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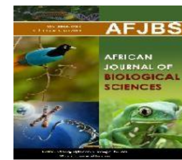

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CAN Spinacea oleracea MODULATE THE BRAIN ANTIOXIDANT LEVEL IN PENICILLIN (PCN) INDUCED EXPERIMENTAL EPILEPTIC RAT MODEL?

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ABSTRACT

Objective: To elucidate the role of antioxidative agents of Spinacea oleracea (SO) on the penicillin-induced epileptic rat model.

Method: Twenty-four adult male Holtzman strain albino rats, weighing between 200-250g, were utilized. These rats were categorized into four groups: Control, Spinacea oleracea (SO) treated control, PCN-induced experimental epileptic rat model, SO pretreated PCN-induced experimental epileptic rat model. These groups received an oral dose of 400 mg/kg body weight of SO aqueous leaf extract for fourteen consecutive days via an orogastric cannula. For the experimental epileptic model, PCN was injected into specific loci of the somatosensory cortex. Subsequently, on the fourteenth day, the rats were euthanized and the tissues of the Cerebral cortex (CC), Cerebellum (CB), Caudate nucleus (CN), Pons and Medulla (PM), and Midbrain (MB) were dissected, which were weighed and homogenized in ice-cold phosphate buffer and prepared for biochemical estimation.

Result: The antioxidant activity was significantly increased ($P<0.001$) in SO pretreated group

Conclusion: Spinacea oleracea may offer antioxidant protection against epilepsy, likely due to its radical scavenging activity.

INTRODUCTION

The penicillin-induced (PCN) seizure serves as a model for acute partial (focal) epilepsy. It is widely utilized to investigate the various alterations occurring during the ictal and interictal stages. The topical application of penicillin to the brains of animals has been shown to induce epileptic convulsions through the antagonism of GABA-mediated inhibition (1,2). In PCN-induced epileptic models, GABA synthesis and uptake, both are evident to reduce in epileptogenic foci (3). The convulsive effects arise from the β-lactam ring binding to GABA receptors, which leads to a decrease in the concentration of inhibitory neurotransmitters and the excitation of cortical afferents, resulting in epileptiform bursts (4).

Evidence indicates that seizures may trigger inflammatory responses. Studies imply that if the pathology of the epileptic brain involves proinflammatory processes, then anti-inflammatory medications could be beneficial. Additionally, various types of brain injuries, such as head trauma, stroke, seizures, or infections, are known to initiate inflammation in the brain. (5). Several studies suggest that the molecular and structural changes seen during and after seizures may be linked to the activation of the brain's innate immune system and subsequent inflammatory responses. (6).

Subsequent studies have also indicated that oxidative stress, characterized by an imbalance between the production of free radicals and the body's internal antioxidant defenses, is implicated in seizure-induced neuronal death (7). Conversely, seizures are commonly observed in mitochondrial diseases that arise from defects in oxidative phosphorylation. (8).

Clinical, experimental, and literature research suggests that epilepsy cases are marked by elevated levels of lipid peroxidation products in cerebrospinal fluid and peripheral blood plasma. (9, 10,11). These instances pertain to the examination of diminished glutathione peroxidase activity (12).

It is vital to maintain a balance between the production of free radicals and the body's ability to counteract their harmful effects with antioxidants. An excess of free radicals, along with a deficiency in antioxidants, results in oxidative stress, a condition associated with extensive cellular damage (13). Our body has its way of neutralizing the damaging effects incurred by free radicals and other reactive oxygen species. The body's endogenous enzyme system, which includes glutathione peroxidases, superoxide dismutases, and catalase, acts as a vital defense mechanism by lowering the levels of the most harmful oxidants in tissues (14). Studies have shown that minerals

such as selenium, copper, manganese, and zinc are crucial for the creation and activity of specific enzymes. Consequently, maintaining sufficient levels of these minerals may bolster enzymatic defenses against free radicals (15). Antioxidants like glutathione are vital in defending against free radical damage due to their stability. They work by donating an electron to stabilize free radicals, minimizing the risk they pose to healthy cells and tissues. Our diet is a significant source of antioxidants, with vitamins E and C, carotenoids, and other nutrients plentiful in the fresh fruits and vegetables we consume. Furthermore, various non-nutrient substances found in food, often phenolic or polyphenolic compounds, have antioxidant effects that support overall health. (16).

