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Research Article
Comparison of Bumetanide, Levetiracetam and Sodium Valproate Effects on Convulsions and Motor Coordination in Experimental Models

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Abstract

Background and Objective: Given the high prevalence of adverse effects with current antiepileptic drugs and limited understanding of their mechanisms, this study aimed to evaluate the anticonvulsant effects, neurotoxic profiles and mechanisms of Bumetanide, Levetiracetam and Valproate (VAL, in rodent epilepsy models). **Materials and Methods:** A study with 72 mice and 24 rats assessed convulsions and motor coordination using MES and PTZ models. Brain biomarkers (GABA, Glutamate, NKCC1 and GABA transaminase) were measured by ELISA. Mice were divided into four groups for MES and Rota rod tests and rats into four groups for biomarker analysis. Drugs were administered intraperitoneally 30 min before testing. Statistical analysis was performed using GraphPad Prism® version 8. **Results:** In the MES test, BUM exhibited moderate anticonvulsant effects but was less effective than LEV and VAL. The LEV and VAL significantly reduced seizure duration, with VAL showing superior efficacy. In the PTZ model, all three drugs delayed convulsion onset, with VAL demonstrating the strongest effect. In the Rota rod test, BUM did not impair motor coordination, while both LEV and VAL caused ataxia, with VAL having the highest effect. The BUM increased GABA levels and decreased GABA transaminase and NKCC1 expression, suggesting chloride homeostasis modulation. The LEV influenced chloride homeostasis without altering GABA levels, while VAL enhanced GABA levels and inhibited GABA transaminase. **Conclusion:** The VAL was the most effective anticonvulsant, followed by LEV, with BUM showing moderate effects. The BUM had the safest neurotoxic profile. The VAL's potency was linked to GABAergic mechanisms, while LEV's effects were likely due to SV2A modulation. Bumetanide's effects are linked to chloride homeostasis regulation.

Key words: Bumetanide, levetiracetam, valproic acid, epilepsy, neurotoxicity, biomarkers

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Epilepsy is a prevalent neurological condition characterized by chronic brain illnesses causing frequent seizures, often accompanied by abnormalities or loss of consciousness[1,2]. It affects more than 70 million individuals worldwide and among those, roughly a quarter of seizure patients have drug-resistant epilepsy[3]. In Saudi Arabia, the incidence of epilepsy is 0.65%[4]. Epilepsy is linked to an elevated death rate despite advances in its therapy over the past few decades[5]. Although the specific mechanism of epileptogenesis is not yet fully known, it is widely accepted that this condition is characterized by an imbalance in the brain's excitatory-inhibitory systems[6,7]. The primary cause of epileptogenesis is a lack of GABA inhibition[8,9]. The NKCC1 (Na-K-2Cl cotransporter) and KCC2 (K-Cl cotransporter) are important for keeping chloride ion homeostasis and controlling how GABA receptors work in neurons[10].

The diuretic drug, BUM, demonstrated anticonvulsant actions in some previous research including clinical trials on newborn seizures[11]. The LEV is one of the recent AEDs. Its exact mechanism of action is unknown. It probably modulates neurotransmitter discharge because of its affinity for synaptic vesicular glycoprotein 2A[12]. The VAL, a traditional antiepileptic drug, was first discovered over 50 years ago in 1962 when it was tested as a solvent for other molecules to determine its potential anticonvulsant activity[13]. It increases GABA levels by inhibiting its degradation, blocks voltage-gated sodium and calcium channels and influences other pathways like histone deacetylase inhibition and glutamatergic transmission[14].

The present study aims to evaluate and compare the effects of BUM, LEV and VAL on the duration of seizures induced by PTZ and MES. Additionally, the study seeks to assess and compare the impact of these three pharmacological agents on motor coordination, using the rotarod test. Furthermore, the study intends to investigate the influence of these drugs on key brain biomarkers, including GABA, glutamate, the NKCC1 transporter and GABA transaminase, to elucidate their potential mechanisms of action.

MATERIALS AND METHODS

Study area: The study was performed at Qassim University, KSA from November, 2024 to January, 2025.

