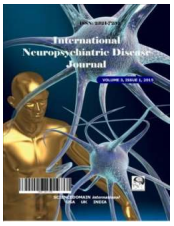

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Bromocriptine Mesylate Protects against Status Epilepticus and Temporal Lobe Epilepsy: Neurobehavioral, Histopathological and Neurochemical Evidences

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Authors' contributions

This work was carried out in collaboration between all authors. Authors FHP and NT designed the study and wrote the protocol. Author FHP carried out main experimental work, statistical analysis, managed the literature review and prepared the first draft of the manuscript with assistance from author NT. Author MMD helped in carrying out histopathological studies and interpretation of the slides. All authors have thoroughly read and approved the final manuscript submitted for publication.

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ABSTRACT

Background: Dopamine (DA) plays a major role in the control of epileptic seizures arising in the limbic system, through D1/D2 receptor-mediated signaling.
Objective: The study was intended to investigate whether bromocriptine mesylate (BRC), a dopamine (D2) receptor agonist, has an anti-seizure property, attenuates oxidative stress and neuronal morphological alterations against pilocarpine induced status epilepticus (SE) in mice, using sodium valproate (SVP) as comparator AED.
Materials and Methods: SVP (300 mg/kg) and BRC (6 &10 mg/kg) were administered orally once daily for a period of 4 weeks followed by pilocarpine (300 mg/kg, i.p) to induce SE. The convulsions

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were monitored for 2 hrs post pilocarpine injection. At 2 hrs post pilocarpine injection hippocampal malondialdehyde, glutathione reduced, glutathione peroxidase, glutathione reductase and catalase activities were measured. While hippocampal histopathological assessment of neuronal injury was performed 24 hrs post pilocarpine injection.

Results: We report that SVP (300 mg/kg) and BRC (6 &10 mg/kg) were able to reduce seizure severity score ($P<0.001$, $P<0.01$, $P<0.05$), attenuate oxidative damage in lipids and proteins, restore depletion in hippocampal glutathione reduced, glutathione peroxidase and glutathione reductase activities compared to toxic/pilocarpine control. Contrary catalase levels were strengthened as like pilocarpine control. In addition SVP (300 mg/kg) and BRC (6 & 10 mg/kg) protected against degeneration in hippocampal CA1, CA2, CA3 & DG regions ($P<0.001$, $P<0.01$, $P<0.05$) compared to pilocarpine control.

Conclusions: The present study highlights the prominent dysregulation of dopaminergic system in seizures and perspectives for the development of novel therapeutic approaches acompassing dopaminergic drugs in amelioration of epileptic seizures especially status-epilepticus and temporal lobe epilepsy.

Keywords: Bromocriptine mesylate; status-epilepticus; temporal lobe epilepsy; oxidative stress; neurodegeneration.

1. INTRODUCTION

Epilepsy is a chronic neurological disorder characterized by reorganization of neuronal architecture to acquire innate ability of generating recurrent seizures. The current antiepileptic drugs act either to modulate sodium and calcium channels or levels of glutamate and GABA resulting only in symptomatic relief while the underlying pathology remains unabated and 20-30% of patients remain refractory to treatment [1]. The unmet need for new anti-epileptic screening immensely lies on choice of appropriate animal models for the initial in vivo testing in rodents. The animal models of temporal lobe epilepsy, involve experimental procedures in which the epileptic condition results as a downstream consequence of brain damage induced by status epilepticus (SE). The SE is induced by: administration of a chemical convulsant, such as pilocarpine or kainic acid, or electrical stimulation [2]. Recent developments in the field have high lightened the role of dopaminergic neurotransmission, oxidative stress and neuroprotection in modulation of epileptic seizures. The effect of dopamine on epileptic seizures depends upon the activation of specific dopamine receptor subtypes [3]. D1 and D2 like receptors play opposite effects on epilepsy i.e stimulation of former results in increased seizure severity and reduction of seizure threshold while stimulation of later exhibits anti-convulsant properties [4,5,3]. In addition blockade of D2 like receptors has proconvulsant effects, while absence of D2R lowers the threshold for seizures induced by both glutamate and acetylcholine [6]. The dopamine (D2) receptor agonist, bromocriptine is reported to possesses anti-epileptic effects in case of self-induced, drug-resistant epilepsy [7], it significantly lowered the spike frequencies in both primary and secondary epileptic foci following implantation of cobalt in conscious rats as recorded by electrocorticogram [8].

The alternative target for anti-epileptic drug development lies with controlling the state of oxidative stress i.e imbalance between generation and disposal of ROS and RNS in epileptic brain given that brain (hippocampus) is particularly vulnerable to oxidative stress because of high levels of polyunsaturated fatty acids and lower antioxidant defense systems [9,10]. During seizures the excess of glutamate released stimulates NMDA receptors resulting in increased neuronal cytosolic Ca^{2+} concentration, overload of mitochondria with Ca^{2+}, generation of free radicals culminating in neuronal degeneration, neuronal damage, and eventually neuronal death mediated via Ca^{2+} triggered opening of mitochondrial permeability transition (MPT) pores associated with apoptosis [11]. Yet another putative target for preventing epileptic seizures is to prevent neurodegeneration following seizures which critically alters neuronal and network functions resulting in clinical onset of pharmacoresistant epileptic seizures sometimes accompanied by cognitive decline [12,13]. The neuroprotective potential of AEDs like phenytoin, phenobarbital, and carbamazepine has been determined in ischemic/hypoxic model of neuronal injury while the extrapolation of this data in epileptic conditions has been questioned [14,15]. Thus mitigating the neurodegenerative processes following epileptic seizures aim not only to

improve seizure control, but also to promote compensatory processes in repairing, modifying or blocking the active changes triggered by the initial injury. Based on the above background the central focus of our research was to raise threshold to epileptic seizures either by modulating brain dopaminergic transmission or through possible antioxidant and neuroprotective activity of bromocriptine mesylate.