The adverse health effects of long-term medication use have prompted scientists to investigate dietary remedies. As a result, researchers have identified several flavonoid compounds in spinach, or Spinacia oleracea (SO), which serve as natural antioxidants. Nutrients found in spinach, such as beta-carotene, vitamin C, and vitamin K, are noted for their anti-inflammatory properties and could potentially relieve symptoms in certain patients.

Our study is designed to clarify the antioxidative effects of SO in a penicillin-induced experimental model of epilepsy in rats.

Materials and methods

Animals used and their maintenance:

In this experiment, forty-eight adult male Holtzman albino rats of pure strain, each weighing between 200-250 grams, were utilized. The rats were kept under standard laboratory conditions before the experiments adhering to the 'Institutional Ethical Committee' guidelines. They received a standardized laboratory diet, rich in essential proteins, carbohydrates, and minerals. Water was made available ad libitum. Daily monitoring and recording of the rats' body weight occurred throughout the experimental period. Behavioral procedures took place from 12:00 to 14:00 hours.

Collection and preparation of water extract from the leaves of SO:

The SO leaves obtained from local markets were authenticated, shade-dried, and ground into a fine powder from which an extract was obtained that was filtered and vacuum-dried at 40-50°C to obtain a dry powder. This powder was dissolved in double-distilled water to create the final product which was used throughout the experimental study.

Treatment:

The SO leaf extract was administered at a dosage of 400 mg/kg body weight for fourteen consecutive days (between 10:00 and 11:00 hrs) through an orogastric cannula. This dosage was standardized according to the study of Das et al (17). Behavior-related parameters, including the seizure score, ictal phase, and interictal phase, were recorded between 12:00 and 1:00 hrs. After the 14th day of treatment, the animals were euthanized through cervical dislocation. Various brain regions such as the Cerebral Cortex (CC), Cerebellum (CB), Caudate Nucleus (CN), Pons and Medulla (PM), and Midbrain (MB) were then collected for the assessment of antioxidant enzymes and neurotransmitters.

Grouping of Animal:

The rats were divided into four groups, with each group consisting of 16 animals, viz; (1) Control (2) Only SO treated control (3) PCN-induced experimental epileptic rat model (4) SO pretreated PCN-induced experimental epileptic rat model.

Preparation of experimental Epileptic rat model by Penicillin:

Before initiating PCN-induced epileptogenesis preparation, all animals underwent fasting for the night before the surgery, totaling 18 hours. The animals were anesthetized by an intraperitoneal injection of sodium pentobarbital at a dosage of 40 mg/kg body weight prior to the surgery.

Topical application of penicillin (PCN):

A stereotaxic apparatus was used to administer the PCN solution with a Hamilton syringe, delivering it slowly in a volume of 0.05 ml (100 IU) over 5 minutes. The injection was made at specific points in the somatosensory cortex (3.2 mm posterior to the bregma, 2.25 mm lateral to the midline, and 1 mm deep) perpendicular to the cortical surface. Following this, antibiotic prophylaxis was provided for three days via intramuscular injections of penicillin, dosed at 10,000 IU (19).

Control animals that underwent sham procedures received treatment through the administration of a vehicle, namely isotonic saline, in a volume equivalent to that used at the same site.

The PCN-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. (20) and Dybdal and Gale (21).

No response – ‘0’;

Gustatory movement / Fictive scratching – ‘1’;

Tremor – ‘2’;

Head bobbing – ‘3’;

Forelimb clonus – ‘4’

Rearing, falling, and clonus – ‘5’

Tissue preparation for the biochemical estimation:

On the fourteenth day, immediately after the behavioral study, the animals were euthanized through cervical dislocation. The cerebral cortex(CC), cerebellum(CB), caudate nucleus(CN), pons and medulla(PM), and midbrain(MB) were meticulously removed.

Biochemical Estimation:

The isolated brain tissues were weighed, homogenized in an ice-cold phosphate buffer, and prepared for subsequent biochemical analysis.

