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# Estimation of the Effect of Chrysin and Diosmin in Reducing Oxidative Stress-Induced Seizures

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**ABSTRACT**

Seizures are one of the important neuro disorders affecting Human beings across the world. Several synthetic drugs used for convulsions have shown side effects and drug interactions. Nature is a major source of safe and effective drugs. The main objective of the study is to estimate the effect of natural drugs Chrysin and Diosmin in reducing oxidative stress-induced seizures. For this we have used the Wistar mice, divided into five groups of six animals each. Seizures were induced by the maximal electrical convulsive meter. Chrysin (25mg/kg) and Diosmin (30mg/kg) were tested against MES-induced seizures. Phenytoin (100mg/kg) was taken as a standard. Mice were sacrificed after 24hrs of their last dose and estimated for biogenic amines i.e. adrenaline, dopamine, and serotonin in the brain. Biochemical parameters i.e. Malondialdehyde, Total Glutathione, and Superoxide dismutase were estimated. Statistical analysis was performed using one-way ANOVA followed by the Bonferroni test, $p<0.05$ was considered statistically significant. Finally, found that Chrysin at doses of 25mg/kg and Diosmin at a dose of 30mg/kg showed a significant ($p<0.05$) anti-epileptic action which is comparable with that of Phenytoin at a dose of 25mg/kg. Before drug treatment, the biogenic amine levels of noradrenaline, dopamine, and serotonin and anti-oxidant enzyme levels decreased compared to MES-induced group mice. After drug treatment, these levels significantly increased. The results suggest that chrysin (25 mg/kg) and diosmin (30mg/kg) have a considerable and reliable effect in reducing seizures in mice. Therefore, these can be developed as safe alternatives for synthetic drugs for convulsions.

## INTRODUCTION

The term seizure refers to a transient alteration of behavior due to the disordered, synchronous, and rhythmic firing of populations of brain neurons. The term Epilepsy refers to a disorder of brain function characterized by the periodic and unpredictable occurrence of seizures (Hardman JD et al., 2006). The word epilepsy is derived from the Greek verb *Epilamvanein* "to be seized", "to be taken hold off", or "to be attacked" indicating that the person having a seizure is 'possessed' or at least out of control (JJ.Engell et al., 1997). Epilepsy includes a group of heterogeneous and diverse conditions. The terms epilepsy and seizure are not synonymous and the distinction must be made clear. 'A seizure is an abnormal behavior (with symptoms or signs) resulting from abnormal discharges of cortical neurons and it is an observable phenomenon that is finite in time. Epilepsy refers to chronic conditions characterized by recurrent seizures (Selim RB et al., 2009).

Despite intensive investigations, the pathophysiology is still poorly understood. Studies with various animal models have provided ample evidence for heterogeneity in the mechanism of epileptogenesis (Vogel HG et al., 1997). Several biochemical hypotheses suggest the involvement of decreased activity of the inhibitory GABAergic system and/or increased activity of excitatory amino acids (glutamate and aspartate system) in epilepsy. According to the latest study by WHO, there are over 50 million sufferers in the world today, of which 85% live in developing countries. Epilepsy increases a person's risk of premature death by about two to three times compared to the general population It is the most common serious brain disorder worldwide with no age, racial, social class, or national or geographic boundaries (Williamson EM et al., 2002).

The flavonoids which are present in these drugs are mainly thought to be responsible for their anti-epileptic activity. All flavonoid compounds have antioxidant activity. It is shown that Chrysin and Diosmin inhibit the onset and incidence of convulsions. Oxidative stress (OS) is the condition that occurs when the steady-state balance of prooxidants to antioxidants is shifted in the direction of the former, creating the potential for organic damage. Prooxidants are by definition free radicals, atoms, or clusters of atoms with a single unpaired electron (Jaraki O *et al.,* 1992).

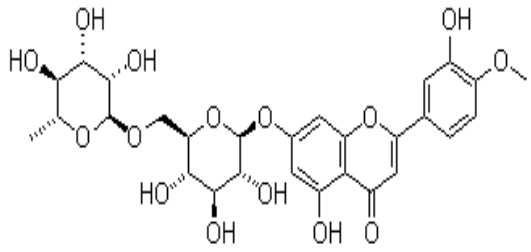

**Figure 1. Diosmin**

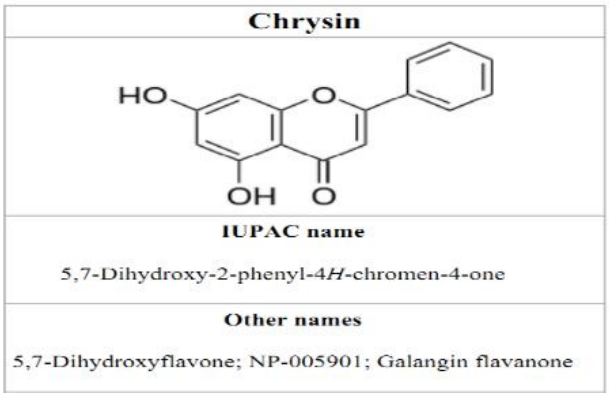


**Figure 2. Chrysin**

The purpose of this study is to know the Oxidative stress induces seizures ameliorated with Chrysin and diosmin.

## MATERIALS AND METHODS

### Collection of Material:

### Collection of chemicals and drugs:

All other chemicals and drugs of pure analytical grade like Chrysin and Diosmin will be obtained from Hi-media suppliers.

**Animals: Mice,** weighing 150-180 g were procured from Sanzyme Ltd., Hyderabad, India, and used for the experiment. They were housed in cages with a maximum of 6 rats and were maintained in an air-conditioned room (25±2°C) with a normal night and day cycle. They were fed with a semi-purified basal diet and drinking water *ad libitum*. The rats were allowed to acclimatize to the laboratory environment for a week before the start of the experiment. All experimental procedures were conducted in approval with the Institutional Animal Ethics committee (IAEC) (Approval No 11353P10**20** / JCP/IAEC/2012, Dt 07-07-2012) for the care and use of animals and were strictly followed throughout the study.

### Experimental Methods: Electroshock induced convulsions.

**Experimental Design**: Electro-convulsive shock, induce Hind Limb Tonic Extension (HLTE) in 99% of the animals. The electrical stimulus (50 mA; 50 Hz; 1-s duration) will be applied through corneal electrodes using a stimulator apparatus. Animals will be divided into 4 groups of 6 each. A Group of 6 mice will be pretreated with test drug (low, high dose), phenytoin (25

mg/kg, as positive control), saline (10 ml/kg, as control) and after 30 min will receive the transauricular electroshock. The criterion for the anticonvulsant effect will be the absence of HLTE within 10 s after delivery of the electroshock (Gilani AH et al., 2000).

Group I: Vehicle control (0.9% saline).

Group II: Phenytoin (25mg/kg) + electrical stimulus (50mA; 50Hz; 1sec duration).

Group III : Chrysin (25mg/kg) + electrical stimulus (50mA; 50Hz;1sec duration)

Group IV: Diosmin (35mg/kg) + electrical stimulus (50mA; 50Hz; 1sec duration).

**Evaluation:**

The animals will be observed closely for 2 min. The disappearance of the hind leg extensor tonic convulsion is used as a positive criterion. Percent of inhibition of seizures relative to controls will be calculated.