2. EXPERIMENTAL PROCEDURES

2.1 Experimental Animals

This study utilized swiss albino mice (male and females, 10 –12 weeks old, 25-35 g) obtained from central animal house facility IIM Jammu, J&K, India. Animals were housed in polypropylene cages with dust free rice husk as a bedding material. The animals were provided with a commercial diet and water *ad libitum* under controlled temperature, humidity and lighting conditions (25±2℃, 60±10% and a 12:12 light/dark cycle with lights). Procedures involving animals and their care were conducted as per the approved guidelines of Institutional Animal Ethical Committee, PG Department of Pharmaceutical Sciences, University of Kashmir, Srinagar, JK, India [Under approval no: F-IAEC(Pharm. Sc.) APPROVAL/2013/15]. Utmost care was taken to ensure that animals were treated in the most humane and ethically acceptable manner.

2.2 Drugs

The drugs used were sodium valproate (Sun Pharmaceuticals Ltd., India), bromocriptine mesylate (Monarch Pharmaceuticals, India). All drugs were freshly suspended in 2% Tween 80 prior to their administration via oral route. Pilocarpine hydrochloride (Himedia, India) and diazepam (Sahil, India) were freshly prepared in 0.9% saline for i.p injection in a volume not exceeding 10 ml/kg.

Sodium valproate (300 mg/kg) reportedly produced statistically significant seizure protection against PTZ induced seizures in mice [16] while bromocriptine mesylate (10 mg/kg) reportedly protected against MPTP - induced neurotoxicity in mice [17].

2.3 Dosing Schedule and Experimental Design

All drugs were administered orally for a period of 4 weeks prior to their evaluation against pilocarpine induced SE. Fifty swiss albino mice were randomly selected and divided into five experimental groups with ten mice in each group. The treatment schedule followed was; **Group 1:** Normal Control (Vehicle), **Group 2:** Toxic Control/Pilocarpine Control (300 mg/kg), **Group 3:** Sodium Valproate (300 mg/kg) + Pilocarpine, **Group 4:** Bromocriptine mesylate (6 mg/kg) + Pilocarpine, **Group 5:** Bromocriptine mesylate (10 mg/kg) + Pilocarpine.

2.4 Pilocarpine Induced SE

Animals were given pilocarpine (300 mg/kg; i.p) for induction of status epilepticus [18]. This dose was also standardized in our laboratory to induce SE in 100% of normal animals tested. Diazepam (10 mg/kg, i.p) was used to reduce pilocarpine induced mortality [19]. The animals were then placed in plexiglas chambers for behavioral monitoring of seizure progression for 120 min. The animals that exhibited SE did so within first 15-30 min of pilocarpine injection and SE recurred several times in them. The continuous seizure activity was recorded as per slight modifications from racine classification, 1972 into stages 6 stages as **Stage 1:** immobilization and staring; **Stage 2:** head nodding; **Stage 3:** rearing accompanied by forelimb clonus and wet dog shakes; **Stage 4:** falling and wobbling; **Stage 5:** jumping, circling, or rolling; **Stage 6:** severe tonic-clonic seizures. Seizure grades 1–4 were regarded as low-grade or mild seizures and 5–6 as high-grade or severe seizures. A score was recorded when the length of a particular stage sustained for > 4 s [20].

2.5 Biochemical Studies

2.5.1 Tissue preparation for biochemical estimations

After behavioral monitoring of seizures (i.e 2 hrs post pilocarpine injection), six mice (3M + 3F) from all groups (I-V), were decapitated under light anesthesia using ketamine (Laborate Pharmaceuticals, India), their brains were dissected on ice, hippocampus was isolated, stored at -80℃ and within next 12 hours processed for determination of some nonenzymatic and enzymatic oxidative stress indices. For antioxidant estimations the hippocampus was homogenized (10% w/v) in 0.01 M phosphate buffer (PH 7.0) and centrifuged at 10,500 rpm for 20 min at 4℃ to obtain post mitochondrial supernatant (PMS). Hippocampal PMS was used for estimation of

protein levels, TBARS, glutathione reduced, glutathione Peroxidase, glutathione reductase and catalase levels.

2.5.2 Assay for protein estimation

The method of Lowry et al. [21] was followed for estimation of protein in the hippocampus of mouse brain. The method is based on reaction of phenolic group of tyrosine and trytophan residues (amino acid) in a protein with folin-ciocalteau reagent, to produce a blue purple color complex, with maximum absorption in the region of 660 nm wavelength. The intensity of color developed varies with the amount of protein present in different samples. Hence amount of protein present in different samples down to 10 µg/ml is determined. The reaction is PH sensitive and a desirable range of pH 9 to 10.5 is essential.

2.5.3 Assay for thiobarbituric acid reactive substances, a marker of lipid peroxidation

The method of Ohkawa et al. [22] was followed for assay of lipid peroxidation in hippocampus of mouse brain. Briefly, 1 ml of PMS (10% w/v) was pipetted off in a fresh autoclaved calibrated glass tube, followed by addition of 1.0 ml of TCA (10%) and 1.0 ml of TBA (0.67%). The tubes were covered with aluminum foil and incubated in boiling water for 45 min. After cooling the tubes were centrifuged at 2500 rpm for 10 min. The absorbance of supernatant was read spectrophotmetrically at 525 nm against appropriate blank. Rate of LPO was estimated by measuring MDA content as nmol MDA formed/gm wet tissue at 37℃ by using a molar extinction coefficient of $1.56 \times 10^5\ M^{-1}\ cm^{-1}$.