Estimation of antioxidant enzymes and lipid peroxidation:

(i) Measurement of Catalase (CAT):

Brain tissue samples were homogenized in a phosphate buffer, and then centrifuged at 3000 rpm for 10 minutes. The resultant precipitate was stirred, followed by the addition of 9 ml of H2O2. The rate of H2O2 decomposition was measured spectrophotometrically by monitoring the change in absorbance at 350 nm. Catalase activity was quantified as the percentage of inhibition unit (22).

(ii) Measurement of Superoxide dismutase (SOD):

Brain tissue samples were homogenized in a phosphate buffer and then centrifuged at 3000 rpm for 10 minutes. Trehalose-6,6-dibehenate (TDB) was introduced, followed by the addition of NADH. With the onset of the reaction, a mixture of ethylenediaminetetraacetic acid and manganese chloride (EDTA-MnCl2) was added. Spectrophotometric readings were subsequently recorded at 340 nm, and another reading was taken following the introduction of Mercaptoethanol. (23).

(iii) Measurement of Reduced glutathione (GSH):

An equal volume of homogenate was mixed with trichloroacetic acid (TCA) and then centrifuged to isolate proteins. To the supernatant, phosphate buffer, 5,5-dithiobis (2-nitrobenzoic acid), and distilled water were added. The mixture was vortexed, and the absorbance at 412 nm was

measured within 15 minutes. The concentration of reduced glutathione was expressed was expressed as μg/g of tissue (24).

(iv) Measurement of Lipid peroxidation (LPO):

Brain tissue samples were homogenized in a phosphate buffer and then centrifuged. The resulting homogenate was mixed with TDB and incubated. Afterward, trichloroacetic acid (TCA) was introduced, the solution was vortexed, and its absorbance was measured at 350 nm. A subsequent spectrophotometric reading was taken followed by the addition of Mercaptoethanol (25).

Inclusion criteria

In our study, we utilized adult male albino rats of the Holtzman strain, with body weights ranging from 200 to 250 grams.

Exclusion criteria

Our study did not include female rats due to the variability in their hormone levels throughout the estrous cycle. Additionally, the dosage of SO was standardized for rats weighing between 200-250 grams; therefore, rats above 250 grams or below 200 grams were not considered for inclusion in our study.

Ethical committee approval

Approval from the Animal Ethical Committee was secured before the commencement of our experiment, specifically from the S. N. Pradhan Center for Neurosciences' ethical committee. The research adhered to the most recent version of the Declaration of Helsinki.

Sample size calculation

We employed the hypothesis testing for two means (equal variances) sample size calculation formulae. Following a pilot study involving 12 rats across four groups, which achieved statistical significance in all groups, we established the standard deviation in the PCN group at 0.035 and in the SO+PCN group at 0.045. With a mean difference of 2.8, an effect size of 70, an alpha error of 1%, a power of 99%, and a two-sided test, the required sample size per group was determined to be 5.

Statistical analysis:

The data are displayed as the mean ± standard error of the mean (SEM). A two-tailed Student's t-test was utilized to determine the significance of differences between the means. A probability level below 0.01 was deemed to indicate statistical significance.

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RESULTS

Fourteen days post-administration of PCN, the levels of SOD, CAT, reduced glutathione, and lipid peroxidation were measured. The pretreatment with SO leaf extract markedly modified the levels of endogenous antioxidants. The results depict an increase in the concentrations of SOD, CAT, and GSH in various brain regions of the group that received SO leaf extract before PCN induction.

Pretreatment with SO in the experimental epilepsy group resulted in a notable reduction in behavioral disturbances when compared to the untreated epilepsy group. There was a significant decrease in both the seizure score and the duration of the ictal phase, alongside a marked increase in the duration of the interictal phase in the SO pretreated group relative to the control group. (Table 1).

A marked increase in lipid peroxidation levels was observed in the PCN-induced group compared to the control group, as indicated by a significant p-value ($p<0.001$) shown in Table 2. Similarly, there was a notable decrease in reduced glutathione levels in the PCN-induced group when compared to the control group, with a significant p-value ($p<0.001$) detailed in Table 3. Additionally, substantial alterations were noted in the SOD and CAT levels in the PCN-induced group in contrast to the control group, also with a significant p-value ($p<0.001$) as depicted in Tables 4 and 5.