Drugs and chemicals: The LEV was obtained from Jazeera (RI, SA). The BUM was purchased from Sandoz (AG, CHE). The VAL was acquired from Sanofi (PA, FR). Diazepam was obtained from Hameln (HM, DE). Pentylenetetrazol (PTZ) was acquired from Sigma-Aldrich (Massachusetts, USA). The doses of these drugs were consistent in all models. Rat GABA Cat. No. MBS269152, Glutamate Cat No. MBS756400 and NKCC1 Cotransporter Elisa kits Cat. No: MBS9906177 was obtained from MyBioSource (South Dakota, USA). The GABA transporter EU Commodity Code 38220000 was acquired from US Biological (Massachusetts, USA).

Dose and administrated drugs: The following drug doses were used in animals: LEV 200 mg/kg[17], BUM 2 mg/kg[18], VAL 300 m/kg[19] and Diazepam 5 mg/kg[20], all these drugs were given by IP before the induction of convulsion. The PTZ dose was used at 85 mg/kg, IP, after the mice took the medicine.

Animal and work design: The 72 male Wistar albino mice (weighing 23.4±3 g and 23±2 weeks old) were utilized for the Rota rod, pentylenetetrazol and maximal electroshock model. The biomarker model was conducted using 24 male Sprague Dawley rats (weighing 190±10 and 8±2 weeks old). The experiment involved a temperature-controlled less than 25°C in an animal facility at Qassim University, with water and food available 24/7, maintaining a humidity of 50-60%. This study was approved by Qassim University (Ethics Committee) with approval No.: 24-14-02.

Maximum electroshock (MES) induced convulsions: The 24 mice were used in this model divided into 4 groups, 6 mice/each. Group I received distal water, whereas Groups II, III and IV received LEV, BUM and VAL, respectively. Mice received electroshock separately using ear-clip electrodes 30 min after drug administration. The current frequency was 45 mA (60 Hz) and the stimulus duration was 0.2 sec. Animals were monitored for tonic hind limb extension and mortality for 15 min[15].

Pentylenetetrazole (PTZ) induced convulsion: The 24 mice were used in this model. Animals were divided into four groups, each containing six mice. Group I was given distal water, Group II was given standard medication (diazepam 5 mg/kg) and Groups III, IV and V were given LEV, BUM and VAL, respectively. Administered an IP injection of PTZ 85 mg/kg to the mice 30 min after treating them or giving them a vehicle. Mice were monitored for the onset of convulsions (duration between the PTZ injection and the convulsion) for 30 min[16]. All groups were statistically compared.

Assessment of motor coordination in mice: This model used 24 mice, arranged into four groups of six mice each. Group I mice were given distal water, whereas Groups II, III and IV were given LEV, BUM and VAL, respectively. After administering these drugs, conducted three-time assessments of motor coordination: The first after 30 min, the second after 60 min and the final assessment after 90 min. Rotarod was used to train the mice for three days. For 10 min the device operated at a steady 10 rpm. As a measure of coordination, the time the animal spends in each section (TS) from the start of the test until it falls off the drum.

Collection of brain sample: Administered distilled water to the first group, treated the second group with LEV, treated the third group with BUM and treated the last group with VAL. The animals were anesthetized and euthanized by cervical decapitation 1 hr after drug administration. The brain was removed and homogenized using a tissue homogenization machine in ice-cold phosphate buffer saline (1 g/10 mL) and centrifuged for 10 min at 5000 rpm. The supernatant was removed and stored in -80°C until biochemical assays.

Laboratory analysis: The GABA analysis was measured using ELISA kits according to the manufacturer's instructions. The procedure involved the preparation of reagents, samples and standards, followed by incubation at 37°C for 90 min. The plate was then washed twice before biotinylated antibody solution was added and the incubation continued at 37°C for 60 min. After three additional washes, an enzyme-working solution was introduced and incubated at 37°C for 30 min. Following five washes, the color reagent solution was added and the plate was incubated at 37°C for up to 30 min. Optical density was measured at 450 ram using an ELISA plate reader and the GABA sample concentration was subsequently calculated through the standard curve.

