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ANTICONVULSANT ACTIVITY OF DIVALPROEX SODIUM AND EFFECT OF GLIMEPIRIDE ON PHARMACOKINETIC: AN EXPERIMENTAL DRUG INTERACTION STUDY

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ABSTRACT

Background and Objectives: Epilepsy is a serious and common chronic neurological disorder caused by abnormal synchronized neuronal discharges. Divalproex is suggested to increase GABA concentration in brain. Both Divalproex and Glimepiride used for long duration indicate for CNS disorder and Diabetes mellitus. The study was conducted to find the influence of Glimepiride on pharmacokinetic and anticonvulsant activity of Divalproex.**Methods:** Four healthy rabbits of either sex were used to study the effect of Glimepiride on pharmacokinetic parameters of Divalproex. The concentration of Valproic acid (VPA) in serum was estimated by HPLC coupled with Mass Spectroscopy (LC-MS/MS). Anticonvulsant activity was studied using Maximal Electroshock (MES) method and Pentylenetetrazole (PTZ) test in healthy albino rats.**Results:** The serum concentration of VPA was significantly increased after Glimepiride treatment for 7 days. Pharmacokinetic parameters like AUC, AUMC, $T_{1/2}$ and C_{max} of VPA showed significant change after Glimepiride treatment in healthy albino rabbits. Glimepiride also exhibited significant increase in duration of hind limb extensor time and onset of clonic convulsion time in MES and PTZ induced seizure test respectively. The percentage prolongation of onset of clonic convulsion was decreased to 49.6% in combination treatment. **Conclusion:** The drug-drug interaction between Divalproex and Glimepiride could be due to metabolism of both the drugs at the same site and protein binding to albumin.

Keywords: Anticonvulsant, Divalproex, Glimepiride, Maximal Electroshock Method, Pentylenetetrazole induced convulsion.

INTRODUCTION

Coexistence of multiple medical conditions as well as the need and practice of polypharmacy play role in occurrence of drug interactions which represents an important and widely under recognized source of medication errors [1, 2]. Drug interaction is an alteration in the nature or effect of drugs due to concurrent administration of one or more drugs, foods or beverages which has also been a known factor affecting response to drugs and a prominent cause of adverse drug reactions [2] i.e. synergistic (when the drug's effect is increased) and antagonistic (when the drug's effect is decreased) [3]. Drug interactions are often categorized as pharmacodynamic (drug's effect on the body) or pharmacokinetic (drug effect to the body) in nature [4].

Drug effects result from the interplay of multiple processes that influence drug absorption, metabolism and excretion as well as drug response [5]. Anticonvulsants are a family of drugs that depress abnormal nerve activity in the brain, thereby blocking seizures commonly used to prevent and treat seizure disorders, as well as other conditions. A seizure is the clinical manifestation of an abnormal, excessive, hyper synchronous discharge of a population of cortical neurons. The neuro-chemical basis of abnormal, excessive, hypersynchromous discharge is not well understood [6]. Several susceptibility genes, mainly encoding neuronal ion channels have been identified in seizures [7].

Epilepsy is a serious common chronic neurological disorder seen by neurologists after stroke in all age groups characterized by recurrent seizures, which are caused by abnormal synchronized neuronal discharges [8-10]. In developed countries, annual new cases are between 40-70/1,00,000 people in the general population. In developing countries, this figure is often close to twice as high due to the higher risk of experiencing conditions that can lead to permanent brain damage. Close to 80% of epilepsy cases worldwide are found in developing regions. The risk of premature death in people with epilepsy is two to three times higher than it is for the general population [11].

Though some people are maintained on a single drug, most take two or more anticonvulsant medications to prevent seizures [12]. Divalproex sodium (DS) is an anticonvulsant (antiepileptic) drug which is used to treat mania and preventing migraine headaches. It is effective in the treatment of epilepsy, particularly for preventing simple, complex (petitmal), absence, mixed, tonic-clonic (grandmal) seizures, manic phase of bipolar disorder i.e. manic-depressive disorder in adults [13].

