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Anticonvulsant effect of *Boswellia serrata* by modulation of endogenous biomarkers

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ABSTRACT

Epilepsy is a group of disorders of CNS bearing its pathological origin in paroxysysmal cerebral dysrhythmia, clinically consisting episodes (seizure) of loss of consciousness. Boswellia serrata is a tetrahydroxy flavone that possess antiyperlipidemic, antioxidant, anti-inflammatory and antidiabetic potential. The aim of present investigation was to investigate the anticonvulsant activity of Boswellia serrata (5, 10 and 200 mg/kg) against Pentylenetetrazole (PTZ), Strychnine (STR), Isoniazid (INH) and maximal electroshock (MES) induced convulsions in mice as well as Electrical kindling seizures in rats. Diazepam and Phenytoin were used as reference anticonvulsant drugs for comparison. Intraperitoneal administration of PTZ (90mg/kg), Strychnine (50 mg/kg) and Isoniazid (300 mg/kg) resulted in hind-limb, tonic-clonic convulsion along with lethality in mice, whereas twice daily auricular stimulation resulted progressive severity of seizures in rats. It also significantly altered levels of brain Gamma amino butyric acid (GABA) along with nitric oxide (NO) and xanthine oxidase (XO) in mice. Treatment with Boswellia serrata (10 and 200 mg/kg) delayed onset of convulsion along with duration of tonic-clonic convulsions as well as it significantly reduced PTZ and STR-induced mortality in mice ($P < 0.05$ - $P < 0.001$). It also significantly ($P < 0.001$) reduced severity of electrically kindled seizures in rats and total number of rats seizure per group. Mice treated with Boswellia serrata (10 and 200 mg/kg) significantly increased level of brain GABA whereas it significantly decreased elevated level of brain NO and XO. In conclusion, the findings of present study provide pharmacological credence to anticonvulsant profile of Boswellia serrata. The protection against the convulsions and restoration of endogenous enzyme level give an innuendo to its probable mechanism of action which may be mediated through the GABAergic pathway and inhibition of oxidative injury.

Keywords: Anticonvulsant, Brain GABA, *Boswellia serrata*, Nitric oxide, Xanthine oxidase

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INTRODUCTION

Epilepsy is demonstrated by a number of diseases of central nervous system which are the manifestations of the severe aberration and discharge in regions of brain, at a focal point, leading epileptic symptomatology [2]. It has been well reported that over stimulation of excitatory amino acids like AMPA and NMDA receptors provoke reactive oxygen species (ROS) which in turn lead to seizures and neuronal death.[3, 4].

The present antiepileptic drugs bear many untoward adverse reactions and toxicities [5]. Hence, there is still room for further research to elucidate drugs with less side effects. The aim of present investigation was to unravel the anticonvulsant potential of *Boswellia serrata* in animal models of convulsions by recording various behavioral and biochemical parameters.

MATERIALS AND METHODS

2.1 Animals:

Adult male Swiss albino mice (18-22 g) and male Wistar rats (180-200 g) were purchased from National Institute of Biosciences, Pune and kept in quarantine for one week in housed at the institute animal house in groups of six animals per cage at standard laboratory conditions at a temperature of $24^0C \pm 1^0C$, relative humidity of 45–55% and 12:12 h dark and light cycle. The experiments were carried out between 10:00 am to 5:00 pm. Animals had free access to food (standard chaw pellet, Pranav Agro industries Ltd., Sangli, India) and water ad libitum. Experimental protocols and procedures were approved by the Institutional Animal Ethics Committee. Animals were brought to testing laboratory 1 hour before the experimentation for adaptation purpose. The experimentation was carried out in noise free area.

2.2 Drugs and solutions:

Boswellia serrata (BS) (Natural Remedies, India), Pentylenetetrazole (PTZ) (Sigma Aldrich, India), Strychnine (STR) (Sigma Aldrich India), Phenytoin (PHY) (Eptoin®, Sun Pharma Ltd., India), Diazepam (DZP) (Calmpose®, Ranbaxy Ltd., India) and Isoniazid (INH) (Solonex®, Macleods, Mumbai, India) were used in present study. All chemicals were dissolved in saline except *Boswellia serrata* was dissolved in 1% DMSO. The doses of *Boswellia serrata* were selected on the basis of previous studies [16]. All other reagents were purchased from S.D. Fine Chemicals, Mumbai, India.

2.3 Assessment of Anticonvulsant activity:

2.1.1 Pentylenetetrazole (PTZ) induced convulsions:

The mice were randomly divided into five groups containing six mice in each group as follows:

Group I: *Boswellia serrata* (50 mg/kg, i.p.);
Group II: *Boswellia serrata* (100 mg/kg, i.p.);
Group III: *Boswellia serrata* (200 mg/kg, i.p.);
Group IV: Diazepam (50 mg/kg, i.p.);
Group V: Vehicle control (1 % DMSO. i.p.).

A previously reported protocol was followed to induced convulsion using PTZ [17]. Vehicle, test drug and standard drugs were administered by intraperitoneal (i.p.) route. PTZ (90 mg/kg) was injected intraperitoneally to mice 45 min after intraperitoneal (i.p.) administration of vehicle (1% DMSO) or test drug (*Boswellia serrata*) and 30 min after the standard (diazepam) drug. Immediately after PTZ administration mice were observed for next 30 min for following symptoms:

- Onset of convulsion
- Duration of clonic convulsion
- Duration of tonic convulsion
- Incidence (number of mice showing convulsions);
- Mortality

2.1.2 Strychnine (STR) induced convulsions:
2.1.3
The mice were randomly divided into five groups containing six mice in each group as follows:

Group I: *Boswellia serrata* (50 mg/kg, i.p.);
Group II: *Boswellia serrata* (100 mg/kg, i.p.);
Group III: *Boswellia serrata* (200 mg/kg, i.p.);
Group IV: Phenytoin (200 mg/kg, i.p.);
Group V: Vehicle control (1 % DMSO. i.p.).

A previously reported protocol was followed to induced convulsion using STR [18]. Vehicle, test drug and standard drugs were administered by intraperitoneal (i.p.) route. STR (50 mg/kg) was injected intraperitoneally to mice 45 min after intraperitoneal (i.p.) administration of vehicle (1% DMSO) or test drug (*Boswellia serrata*) and 30 min after the standard (phenytoin) drug. Immediately after STR administration mice were observed for next 60 min for following symptoms:

- Onset of convulsion
- Duration of clonic convulsion
- Duration of tonic convulsion
- Incidence (number of mice showing convulsions);
- Mortality

2.1.4 Isoniazid (INH) induced convulsions:

The mice were randomly divided into five groups containing six mice in each group as follows:

Group I: *Boswellia serrata* (50 mg/kg, i.p.);
Group II: *Boswellia serrata* (100 mg/kg, i.p.);
Group III: *Boswellia serrata* (200 mg/kg, i.p.);
Group IV: Diazepam (50 mg/kg, i.p.);
Group V: Vehicle control (1 % DMSO. i.p.).