The Glutamate levels in brain tissue were determined using ELISA kits in accordance with the manufacturer's guidelines. The samples, standards and blank were added in the coating microplate wells; with PBS, balance solution and conjugate and incubated for 1 hr at 37°C. The plate was washed and blotted and then substrate A and substrate B were added, incubated for 15-20 min and the optical density (O.D.) was measured at 450 nm using a microplate ELISA reader (BioTek Instruments, Santa Clara, California, USA).

The ELISA Kit for Na-K-Cl Cotransporter 1 the experiment was conducted according to the company's instructions, 50 µL of standards, 50 µL of sample and 100 µL of HRP-conjugate reagent. After washing the plate four times and incubating it for 60 min at 37°C, 50 µL of Chromogen Solutions A and B were added. The optical density was assessed after the plate had been incubated for 15 min.

As stated in the manufacturing instructions, the GABA-T analytical method required the preparation of reagents, samples and standards. After an hour of incubation at 37°C, 100 µL of detection reagent A was aspirated into each well. The plate was incubated at 37°C for 1 hr. The wells were aspirated and washed three times. As 100 µL of detecting reagent B was then added. The plate was incubated at 37°C for 30 min. The wells were aspirated and washed five times. After adding 90 µL of substrate solution, the mixture was incubated at 37°C for 10-20 min followed by the addition of 50 µL of stop solution. The optical density (O.D.) was measured at 450 nm and the concentration of the samples was calculated through the standard curve.

Statistical analysis: Used GraphPad prism® version 8 to perform statistics on the resultant data, which was expressed as mean SD (California, USA). The significant differences were determined for all sets using a One-way ANOVA test with Tukey's post-ANOVA performed for multiple comparisons and differences were deemed significant at probability<0.05.

RESULTS

Effect of LEV 10 mg/kg, BUM 2 mg/kg and VAL 300 mg/kg on the duration of electroshock-induced seizures (sec) in mice: The mean value of the durations of convulsions in the control group was 37.10±1.56 sec. Administration of the three antiepileptic drugs (LEV, BUM and VAL) produced a significant reduction ($p<0.05$) in the durations of convulsions to (11.71±0.89, 12.73±1.60 and 8.46±1.53 sec, respectively) about the control group (with percentage reductions 68, 65 and 77%, respectively). The mean of the durations of convulsions of valproate treated group was significantly decreased in relation to LEV and BUM treated groups. However, no significant change was demonstrated between the durations of convulsions of LEV, BUM treated groups as demonstrated in Fig. 1.

Effect of Diazepam 4 mg/kg, LEV 200 mg/kg, BUM 2 mg/kg and VAL300 mg/kg on the onset of PTZ induced seizures (min): The mean value of the onset of convulsions in the control group was 0.5917±0.235 min. Administration of the

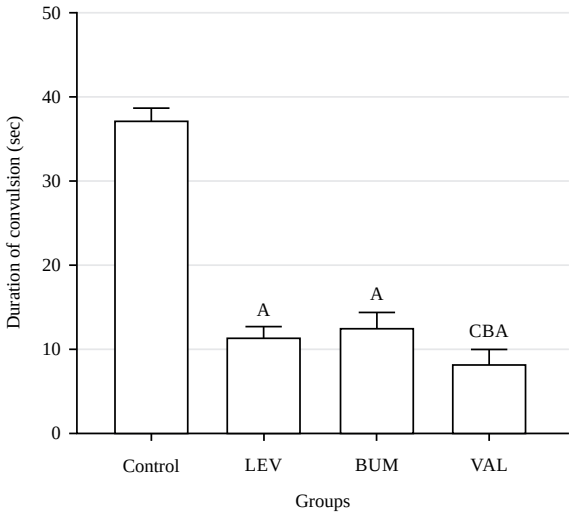

Fig. 1: Effect of LEV 10 mg/kg, BUM 2 mg/kg and VAL 300 mg/kg on duration of seizures (sec) by using maximum electroshock