2.5.4 Assay for reduced glutathione content

The method of Jollow et al. [23] was followed for assay of reduced glutathione (GSH) in hippocampus of mouse brain. Briefly, 1 ml of PMS (10% w/v) was pipetted off in a fresh autoclaved calibrated glass tube and mixed with 1.0 ml of sulphosalicylic acid (4%). The samples were incubated at 4℃ for an hour and then centrifuged at 1200 rpm for 15 min at 4℃. Then to 0.4 ml of supernatant was added 2.2 ml of phosphate buffer (0.1 M, PH 7.4) and 0.4 ml of DTNB (4 mg/1 ml). The yellow color developed was read spectrophotmetrically at 412 nm against appropriate blank. The GSH concentration was calculated as nmol DTNB conjugate formed/ mg of protein.

2.5.5 Assay for glutathione peroxidase activity

The method of Mohandas et al., (1984) [24] was followed for assay of glutathione peroxidase (GPx) activity in hippocampus of mouse brain. Briefly, 0.1 ml of PMS (10% w/v), 1.44 ml of phosphate buffer (0.1 M, PH 7.4), 0.1 ml EDTA (1.0 mM), 0.1 ml of sodium azide (1.0 mM), 0.05 ml of GR (1 eu/ml), 0.05 ml of GSH (1.0 mM), 0.1 ml of NADPH (0.2 mM) and 0.01 ml of H_20_2 (0.25 mM) were pipetted off and mixed in a fresh autoclaved calibrated glass tube to make total assay mixture of 2.0 ml. Disappearance of NADPH at 340 nm was recorded spectrophotometrically. The enzyme activity was calculated as nmol NADPH oxidized/ min/mg protein using molar extinction coefficient of $6.22 \times 10^3\ M^{-1}\ cm^{-1}$.

2.5.6 Assay for glutathione reductase activity

The method of Carlberg and Mannervik ,1975 [25] was followed for assay of glutathione reductase (GR) activity in hippocampus of mouse brain. Briefly, 0.1 ml of PMS (10% w/v), 1.65 ml of phosphate buffer (0.1 M, PH 7.6), 0.1 ml EDTA (0.5 mM), 0.05 ml oxidized glutathione (1.0 mM) and 0.1 ml of NADPH (0.1 mM) were pipetted off and mixed in a fresh autoclaved calibrated glass tube to make total assay mixture of 2.0 ml. The enzyme activity was quantitated at 25℃ by measuring the disappearance of NADPH at 340 nm and was calculated as nmol NADPH oxidized/ min/ mg of protein, using molar extinction coefficient of $6.22 \times 10^3\ M^{-1}cm^{-1}$

2.5.7 Assay for catalase activity

The method of Maehly (1954) [26] was followed for assay of catalase (CAT) activity in hippocampus of mouse brain. Briefly, 0.05 ml of PMS (10% w/v), 1.95 ml of phosphate buffer (0.1 M, PH 7.4),1.0 ml of H_2O_2 (0.019 M) were pipetted off and mixed in a fresh autoclaved calibrated glass tube to make total assay mixture of 3.0 ml. Changes in absorbance were recorded spectrophotometrically at 240 nm. Catalase activity was calculated as nmol H_2O_2 consumed/min/ mg of protein.

2.6 Histopathological Studies and Quantification of Neuronal Damage

Quantification of hippocampal cell densities was performed at 24 hrs post pilocarpine injection on remaining four mice in all groups (I-V). Animals were lightly anesthetized with ketamine (10

mg/kg, i.p) then decapitated for removal of brains, washed of blood in normal saline and immediately preserved in 10% buffered formalin solution. Thereafter, tissues were sliced, routinely processed, and embedded in paraffin wax. The series of 8-µm thick coronal sections were cut every 100-µm from 2.3 to 4.3 mm posterior to the bregma with the help of microtome and processed for hematoxylin eosin staining. Slides were examined under light microscope and digital photographs were taken by a pathologist unaware of treatment regimen from hippocampal CA1, CA2, CA3 and DG areas of both hemispheres. Healthy neurons were easily distinguished from injured neurons by their characteristic large size, medium-intensity staining, and dark nucleoli. After seizures pyramidal cells often were shrunken, pyknotic or some had completely disappeared. The quantitative analysis of neurons was performed with a 1-cm^2 10 × 10-box microscopic grid. The grid of counting was placed on a well defined area of hippocampus understudy i.e CA1, CA2, CA3 and DG, and counting was carried out with a microscopic enlargement of 200- or 400- fold defined for each single hippocampal subfield. Neurons touching the inferior and right edges of the grid were not counted. The amount of damage in all hippocampal subfields were scored with slight modifications from Borges et al., (2003) for each mouse on 0-10 scale, where increments in score was attributed to reciprocal relationship between healthy hippocampal pyramidal neurons and pyknotic neurons by an amount of 10%. The attribution of scores followed was: **Score 0:** Healthy neurons ≤ 100%, Pyknotic neurons ≤ 1%; **Score 1:** Healthy neurons ≤ 90%, Pyknotic neurons ≥ 10%; **Score 2:** Healthy neurons ≤ 80%, Pyknotic neurons ≥ 20%; **Score 3:** Healthy neurons ≤ 70%, Pyknotic neurons ≥ 30%; **Score 4:** Healthy neurons ≤ 60%, Pyknotic neurons ≥ 40%; **Score 5:** Healthy neurons ≤ 50%, Pyknotic neurons ≥ 50%; **Score 6:** Healthy neurons ≤ 40%, Pyknotic neurons ≥ 60%; **Score 7:** Healthy neurons ≤ 30%, Pyknotic neurons ≥ 70%; **Score 8:** Healthy neurons ≤ 20%, Pyknotic neurons ≥ 80%; **Score 9:** Healthy neurons ≤ 10%, Pyknotic neurons ≥ 90%; **Score 10:** Healthy neurons ≤ 1%, Pyknotic neurons ≤ 100% [27].