A previously reported protocol was followed to induced convulsion using INH [19]. Vehicle, test drug and standard drugs were administered by intraperitoneal (i.p.) route. INH (300 mg/kg) was injected intraperitoneally to mice 45 min after intraperitoneal (i.p.) administration of vehicle (1% DMSO) or test drug (*Boswellia serrata*) and 30 min after the standard (diazepam) drug. Immediately after INH administration mice were observed for next 120 min for following symptoms:

- Onset of convulsion
- Duration of clonic convulsion
- Duration of tonic convulsion
- Incidence (number of mice showing convulsions);
- Mortality

2.1.5 Maximal electroshock (MES) induced convulsions:

The mice were randomly divided into five groups containing six mice in each group as follows:

Group I: *Boswellia serrata* (50 mg/kg, i.p.);
Group II: *Boswellia serrata* (100 mg/kg, i.p.);
Group III: *Boswellia serrata* (200 mg/kg, i.p.);
Group IV: Phenytoin (50 mg/kg, i.p.);
Group V: Vehicle control (1 % DMSO. i.p.).

A previously reported protocol was followed to induced convulsion by MES [20]. Convulsions were induced in mice 45 min after intraperitoneal (i.p.) administration of vehicle (1% DMSO) or test drug (*Boswellia serrata*) and 30 min after the standard (phenytoin) drug by giving a current stimulus (45 mA, 60 Hz for 0.2 s) by through auricular electrodes by an electro-convulsometer (Dolphin, India). All animals were observed for duration of HLTE (hind limb tonic extension) and percent protection against HLTE.

2.4 Biochemical evaluation:

2.4.1 Brain GABA estimation:

2.4.1.1 Sample preparation:
Forty-five min after vehicle (1 % DMSO) or *Boswellia serrata* and 30 min after diazepam (50 mg/kg), Phenytoin (200 mg/kg) mice were sacrificed. PTZ (90 mg/kg), INH (300 mg/kg) and STR (3 mg/kg) treated animals were sacrificed as soon as onset of convulsions occurs. Animals which received PTZ (90 mg/kg), INH (300 mg/kg) and STR (3 mg/kg) after 45 min of *Boswellia serrata* (10 and 200 mg/kg) and sacrificed at the exact time of onset of convulsions. Brain was isolated immediately and transferred to homogenization tube containing 5 ml of 0.01N hydrochloric acid and homogenized. Brain homogenate was transferred to bottle containing 8 ml of ice cold absolute alcohol and kept for 1 hour at 0 ^{0}C. The content was centrifuged for 10 min at 16000 rpm, supernatant was collected in petridish. Precipitate was washed with 3-5 ml of 75% alcohol for three times and washes were combined with supernatant. Contents in petridish were evaporated to dryness at 70-90 ^{0}C on water bath under stream of air. To the dry mass 1 ml water and 2 ml chloroform were added and centrifuged at 2000 rpm. Upper phase containing GABA was separated and 10 µl of it was applied as spot on Whatman paper (No. 41).

2.4.1.2 Chromatographic conditions:
The mobile phase consisted of n-butanol (50 ml) acetic acid (12 ml) and water (60 ml). The chamber was saturated for half hour with mobile phase. The paper chromatogram was developed with ascending technique. The paper was dried in hot air and then spread with 0.5% Ninhydrin solution in 95% ethanol. The paper was dried for 1 hr at 90 ^{0}C. Blue color spot developed on paper was cut and heated with 2 ml ninhydrin solution on water bath for 5 min. water (5 ml) was added to solution and kept for 1h Supernatant was decanted and absorbance was measured at 570 nm.

2.4.1.3 Standards and calculations:
Stock solution of standard GABA, 1 mg/ml was prepared in 0.01N HCl. Serial dilutions were prepared to get concentrations 1ng/10µl to 1000ng/10µl. To obtain a standard concentration curve for GABA same procedure was followed replacing brain homogenate with standard GABA solutions [22].

2.4.2 Estimation of total protein:
Protein concentration was estimated according to previously described method [23], using BSA (bovine serum albumin) as a standard.

2.4.3 Estimation of nitrite/nitrate level:
The NO level was estimated as nitrite by the acidic Griess reaction after reduction of nitrate to nitrite by vanadium trichloride according to previously described method [24].

The Griess reaction relies on a simple colorimetric reaction between nitrite, sulfonamide and N-(1- naphthyl) ethylenediamine to produce a pink azo-product with maximum absorbance at 543 nm. The concentrations were determined using a standard curve of sodium nitrate and the results were expressed as μg/ml.

2.4.4 Estimation of Xanthine oxidase (XO) level:

Xanthine oxidase activity was measured spectrophotometrically by the formation of uric acid from xanthine through the increase in absorbency at 293 nm, according to previously described method [25]. The concentrations were determined using a standard curve of XO solutions and results were expressed as units per gram protein in brain homogenate.

2.5 Statistical analysis:

Data were expressed as mean ± standard error mean (SEM). The data of 'Brain GABA', 'Nitric oxide' and 'Xanthine oxidase' was analyzed using one-way analysis of variance (ANOVA), Dunnett's multiple range test was applied for post hoc analysis. Data of 'incidence of convulsion' was analyzed by nonparametric Kruskal–Wallis ANOVA. Data of 'mortality' was analyzed by Fisher's exact test. Analysis of all the statistical data was performed using GraphPad Prism 5.0 (GraphPad, San Diego, USA). $P < 0.05$ was considered as statistically significant.

RESULTS

3.1 Effects of Boswellia serrata and diazepam on pentylenetetrazole induced convulsions in mice:

Intraperitoneal administration of PTZ (90mg/kg) lead to hind-limb, tonic-clonic convulsion along with death in mice. Pretreatment with *Boswellia serrata* (5, 10 and 200 mg/kg) significantly and dose dependently ($P < 0.05$, $P < 0.01$ and $P < 0.001$ respectively) procrastinated the onset of convulsion and downregulated the PTZ-induced death in mice as compared to vehicle control mice.