Data represent the Mean±SD, one-way ANOVA and Tukey-Kramer post ANOVA were used for multiple comparisons, (A) Indicates a significant difference from the control value at p<0.05, (B) Indicates a significant difference from the LEV value at p<0.05 and (C) Indicates a significant difference from the BUM value at p<0.05

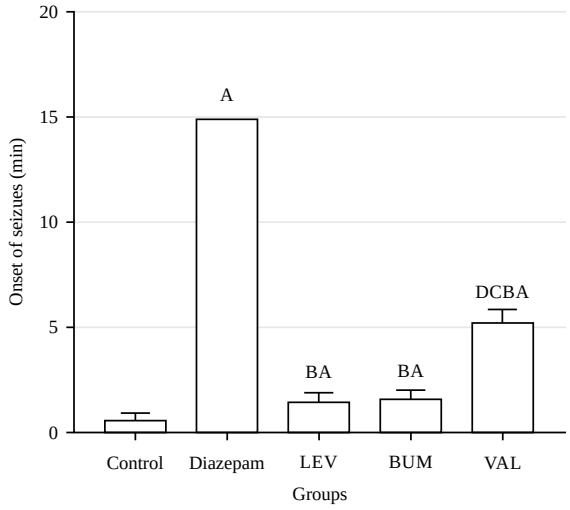

Fig. 2: Effect of diazepam 4 mg/kg, LEV 200 mg/kg, BUM 2 mg/kg and VAL 300 mg/kg on onset of Pentylenetetrazole induced seizures (min) in mice

Data represent the Mean±SD, one-way ANOVA and Tukey-Kramer post ANOVA for multiple comparisons, (A) Indicates a significant difference from the control value at p<0. 05, (B) Indicates a significant difference from the Diazepam value at p<0. 05, (C) Indicates a significant difference from the LEV value at p<0.05 and (D) Indicates a significant difference from the BUM value at p<0.05

standard drug (Diazepam 5 mg/kg) produced 100% protection. The three antiepileptic drugs (LEV, BUM and VAL) produced a significant increase (p<0.05) in time to the onset of convulsions to (1.550±0.37, 1.708±0.32 and 5.325±0.58 min, respectively) about the control group (with percentage increase 61, 65 and 88%, respectively) as shown in Fig. 2.

The mean of the onset of convulsions in VAL treated group significantly increased concerning LEV, BUM treated groups. However, the results of both LEV and BUM treated groups were insignificantly different.

The results of VAL, LEV and BUM-treated groups were significantly reduced to the diazepam-treated group as shown in Fig. 2.

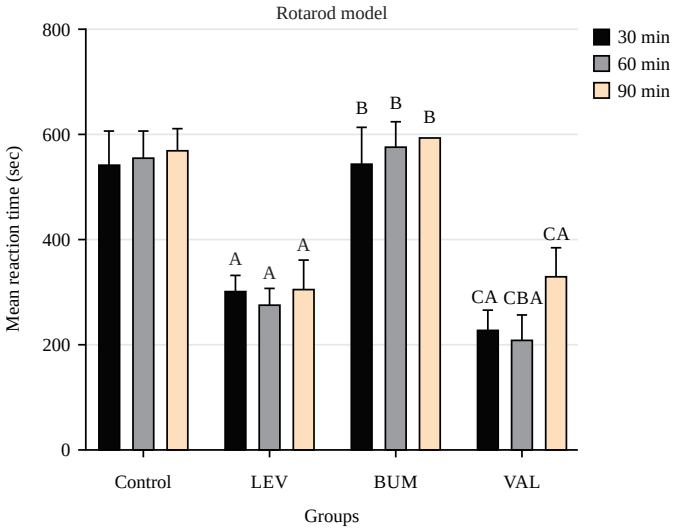

Fig. 3: Effect of LEV 200 mg/kg, BUM 2 mg/kg and VAL 300 mg/kg on motor coordination in mice