**Estimation of Biogenic Amines:** It has been well established that monoamines play an integral role in epileptic phenomena. Alterations in both catecholaminergic and dopaminergic activity may lead to seizure activity (schlumpf M et al., 1974). Spontaneous deficiencies in noradrenaline (NA), dopamine (DA), and/or Serotonin (5-hydroxy- tryptamine or 5-HT) levels were observed when epilepsy is induced experimentally.

The effect of the extract to bring back the normal level of these biogenic amines is an indication of the anticonvulsant activity of the plant extract (Mohan EN et al., 2010).

From the tissue homogenate, biogenic amines were extracted from the butanol. For the estimation of noradrenaline and dopamine, the trihydroxide method was employed whereas for the estimation of serotonin O-phthalaldehyde method was employed. In the trihydroxide method, fluorescent derivatives of noradrenaline and dopamine were prepared by oxidation with iodine solution in ethanol followed by condensation with ethylene diamine. In the O-phthalaldehyde method, a powerful fluorophore was prepared by the reaction of O-phthalaldehyde with histamine (Baker EN et al., 1982).

**Instruments required:** Electro convulsometer, corneal electrodes, and spectrofluorometer

**Grouping of animals:**

The animals will be divided into four groups containing six animals in each group.

Group 1-was treated as a control in which seizures will not be induced.

Group 2-was treated with chrysin (25mg/kg, i.p)

Group 3-was treated with diosmin *(*35 mg/kg, i.p)

Group 4-was treated with phenytoin (25mg/kg, i.p)

**Induction of seizures:** On the 15th day, seizures were induced in all the groups except Group 1 animals using an electroconvulsometer and biogenic amines in the forebrain of the mice were estimated.

**Brain dissection technique:** The conscious mice were decapitated and the brain was removed within 30 seconds from the skull. The skin covering the skull was cut along the midline and removed to expose the dorsal skull plates. The plates were split by introducing one blade of the paired scissors along the midline. The plates were then twisted and turned across the lateral border to expose the brain.

The membrane covering the brain was removed with the help of fine forceps. The brain then was taken out using a spatula washed in cold saline and the forebrain region was dissected (Jobe PJ et al., 1972).

**Extraction of biogenic amines:** Weighed quantity of tissue and was homogenized in 0.1 mL hydrochloric acid -butanol, (0.85 ml of 37% hydrochloric acid in one-liter *n*- butanol) for 1 min in a cool environment. The sample was then centrifuged for 10 min at 2,000 rpm. 0.08 mL of supernatant phase was removed and added to an Eppendorf reagent tube containing 0.2 mL of heptane and 0.025 mL 0.1 M hydrochloric acid. After 10 min of vigorous shaking, the tube was centrifuged under the same conditions to separate the two phases. The upper organic phase was discarded and the aqueous phase (0.02 mL) was used for the estimation of Serotonin, Noradrenaline, and Dopamine assay (Onyeyili PA et al., 2001).

**Estimation of noradrenaline and dopamine:** The assay represents a miniaturization of the trihydroxide method. To 0.02ml of hydrochloric acid phase, 0.05ml of 0.4M hydrochloric acid and 0.01ml EDTA/Sodium acetate buffer (pH 6.9) were added, followed by 0.01ml 0.1 M

iodine solution in ethanol for oxidation. The reaction was stopped after two minutes by the addition of 0.01ml sodium sulfite in 5m sodium hydroxide (0.5 g sodium sulfite in 2 mL deionized water + 18 mL 5 M sodium hydroxide). Acetic acid (0.01 mL, 10 M) was added 1.5 minutes later. The solution was then heated to 100$^{o}$ C for 6 minutes. When the sample again reached room temperature, excitation and emission spectra were read in the microcuvette at 395-485nm for noradrenaline and 330-375nm for dopamine uncorrected instrument values (Ayanwuyi LO et al., 1996).

**Estimation of serotonin:** For 5-HT determination, the O-pthaldialdehyde (OPT) method was employed. From the OPT reagent 0.025ml were added to 0.02ml of the hydrochloric acid extract. The fluorophore was developed by heating at 100°C for 10 min. After the samples reached equilibrium with the ambient temperature, excitation estimation spectra or intensity readings at 360-470 nm were taken in the micro cuvette (Osunkwo UK et al., 2001).

**Preparation of standards:** Standard stock containing 100 microgram/ml of stock solutions of noradrenaline hydrochloride, dopamine hydrochloride, and serotonin hydrochloride in deionized water was in eppendorf tubes. At the time of assay 0.05, 0.1, 0.15, and 0.2 ml of stock standards were diluted to 10 ml with deionized water.

**Calibration curves:** The calibration curve was made by treating the standards in the same way as described for the brain samples. 1ml of deionized water treated in the same way was used as a blank reagent.

**Antioxidant Activity Of Chrysin And Diosmin:** Animals were sacrificed and the brain was removed. A 10% brain tissue homogenate was used to estimate lipid peroxidation (LPO) and antioxidants enzymes such as Superoxide Dismutase (SOD), Glutathione Peroxidation (Gpx), were done (Malaya Gupta et.al., 2004).

**Estimation of Lipid Peroxidation (LPO)**: The amount of lipid peroxidation end products present in the brain homogenate was estimated by the thiobarbituric acid reactive substances (TBARS) method (Ohkawaka et al., 1979), which measures the malondialdehyde (MDA) reactive products by using spectrophotometrically. The reaction of thiobarbituric acid (TBA) with malondialdehyde (MDA), a secondary product of lipid peroxidation has been widely adopted as a sensitive assay method for the measurement of lipid peroxidation in biological fluids. It is widely used as an index of the extent to which lipid peroxidation has progressed. Since the assay procedure estimates the amount of TBA reactive substances e.g. MDA, it is

also referred to as TBARS (Thiobarbituric acid reactive substance) test. To 0.5 ml liver homogenate, 0.5 ml of 30% trichloro acetic acid (TCA) was added to precipitate the proteins and vortexed for 30 sec. The clear supernatant was taken after centrifuging at 3000 rpm for 10 min. To the supernatant, 500µl of 1%TBA solution and 500µl of water was added and this solution was heated for 1hr at 98°C.

Cool the solutions to room temperature and kept them on ice for 5 minutes. Then read the pink color at 532 nm using a spectrophotometer. The standard graph was plotted using TEP (1, 1, 3, 3-tetra ethoxy propane).

**Estimation of *Superoxide Dismutase***: The enzyme superoxide dismutase (SOD) was determined in brain homogenate using the photo-oxidation method (Misra and Fridowich, 1977; Arutla et al., 1998), which is briefly described below. In this assay, free radicals are generated by photo-oxidation of *o*-dianisidine sensitized by riboflavin.

The photo-oxidation of O-dianisidine involves a complex series of free radical chain reactions involving the superoxide anion ($O_2^{\bullet -}$) as the propagating series (Figure 1). A general free radical scavenging compound has an inhibitory effect on this reaction leading to a decrease in the oxidized dianisidine measurable spectrophotometrically. In contrast, any compound which specifically scavenges $O_2^{\bullet -}$ will remove the $O_2^{\bullet -}$ from steps 3 and 4 in Figure 4 thus increasing the amount of oxidized dianisidine and hence will have an augmented effect in this reaction. This assay can thus be used to determine whether a compound is a general, free radical, or a scavenger specific for the superoxide anion. A substance with no free radical scavenging activity will not affect the assay.