Mice receiving a pretreatment with *Boswellia serrata* (10 and 200 mg/kg) significantly reduced ($P < 0.01$ and $P < 0.001$ respectively) duration of tonic convulsion and significantly reduced duration of clonic convulsions ($P < 0.05$ and $P < 0.01$ respectively) when compared to vehicle control mice. It also significantly inhibited the total number of animals experiencing convulsions per group ($P < 0.01$ and $P < 0.001$ respectively) when compared to vehicle control mice. When compared with vehicle control mice, diazepam (50 mg/kg) administered mice demonstrated significant procrastinated ($P < 0.001$) onset of convulsion and it significantly ($P < 0.001$) donregulated the duration of tonic and clonic convulsions. Mortality induced by PTZ was also significantly reduced ($P < 0.001$) by administration of diazepam (50 mg/kg). (Fig1-3)

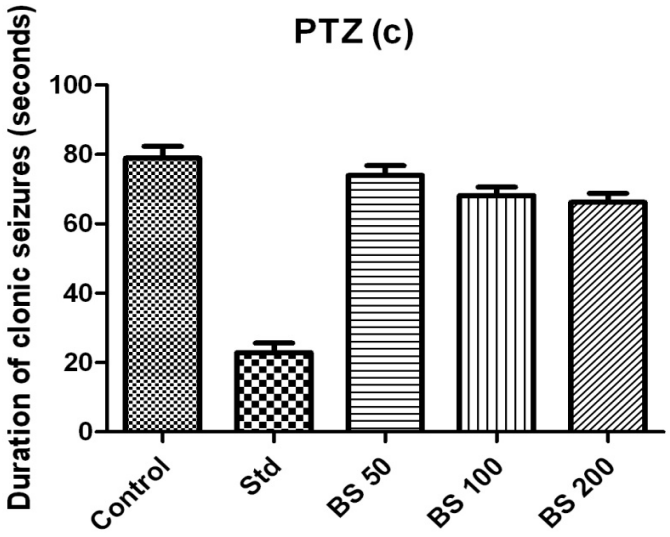

Fig 1 All data analysed using one way ANOVA followed by Dunnet's post hoc test. *p<0.05,p<0.01,***p<0.001 when all the experimental groups were compared with vehicle control**

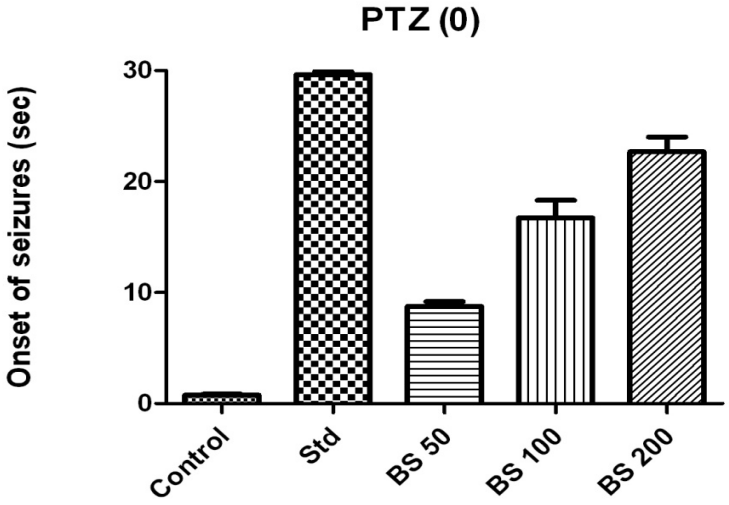

Fig 2 All data analysed using one way ANOVA followed by Dunnet's post hoc test. *p<0.05,p<0.01,***p<0.001 when all the experimental groups were compared with vehicle control**

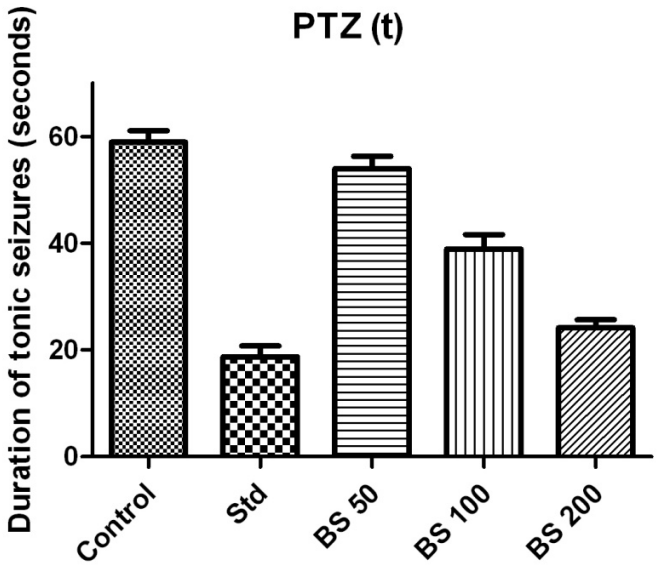

Fig 3 All data analysed using one way ANOVA followed by Dunnet's post hoc test. *p<0.05,p<0.01,***p<0.001 when all the experimental groups were compared with vehicle control**

3.2 Effects of Boswellia serrata and phenytoin on strychnine induced convulsions in mice:

When compared to vehicle control mice, *Boswellia serrata* (10 and 200 mg/kg) treated mice demonstrated significant procrastinate in convulsion onset as well as significant reduction in the strychnine attributed mortality in the mice ($P < 0.01$ and $P < 0.001$ respectively).

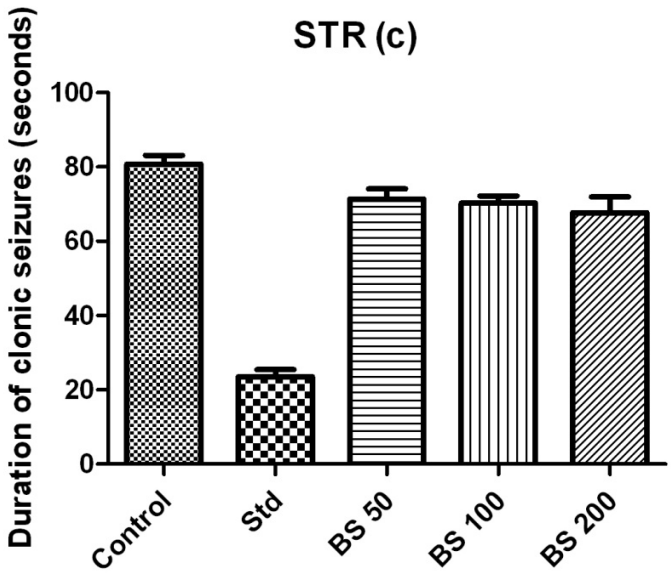

Fig 4 All data analysed using one way ANOVA followed by Dunnet's's post hoc test. *p<0.05,p<0.01,***p<0.001 when all the experimental groups were compared with vehicle control**

Total mice that underwent convulsions by treatment of strychnine (50 mg/kg, i.p.) was also significantly reduced by pretreatment with *Boswellia serrata* (10 and 200 mg/kg, $P < 0.01$ and $P < 0.001$ respectively).

