±0.07	0.000†	3.72±0.12	0.000†
PTZ	8.52±0.26	0.000†	7.46±0.34	0.000†	7.59±0.26	0.000†	7.70±0.25	0.000†	7.19±0.32	0.000†
SO	2.93±0.11	0.000†	2.90±0.07	0.000†	3.19±0.34	0.000†	2.89±0.18	0.000†	3.35±0.28	0.000†
SO + PTZ	5.29±0.16	0.000†	4.63±0.25	0.000†	4.20±0.24	0.000†	4.68±0.28	0.000†	4.60±0.23	0.000†

†P<0.01 statistically significant

Table - 5 Effect of SO on CAT activity on PTZ induced seizure (mean±SEM)

Group	CAT [% inhibition unit]									
	CC	P value	CB	P value	CN	P value	MB	P value	PM	P value
Control	13.98±0.09	0.000†	12.30±0.14	0.000†	12.54±0.19	0.000†	13.19±0.22	0.000†	12.16±0.17	0.000†
PTZ	21.65±0.17	0.000†	20.35±0.19	0.000†	19.69±0.29	0.000†	20.77±0.40	0.000†	20.13±0.79	0.000†
SO	12.27± 0.25	0.000†	10.81±0.15	0.000†	10.66±0.16	0.000†	11.79±0.18	0.000†	10.55±0.18	0.000†
SO + PTZ	15.72± 0.25	0.000†	14.23± 0.29	0.000†	14.82± 0.40	0.000†	14.40± 0.39	0.000†	13.97±0.29	0.000†

†P<0.01 statistically significant

Table-6. Effect of SO on GSH level on PTZ induced seizure [Reduced glutathione (µg/g of tissue)(mean ± SEM)]

Group	CC	CB	CN	MB	PM
Control	30.54±0.76	29.62±0.81	29.61±0.44	23.9±0.26	26.65±0.43
PTZ	19.96±0.5	18.78±0.64	15.26±0.48	16.15±0.33	17.17±0.46
SO	31.21±0.77	31.2±0.61	28.08±0.58	26.71±0.41	27.68±0.81
SO + PTZ	26.8±0.61	26.81±0.61	24.87±0.7	25.33±0.67	25.17±0.63

We found that aqueous solution of SO significantly altered DNA fragmentation of brain tissue, thereby protecting the brain from further damage from oxidative stress in epilepsy.

PTZ –a convulsive agent

PTZ was used as a convulsant in our work in accordance with the other experimental studies of seizures activity, to produce experimental epileptic animals. PTZ is capable to activate the excitatory synapses or deactivate the inhibitory synapses (*i.e*; by a synergistic action with glutamate or an antagonistic action with α – aminobutaric acid, or GABA) [34]. Our study showed the development of epilepsy in rats with the intraperitoneal administration of PTZ which was prominent by seizures. On the whole, the outcomes point to the fact that seizure may be associated with an altered neurotransmitter balance and an increase in neuronal loss and gliosis [35].

DNA fragmentation through modulation of NO levels

Seizures induce brain inflammation. Research theories stated that inflammatory reactions are associated with activation of the innate immune system that is mediated through some of the molecular and structural changes occurring during and after seizure activity. The onset of inflammation is characterized by release of reactive oxygen species (ROS)[36].

Following a line of investigation it is proved that ROS reacts with reactive nitrogen species (RNS) such as NO and causes disruption of protein S-nitrosylation and disrupts signaling pathways [37]. The harmful effects of this; most often, lead to damage of DNA.

Increased NO level was observed during the onset of generalized seizures in rat brain. So the salient findings of present study, after intraperitoneal administration of PTZ, possibly increased the generation of ROS and reactive nitric oxide species (RNOS) and through receptor mechanism thereby increased serum NO production. This in turn, increased the seizure score, ictal phase and decreased the interictal phase. SO leaf extracts is found to exert a reverse effect, probably by increasing the antioxidant activities and decreasing the ROS formation. This protects the brain tissue from damages during epilepsy by controlling the DNA fragmentation. It may be due to the protection by SO or vitamin E, C, carotenoids, beta-carotene, bioflavonoid and quercetin, which are present in SO leaf extract with a decreases in the possibility of DNA fragmentation.