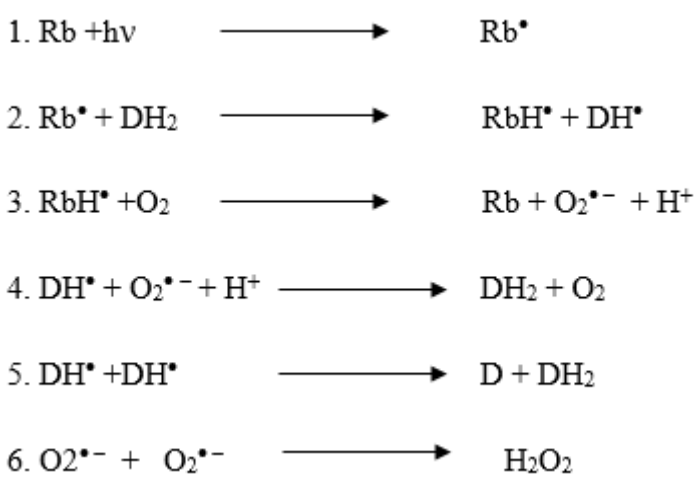


**Figure 3: Photo-oxidation of *o*-dianisidine**

Rb - Riboflavin; hν - the energy of photon light; $Rb^{\bullet}$ - exited riboflavin; $DH_2$ -*o*-dianisidine; $O_2^{\bullet -}$ - superoxide anion; D - product formed by photo-oxidation measured at 460nm.

0.88ml of riboflavin solution ($1.3 \times 10^{-5}$ M in 0.01M potassium phosphate buffer, pH 7.5) was added to 60µl of O-dianisidine solution ($10^{-2}$ M in ethanol), and to this 100µl of supernatant of liver homogenate was added and optical density was measured at 460nm. Then the cuvette containing the reaction mixture was transferred to the illuminating box, illuminated for 4min., and optical density was remeasured against a blank containing ethanol in place of the enzyme. The change in the optical density was determined. The SOD content was determined from the standard graph prepared using pure bovine erythrocyte SOD.

**Estimation of Glutathione:** Glutathione forms a colored complex with DTNB, which is measured spectrophotometrically (Beulter et al., 1963). Glutathione (GSH) is the most abundant thiol (SH) compound in animal tissues, plant tissues, bacteria, and yeast. GSH plays many different roles such as protection against reactive oxygen species and maintenance of protein SH groups.

During these reactions, GSH is converted into glutathione disulfide (GSSG: an oxidized form of GSH). Since GSSG is enzymatically reduced by glutathione reductase, GSH is the dominant form in organisms. DTNB (5, 5'- Dithiobis (2-nitrobenzoic acid)), known as Ellman's Reagent, was developed for the detection of thiol compounds. In 1985, Dr. Anderson suggested that the glutathione recycling system by DTNB and glutathione reductase created a highly sensitive glutathione detection method. DTNB and glutathione (GSH) react to generate 2-nitro-5-

thiobenzoic acid and glutathione disulfide (GSSG). Since 2-nitro-5-thiobenzoic acid is a yellow-colored product, GSH concentration in a sample solution can be determined by the measurement at 412 nm absorbance. GSH is generated from GSSG by *glutathione reductase* and reacts with DTNB again to produce 2-nitro-5-thiobenzoic acid. Therefore, this recycling reaction improves the sensitivity of total glutathione detection (Figure 4).

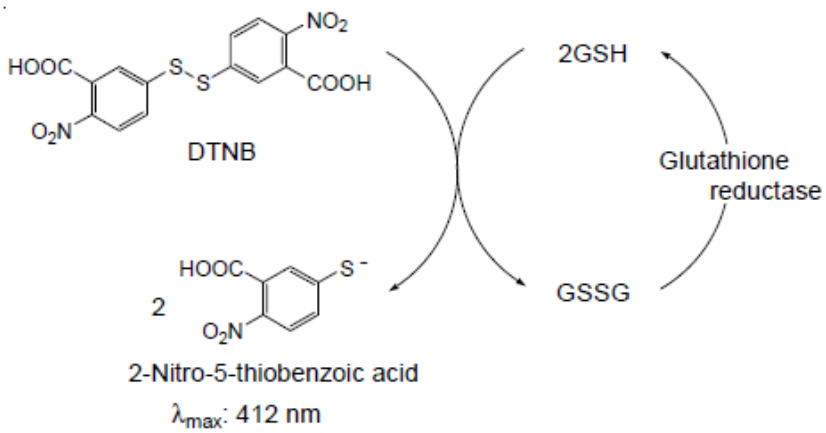


**Figure 4: Principle of Total Glutathione**

To 0.5 ml of homogenated tissue was taken, 0.5 ml of 5% trichloroacetic acid (TCA) solution was added to precipitate the proteins and centrifuged at 3000 rpm for 20 minutes. To 0.1 ml of supernatant, 1 ml of sodium phosphate buffer and 0.5 ml of DTNB reagent were added. The absorbance of the yellow color developed was measured at 412 nm. The glutathione content was determined from the standard graph by using pure glutathione.

**Statistical Analysis:** The results for electrically induced seizures were expressed as Mean ± Standard deviation. One-way ANOVA was used to analyze the level of significance. A *p*-value of <0.05 was considered statistically significant. The results for biogenic amines were expressed as Mean ± Standard Standard deviation. The Significance of differences among the group was assessed using a one-way analysis of variance (ANOVA). The test followed by the Bonferroni test, p values less than 0.05 were considered statistically significant. It was done using Graph pad 5.0 software versions. The results for biogenic antioxidants were expressed as Mean ± Standard deviation. The Significance of differences among the group was assessed using a one-way analysis of variance (ANOVA). The test followed by Bonferroni's Multiple Comparison Test p values less than 0.05 were considered statistically significant. It was done using Graph pad 5.0 software versions.

## RESULTS AND DISCUSSIONS

### Effect of Chrysin and Diosmin on Maximal Electroshock-Induced Seizures in Mice

**Table 1: Effect Of Chrysin and Diosmin On Maximal Electroshock-Induced Seizures In Mice**

| S.No | Treatments | Dose(mg/kg) | Duration of HLTE (sec) | Quantal protection | Percentage of Protection |
|---|---|---|---|---|---|
| 1 | Control | - | 20.24±2.29 | 4/6 | 66.67 |
| 2 | Diosmin | 100 | 11.20±1.53** | 5/6 | 88.33 |
| 3 | Chrysin | 25 | 1.169±1.66*** | 6/6 | 100 |
| 4 | Phenytoin | 25 | 5.50±1.33*** | 6/6 | 100 |

Values are Mean±SD (n=6);

***P< 0.0001 compared with control using ANOVA followed by Bonferroni.

**P< 0.001 compared with MES using ANOVA followed by Bonferroni.

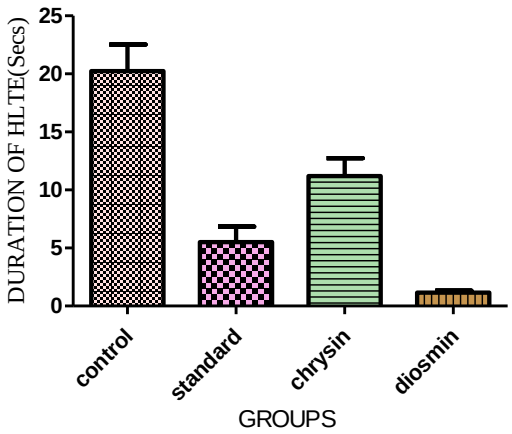


**Fig 5: Effect of chrysin and diosmin on maximal electroshock induced seizures in mice Data are expressed as are Mean ± SD from six mice.**

MES produced hind limb tonic extension seizures in all the animals used. The control mice showed tonic limb extension for the duration of 20.24±2.29sec Diosmin protected 83.33 % of

mice and alter the incidence of seizures elicited by MES to a significant extent. At a dose of 25 mg/kg, the Chrysin protected 100% of the animals and significantly reduced the duration of the seizure.