Duration of the convulsion of clonic nature was significantly reduced by administration of *Boswellia serrata* (200 mg/kg, $P < 0.05$) whereas duration of tonic convulsion also significantly reduced by *Boswellia serrata* treatment (10 and 200 mg/kg, $P < 0.05$ and $P < 0.01$ respectively) when compared to mice treated with vehicle.

Treatment with phenytoin (200 mg/kg) significantly procrastinated onset of convulsion as well as it significantly reduced duration of tonic clonic convulsions ($P < 0.001$). It also significantly reduced the strychnine induced death rate in mice ($P < 0.001$). (Fig4-6)

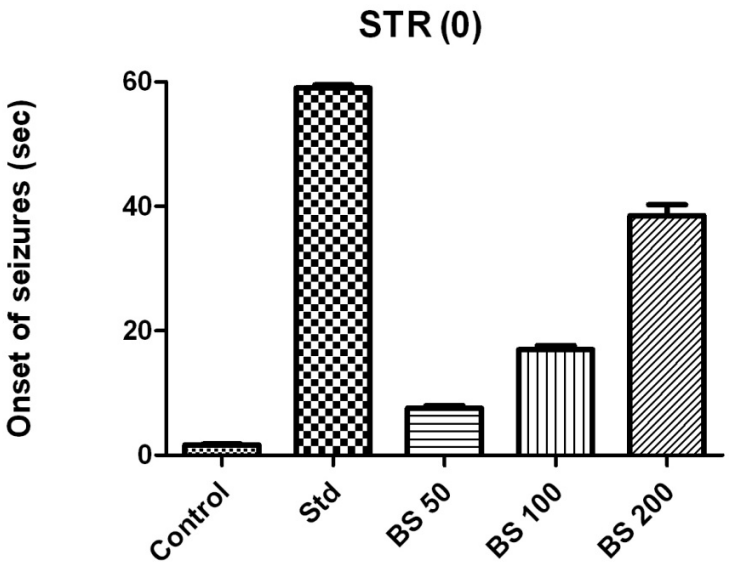

Fig 5 All data analysed using one way ANOVA followed by Dunnet's's post hoc test. *p<0.05,p<0.01,***p<0.001 when all the experimental groups were compared with vehicle control**

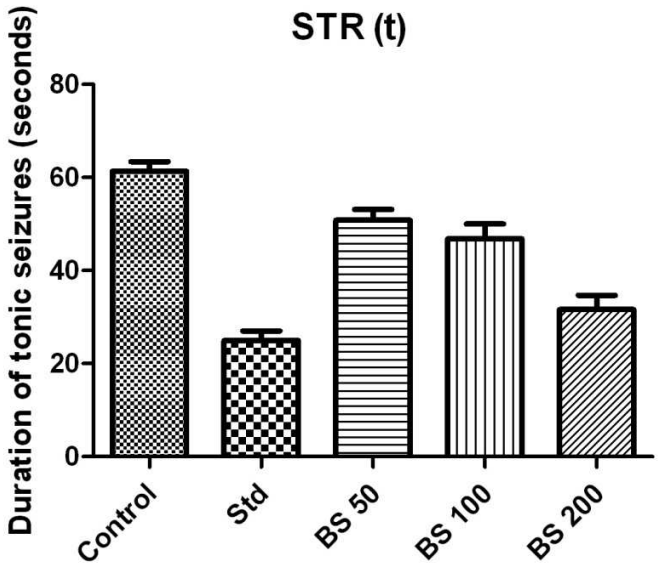

Fig 6 All data analysed using one way ANOVA followed by Dunnet's's post hoc test. *p<0.05,p<0.01,***p<0.001 when all the experimental groups were compared with vehicle control**

3.3 Effects of Boswellia serrata and diazepam on isoniazid induced convulsions in mice:

Treatment with *Boswellia serrata* (200 mg/kg) significantly reduced ($P < 0.05$) the INH induced death rate in the mice and it also significantly procrastinated ($P < 0.05$) onset of convulsions when compared to vehicle treated mice.

Mice administered with *Boswellia serrata* (100 mg/kg) significantly reduced ($P < 0.05$) time of clonic convulsions but it did not produce any significant reduction in duration of tonic convulsion at any dose when compared with vehicle treated mice.

Treatment with *Boswellia serrata* (10 and 200 mg/kg) significantly diminished total number of mice experiencing convulsions per group ($P < 0.05$) when compared with vehicle treated mice.

When compared with vehicle control mice, diazepam (50 mg/kg) treated mice demonstrated significant protection against INH induced morality as well as it significantly procrastinated onset of the convulsion epidsodes and significantly diminished duration of tonic clonic convulsions ($P < 0.001$). (Fig7-9)

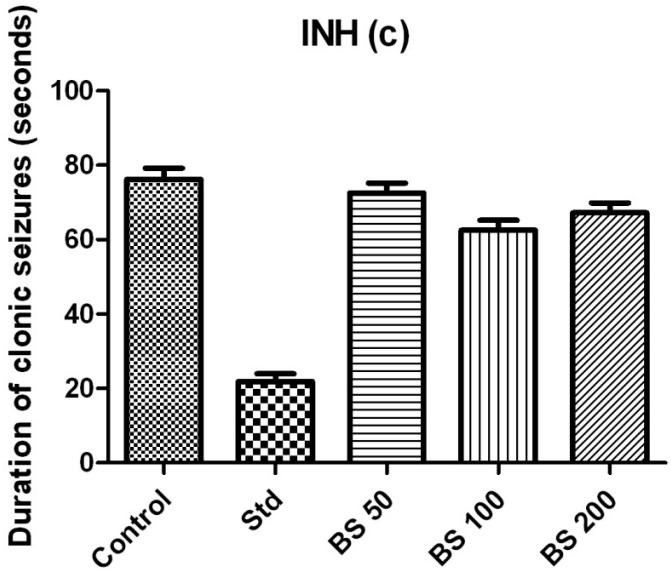

Fig 7 All data analysed using one way ANOVA followed by Dunnet's's post hoc test. *p<0.05,p<0.01,***p<0.001 when all the experimental groups were compared with vehicle control**