In addition, there was a marked increase ($p<0.001$) in lipid peroxidation levels in the group induced with PCN compared to the group pre-treated with SO before PCN induction (Table 2). Similarly, significant changes ($p<0.001$) were noted in the levels of reduced glutathione in the PCN-induced group relative to the SO pre-treated and PCN-infused group (Table 3). Furthermore, Tables 4 and 5 illustrate that the levels of SOD, CAT, and GSH were significantly elevated, while LPO activity was notably reduced ($p<0.001$) in the SO pre-treated control groups compared to the control groups (Table 2).

Table – 1. Changes in duration of seizure score, ictal phase and interictal phase of SO pretreated PCN induced experimental epileptic rat model.

GROUP	LATENCY PERIOD	Seizure score	Ictal phase (in sec.)	Interictal phase (in sec.)	PERCENTILE PROTECTION (%)
PCN		**96.00±0.92**	**82.00±0.21**	**125.00±0.38**	**0**
SO+PCN		**42.00±0.41#**	**30.00±0.23#**	**365.00±0.42#**	**71. 52**

Values are mean ± SEM; #p < 0.001, when compared with PCN induced group.

Table – 2. Effect of SO on LPO level on PCN-induced seizure

GROUP	LPO (nmol of TBARS / gm mol of tissue)				
	CC	CB	CN	MB	PM
Control	4.03±0.10	3.99±0.46	3.46±0.07	3.89±0.07	3.72±0.12
PCN	9.11±0.23***	6.98±0.24***	7.56±0.36***	7.67±0.32***	6.98±0.23***
SO	2.93±0.11***	2.90±0.07*	3.19±0.34	2.89±0.18**	3.35±0.28
SO + PCN	5.19±0.16#	3.83±0.25#	4.19±0.32#	4.53±0.35#	4.45±0.23#

Values are mean ± SEM; ***p < 0.001 when compared with the control group. #p < 0.001 when compared with PCN treated group.

Table – 3. Effect of SO on GSH level on PCN-induced seizure

GROUP	Reduced glutathione (μg/g of tissue)				
	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
PCN	20.11±0.4**	18.18±0.42**	14.86±0.12**	15.25±0.44**	17.12±0.45**
SO	31.21±0.77	31.2±0.61 **	28.08±0.58**	26.71±0.41**	27.68±0.81*
SO + PCN	25.11±0.54#	25.11±0.61#	23.97±0.17#	25.45±0.72#	25.12±0.63#

Values are mean ± SEM; ***p < 0.001 when compared with the control group. #p < 0.001 when compared with PCN treated group.

Table – 4. Effect of SO on SOD activity on PCN-induced seizure

GROUP	**SOD (% inhibition unit)**				
	CC	CB	CN	MB	PM
Control	13.30±0.41	11.28±0.32	11.31±0.28	11.12±0.14	12.47±0.19
PCN	22.68±0.36***	18.91±0.55***	20.11±0.42***	19.58±0.36***	24.85±0.32***
SO	10.31±0.22***	9.82±0.18 **	9.96±0.11*	9.79±0.21**	10.27±0.26***
SO + PCN	18.34±0.17#	13.89±0.64#	13.91±0.36#	15.11±0.41#	15.86±0.32#

Values are mean ± SEM; ***$p < 0.001$ when compared with the control group. #$p < 0.001$ when compared with PCN treated group.

Table – 5. Effect of SO on CAT activity on PCN-induced seizure

GROUP	CAT (% inhibition unit)				
	CC	CB	CN	MB	PM
Control	13.98±0.09	12.30±0.14	12.54±0.19	13.19±0.22	12.16±0.17
PCN	21.35±0.12***	21.10±0.29***	18.99±0.29***	21.72±0.30***	20.12±0.68***
SO	12.27± 0.25***	10.81±0.15***	10.66±0.16***	11.79±0.18**	10.55±0.18*
SO + PCN	14.12± 0.15#	12.83± 0.56#	12.45± 0.20#	14.30± 0.34#	12.97±0.19#

Values are mean ± SEM; ***$p < 0.001$ when compared with the control group. #$p < 0.001$ when compared with PCN treated group.

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Discussion

Epilepsy, a prevalent and diverse neurological condition, stems from biochemical and molecular processes that remain partially elusive. Oxidative stress is significantly implicated in the pathogenesis of seizure-related neuronal death (26). Additionally, seizures frequently manifest in mitochondrial disorders, which are linked to impairments in oxidative phosphorylation. (8). The study highlights the emerging significance of oxidative stress as both a result and a contributing factor to epileptic seizures.