Data represent the Mean±SD, statistical calculations were conducted using one-way ANOVA and Tukey-Kramer post ANOVA for multiple comparisons, (A) Indicates a significant difference from the control value at $p<0.05$, (B) Indicates a significant difference from the LEV value at $p<0.05$ and (C) Indicates a significant difference from the BUM value at $p<0.05$

Effect of intraperitoneal injection of LEV 200 mg/kg, BUM 2 mg/kg and VAL 300 mg/kg on motor coordination in mice: The effect of LEV, BUM and VAL on motor coordination is shown in Fig. 3. The means of TS values of the Control group after 30, 60 and 90 min of IP injection of distilled water were 546.7±63.14, 561.7±47.50 and 576.7±38.30 sec, respectively. These results were insignificantly different in relation to each other.

In LEV treated mice, the means of TS values at 30, 60 and 90 min (329.3±56.41, 285.7±77.58 and 305.2±90.08, respectively) were significantly decreased ($p<0.05$) in relation to the corresponding values of the control group with percentage reductions (40, 49 and 47%, respectively) as shown in Fig. 3. Moreover, the value at 60 min was insignificant in relation to those at 30 and 90 min however, the value at 30 min was significant in relation to that at 90 min.

Also in Fig. 3 the BUM treated group the means of TS values at 30, 60 and 90 min (545.2±68.76, 581.7±44.91 and 600.0±0.00, respectively) were insignificantly different in relation to each other and to the control group. However, the value of BUM at 30 min were significantly differences with LEV and VAL. Addition the value at 60 min were significantly differences between LEV and VAL. Also at 90 min, the values were significantly differences with LEV and VAL.

Meanwhile, the means of TS values of VAL treated group at 30, 60 and 90 min (226.5±55.71, 215.2±43.49 and 342.0±63.92 sec, respectively) were significantly decreased in relation to the corresponding values of the control group with percentage reduction (58, 62 and 41%, respectively) as shown in Fig. 3. On the other hand, statistical analysis revealed that the means of the TS values of valproate treated group were significant at 30 min with BUM but insignificantly differences with LEV, in addition, the values at 60 min were significant with LEV and BUM. Moreover, the value at 90 min were significantly decreased with BUM but insignificant with LEV.

Effect of LEV 200 mg/kg, BUM 2 mg/kg and VAL 300 mg/kg on brain sample biomarkers (GABA, Glutamate, NKCC1 transporter and GABA transaminase): The means values of GABA level after 60 min of IP injection of distilled water, LEV, BUM and VAL were (128±10.24, 159.1±7.04, 250.9±28.95 and 265.3±23.10 pg/mL), respectively. The results of BUM and VAL treated groups were significantly increased ($p<0.05$) in relation to the corresponding values of the control group with percentage increase (50 and 52%, respectively). However, the results of LEV treated group were insignificantly different in relation to the results of the control group in Fig. 4a.

The means values of Glutamate level after 60 min of IP injection of distilled water, LEV, BUM and VAL were 23.58±9.08, 22.35±2.59, 20.63±1.50 and 23.09±3.10 nmol/g, respectively. These results were insignificantly different in relation to the control group in Fig. 4b.