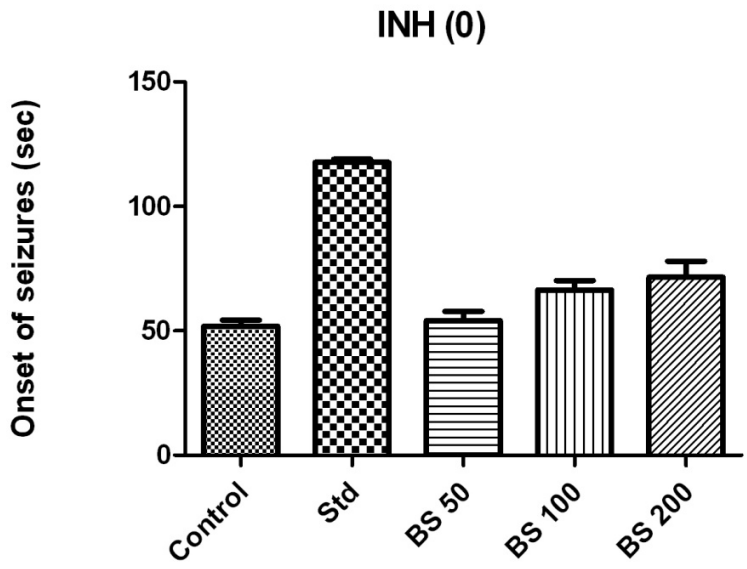

Fig 8 All data analysed using one way ANOVA followed by Dunnet's's post hoc test. *p<0.05,p<0.01,***p<0.001 when all the experimental groups were compared with vehicle control**

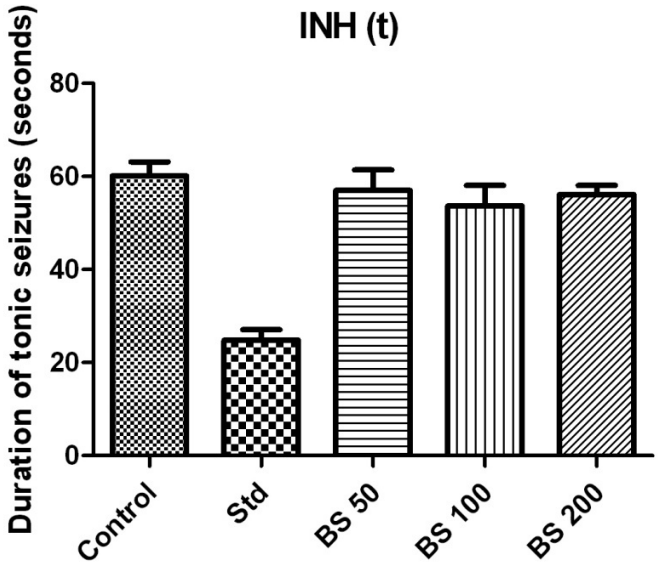

Fig 9 All data analysed using one way ANOVA followed by Dunnet's's post hoc test. *p<0.05,p<0.01,***p<0.001 when all the experimental groups were compared with vehicle control**

3.4 Effects of Boswellia serrata, diazepam and phenytoin on PTZ, STR and INH induced alteration in brain GABA level:

Intraperitoneal administration of PTZ (90 mg/kg) and INH (300 mg/kg) led to significant reduction ($P < 0.001$) GABA levels in brain (26.23 ± 1.11 and 26.03 ± 1.33 ng/gm respectively) as compared to normal mice (57.79 ± 1.69 ng/gm).

Mice treated with *Boswellia serrata* (200 mg/kg) significantly reduced the brain GABA level in PTZ treated rats (44.50 ± 1.02 ng/gm, $P < 0.001$) when compared to vehicle treated mice. In
Boswellia serrata (100 mg/kg) treated rats there was significant attenuation and decrease in levels of brain GABA in PTZ treated rats (37.09 ± 1.11 ng/gm, $P < 0.01$) when compared to vehicle treated mice.

Mice administered with *Boswellia serrata* (10 and 200 mg/kg) did not produce any significant alteration in brain GABA level in INH (300 mg/kg, i.p.) adminstered mice when compared to vehicle treated mice. When compared to vehicle control mice, diazepam (50 mg/kg) and phenytoin (200 mg/kg) administered mice demonstrated significant elevation in GABA levels of brain GABA in PTZ and INH treated mice (49.78 ± 1.45and 44.05 ±1.65 ng/gm respectively, $P < 0.001$). (Fig. 10)

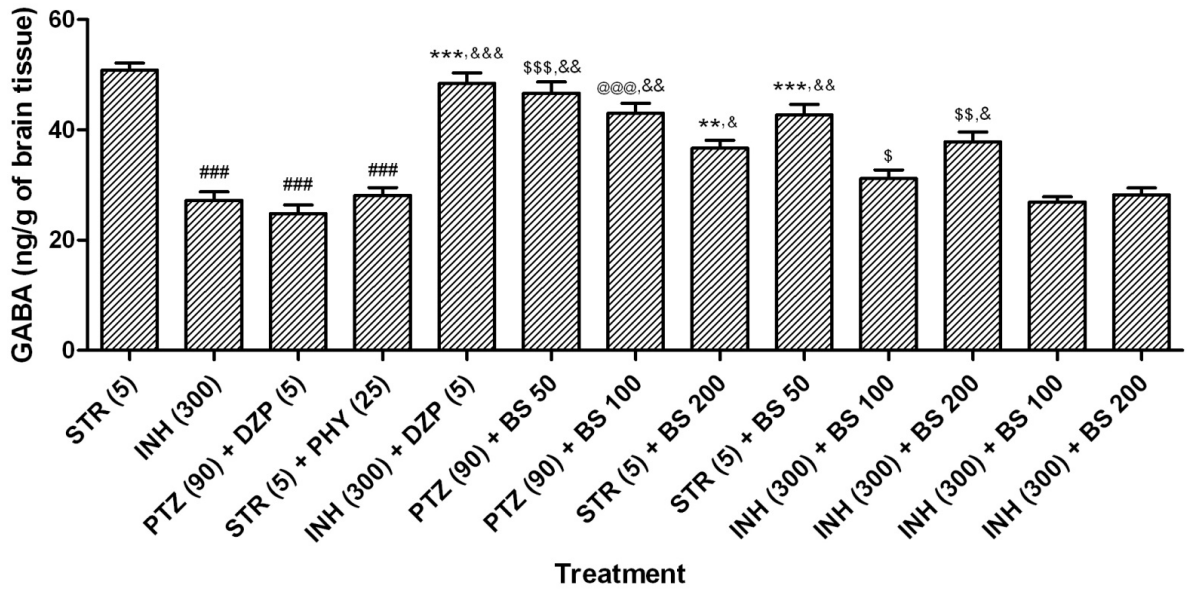

Fig 10

All data analysed using one way ANOVA followed by Tukey's multiple range test. *p<0.05,**p<0.01,***p<0.001 when all the experimental groups were compared with

vehicle control., $p<0.05,$$p<0.01,$$$ when all the drug treated groups were compared to one another p<0.001,### p<0.001 when vehicle control compared with healthy control group compared to normal group.