There is a documented association between free radicals and scavenger enzymes in epilepsy, with reactive oxygen species being linked to seizure-induced neurodegeneration. Research indicates significant alterations in superoxide dismutase and catalase activities during the chronic phase of epilepsy. Concurrently, there is an increase in lipid peroxidation and nitrite levels in the hippocampus of animals experiencing spontaneous recurrent seizures. Comparative studies reveal that, following 24 hours of status epilepticus, animals exhibit normal superoxide dismutase levels but heightened catalase activities, along with increased lipid peroxidation and nitrite concentrations in the hippocampus. These findings provide concrete evidence of lipid peroxidation and nitrite presence during seizures, which may be the cause of neuronal damage in the hippocampus of rats' model of epilepsy (27).

Topical administration of penicillins to the brains of animals has been shown to induce epileptiform convulsions, potentially due to the antagonism of GABA-mediated inhibition (1). Studies have demonstrated that vitamin E and β-carotene can safeguard rat neurons from oxidative stress (28).

In our current study, rats injected with penicillin exhibited a reduction in SOD, CAT, and glutathione levels, alongside an increase in LPO levels. During a series of 10 daily trials, the number of correct choices made by the rats decreased, and the latency period increased significantly in those with PCN-induced conditions. However, administering SO leaf extract for fourteen consecutive days appeared to enhance the locomotor activity.

Some studies have observed that in an epileptic rat model, induced by the administration of PCN, there is a generation of free radicals. This leads to a significant decrease in SOD and CAT

activities, as well as a reduction in glutathione levels, while simultaneously increasing LPO levels. The administration of PCN likely produces reactive oxygen species (ROS), initiating lipid peroxidation (LPO) reactions. This triggers a chain reaction beginning with the oxidation of neighboring polyunsaturated fatty acids (PUFAs). The oxidized PUFAs then degrade into toxic compounds, including 4-hydroxy-2-nonenal (HNE), acrolein, malondialdehyde (MDA), and other short-chain aldehydes, which have been partially implicated in exerting neurotoxic effects. (29, 30).

Glutathione, an intrinsic antioxidant, predominantly exists in its reduced form within cells. The most pronounced and critical change in antioxidant defense mechanisms is the diminution of GSH levels. In conditions of oxidative stress, glutathione peroxidase catalyzes the transformation of reduced glutathione into its oxidized form (GSSG). The observed reduction in glutathione levels in our study suggests an escalation in free radical production, leading to the depletion of reduced glutathione as it is utilized in neutralizing oxidative stress. (31).

Fourteen-day treatment with SO leaf extract markedly enhanced the activities of SOD and CAT, elevated the level of reduced glutathione, and significantly reduced the LPO level. Vitamin C, an exogenous antioxidant, may be utilized in the management of seizures. It has the potential to modify oxidative stress and mitigate neuronal damage caused by seizures; as was suggested in a study earlier (32). Furthermore, vitamin E, in an animal model, has demonstrated the ability to accumulate in the brain and reduce lipid peroxidation. (33).

Spinach is a nutrient-dense leafy green, packed with vitamins A and C, thiamin, riboflavin, and niacin. It also contains a wealth of minerals such as calcium, phosphorus, iron, sodium, and potassium. Rich in carotenoids like beta-carotene and lutein, spinach is also an excellent source of the bioflavonoid quercetin, which endows it with antioxidant properties, among its numerous other health benefits (34).

Thus, SO leaf extract helped to improve the epileptic condition, by enhancing the activity of SOD, CAT, and reduced glutathione level and by depleting the LPO level, which is evident from the study of seizure score.

Conclusion

Based on current observations, it is evident that SO offers antioxidant defense against penicillin-induced epilepsy through its free radical scavenging activity. Consequently, this research indicates that SO boosts the body's own antioxidant capacity, suggesting a potential herbal solution for the control and management of epilepsy.

Future scope of the study

It is essential to isolate the active component of SO and conduct advanced studies to evaluate its therapeutic significance. Furthermore, clinical trials must be conducted on humans to assess the efficacy.