The standard antiepileptic drug, phenytoin (25mg/kg) also protected all the animals and significantly reduced the duration of HLTE.

**Biogenic amines on seizures induced mice**

**Table 2: Effect Of Chrysin and Diosmin On The Levels Of Biogenic Amines On Seizures Induced Mice**

| S.no | Treatments | Noradrenaline (ng/g of wet tissue) | Dopamine (ng/g of wet tissue) | Serotonin (ng/g of wet tissue) |
|---|---|---|---|---|
| 1 | Control | 270.2 ±2.93 | 357.5±1.046 | 234.4±0.056 |
| 2 | MES | 91.31±2.574 | 123.3±1.091 | 86.6±0.549 |
| 3 | Chrysin | 226.4 ±1.96*** | 217.3±1.095** | 172.1±0.897*** |
| 4 | Diosmin | 159.2 ±2.01** | 209 ±1.374** | 136.8 ±0.089** |
| 5 | Phenytoin | 199.2 ±2.96*** | 312.5 ±0.095*** | 209.8±0.036*** |

Values are Mean±SD (n=6);

***P< 0.0001 compared with control using ANOVA followed by Bonferroni test.

**P< 0.001 compared with MES using ANOVA followed by Bonferroni test.

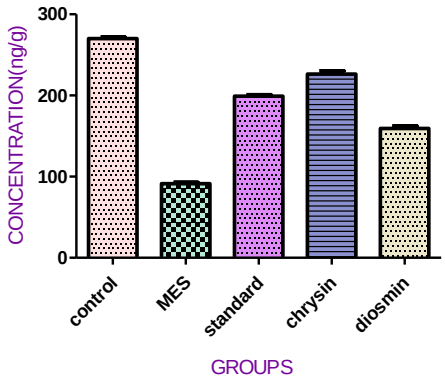


**Fig 6: Effect of chrysin and diosmin on the levels of noradrenaline on seizures induced mice. Data are expressed as are Mean ± SD from six mice.**

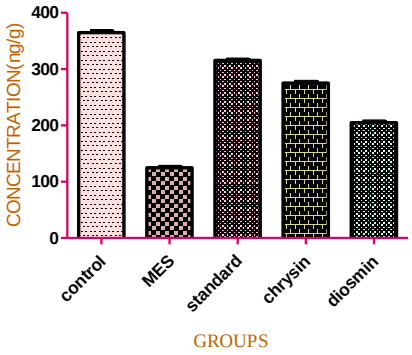


**Fig 7: Effect of chrysin and diosmin on the levels of dopamine on seizures induced mice. data are expressed as are mean ± sd from six mice.**

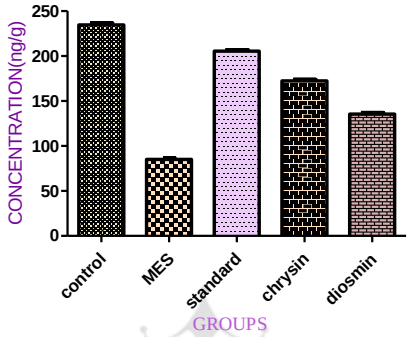


**Fig 8: Effect Of Chrysin And Diosmin On The Levels Of Serotonin On Seizures Induced Mice. Data are expressed as are Mean ± SD from six mice.**

A significant increase in the noradrenaline, dopamine and serotonin concentration was noted in the fore brain region of chrysin and diosmin treated animals.

**Table 3: Effect of Chrysin and Diosmin on Antioxidant Activity**

| **Parameters** | **Control** | **standarad** | **chrysin** | **Diosmin** |
|---|---|---|---|---|
| LPO (n.mol of MDA/mg protein) | 0.7517±0.0198 | 0.2060±0.004*** | 0.3527±0.028*** | 0.4503±0.007** |
| SOD (n.mol/mg protein) | 10.71±0.4078 | 19.43±0.2818*** | 15.91±0.060*** | 14.01±0.2501** |
| GSH (n.mol/mg protein ) | 9.450±4917 | 14.79±0.440*** | 13.08±0.6701*** | 12.29 ±0.7068** |

Values are Mean±SD (n=6);

***P< 0.0001 compared with control using ANOVA followed by Bonferroni.

**P< 0.001 compared with MES using ANOVA followed by Bonferroni.

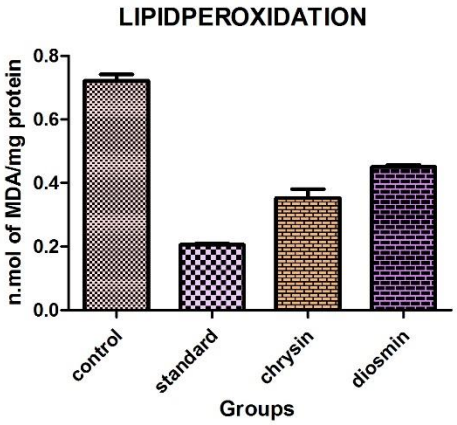


**Fig 9: Effect of chrysin and diosmin on lipidperoxidation. Data are expressed as are mean ± SD from six mice.**

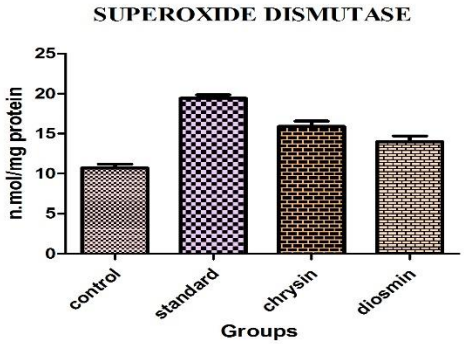


**Fig 10: Effect Of Chrysin And Diosmin On Superoxide Dismutase. Dataare expressed as are Mean ± SD from six mice.**

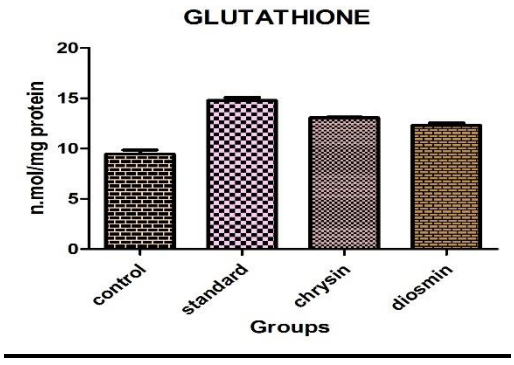


**Fig 11: Effect of chrysin and diosmin on glutathione. Data are expressed as Mean ± SD from six mice.**

**Effect of chrysin and diosmin on GSH, SOD, and lipid peroxidation:** MES-induced mice showed a significant decrease in the levels of GSH, SOD and a significant increase in the levels

of MDA ($p<0.0001$) of brain homogenate. The mice administered chrysin (25mg/kg) had shown a significant increase in the GSH ($P<0.001$), SOD ($P<0.001$), total protein ($P<0.0001$) levels, and a significant decrease in the MDA ($p<0.0001$) levels of brain homogenate compared to MES induced control group.