3.5 Effects of Boswellia serrata , diazepam and phenytoin on PTZ, STR and INH induced alteration in brain nitric oxide level:

Levels of brain NO in PTZ (90 mg/kg, i.p.), STR (50 mg/kg, i.p.) and INH (300 mg/kg, i.p.) treated mice (0.17 ± 0.0019, 0.31 ± 0.015 and 0.19 ± 0.009 µmole/g respectively) was significantly elevated (P < 0.001) when compared to normal mice (0.14 ± 0.02 µmole/g). Treatment with *Boswellia serrata* (200 mg/kg) significantly attenuated this elevated level of brain NO in PTZ and STR treated rats (0.17 ± 0.014 and 0.12 ± 0.006 µmole/g, P < 0.01 and P < 0.001 respectively) when compared to vehicle control mice.

Mice administered with *Boswellia serrata* (100 mg/kg) significantly reduced brain NO level (0.160 ± 0.001 µmole/g, P < 0.01) in STR adminstered mice, but in PTZ treated mice it could not do so when compared to vehicle treated mice. When compared to vehicle treated mice, mice administered with *Boswellia serrata* (10 and 200 mg/kg) did not produce any significant decrease in brain NO level in INH (300 mg/kg, i.p.) treated mice as compared to vehicle control mice. Mice treated with diazepam (50 mg/kg) and phenytoin (200 mg/kg) significantly diminished the elevated level of NO in brain in PTZ, INH and STR administered mice (0.12 ± 0.003, 0.12± 0.003 and 0.12 ± 0.04 µmole/g respectively, P < 0.001). (Fig. 11)

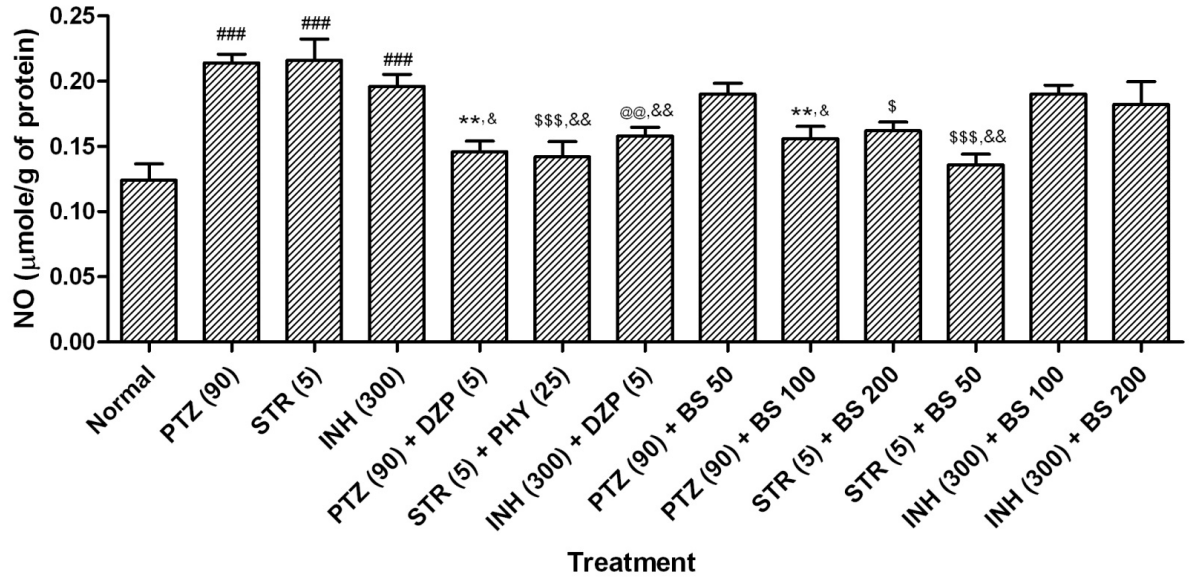

Fig 11

All data analysed using one way ANOVA followed by Tukey's multiple range test. *p<0.05,**p<0.01,***p<0.001 when all the experimental groups were compared with vehicle control., $p<0.05,$$p<0.01,$$$ when all the drug treated groups were compared to one another p<0.001,### p<0.001 when vehicle control compared with healthy control group compared to normal group.

3.6 Effects of Boswellia serrata , diazepam and phenytoin on PTZ, STR and INH induced changes in brain xanthine oxidase level:

Mice administered with PTZ (90 mg/kg, i.p.), STR (50 mg/kg, i.p.) and INH (300 mg/kg, i.p.) results significant increase (7.07 ± 0.21, 6.02 ± 0.29 and 6.07 ± 0.91 U/g respectively, $P < 0.001$) in brain XO level when compared to normal mice (3.17 ± 0.59 U/g).

Treatment with *Boswellia serrata* (200 mg/kg) led to significant reduction of brain XO levels (4.03 ± 0.34 and 4.34 ± 0.42 U/g, $P < 0.01$ and $P < 0.05$ resp.) when compared with PTZ and STR control group mice.