2.7 Statistical Analysis

The seizure severity was represented as mean seizure ranks and analyzed by Kruskal Wallis ANOVA followed by post hoc Bonferroni's comparison test. Data was represented as Mean ± SEM and analyzed by one-way ANOVA followed by Tukey Kramer's test for all biochemical estimations. The neuronal damage was analyzed by Fischer's exact test. $P < 0.05$ was considered as significant in all the cases. The data was analyzed using **SPSS** computer software package, version 1.6 (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA).

3. RESULTS

3.1 Effect of Bromocriptine Mesylate on Pilocarpine Induced Status-epilepticus in Mice: Comparison with Sodium Valproate

Pilocarpine administration (300 mg/kg, i.p.) induced status epilepticus (SE) in mice. Administration of SVP (300 mg/kg for 4 weeks, p.o), BRC (6 & 10 mg/kg for 4 weeks, p.o) markedly reduced the mean seizure severity rank ($P<0.001$), ($P<0.01$, $P< 0.05$) compared to pilocarpine control group. The lower dose of BRC was more protective than its corresponding higher dose (Fig. 1).

3.2 Effect of Bromocriptine Mesylate on Hippocampal Anti-oxidant Enzyme Levels Post Pilocarpine Induced Status Epilepticus in Mice: Comparison with Sodium Valproate

3.2.1 Lipid peroxidation level

The lipid peroxidation level (TBARS) in the hippocampus markedly increased in pilocarpine control group compared to normal control group ($P<0.001$). SVP (300 mg/kg for 4 weeks, p.o), BRC (6 & 10 mg/kg for 4 weeks, p.o) significantly decreased TBARS ($P<0.001$), ($P<0.01$, $P< 0.05$) compared to pilocarpine control group (Fig. 2A).

3.2.2 Glutathione reduced, glutathione peroxidase and glutathione reductase levels

A highly significant depletion of hippocampal glutathione reduced (GSH), glutathione peroxidase (GPx) and glutathione reductase (GR) was observed in pilocarpine control group compared to normal control group ($P<0.001$, $P< 0.001$, $P<0.001$). SVP (300 mg/kg for 4 weeks, p.o), BRC (6 & 10 mg/kg for 4 weeks, p.o) significantly restored the levels of GSH, GPx and GR ($P<0.001$, $P<0.01$, $P< 0.05$), ($P<0.001$, $P<0.01$, $P< 0.05$), ($P<0.001$, $P<0.01$, $P< 0.05$) (Figs. 2B, 2C, 2D).

3.2.3 Catalase

The CAT level in the hippocampus was markedly increased in pilocarpine control group compared to normal control group (P<0.001). SVP (300 mg/kg for 4 weeks, p.o), BRC (6 &10 mg/kg for 4 weeks, p.o) further increased the levels of CAT (P < 0.001), (P<0.01, P< 0.05) (Fig. 2E).

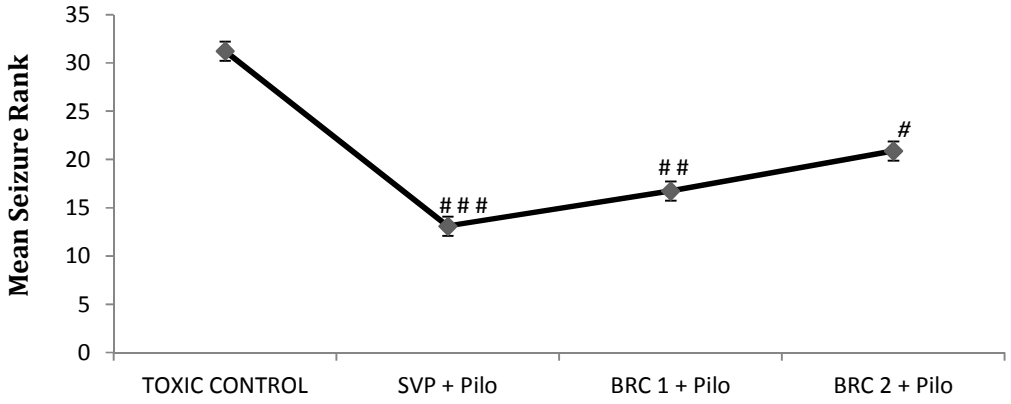
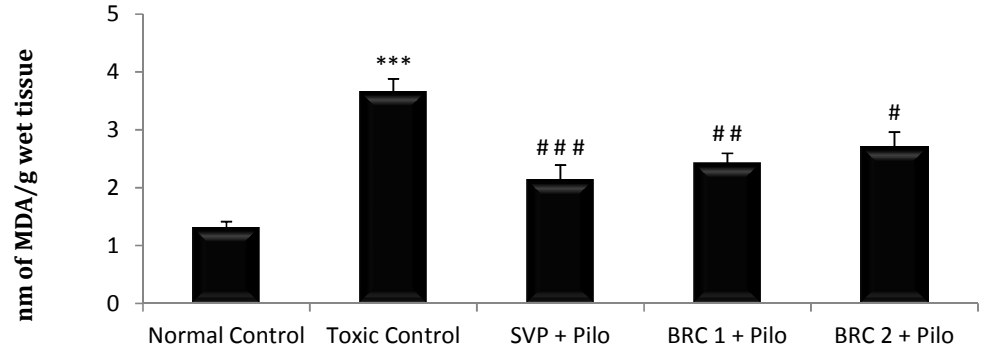