The mice administered diosmin (30mg/kg) had shown a significant increase in the GSH ($P<0.01$), SOD ($P<0.01$), total protein ($P<0.0001$) levels, and a significant decrease in the MDA ($p<0.0001$) levels of brain homogenate compared to MES induced control group MES induced mice were treated with phenytoin (20mg/kg) and the levels of GSH, SOD was found to be significantly ($P<0.0001$) increased and the MDA levels were significantly decreased compared to MES induced control group.

**Effect of chrysin and diosmin on noradrenaline, dopamine, and serotonin levels.**

MES-induced mice showed a significant decrease in the levels of noradrenaline, dopamine, and serotonin of brain homogenate. The mice administered chrysin (25mg/kg) had shown a significant increase in the noradrenaline ($p>0.0001$), dopamine ($p>0.001$), and serotonin ($p>0.0001$) of brain homogenate compare to MES-induced control mice. These levels decreased compared to normal control mice.

The mice administered Diosmin (30mg/kg) had shown a significant increase in noradrenaline ($p>0.001$), dopamine ($p>0.001$), and serotonin ($p>0.001$) of brain homogenate compare to MES-induced control mice. These levels decreased compared to normal control mice. MES-induced mice were treated with phenytoin (20mg/kg) and the levels of noradrenaline, SOD, and serotonin were found to be significantly ($P<0.0001$) increased compared to MES induced control group. But these levels decreased in normal control mice.

It is well known that reactive oxygen species (ROS) formation increases during seizures and the removal of these formed species depends on antioxidant systems. (Packer L et al., 1997) If the rise in the level of ROS exceeds the antioxidant capacity to neutralize them, then cell lipids, proteins, and even DNA material may suffer oxidative damage.

In the present study, we have examined whether the treatment with chrysin and diosmin can alter the lipid peroxidation level, biogenic amine levels, superoxide dismutase, and glutathione activities in the hippocampus observed during seizures induced by maximal electrical shock in mice. The generation of reactive oxygen species is currently viewed as one of the processes through which epileptic activity exerts its deleterious effects on the brain. (Castagne V et It al.,

1999) These reactive oxygen species in the absence of an efficient defense mechanism cause peroxidation of membrane polyunsaturated fatty acids. (Halliwell B et al., 1999) The brain is particularly susceptible to peroxidation due to the simultaneous presence of high levels of polyunsaturated fatty acids and iron, (Halliwell B et al., 1989) which is the target of free radical damage. We have recorded the rise in lipid peroxidation levels in the hippocampus homogenate of mice, after induction of seizures. This is reflected by a rise in TBARS level which may be related to its intermediate free radicals formed during seizures induced by maximal electrical shock.

Literature has shown that seizures induced by maximum electric shock produce changes in nitric oxide metabolism increased the production of their metabolites (nitrite and nitrate). Its metabolites interact with glutaminergic receptors producing a part of its stimulatory action on the CNS.(Maczurek A et al., 2008) The antioxidant action after pretreatment with chrysin and diosmin was readily explained as a consequence of inhibition of the formation of free radicals, lipid peroxidation products as well as scavenging activity on reactive oxygen species.(Michiels C et al., 1994) Increased free radicals and elevated nitrite levels may cause lipid peroxidation in the hippocampus of mice during seizures.(Tejada S et al., 2006)

GABA is the most important inhibitory neurotransmitter in the human central nervous system. GABA is involved in epilepsy, sedation, and anxiety. It works via binding to GABA-A receptors. GABA-A receptors are heteromeric GABA-gated chloride channels. The transmembrane ion channel is opened by a stimulus generated by GABA, which allows an influx of chloride ions. This results in a decrease in the depolarizing effects of excitatory input, thereby depressing excitability (Bruton R et al., 1999). As a result, the cell is inhibited and an anticonvulsant, sedative, or anxiolytic activity is achieved.

Chrysin is an active compound in *Passiflora coerulea* (Passifloraceae) with a Ki-value of 3 µM (Medina J.H et al., 1990). Ki value has a linear relationship concerning affinity for GABA-A receptors. The Ki value of diosmin was 2mM. So chrysin and diosmin activate GABA. The transmembrane ion channel is opened by a stimulus generated by GABA, which allows an influx of chloride ions.

This results in a decrease in the depolarizing effects of excitatory input, thereby depressing excitability (Bruton R et al., 1999). As a result, the cell is inhibited and an anticonvulsant, sedative, or anxiolytic activity is achieved.

Monoamine oxidase (MAO) is a flavoenzyme found in the outer membrane of mitochondria. MAO catalyzes the oxidative deamination of primary, secondary, and some tertiary amines (Edmondson D.E et al., 2004). Two isoforms of MAO exist MAO-A and MAO-B, where MAO-A preferentially oxidizes serotonin (5- hydroxytryptamine) and noradrenaline, whereas MAO-B preferentially oxidizes phenylethylamine (Billett E.E et al 2004).

Dopamine and tyramine appear to be substrates for both isoenzymes. In the CNS, MAO-A is present in the extraneuronal compartment and within the dopaminergic, serotonergic, and noradrenergic nerve terminals, while MAO-B is mainly localized in the glial cells (Edmondson D.E et al., 2004). The primary roles of MAO-A and MAO-B lie in the metabolism of exogenous amines and the regulation of neurotransmitter levels and intracellular amine stores (Billett E.E et al 2004). It is believed that the pathophysiology of epilepsy involves decreased levels of 5-hydroxytryptamine and noradrenaline, and dopamine levels in the brain.

Diosmin and chrysin are flavone components (Edmondson D.E et al., 2004). They inhibit the MAO-A and MAO-B enzymes present in the outer membrane of mitochondria. Diosmin and chrysin prevent the metabolism of exogenous amines and increase the neurotransmitter levels and intracellular amine stores which automatically increase dopamine, serotonin, and noradrenaline levels in the brain.

When mice were treated with maximal electric shock there is an increase in the activity of MAO-A and MAO-B enzymes outer membrane of mitochondria.

The primary roles of MAO-A and MAO-B lie in the metabolism of exogenous amines and the regulation of neurotransmitter levels and intracellular amine stores hence there is a decrease in the levels of noradrenaline, dopamine, and serotonin in the brain. (Billet E.E et al 2004). After drug treatment, there is an increase in the levels of noradrenaline, serotonin, and dopamine which demonstrates the inhibitory action of chrysin and diosmin on MAO-A and MAO-B iso-enzymes.

The present study was designed to evaluate the amelioration of oxidative stress-induced seizures and the antioxidant potential of chrysin and diosmin in experimental mice. A maximal electric shock method was used for screening antioxidant activity.