Endogenous antioxidants and DNA fragmentation

Free radicals are known to participate actively in the pathogenesis of epilepsy. Lipid peroxidation is an indicator of measuring the degree of damage that occurs in membranes of tissue as a result of free radical generation [38]. The results of our study showed a significant elevation of LPO level in PTZ induced experimental Epileptic group. This is possibly due to the generation of free radicals via auto-oxidation or through metal ion or superoxide catalyzed oxidation process. Pretreatment with SO was found to significantly decrease the LPO level. Endogenous antioxidant status in PTZ induced experimental Epileptic model was evaluated by noting the activities of CAT, SOD and GSH which play a chief role for scavenging free radicals [39].

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CAT protects the tissues from highly reactive hydroxyl radical by scavenging H_2O_2 [40]. SOD acts as an important defense system against oxidative damage by contributing to the destruction of superoxide radicals. From our experimental results of the aforesaid antioxidant enzyme activities in brain tissues of PTZ induced experimental models were found to have a significant decreased level of SOD, CAT, GSH as compared to the control group, SO pretreated group and SO pretreated PTZ induced experimental epileptic groups. SO leaf extract with its high antioxidant content, significantly increase SOD, CAT, GSH levels.

Glutathione; an endogenous antioxidant, present mostly in the reduced form within the cells. It prevents the hydroxyl radical generation by interacting with free radicals. During this defensive process, reduced glutathione is converted to oxidized form under the influence of the enzyme glutathione peroxidase (GPX). The decreased level of reduced glutathione in PTZ induced experimental group seen in our study indicates that there was an increased generation of free radicals and the reduced glutathione was depleted during the process of combating oxidative stress [41]. This probably has been modulated either by lowering the level of ROS production or through a rapid dissolution of ROS. This is evidenced from the elevated activities of important antioxidant defense enzymes CAT and SOD, studied in this experiment. Earlier Literature had shown that the SO contains high level of vitamin E and beta-carotene which protects rat neurons against oxidative stress possibly through the presence of both vitamin E and beta-carotene. It may be inferred from the present results, that, SO protects neurons against oxidative stress by modulating the levels of LPO, CAT, SOD and GSH possibly through the action of its high antioxidant contents as well as SO being a rich source of bioflavonoid. The study also provides evidence for the ability of the SO to inhibit seizure-induced increase level of DNA fragmentation.

Conclusion

The present investigation, together with the behavioral and biochemical effects on different antioxidant enzyme activity along with LPO level and DNA fragmentation, lead to the categorization of SO as a nootropic agent. SO showed to exert an immunomodulatory action on serum NO level and in the production of ROS through the alteration of the antioxidant activities. Thereby, this study provides a breakthrough for the management of epileptic cases with herbal remedies like SO.

Limitations & future scope of the study

The active flavonoid of SO need to be isolated and further studies on endogenous antioxidants levels with this active component need to be studied by which the therapeutic importance may be evaluated and the outcome of which can be utilized in the protection of Epilepsy.

This study was done on rodent models, thus further studies need to be carried on human population for the further implementation of this herbal management.

Abbreviations

Catalase (CAT), caudate nucleus (CN), cerebellum (CB), cerebral cortex (CC), deoxyribonucleic acid (DNA), Ethylenediaminetetraacetic acid (EDTA), gamma Aminobutyric cid (GABA), Glutathione peroxidase (GPX), Hydrogen peroxide (H_2O_2), lipid peroxidation (LPO), midbrain (MB), natural antioxidant mixture (NAO), nitric Oxide (NO) pentylenetetrazole (PTZ), pons medulla (PM), reactive nitric oxide species (RNOS), reactive nitrogen species (RNS) reactive oxidative species (ROS), reduced glutathione (GSH) *Spinacea oleracea* (SO), superoxide dismutase (SOD), tricholoroacetic acid (TCA), triethanolamine diethanolamine HCl buffer (TDB).

Competing interests

The authors have no conflict of interest and there was no funding agency that supported this study

Authors' contribution

Dr. Monami Mondal performed the experiments, collected the data and drafted the manuscript. Prof. Debjani Guha, Dr. Swati Sinha and Dr. Sujata Maity directed this research work helped with the critical analysis. Dr. Brijesh Sathian helped with statistical analysis of the data. Final manuscript was accepted by all authors.

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