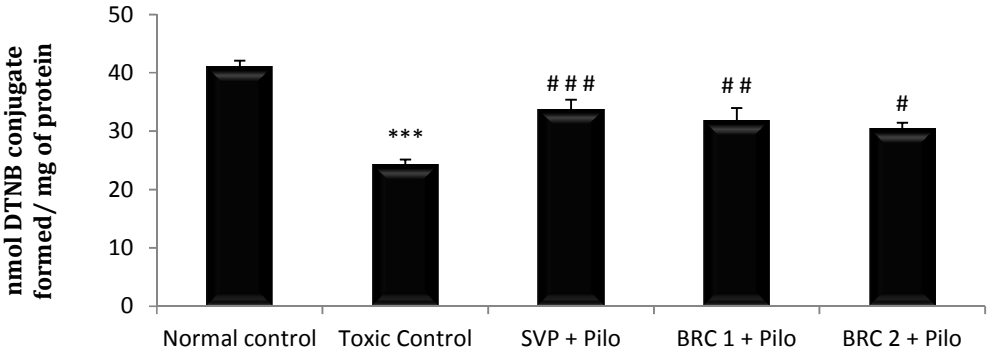
Fig. 1. Effect of sodium valproate and bromocriptine mesylate on pilocarpine induced seizure severity in mice. All drugs were administered for 4 weeks prior to pilocarpine (300 mg/kg, i.p)

Data is represented as mean seizure rank of 10 animals per group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ when compared to toxic control (significant by Kruskal Wallis ANOVA followed by Bonferronis comparison test). Pilo: pilocarpine (300 mg/kg), SVP: sodium valproate (300 mg/kg), BRC: bromocriptine mesylate, BRC 1 (6 mg/kg) and BRC 2 (10 mg/kg)