Apart from the estimation of the concentration of biogenic amine in mice brain the antioxidant potential of chrysin and diosmin was also estimated by lipid peroxidation, superoxide dismutase, and glutathione levels. On MES the results of the present study showed that the

chrysin and diosmin decreased the duration of tonic hind leg extension in maximal electric shock-induced convulsion. Chrysin and diosmin activated the GABA receptors and reduced depolarization and decreased the duration of hind limb extension.

Chrysin and diosmin administration significantly increased the brain levels of serotonin, dopamine, and noradrenaline, which could be attributed to the significant protection offered against MES-induced seizures. They increased the monoamines by inhibiting the MOA-A and MAO-B enzymes in the outer side of mitochondria. Inhibiting the monoamines showed that reduced the metabolism of exogenous amine and increased neurotransmitter release and increased intracellular storage. In the animal group treated with maximal electrical shock, an increase in oxidative stress was observed indicated by the higher MDA levels as well as a decrease in SOD, and glutathione activity which might be responsible for the free radicals and activating the glutamate receptors and formation seizures.

Chrysin and diosmin-protected MES induce seizures and the amounts of SOD, and glutathione was significantly higher in drug-treated animals. The results suggested that chrysin and diosmin have a considerable and reliable effect in reducing MES and oxidative stress-induced seizures.

**CONCLUSIONS:**

The results showed that Chrysin and Diosmin have anti-epileptic and antioxidant activities. Chrysin and Diosmin are flavonoid compounds. All flavonoid compounds show antioxidant activity. So Chrysin and Diosmin are flavonoid compounds both have antioxidant activity. Oxidative stress induces seizures ameliorated by Chrysin and Diosmin. It proved by estimation of biogenic amines and antioxidant enzymes. In the biogenic amine estimation increase, the levels of noradrenaline, dopamine, and serotonin after treatment of MES induce seizures. So it indicates to reduce the seizures. Anti-oxidant enzymes superoxide dismutase and glutathione levels increase after treatment of MES induce seizures. So Chrysin and Diosmin have antioxidant activity.

The anticonvulsant and antioxidant effects of chrysin and diosmin have been investigated in the present study in experimental maximal electroshock-induced convulsion in mice. The anticonvulsant activity was observed experimentally induced convulsion i.e. maximal electroshock (MES) convulsion in mice. In the Maximal electrical shock-induced convulsion in mice model, chrysin and diosmin showed significant anticonvulsant activity by delaying the onset of convulsion, reducing the duration of action, and reducing mortality in mice. In

biogenic amine estimation, chrysin and diosmin showed significant anticonvulsant activity by increasing the levels of noradrenaline, dopamine, and serotonin in the brain. In brain lipid peroxidation, chrysin and diosmin showed significant decreases in malondialdehyde content. Chrysin and diosmin significantly increased superoxide dismutase and glutathione (GSH) levels in the brain as compared to the control. It was observed that Chrysin and Diosmin possess anticonvulsant and antioxidant activity in maximal electroshock convulsion.

## REFERENCES:


1. Aji BM, Onyeyili PA, Osunkwo UA. The central nervous effects of *Mitragyna africanus* (Willd) stembark extract in rats. Journal of *Ethnopharmacology*. 2001; 77(2-3):143-149.
2. Asl MA, Rad SS, Zamansoltani F. Anticonvulsant effects of aerial parts of *passiflora incarnata* extract in mice: involvement of benzodiazepine and opioid receptors. BMC Complement Altern Med 2007, **7**: 1-6.
3. Asuntha G, Prasannaraju Y, Prasad KV. Research Article Effect of Ethanol Extract of *Indigofera tinctoria* Linn (Fabaceae) on Lithium/Pilocarpine-Induced Status Epilepticus and Oxidative Stress in Wistar Rats. Trop J Pharm Res 2010; 9: 149-56.
4. Ayanwuyi LO, Yaro AH, Adamu YS. Studies on anticonvulsant activity of methanol capitulum extract of *Leonotis nepetifolia* linn. Nigerian Journal of Pharmaceutical Sciences. 2009; 8(1)73-79.
5. Baker GB, Coutts RT. Analysis of biogenic amines. The Netherlands: Elsevier scientific publishing company. 1982; Chapter 4 Fluorescence techniques for detection and quantitation; 63-82.
6. Bancaud J, Henriksen O, Rubio-Donnadieu F, Seino M, Dreifuss FE, Penry JK . Proposal for revised clinical and electroencephalographic classification of epileptic seizures. From the Commission on Classification and Terminology of the International League Against Epilepsy. Epilepsia. 1981; 22(4):489–501.
7. Barreiros A. L. B. S, David J. M, and David J. P "Estresse oxidativo: relação entre geração de espécies reativas e defesa do organismo," Química Nova, vol. 29, no. 1, pp. 113–123, 2006.
8. Berger A, Henderson M, Nadoolman W, Duffy V, Cooper D, Saberski L, Bartoshuk L. Oral capsaicin provides temporary relief for oral mucositis pain secondary to chemotherapy/radiation therapy. Journal of Pain and Symptom Management. 1996; 10(3): 243-248.
9. Billett, E.E. Monoamine oxidase (MAO) in human peripheral tissues. Neurotoxicology 2004, 25, 139-148.
10. Bjornstedt M, Hamberg M, Kumar S, Xue J, and Holmgren A, "Human thioredoxin reductase directly reduces lipid hydroperoxides by NADPH and selenocystine strongly stimulates the reaction via catalytically generated selenols," Journal of Biological Chemistry, vol. 270, no. 20, pp. 11761–11764, 1995.
11. Bliznakov E. G "Cardiovascular diseases, oxidative stress and antioxidants: the decisive role of coenzyme Q10," Cardiovascular Research, vol. 43, no. 1, pp. 248–249, 1999.
12. Brunton LL, Goodman LS, Blumenthal D, Buxton I. Goodman and Gilman's manual of pharmacology and therapeutics. United States: McGraw-Hill Professional; 2007. Chapter 19 Pharmacotherapy of the Epilepsies; 321-337.
13. Bruton, R.; Qume, M. [3H]-GABA binding to GABA-A and GABA-B sites on rat brain crude synaptic membranes. Methods Mol. Biol. 1999, 106, 27-35.
14. Capasso A, Piacente S, Pizza C, Sorrentino L. Flavonoids reduce morphine withdrawal in vitro. J Pharm Pharmacol. 1998; 50:561-564
15. Castagne V, Gastschi M, Lefevre K, et al. Relationship between neuronal death and cellular redox status, focus on the developing nervous system. Prog Neurophysiol. 1999;59:397–423.
16. Comhair A. and Erzurum S "Antioxidant responses to oxidant-mediated lung diseases," American Journal of Physiology, vol. 283, no. 2, pp. L246–L255, 2002.
17. Costello D. J, and Delanty N "Oxidative injury in epilepsy: potential for antioxidant therapy?" Expert Review of Neurotherapeutics, vol. 4, no. 3, pp. 541–553, 2004.