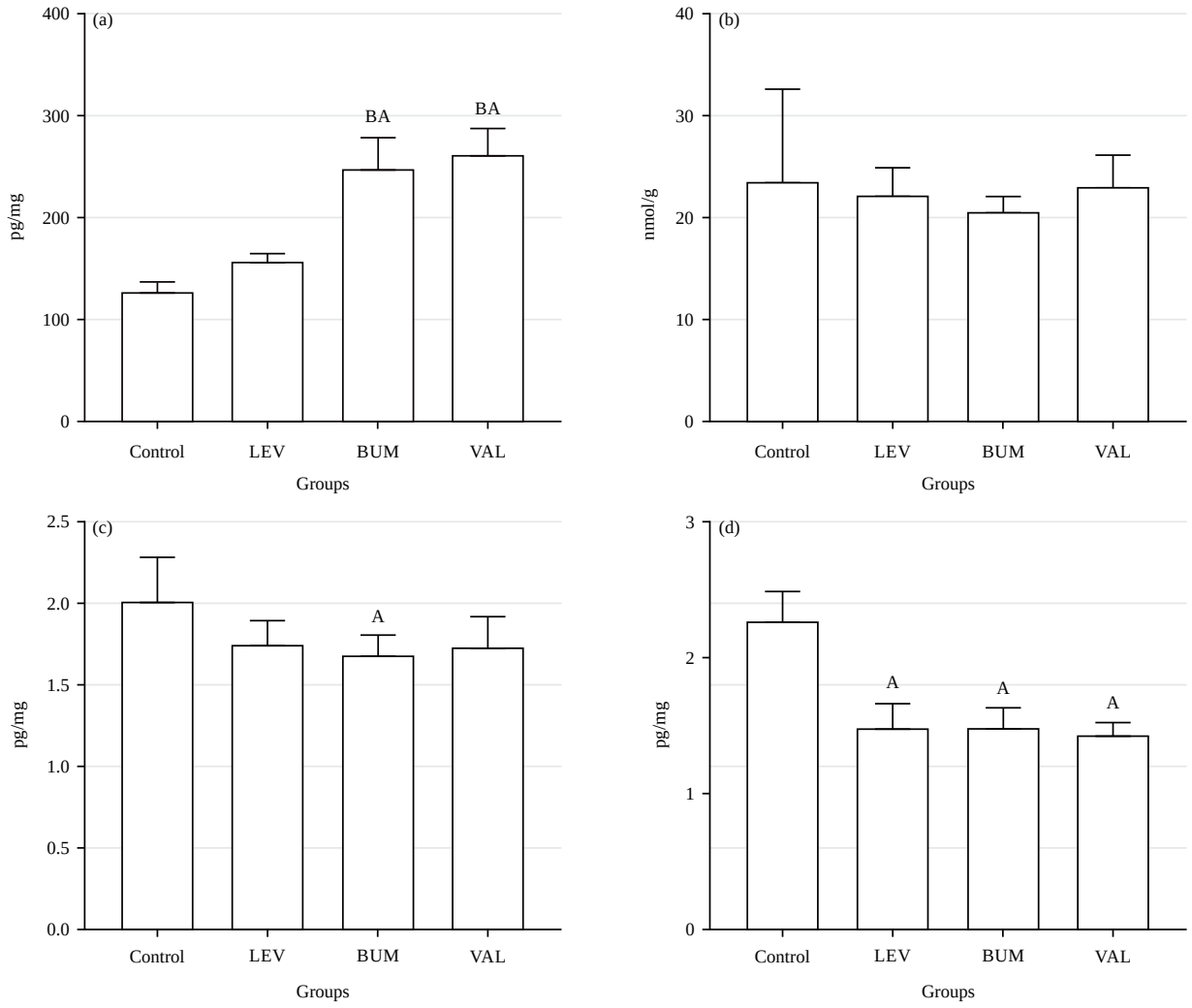

Fig. 4(a-d): Effect of LEV, BUM and VAL on (a) GABA level, (b) Glutamate level, (c) NKCC1 transporter and (d) GABA transaminase in rat brain samples by using ELISA kits

Data represent the Mean±SD, one-way ANOVA and Tukey-Kramer post ANOVA for multiple comparisons, (A) Indicates a significant difference from the control value at $p<0.05$ and (B) Indicates a significant difference from the LEV value at $p<0.05$

The NKCC1 transporter mean values following a 60 min IP injection of pure water, LEV, BUM and VAL were 2.01±0.27, 1.69±0.11, 1.751±0.14 and 1.74±0.18 pg/mg, respectively.

The results of BUM treated group were significantly decreased ($p<0.05$) in relation to the corresponding values of the control group with a percentage reduction 16%, but LEV and VAL results were insignificantly differences when compare with control in Fig. 4c.

The GABA transaminase mean values following an IP injection of distilled water, LEV, BUM and VAL for 60 min were 2.27±0.21, 1.48±0.18, 1.49±0.14 and 1.44±0.09 pg/mg, respectively. The results of the three treated groups were significantly decreased ($p<0.05$) in relation to the corresponding values of the control group with percentage reduction (34.5,34 and 36.6%, respectively) in Fig. 4d.