A



B



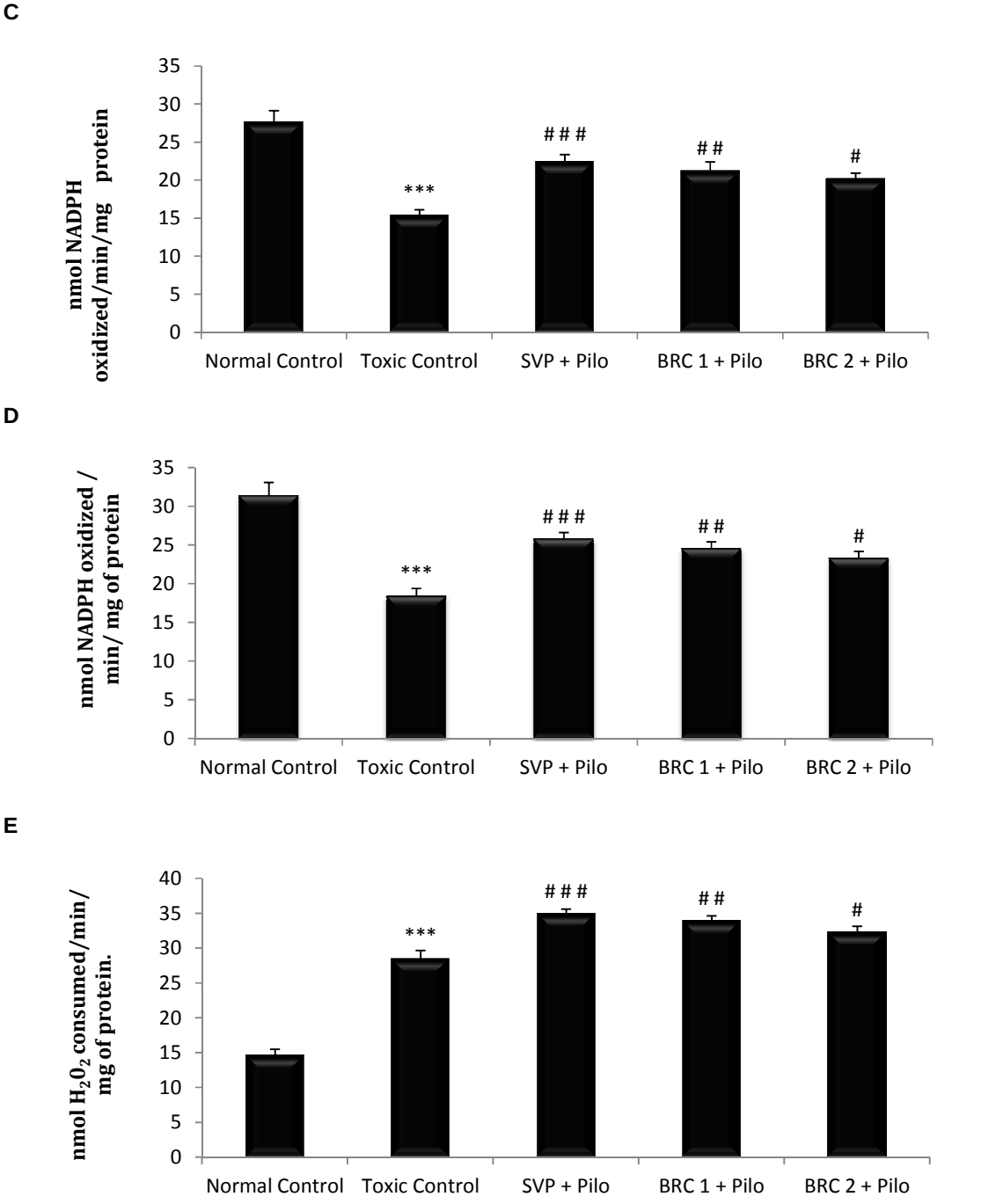


Fig. 2. Effect of sodium valproate and bromocriptine mesylate on lipid Peroxidation (A), reduced glutathione (B), glutathione peroxidase (C), glutathione reductase (D), Catalase (E) activities, 2 hours post Pilo injection. All drugs were administered for 4 weeks prior to pilocarpine (300 mg/kg, i.p)

*Data is represented as Mean ± SEM of 6 animals per group, *P < 0.05, **P < 0.01, ***P < 0.001 when compared to normal control, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ when compared toxic control (significant by one way ANOVA followed by Tukey Kramer's test). Pilo: pilocarpine (300 mg/kg), SVP: sodium valproate (300 mg/kg), BRC: bromocriptine mesylate, BRC 1 (6 mg/kg) and BRC 2 (10 mg/kg)*

3.3 Effect of Bromocriptine Mesylate on Hippocampal Neuronal Degeneration Post Pilocarpine Induced Status-epilepticus in Mice: Comparison with Sodium Valproate

Hippocampal transverse section of normal mice exhibited normal neuronal organization in hematoxylin–eosin stained hippocampal subfields and layers with no evidence of pyknosis (damage score = 0). Pilocarpine hydrochloride (300 mg/kg) induced seizures in normal mice resulted in extreme neuronal degeneration and neuronal loss in the order CA1 > CA2 > CA3 > DG (damage score = 10), sodium valproate (300 mg/kg) protected against extensive neuronal loss, deranged morphology and decreased pyknosis (damage score = 2), while bromocriptine mesylate (6 & 10 mg/kg) again reduced the number of degenerating neurons, pyknotic neurons, ectopic neurons, dark neurons, sunken neurons and resumed normal morphology (damage score = 3 & 4). But protection exhibited by bromocriptine mesylate (6 mg/kg) was more prominent than with bromocriptine mesylate (10 mg/kg), confirming results from neurobehavioral study that bromocriptine mesylate at lower dose (6 mg/kg) offers higher protection than at higher dose (10 mg/kg) against pilocarpine induced SE (Fig. 3 and Fig. 4).

4. DISCUSSION

Although great efforts have been made to prevent epileptic seizures but none has been successful in targeting the epileptogenesis i.e process by which brain becomes epileptic. Strictly speaking none of the drug(s) available in market are anti-epileptic but are only anti-seizure i.e they control seizure onset but not the underlying pathology. Evidence from various preclinical and clinical studies suggests epilepsy as a consequence of dysregulation of brain neurotransmitter systems especially dopaminergic systems, decreased brain antioxidant defense enzymes like GSH, GR, GPx, CAT e.t.c and neuronal degeneration. In the present study the prime focus has been to fill the lacuna in epilepsy treatment by stimulating hippocampal dopamine (D2) receptors, preventing against oxidative stress and protecting hippocampal CA1, CA2, CA3 and DG regions with the motive to restrict duration of SE, given that probability, latency and severity of temporal lobe epilepsy and of neuropathological changes decreases with restricted SE duration [28]. Hence if a drug abolishes the onset of SE or restricts its duration to few minutes the subsequent development of chronic epilepsy can be prevented. Bromocriptine mesylate was chosen as prototype drug from dopamine (D2) receptor agonists and evaluated against pilocarpine induced status epilepticus (SE), using sodium valproate as comparator AED in swiss albino mice, along with its possible antioxidant and neuroprotective effect. In this study, the results suggested that bromocriptine mesylate decreased severity of seizures following pilocarpine administration, reduced oxidative stress and protected against neuronal degeneration in hippocampal CA1, CA2, CA3 and DG, hallmark of status epilepticus and temporal lobe epilepsy although less so than sodium valproate.

The importance of dopaminergic regulation of neurological disorders is no longer constrained to treatment of parkinsonism, schizophrenia and psychosis, but growing evidence suggests putative role of dopaminergic system in modulation of epileptic seizures. The dopaminergic receptors are classified as D1- like (D1 and D5) and D2-like (D2, D3 and D4) which are G-protein-coupled receptors (GPCRs) [29]. Werhahn et al. [30] observed decrease in binding of D2/D3-receptor at the pole and in lateral aspects of the epileptogenic temporal lobe in patients with mesial TLE and HS as analyzed by positron emission tomography (PET) by using the high-affinity dopamine D2/D3-receptor ligand [18F] Fallypride ([18F]FP). Bozzi et al. [31] inactivated D2R gene in two mouse strains known to be previously resistant to KA induced seizures and excitotoxicity and demonstrated their increased sensitivity, susceptibility and hippocampal cell death on kainic acid administration compared to WT mice. The SVP (300 mg/kg) and BRC (6 mg/kg > 10 mg/kg) decreased seizure severity score compared to pilocarpine control group.