18. Danielsson G, Jungbeck C, Peterson K, Norgren L (January 2002). "A randomised controlled trial of micronised purified flavonoid fraction vs placebo in patients with chronic venous disease". Eur J Vasc Endovasc Surg 23 (1): 73–6. doi:10.1053/ejvs.2001.1531. PMID 1174
19. Edmondson, D.E.; Mattevi, A.; Binda, C.; Li, M.; Hubalek, F. Structure and mechanism of monoamine oxidase. Curr. Med. Chem. 2004, 11, 1983-1993.
20. Ellman, G.L. (1959) Tissue sulfhydryl groups. *Arch Biochem Biophys.*, Vol 82, pp. 70–77.
21. Emerit, J. Edeas, M. and Bricaire, F. "Neurodegenerative diseases and oxidative stress," Biomedicine and Pharmacotherapy, vol. 58, no. 1, pp. 39–46, 2004.
22. Engel JJ, Pedley A. Timothy ED, Epilepsy A Comprehensive Textbook. Philadelphia: Lippincott-Raven Publishers; 1997. p. 1-7.
23. Estevez A. Y, Pritchard S, Harper K et al., "Neuroprotective mechanisms of cerium oxide nanoparticles in a mouse hippocampal brain slice model of ischemia," Free Radical Biology and Medicine, vol. 51, pp. 1155–1163, 2011.
24. Evans WJ. Vitamin E, vitamin C and exercise. Am J Clin Nutr. 2000;72:521–647.
25. Fridovich, I. (1997) Superoxide anion radical, superoxide dismutases and related matters. *Journal of Biological Chemistry*, Vol 272, pp. 18515-18517.
26. Gilani AH, Aziz N, Khan MA, Shaheen F, Jabeen Q, Siddiqui BS, Herzig JW. Ethnopharmacological evaluation of the anticonvulsant, sedative and antispasmodic activities of *Lavandula stoechas* L. Journal of Ethnopharmacology. 2000; 71(1-2):161-167
27. Golechha M, Bhatia J, Arya DS. Hydroalcoholic extract of Emblica officinalis Gaertn. afford protection against PTZ- induced sizure, oxidative stress and congnitive in rats. Ind J Exp Biol 2010; 48: 474-78.
28. Halliwell B, Gutteridge JMC. Free Rad Biol Med. Oxford: Clarendon Press; 1989. Lipid peroxidation: a radical chain reaction; pp. 188–276.
29. Halliwell B, Gutteridge JMC. Free Radicals in Biology and Medicine. London: Oxford Science Publications; 1999.
30. Hardman JG, Limbird LE. Goodman & Gilman's The Pharmacological Basis of Therapeutics. 10h ed. New York: McGraw-Hill; 2006. p. 521-47.
31. Harman D, "The biologic clock: the mitochondria?" Journal of the American Geriatrics Society, vol. 20, no. 4, pp. 145–147, 1972.
32. Indranarayana A, Satyanarayana L. Biostatistics for Medical, Nursing and Pharmacy Students. New Delhi: Prientice Hall of India Private Limited. 2006; Chapter 16 General principles of Statistical test; 219-230, chapter 17: Statistical significance parametric and nonparametric test; 231-244.
33. Indranarayana A, Satyanarayana L. Biostatistics for Medical, Nursing and Pharmacy Students. New Delhi: Prientice Hall of India Private Limited. 2006; Chapter 16 General principles of Statistical test; 219-230, chapter 17: Statistical significance parametric and nonparametric test; 231-244.
34. Jeong HJ, Shin YG, Kim IH, Pezzuto JM. Inhibition of aromatase activity by flavonoids. Arch Pharm Res. 1999; 22:309-312
35. Jobe PC, Laird HE. Neurotransmitters and epilepsy. New Jersey: The Humana Press. 1987; Chapter 6 The Role Of Neurotransmitters in Electroshock Seizure Models; 277-297
36. Jones D. P, "Disruption of mitochondrial redox circuitry in oxidative stress," Chemico-Biological Interactions, vol. 163, no. 1-2, pp. 38–53, 2006.
37. Jotisalikorn C, Tettoni LI. Thai Spa Book: The Natural Asian Way to Health and Beauty. Singapore: Periplus Editions (HK) Limited. Chapter 1: The Thai spa experience; 2002:16.
38. Kann O, and Kovács R, "Mitochondria and neuronal activity," *American Journal of Physiology*, vol. 292, no. 2, pp. C641–C657, 2007.
39. Kokate CK, Purohit AP, Gokhale SB. Pharmacognosy. 42nd edition. Pune: Nirali Prakashan; 2008. Chapter 11, Terpenoids, Capsicum annuum; 11.107-11.108.
40. Kuiper GG, Lemmen JG, Carlsson B, et al. Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta. Endocrinology. 1998; 139:4256-4263.
41. Leelavinothan Pari, Subramani Srinivasan, Antihyperglycemic effect of diosmin on hepatic key enzymes of carbohydrate metabolism in streptozotocin-nicotinamide-induced diabetic rats, Biomedicine & Pharmacotherapy, Volume 64, Issue 7, September 2010, Pages 477-481.