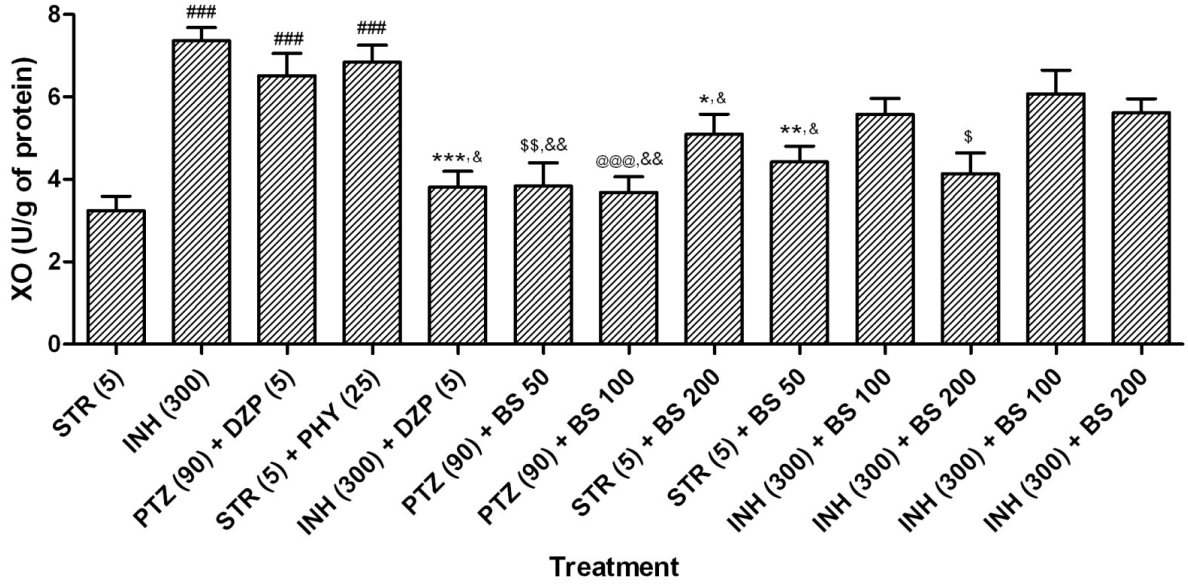

Fig 12

All data analysed using one way ANOVA followed by Tukey's multiple range test. *p<0.05,**p<0.01,***p<0.001 when all the experimental groups were compared with vehicle control., $p<0.05,$$p<0.01,$$$ when all the drug treated groups were compared to one another p<0.001,### p<0.001 when vehicle control compared with healthy control group compared to normal group.

In the PTZ administered mice increased levels of XO in brain was significantly reduced by pretreatment of *Boswellia serrata* (100 mg/kg) (5.44 ± 0.47 U/g, $P < 0.05$) when compared with vehicle treated animals whereas in STR administered mice it did not produce any significant effects in levels of XO in brain.

When compared with vehicle treated mice, treatment with diazepam (50 mg/kg) and phenytoin (200 mg/kg) significantly reduced elevated levels of brain XO in PTZ, INH and STR treated mice (3.64 ± 0.34, 3.23 ± 0.47 and 3.72 ± 0.41 U/g respectively, $P < 0.001$). (Fig. 12)

DISCUSSION

Epilepsy is a CNS disorder depicted by tumours, neurodegenerative aberrations a other related cerebro vascular pathologies. The imbalance of excitatory and inhibitory neurotransmission in the CNS is an indomitable standard of experimental and clinical seizures and convulsions in clinical and preclinical pharmacology [21].

Depolarization of the nerve cell caused due to excitatory postsynaptic potentials (EPSP) which overrules the inhibitory (IPSP) in the brain often leads to epilepsy. Epilepsy is caused due to an array of factors spanning across elevated electrolytes (Na^+, K^+, Ca^{2+}) levels, excitatory amino acids (glutamic acid), and inhibitory amino acids (GABA), and insufficient afferent connections from subcortical brain parts which modulate biochemical routes which lead to discharges of a huge cogregation of neurons and ultimately epileptic convulsions [26].

PTZ induced convulsion in mice has been well studied and often used to screen herbal drugs as it mirrors an variety of features of human epileptiform convulsions [28]. In PTZ induced seizures model, PTZ produces seizures by its derogatory effect on GABA - mediated Cl^- influx [29].

Is well studied in the scientific literature that diazepam potentiates GABAergic neurotransmission and hence protects convulsions induced by PTZ [30].

Boswellia serrata protects against the PTZ induced convulsions providing an innuendo into the anticonvulsant activity by stimulation of GABAergic pathways in brain.

In the present study, it was found that *Boswellia serrata* helps surging of the levels of GABA in brain of mice which directs us to believe that its anticonvulsant activity is via GABA level surge in brain.

STR is competitive antagonist of glycine which acts by blocking the inhibitory effect to lead to convulsions [31]. *Boswellia serrata* and phenytoin exert their protective effects through glycinergic mediated pathway.

INH induces epileptiform convulsions by damaging GABA synthesis and stimulation of glutamic acid decarboxylase (GAD) enzyme mediated action, leading to diminished levels of GABA in the brain tissues [32].

Diazepam causes significant ameliorative effects in INH induced convulsive behavior in mice but *Boswellia serrata* could not protect against INH-induced seizures and death proving its mild potency on GABA synthesis.

It has been experimentally studied that oxidative stress is an pivotal player in central nervous system in various preclinical models of epilepsy in laboratory animals [3, 35]. Xanthine oxidase bears an important role in formation of ROS [37] which lead to an array of reactions harmful to neuronal tissue.

Hence, antioxidants dimish the superoxide anion, hydroxyl and peroxyl radicals and protect the neuronal tissues against nerve damage. *Boswellia serrata* is a proven antioxidant [7]. The mice pretreated with *Boswellia serrata* exhibited downregulation of activity of this xanthine oxidase enzyme, showing that *Boswellia serrata* has the ability to arm the cellular components against seizures exerted by oxidative stress.

Nitric oxide is an intercellular molecule which is produced endogeneously which may have both inhibitory and elevator activity of epilepsy and is involved in a majority of cell signaling pathways.

The present investigation shows that the epileptic seizures are modulated by *Boswellia serrata* via NO dependent pathway.

STR leads to reflex tonic clonic types of convulsions [43]. Hence, the protective action of *Boswellia serrata* against STR induced convulsions gives a conclusive evidence to the theory that it may be of value in treatment of various types of convulsions.

Phytochemical studies have shown the presence of many valuable compounds such as lignans, flavonoids, hydrolysable tannins (ellagitannins), polyphenols, triterpenes, sterols and alkaloids. The extracts and the compounds isolated from P. amarus show a wide spectrum of pharmacological activities including antiviral, antibacterial, antiplasmodial, anti-inflammatory, antimalarial, antimicrobial, anticancer, antidiabetic, hypolipidemic, antioxidant, hepatoprotective nephroprotective and diurectic properties.

CONCLUSION

It could be concluded from the present investigation that *Boswellia serrata* protects endogenous enzyme level, inhibits oxidative damage and modulates GABAergic transmission to exhibit anticonvulsant effect. Further studies are needed to unravel its mechanism of action.