Conflict of interests

This study was not supported by any funding agency and there were no conflicts of interest.

Authors' contribution

Monami Mukherjee Mondal performed the experiments, collected and analyzed the data, and drafted the manuscript.

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References:

1. Hill RG, Simmonds MA, and Straughan DW. A comparative study of some convulsant substances as gamma-aminobutyric acid antagonists in the feline cerebral cortex. Br. J. Pharmac., 49, 37-5 1,1973)

2. Kandratavicius L, Balista PA, Lopes-Aguiar C, et al. Animal models of epilepsy: use and limitations. Neuropsychiatr Dis Treat. 2014;10:1693-1705. Published 2014 Sep 9. doi:10.2147/NDT.S50371.

3. Gottesfeld and Elazar Z. GABA synthesis and uptake in penicillin focus. Brain Research, Volume 84, Issue 2, 1975, Pages 346-350,10.1016/0006-8993(75)90992-0.

4. K.M. Chow, A.C. Hui, C.C. Szeto. Neurotoxicity induced by beta-lactam antibiotics: from bench to bedside. Eur J Clin Microbiol Infect Dis, 24 (2005), pp. 649-653, 10.1007/s10096-005-0021-y

5. Vezzani A, Granata T. Brain inflammation in epilepsy: experimental and clinical evidence. Epilepsia. 2005;46(11):1724-1743. doi:10.1111/j.1528-1167.2005.00298.x

6. Löscher W, Howe CL. Molecular Mechanisms in the Genesis of Seizures and Epilepsy Associated With Viral Infection. Front Mol Neurosci. 2022;15:870868. Published 2022 May 9. doi:10.3389/fnmol.2022.870868.

7. Sudha K, Rao AV, Rao A. Oxidative stress and antioxidants in epilepsy. Clin Chim Acta. 2001;303(1-2):19-24. doi:10.1016/s0009-8981(00)00337-5.

8. Patel MN. Oxidative stress, mitochondrial dysfunction, and epilepsy. Free Radic Res. 2002;36(11):1139-1146. doi:10.1080/1071576021000016391.

9. Kryzhanovskiĭ GN, Nikushkin EV, Voronko VA, et al. Concentration of lipid peroxidation products and free fatty acids in the plasma and cerebrospinal fluid of epileptics. Zh Nevropatol Psikhiatr Im S S Korsakova. 1984;84(6):806-809.

10. Willmore LJ, Hiramatsu M, Kochi H, et al. Formation of superoxide radicals after FeCl3 injection into rat isocortex. Brain Research, 1983;277(2):393-396. 10.1016/0006-8993(83)90954-X.

11. Panter SS, Sadrzadeh SM, Hallaway PE, et al. Hypohaptoglobinemia associated with familial epilepsy. J Exp Med. 1985;161(4):748-754. doi:10.1084/jem.161.4.748

12. Yavuzer S, YurtaslanI Z, Karan O, et al. Anticonvulsive effects of superoxide dismutase and catalase. Hemobiochemistry, Journal of Islamic Academy of Sciences. 1989; 2(1), 41-43.

13. Chaudhary P, Janmeda P, Docea AO, et al. Oxidative stress, free radicals and antioxidants: potential crosstalk in the pathophysiology of human diseases. Front Chem. 2023;11(11)58198. Published 2023 May 10.10.3389/fchem.2023.1158198

14. Vašková J, Kočan L, Vaško L, Perjési P. Glutathione-Related Enzymes and Proteins: A Review. Molecules. 2023;28(3):1447. 10.3390/molecules28031447

15. Saleh SAK, Adly HM, Abdelkhaliq AA, Nassir AM. Serum Levels of Selenium, Zinc, Copper, Manganese, and Iron in Prostate Cancer Patients. Curr Urol. 2020;14(1):44-49. 10.1159/000499261

16. Hajhashemi V, Vaseghi G, Pourfarzam M, Abdollahi A. Are antioxidants helpful for disease prevention? Research in Pharmaceutical Sciences. 2010 Jan;5(1):1-8. PMID: 21589762; PMCID: PMC3093095.

17. Das S, Guha D. CNS depressive role of aqueous extract of Spinacia oleracea L. leaves in adult male albino rats. Indian Journal of Experimental Biology. 2008;46(3):185-190. PMID: 18432058.