Oxidative stress is defined as deficit between generation and neutralization of ROS species such as hydroxyl radical ($HO^{\bullet}$), superoxide anion radical ($O_2^{\bullet}$), hydrogen peroxide (H_2O_2), peroxyl radicals ($HOO^{\bullet}$), and high amounts of nitric oxide ($NO^{\bullet}$) and its derivative reactive nitrogen species (RNS) [32] which play an important role in the generation and progression of epileptic seizures [33-35]. Anomalous levels of oxidant and antioxidant enzymes have been reported in children with refractory epilepsies [36].

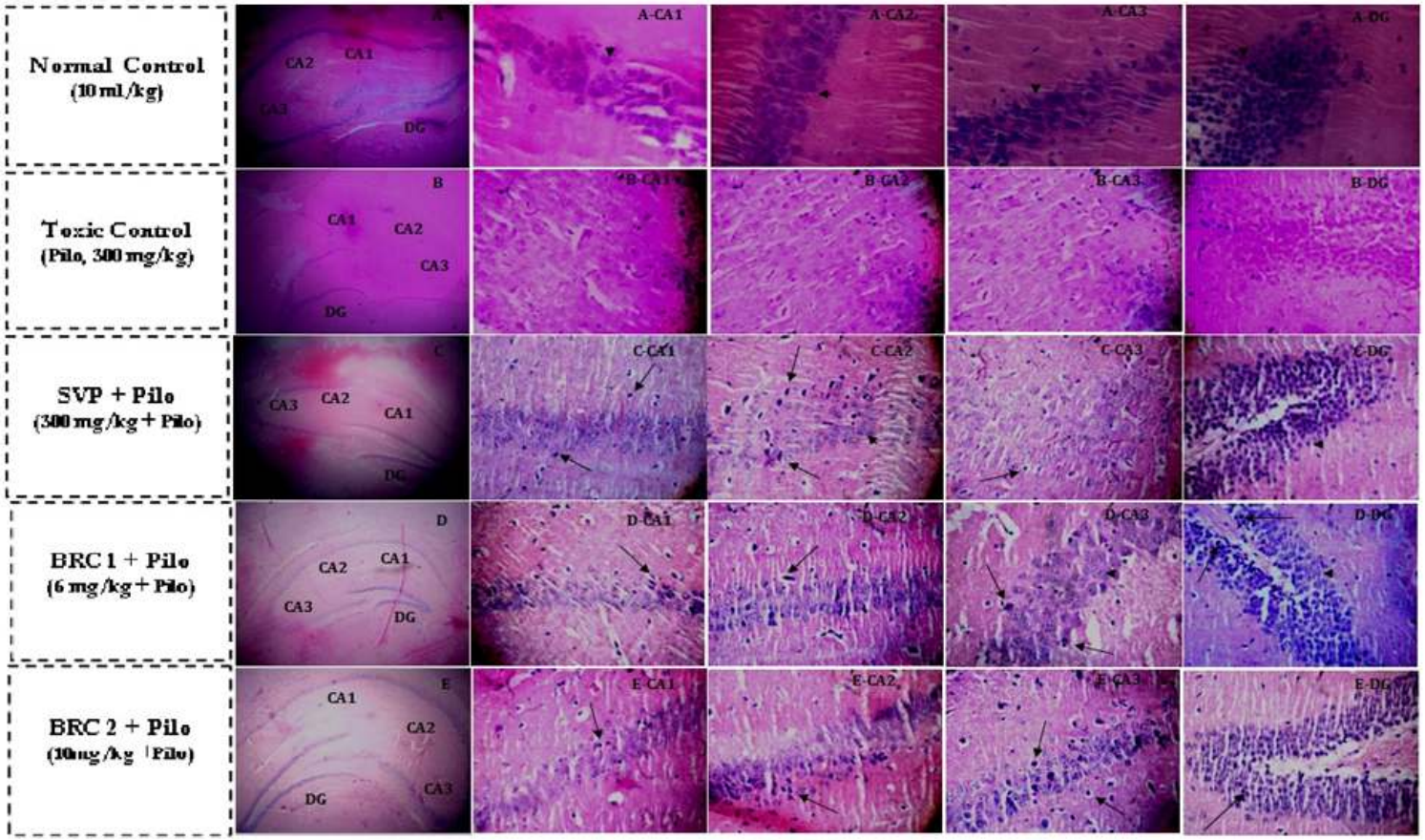


Fig. 3. Photomicrographs of mice hippocampus, 24 hours post Pilocarpine injection A, B, C. D, E represent low-power (4X)

While their respective CA1 to DG represent (40X) views of CA1, CA2, CA3 & DG subregions. (A-CA1 to A-DG): Illustrate intact morphology, healthy neurons and absence of pyknotic neurons, damage score = 0. (B-CA1 to B-DG): illustrate complete loss of neurons, damage score = 10. However increment in healthy neurons (◂) and decrement in pyknotic neurons (↑) with overall intact morphology is observed with all drug treated groups in the order (C-CA1 to C-DG, damage score = 2) > (D-CA1 to D-DG, damage score = 3) > (E-CA1 to E-DG, damage score = 4). H/E staining; Transverse sections