42. Limón-Pacheco J, and Gonsebatt M. E, "The role of antioxidants and antioxidant-related enzymes in protective responses to environmentally induced oxidative stress," Mutation Research, vol. 674, no. 1-2, pp. 137–147, 2009.
43. Maczurek A, Hager J, Kenklies M, et al. Vitamin C as an anti-inflammatory and neuroprotective treatment for Alzheimer's disease. Adv Drug Deliver Rev. 2008;60:1463–1470.
44. Maes M. , Galecki P, Chang Y. S, and Berk M, "A review on the oxidative and nitrosative stress (O&NS) pathways in majordepression and their possible contribution to the (neuro)degenerative processes in that illness," Progress in Neuro-Psychopharmacology and Biological Psychiatry, vol. 35, no. 3, pp. 676–692, 2011.
45. Malaya, G. *et al*., (2004) Antitumor Activity and Antioxidant Status of Caesalpinia bonducella Against Ehrlich Ascites Carcinoma in Swiss Albino Mice. *J Pharmacol Sci.*, Vol 94, pp. 177 -184.
46. Matés J. M, and Sánchez-Jiménez F, "Antioxidant enzymes and their implications in pathophysiologic processes," Frontiers in Bioscience, vol. 4, pp. D339–D345, 1999.
47. May M, Morrow J. D, and Burk R. F "Thioredoxin reductase reduces lipid hydroperoxides and spares α-tocopherol," Biochemical and Biophysical Research Communications, vol. 292, no. 1, pp. 45–49, 2002.
48. Medina, J.H.; Paladini, A.C.; Wolfman, C.; Destein, M.L.; Calvo, D.; Diaz, L.E.; Pena, C. Chrysin (5,7-Di-Oh-Flavone), A naturally occurring ligand for benzodiazepine receptors, with anticonvulsant properties. Biochem. Pharmacol. 1990, 40, 2227-2231.
49. Menéndez L, Lastra A, Hidalgo A, Baamonde A. The analgesic effect induced by capsaicin is enhanced in inflammatory states. Life Sciences. 2004; 74 (26): 3235-44.
50. Michiels C, Raes M, Toussaint O, et al. Importance of Se-glutathione peroxidase, catalase, and Cu/Zn-SOD for cell survival against oxidative stress. Free Radic Biol Med. 1994;17:235–248. [ Pu
51. Milder M and Patel J "Modulation of oxidative stress and mitochondrial function by the ketogenic diet," Epilepsy Research,2001.
52. Miyagawa T , Ohtsuki T, Koyano T, Kowithayakorn T, Ishibashi M. Cardenolide glycosides of Thevetia peruviana and triterpenoid saponins of *Sapindus emarginatus* as TRAIL resistance-overcoming compounds. Journal of Natural Products. 2009; 72:1507-1511.
53. Mohan EM, Mohan CK, Amuda P. Effects of *Indigofera tinctoria* extracts on neurotransmitters concentrations in rat brain after induction of seizures. International Journal of Phytopharmacology. 2010; 1:23-27.
54. Nohl H, Kozlov A. V, Staniek K, and Gille L "The multiple functions of coenzyme Q,Bioorganic Chemistry, vol. 29, no. 1, pp. 1– 13, 2001
55. Okhawa, H., Ohishi, N. and Yagi K. (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Ann Biochem.*, Vol 95, pp. 351–8.
56. Otake Y, Walle UK, Walle T. Extensive metabolism of the flavonoid chrysin by human Caco-2 and Hep G2 cells. Xenobiotica. 1999; 29:1241-1256
57. Paladini AC, Marder M, Viola H, et al. Flavonoids and the central nervous system: from forgotten factors to potent anxiolytic compounds. J Pharm Pharmacol. 1999; 51:519-526.
58. Patel M "Mitochondrial dysfunction and oxidative stress: cause and consequence of epileptic seizures," Free Radical Biology and Medicine, vol. 37, no. 12, pp. 1951–1962, 2004.
59. R. A. Webster. Neurotransmitters, drugs, and brain function. Chichester: John Wiley and Sons Ltd; 2001. Chapter 16, The epilepsies; 325-350.
60. Rahman I, Biswas S. K, and Kode A "Oxidant and antioxidant balance in the airways and airway diseases," European Journal of Pharmacology, vol. 533, no. 1–3, pp. 222–239, 2006.
61. Rowan A. J, "Epilepsy and the elderly," Epilepsy and Behavior, vol. 1, supplement 1, pp. S12–S14, 2000.
62. Sanjay L.Dholakiya, Kenza E. Benzeroual, Protective effect of Diosmin on LPS-induced apoptosis in PC12 cells and inhibition of TNF- [alpha] expression, Toxicology in Vitro, In Press, Accepted Manuscript, Available online 6 April 2011.
63. schlumpf M, Lichtensteiger W, Langemann H, Waser PG, Hefti F. A fluorimetric micromethod for the simultaneous determination of serotonin, noradrenaline and dopamine in milligram amounts of brain tissue. Biochemical Pharmacology. 1974; 23:2337-2346.
64. Schulz JB, Lindenau J, Seyfried J, Diechgans J. Glutathione, oxidative stress and neurodegeneration. Eur J Biochem 2000: 267: 4904-09.
65. Selim RB. Epileptic seizures and syndromes. Neurologic clinics 2001. 19(2): p. 251-70.

66. Silva R. C, and Goncalves A. A "Espécies reativas do oxigênio e as doençaas respiratórias em grandes animais," Ciência Rural, vol. 40, pp. 994–1002, 2010.
67. Stamler, J. S. Simon, D. J. Jaraki, O et al., "S-nitrosylation of tissue-type plasminogen activator confers vasodilatory and antiplateletn properties on the enzyme," Proceedings of the National Academy of Sciences of the United States of America, vol. 89, no. 17, pp. 8087– 8091, 1992.
68. Tandan R, Lewis GA, Krusinski PB, Badger GB, Fries TJ. Topical capsaicin in painful diabetic neuropathy. Controlled study with long-term follow-up" Diabetes Care. 1992; 15(1): 8-14.
69. Tejada S, Roca C, Sureda A, et al. Antioxidant response analysis in the brain after pilocarpine treatments. Brain Res Bull. 2006;69:587–592. [PubMed ]
70. The Taxonomicon. The Taxonomicon & Systema Naturae 2000 [Internet]. 2009 [cited on 2009 December 9].
71. Usui S, Komeima K, Lee S. Y et al., "Increased expression of catalase and superoxide dismutase 2 reduces cone cell death in retinitis pigmentosa," Molecular Therapy, vol. 17, no. 5, pp. 778–786, 2009
72. Valko M, Rhodes C. J, Moncol J, Izakovic M, and Mazur M "Free radicals, metals and antioxidants in oxidative stress-induced cancer," Chemico-Biological Interactions, vol. 160, no. 1, pp. 1–40, 2006.
73. Valko M, Rhodes C. J, Moncol J, Izakovic M, and Mazur M, "Free radicals, metals and antioxidants in oxidative stress-induced cancer," Chemico-Biological Interactions, vol. 160, no. 1, pp. 1–40, 2006.
74. Veverková L, Kalac J, Jedlicka V, Wechsler J (August 2005). "[Analysis of surgical procedures on the vena saphena magna in the Czech Republic and an effect of Detralex during its stripping]" (in Czech). Rozhl Chir 84 (8): 410–2, 414–6. PMID 16218350
75. Vogel HG, Wolfgang H. Drug Discovery and Evaluation. Berlin: Springer Verlag; 1997. p. 246-266.
76. Voss P, Engels M, Strosova M, Grune T, and Horakova L "Protective effect of antioxidants against sarcoplasmic reticulum (SR) oxidation by Fenton reaction, however without prevention of Ca-pump activity," Toxicology in Vitro, vol. 22, no. 7, pp. 1726–1733, 2008.
77. Waldbaum S, Liang L. P, and Patel M "Persistent impairment of mitochondrial and tissue redox status during lithium-pilocarpine-induced epileptogenesis," Journal of Neurochemistry, vol. 115, no. 5, pp. 1172–1182, 2010.
78. Waldbaum S, Liang L. P, and Patel M, "Persistent impairment of mitochondrial and tissue redox status during lithium-pilocarpine -induced epileptogenesis," Journal of Neurochemistry, vol. 115, no. 5, pp. 1172–1182, 2010.
79. Walle UK, Galijatovic A, Walle T. Transport of the flavonoid chrysin and its conjugated metabolites by the human intestinal cell line Caco-2. Biochem Pharmacol. 1999; 58:431-438.
80. Walz R, Moreira JCF, Benfato MS, Quevedo J, Schorer N, Vianna MMR, et al. Lipid peroxidation in hippocampus early and late after status epilepticus induced by pilocarpine of kainic acid in Wistar rats. Neuroscience Letters. 2000;291:179–182.
81. Wassmann S, Wassmann K, and Nickenig G "Modulation of oxidant and antioxidant enzyme expression and function in vascular cells," Hypertension, vol. 44, no. 4, pp. 381–386, 2004
82. Whiteman M, Spencer J. P. E, Jenner A, and Halliwell B "Hypochlorous acid-induced DNA base modification: potentiation by nitrite: biomarkers of DNA damage by reactive oxygen species," Biochemical and Biophysical Research Communications, vol. 257, no. 2, pp. 572–576, 1999.
83. Williamson EM. Major herbs of ayurveda. 1st ed. Churchill livingstone: Elsevier science limited; 2002. P. 233.
84. Packer L. Oxidants, antioxidants, nutrients and the athlete. J Sports Sci. 1997;15:353–363.