18. Hazra R, Ray K, Guha D. Inhibitory role of Acorus calamus in ferric chloride–induced epileptogenesis in rat. Human & Experimental Toxicology. 2007;26(12):947-953. doi:10.1177/0960327107087791

19. Purkayastha S and Guha D. Monoaminergic correlates of penicillin-induced epilepsy. Biogenic Amines. 2003;18:29-40.

20. Patel S, Meldrum BS, Fine A. Susceptibility to pilocarpine-induced seizures in rats increases with age. Behav Brain Res. 1988;31(2):165-167. 10.1016/0166-4328(88)90019-8

21. Dybdal DJ and Gale KN. Postural and Anticonvulsant Effects of Inhibition of the Rat Subthalamic Nucleus. The Journal of Neuroscience. 2000:20:6728 - 6733.

22. Cohen G, Dembiec D, Marcus J. Measurement of catalase activity in tissue extracts. Anal Biochem. 1970;34:30-38. 10.1016/0003-2697(70)90083-7

23. Misra HP, and Fridovich I. The Generation of Superoxide Radical during the Autoxidation of Hemoglobin. Journal of Biological Chemistry. 1972;247(21):6960-6962. 10.1016/S0021-9258(19)44679-6.

24. Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys. 1959;82(1):70-77. 10.1016/0003-9861(59)90090-6.

25. Bhattacharya SK, Bhattacharya A, Das KR, et al. (). Further Investigations on the Antioxidant Activity of Ocimum sanctum Using Different Paradigms of Oxidative Stress in Rats. Journal of Natural Remedies, 2001;1:6-16. 10.18311/JNR/2001/54.

26. Mueller SG, Trabesinger AH, Boesiger P, Wieser HG. Brain glutathione levels in patients with epilepsy measured by in vivo (1)H-MRS. Neurology. 2001;57(8):1422-1427. 10.1212/wnl.57.8.1422

27. Freitas RM. Investigation of oxidative stress involvement in hippocampus in epilepsy model induced by pilocarpine. Neurosci Lett. 2009;462(3):225-229. 10.1016/j.neulet.2009.07.037

28. Mitchell JJ, Paiva M, Heaton MB. Vitamin E and beta-carotene protect against ethanol combined with ischemia in an embryonic rat hippocampal culture model of fetal alcohol syndrome. Neurosci Lett. 1999;263(2-3):189-192. 10.1016/s0304-3940(99)00144-5

29. Neely MD, Sidell KR, Graham DG, Montine TJ. The lipid peroxidation product 4-hydroxynonenal inhibits neurite outgrowth, disrupts neuronal microtubules, and modifies cellular tubulin. J Neurochem. 1999;72(6):2323-2333. 10.1046/j.1471-4159.1999.0722323.x

30. Picklo MJ, Montine TJ. Acrolein inhibits respiration in isolated brain mitochondria. Biochim Biophys Acta. 2001;1535(2):145-152. 10.1016/s0925-4439(00)00093-4

31. Reiter RJ. Melatonin: Lowering the High Price of Free Radicals. News Physiol Sci. 2000;15:246-250. doi:10.1152/physiologyonline.2000.15.5.246.

32. Santos LF, Freitas RL, Xavier SM, Saldanha GB, Freitas RM. Neuroprotective actions of vitamin C related to decreased lipid peroxidation and increased catalase activity in adult rats after pilocarpine-induced seizures. Pharmacology, Biochemistry, and Behavior. 2008;89(1):1-5. 10.1016/j.pbb.2007.10.007. PMID: 18096215.

33. Clément M, Bourre JM. Graded dietary levels of RRR-gamma-tocopherol induce a marked increase in the concentrations of alpha- and gamma-tocopherol in nervous tissues, heart, liver and muscle of vitamin-E-deficient rats. Biochim Biophys Acta. 1997;1334(2-3):173-181. 10.1016/s0304-4165(96)00090-6

34. Umeo Takahama. Oxidation of Quercetin by Carotenoid Radical Generated in Illuminated Spinach Chloroplasts: The Effect of Ascorbate on Quercetin Oxidation, Plant and Cell Physiology. 1984;25(7): 1181–1185. 10.1093/oxfordjournals.pcp.a076825