Disclosure of interest:
There is no conflict of interest between any of the authors.

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REFERENCES

[1] Jin HG, Sun XY, Chai KY, Piaob HR, Quan ZS. *Bioorg Med Chem* **2006**;14:6868-73.
[2] De Sousa DP, Goncalves JCR, Junior LQ, Cruz JS, Araujo DAM, De Almeida RN. *Neurosci Lett* **2006**;40:231-35.
[3] Kaneko K, Itoh K, Berliner LJ, Miyasaka K, Fujii H. *Magn Reson Med* **2002**;48:1051-6.
[4] Liang LP, Patel M. *Free Radical Biol Med* **2004**;36:542-4.
[5] Samren EB, Van Duijn CM, Koch S, Hiilesmaa VK, Klepel H, Bardy AH, et al. *Epilepsia* **1997**;38:981-90.
[6] Akaishi T, Morimoto T, Shibao M, Watanabe S, Sakai-Kato K, Utsunomiya-Tate N, Abe K. *Neurosci Lett* **2008**;444:280-5.
[7] Ke Y, Li Z, Yi Dong Z, PanPan Y, Ning Z. *Mole Vision* **2008**;14:1865-71.
[8] Sriram G, Subramanian S. *Inter J Pharma Sci Review Res* **2011**;6(1):68-74.
[9] Suh Y, Afaq F, Khan N, Johnson JJ, Khusro FH, Mukhtar H. *Carcinogenesis* **2010**;31(8):1424-33.
[10] Tordera M, Ferrandiz ML, Alcaraz MJ. *Z Naturforsch* [C] **1994**; 22(3):409-14.
[11] Laughton MJ, Evans PJ, Moroney MA, Hault JR, Halliwell B. *Biochem Pharmacol* **1991**;42:1673-81.
[12] Eaton EA, Walle UK, Lewis AJ, Hudson T, Wilson AA, Walle T, *Drug Metab Dispos* **1996**;24(2):232-7.
[13] Kuppusamy UR, Das NP. *Biochem Pharmacol* **1994**;47:521-9.
[14] Jimenez M, Cebrian JE, Carmona FG. *Biochimica et Biophysica Acta* **1998**;1425:534-42.
[15] Mazzio EA, Close F, Soliman KFA. *Int J Mol Sci* **2011**;12:506-569.
[16] Maher P, Akaishi T, Abe K. *PNAS* **2006**;103(44):16568-73.
[17] Nisar M, Khan I, Simjee SU, Gilani AH, Obaidullah, Perveen H.. *J Ethnopharmacol* **2008**;116:490-4.
[18] Quintans-Junior LJ, Souza TT, Leite BS, Lessa NMN, Bonjardim LR, Santos MRV, et al. *Phytomedicine* **2008**;15:619-24.
[19] Vogel HG, Vogel WH, editors. Drug discovery and evaluation: pharmacological assays. 2nd ed New York: Springer; **2002,**:421-4.
[20] Bum EN, Schmutz M, Meyer C, Rakotonirina A, Bopelet M, Portet C, et al. *J Ethnopharmacol* **2001**;76:145-50.
[21] McNamara JO. *J Neurosci* **1994**;14:3413-25.
[22] Maynert EW, Klingman GI, Kaji HK. *J Pharmacol Exp Ther* **1962**;135:296-9.
[23] Lowry OH, Rosenbrough NJ, Farr AC, Randell RJ. *J Bio Chem* **1951**;193:265-75.
[24] Miranda K, Espy MG, Wink DA. A rapid and simple spectrophotometric method for simultaneous detection of nitrate and nitrite. Nitric Oxide **2001**;5:62-71.
[25] Prajda N, Weber G. *FEBS Lett* **1975**;59:245-9.

[26] Rohkamm R. Epilepsy: seizures and types. In: Colour Atlas of Neurology, Malestrom. Thieme Flexi book. 2nd ed Stuttgart, Germany: Georg Thieme Verlag; **2004**,:192–205.
[27] McNamara JO. Pharmacotherapy of the epilepsies. In: Goodman, Gilman's (Eds.), 'The Pharmacological Basis of Therapeutics', eleventh ed. McGraw-Hill Medical Publishing Division, New York, London, Toronto. **2006,**:501–525.
[28] Olsen RW, Avoli M. *Epilepsia* **1997**;38:399-407.
[29] Corda MG, Giorgi O, Longoni B, Orlandi M, Biggio G *J Neurochem* **1990**;55:1221-61.
[30] Rang HP, Dale MM, Ritter JM, Moore PK. Pharmacology, fifth ed. Edinburgh, Churchill Livingstone, **2003**,:550–560.
[31] Parmar NS, ShivPrakash. Screening Methods in Pharmacology. Narosa Publishing House, New Delhi. **2006**,:90-91.
[32] Vergnes M, Boehrer A, Reibel S, Simler A, Marescaux C. *Exp Neurol* **2000**;161:714-23.
[33] Holmes GL, Zhao Q. *J Pediatr Neurol* **2007**;38(3):151-62.
[34] Loscher W, Schmidt D. *Epilepsy Res* **1988**;2:145-81.
[35] Kudin AP, Kudina TA, Seyfried J, Vielhaber S, Beck H, Elger CE, et al. *Eur J Neurosci* **2002**;15:1105-14.
[36] Arnaiz SL, Travacio M, Llesuy S, Arnaiz G. *Neurochem Res* **1998**;23:1477-83.
[37] Fadillioglu E, Yilmaz HR, Erdogan H, Sogut S. *Toxicol* **2003**;191:153-8.
[38] Dawson TM, Snyder SH. *J Neurosci* **1994**;14:5147-59.
[39] Lallement G, Shih TM, Pernot Marino I, Baubichon D, Foquin A, McDonough JH. *Pharmacol Biochem Behav* **1996**;54,731-7.
[40] Przegalinski E, Baran L, Siwanowicz J. *Neurosci Lett* **1996**;217,145-8.
[41] Giorgi O, Orlandi M, Geic M, Corda MG. *Eur J Pharmacol* **1991**;193:363-5.
[42] Kupferberg HJ, Schmutz M. Screening of new compounds and the role of the pharmaceutical industry. In: Engel J, Pedley TA (Eds.), Epilepsy: A Comprehensive Textbook. Lippincott- Raven, Philadelphia, PA. **1998**,:42-47
[43] Tripathi KD. Essential of medicinal Pharmacology, 5th Edn, Jaypee brothers medical publication (P) Ltd. New Dehli. **2005**,:371-373