DISCUSSION

The primary aim of the present study was to assess and compare the anticonvulsant effects of three antiepileptic drugs (AEDs): BUM, LEV and VAL, in both mice and rat models of epilepsy. This was achieved through the use of the maximal electroshock (MES) test and the PTZ Test. Additionally; the acute neurotoxic effects of these AEDs were evaluated using the Rota rod test, while the potential underlying mechanisms of action were explored by measuring key brain biomarkers involved in neurotransmission, including GABA, Glutamate, NKCC1 and GABA transaminase.

The MES test is an animal model for assessing the efficacy of AEDs, particularly for generalized tonic-clonic seizures. In the present study, the control group exhibited the longest seizure durations. Among the treatments, BUM demonstrated a moderate anticonvulsant effect, significantly reducing seizure duration compared to the control group, but was less potent than the other two drugs, levetiracetam and valproic acid. These results are consistent with those of Luszczki *et al.*[17], who similarly reported that bumetanide exhibited moderate anticonvulsant effects in the MES model. The finding that bumetanide is less effective than LEV and VAL in this model highlights its potential as a supplementary or adjunctive treatment in epilepsy, rather than a first-line option.

The LEV and VAL both demonstrated a more significant reduction in seizure duration, with VAL proving to be the most effective. This finding is consistent with previous research by Johannessen and Johannessen[18], who indicated that valproic acid exerts a potent anticonvulsant effect through the inhibition of voltage-gated sodium channels and suppression of T-type calcium channels. The superiority of VAL in reducing seizure duration across both MES and PTZ models underscores its broad-spectrum mechanism of action, making it one of the most widely prescribed AEDs. The results of the present study also align with the work of Jafari and Hassanpourezatti[19], who reported significant reductions in seizure duration following VAL administration in both MES and PTZ models.

On the other hand, LEV demonstrated moderate effects in the MES test. While it was effective in reducing seizure duration, it was not as potent as VAL, in line with the findings of Klitgaard *et al.*[20]. The LEV is known to modulate synaptic vesicle protein 2A (SV2A) to inhibit abnormal neuronal firing, which likely explains its moderate anticonvulsant effects in this model. Interestingly, LEV's efficacy may be influenced by genetic factors, such as SV2A deficiencies in certain rodent models, as suggested by Kaminski *et al.*[21]. This could explain some of the variability in LEV's effectiveness, particularly in different experimental settings.

The PTZ model, a chemically induced model of epilepsy, was used to further assess the anticonvulsant effects of BUM, LEV and VAL. The results demonstrated that all three drugs significantly delayed the onset of convulsions compared to the control group. This is consistent with previous research, such as that by Rehman *et al.*[22], who reported that LEV has a protective effect against PTZ-induced seizures. The ability of these drugs to delay the onset of seizures in the PTZ model further supports their potential as anticonvulsant agents in clinical settings. Notably, diazepam, a GABAergic agonist, showed the most profound effect in delaying seizure onset, which aligns with its established use as a first-line treatment for acute seizures and status epilepticus.

Although not as effective as diazepam, VAL demonstrated strong protective effects in this model, likely due to its multi-target mechanisms, including the enhancement of GABAergic inhibition and the modulation of sodium channels. This finding is consistent with the work of Blanco *et al.*[23], who showed that VAL-delayed PTZ-induced seizures. Similarly, LEV showed a modest delay in the onset of convulsions, which is consistent with findings from Coppola *et al.*[24], who demonstrated that LEV had protective effects in the PTZ model. Bumetanide also delayed the onset of seizures, which is in line with the results of Kharod *et al.*[25], who showed that bumetanide enhanced anticonvulsant efficacy in PTZ models.