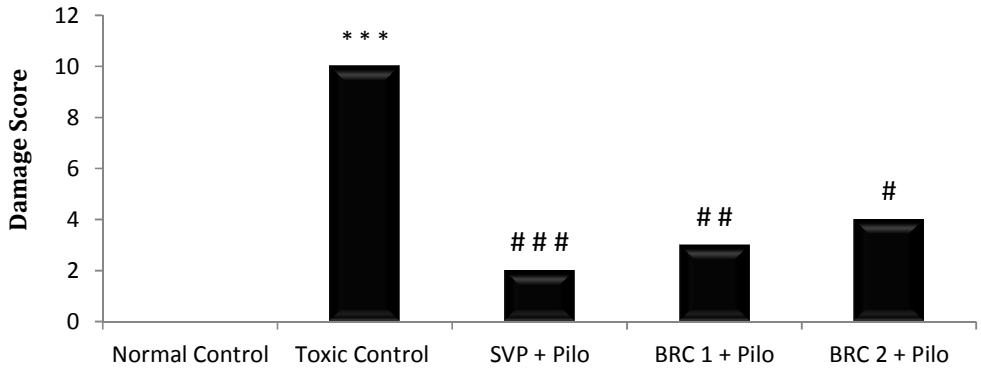


Fig. 4. Hippocampal damage score of mice hippocampus, 24 hours post Pilocarpine injection the amount of damage for each mouse hippocampal section was scored on a 0–10 scale by estimating decrease in healthy neurons over pyknotic neurons by a factor of 10% for 4 animals per group

**P<0.05, **P<0.01, ***P<0.001 when compared with normal control group. #P<0.05, ##P<0.01, ###P<0.001 when compared with Toxic control (Significant by Fischer's exact test). Pilo: pilocarpine (300 mg/kg), SVP: sodium valproate (300 mg/kg), BRC: bromocriptine mesylate, BRC 1 (6 mg/kg) and BRC 2 (10 mg/kg)*

In our study, there was an increase in lipid peroxidation in the hippocampus of mice tested against pilocarpine induced SE. Our data indicate that sodium valproate (300 mg/kg) and bromocriptine mesylate (6 mg/kg > 10 mg/kg) prevented the rise in hippocampal lipid peroxidation as revealed by decreased MDA levels against pilocarpine induced SE. The hippocampus (Brain) contains natural antioxidants (free radical scavengers) like glutathione reduced (GSH), gluathatione peroxidase (GPx), glutathione reductase (GR), catalase (CAT) e.t.c to protect against oxidative stress, a mediator of neurodegeneration and epileptic seizures. Glutathione reduced (GSH) is an endogenous intracellular nonprotein thiol antioxidant [37,38], the modification of levels of which have been shown to regulate seizure susceptibility and neuronal survival [39]. Glutathione peroxidase act against oxidative stress by reducing hydrogen peroxide and alkyl hydroperoxides at the cost of glutathione reduced and form H_2O and O_2 from H_2O_2 as like catalases [40]. Glutathione reductase is a flavoprotein found in cytoplasm which catalyzes the NADPH-dependent reduction of oxidized glutathione (GSSG) to glutathione (GSH) [41]. This enzyme is critical for the GSH redox cycle which maintains adequate levels of reduced cellular GSH. A high GSH/GSSG ratio is essential for protection against oxidative stress. Catalase is an enzyme present mainly in mitochondria and peroxisomes that catalyzes the decomposition of hydrogen peroxide to water and oxygen [42]. The levels of glutathione reduced (GSH), glutathione peroxidase (GPx) and glutathione reductase (GR) showed a decrease in pilocarpine control group while sodium valproate (300 mg/kg) and bromocriptine mesylate (6 mg/kg > 10 mg/kg) restored the levels of same. The levels of CAT was substantially increased in hippocampus of pilocarpine treated mice which is in agreement with previous studies [43,44] as it has been suggested that increase in ROS generated because of pilocarpine administration leads to increase in CAT as compensatory mechanism. The levels of CAT were further elevated by sodium valproate (300 mg/kg) and bromocriptine mesylate (6 mg/kg > 10 mg/kg).

The significant component of epileptogenesis remains rewiring of neuronal networks following an initial insult or SE caused by neuronal damage especially in the hippocampus. In addition the neurodegeneration by altering the properties of neurons impairs the anti-epileptic profile of anti-epileptic drugs [45]. Thus treatment regimens ameliorating seizure induced neuronal damage may become critical components, preventing the deteriorating process of epileptogenesis [46]. In our study bromocriptine mesylate (6 mg/kg > 10 mg/kg) was found to reduce neurodegeneration of hippocampal CA1, CA2, CA3 and DG subregions in pilocarpine treated mice although less so than sodium valproate, thus substantiating behavioral results with histomorphologic findings which is in agreement with starr's hypothesis which suggests a low dose of a D2R agonist would act through stimulation of presynaptic D2 autoreceptors leading to decreased DA release,

while preventing the downregulation of postsynaptic D2R [47].

5. CONCLUSION

The results of this study revealed that bromocriptine mesylate exerts an anticonvulsant effect against pilocarpine induced seizures in mice although less so than sodium valproate. The anti-convulsant effects may in part be mediated due to its potential to mitigate oxidative stress and neuronal damage.

The advantages of using bromocriptine mesylate over sodium valproate could be treatment of drug resistant epileptic seizures, aimed at potential of bromocriptine mesylate in restricting the process of epileptogenesis by interfering with route cause of seizure generation i.e dysregulation of dopaminergic system. Apart from this bromocriptine mesylate is nonteratogenic [48] hence can be used in cases where sodium valproate poses teratogenic threat [49], Further bromocriptine mesylate could be used as DOC for epilepsy associated with parkinsonism.

CONSENT

It is not applicable.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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