Regarding the effect of drugs on motor coordination, bumetanide (BUM) did not significantly impair motor coordination, as evidenced by similar performance to the control group. This finding is consistent with those of Egawa *et al.*[26] and Cheung *et al.*[27], they reported that bumetanide does not induce neurological impairment despite its anticonvulsant effects. In contrast, both LEV and VAL resulted in notable reductions in motor coordination. The LEV-treated mice showed a significant decline in motor performance, which may be indicative of mild sedation, transient coordination deficits or ataxia. These findings are consistent with previous studies by Klitgaard[28], which observed dose-dependent impairment of motor coordination in animals treated with LEV. Similarly, VAL resulted in the most significant motor incoordination, likely due to its sedative effects at the tested dose, which aligns with the study by Bath and Pimentel[29].

The current study also investigated the effects of BUM, LEV and VAL on brain biomarkers involved in neurotransmission. Bumetanide was found to significantly reduce the levels of GABA transaminase and NKCC1 transporter while increasing GABA levels with no significant effect on Glutamate levels. These findings suggest that bumetanide's therapeutic effect may be partially mediated through its action on GABAergic neurotransmission and chloride homeostasis. Specifically, the reduction in NKCC1 expression, which is responsible for the regulation of chloride ions across cell membranes, is consistent with previous research by Jantzie *et al.*[30], which suggested that bumetanide inhibits NKCC1 in the rat brain. These results support the idea that bumetanide may help restore GABAergic activity, which could contribute to its therapeutic effects in neurological disorders, particularly autism and developmental epilepsy, as noted by Wang and Kriegstein[31].

The LEV did not significantly affect GABA levels, which is consistent with previous studies by Kuzniecky *et al.*[32]. However, LEV was found to modestly decrease NKCC1 transporter expression, suggesting that its anticonvulsant effects may be mediated indirectly through modulation of chloride homeostasis. This is in line with the mechanism of action outlined by Meehan *et al.*[33], which suggests that LEV primarily acts on synaptic vesicle protein SV2A, modulating neurotransmitter release rather than directly affecting GABAergic or glutamatergic neurotransmission.

In contrast, VAL was found to significantly enhance GABA levels, aligning with earlier studies by Mesdjian *et al.*[34], who reported that valproate increases GABA concentrations and enhances GABAergic inhibition in the brain. Additionally, VAL inhibited GABA transaminase, supporting its well-established role as a GABAergic drug. This mechanism contributes to its potent anticonvulsant effects and its use in a wide range of seizure types. The minimal effect of VAL on Glutamate levels further suggests that its primary mode of action involves enhancing GABAergic transmission rather than directly modulating excitatory neurotransmitter systems.

CONCLUSION

Bumetanide exhibited moderate anticonvulsant effects but was less potent than LEV and VAL, with VAL showing superior efficacy in reducing seizure duration. The LEV was effective but caused mild sedation and transient motor coordination deficits. VAL was the most potent anticonvulsant, likely due to its multifaceted mechanisms involving GABAergic enhancement and modulation of sodium and calcium channels. Bumetanide did not significantly impair motor coordination, while both LEV and VAL induced motor incoordination, with VAL causing the most pronounced effect. Bumetanide increased GABA levels and decreased GABA transaminase and NKCC1 expression, suggesting a modulation of chloride homeostasis. The LEV did not significantly alter GABA levels but influenced chloride homeostasis, while VAL enhanced GABA levels and inhibited GABA transaminase, consistent with its role as a GABAergic drug. Overall, these results suggest that VAL is the most effective anticonvulsant drug, followed by LEV, with bumetanide being the least potent however bumetanide had the safest side effect profile on the central nervous system.

SIGNIFICANCE STATEMENT

The search for more effective and safer treatments for epilepsy continues unabated. While traditional AEDs are generally quite effective at controlling seizures, they too often come with a litany of side effects and are ineffective in a significant portion of the population. The study looks at how Bumetanide, Levetiracetam and Valproic acid treat seizures and damage nerve cells in animal models of epilepsy. Bumetanide is characterized by the least neurocentral side effects. The study suggests that VAL is a first-line anticonvulsant, with LEV serving as a viable alternative for specific patient profiles. More research is needed to find out how to best use bumetanide, LEV and VAL in people with different types of epilepsy and at different stages of development.

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