Glimepiride is effective as initial drug therapy and is the first III generation potent sulphonylurea with long duration of action and an oral blood glucose-lowering drug which stimulates the release of insulin from functioning pancreatic-β- cells. "Diabetes mellitus" describes a metabolic disorder of multiple etiologies characterized by chronic hyper-glycaemia with disturbances of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action, or both. The effects of diabetes mellitus include long–term damage, dysfunction and failure of various organs [14].

The patient-related factors include drug clearance in a particular patient, age, genetic factors, gender, concurrent diseases, environmental factors and diet [15, 16]. Drug interaction becomes more crucial in patients with extremes of age (too old and too young age), immune-compromised hosts, patients receiving medications pertaining to or affecting cardiovascular or central nervous systems, also in patients with chronic diseases, multiple illnesses and in those having renal or hepatic impairment. The post-transplant patients, patients with severe illnesses and those with AIDS related disease also are more susceptible to occurrence of drug interactions [17].

Literatures of drug-drug interactions (DDIs) in the 1960s were based primarily on animal experiments, with a few case reports. Clinical reports seemed to focus on oral anticoagulants or on interactions of the monoamine oxidase inhibitors only. With practitioners noting more drug interactions in 1970s and 1980s, publication of both case reports and clinical studies increased [18]. In the modern era of medicine, it is surprising if one comes across a patient receiving just one or two medications at a time [2]. Patients have many concerns when multiple medications are started, including prescribing errors, the cost of medications, and possible adverse effects. Significantly, 58% of patients worry that they will be given medications having drug interactions will adversely affect their health [19]. Therefore, the present study was

aimed to investigate the possible drug interaction between Glimepiride and Divalproex sodium, and its influence of repeated administration of Glimepiride on anti-convulsant activity of Divalproex sodium in healthy albino rats and Glimepiride on pharmacokinetic parameters of Divalproex sodium in healthy albino rabbits.

MATERIALS AND METHODS

Study Design

The descriptive and experimental research based study was conducted at Mallige College of Pharmacy, Banglore, Karnataka, India in 2015 AD.

This experimental research was carried out into two parts: In the first part, the pharmacokinetic parameters of Divalproex sodium (6 mg/kg, *p.o.*) were established in healthy albino rabbits. After this, animals were left for a washout period of 15 days. In the second part, the same animals were administered with Glimepiride (0.30 mg/kg, *p.o.*) once daily for a week. On the 8th day, the Divalproex sodium (6 mg/kg, *p.o.*) was administered along with the Glimepiride and the pharmacokinetic parameters were established.

Study Population and its Housing

Healthy adult male albino rabbits and healthy albino rats weighing 2.0 to 2.5 kg and 180 to 220g respectively were selected for the study. Rabbits were housed in stainless steel cage with a fenestrated floor to allow feces to drop through into a pan and were provided with regular rabbit chow, carrot, cucumber and green leaves. Rats were housed in separate clean cages. The bed ding material of the cages for rats were removed and replaced thrice a week with fresh materials as often as necessary to keep the animals clean and dry. Bedding materials used insufficient amount to keep animals dry between cage changes without coming into contact with watering tubes. The animals were provided with distilled water and libitum throughout the experiment. They were fed with standard pellet diet. The animals were acclimatized to standard laboratory conditions of temperature (25±3^{0}C) and maintained on 12:12 hour natural light dark cycle. Cage cards were utilized to identify the strain of rabbit, sex, number, principal investigator and research protocol. Temporary identification of individual rabbits was accomplished by dyeing the fur. Swiss albino rats were housed separately in cages with free access to food and water.

Preparation of Standard Solution

Glimepiride Solution

Accurately weighed 3.64 mg of pure sample of Glimepiride and was dissolved with 2% polyvinyl pyrrolidone (PVP) in water and was transferred in to a clean dry 20 ml volumetric flask. Final volume was adjusted with distilled water to get 2 mg/ml stock solution.

Divalproex Sodium Solution

Accurately weighed 100 mg of pure sample of Divalproex sodium was dissolved with 0.5 ml of 0.9% NaCl and was transferred in to a clean dry 10 ml volumetric flask. Final volume was adjusted with distilled water to get 10mg/ml stock solution.

Pentylenetetrazole (PTZ) solution

Accurately weighed 800 mg of pure sample of PTZ was transferred in to a clean dry 10 ml volumetric flask. Dissolved it and final volume was adjusted with sterile water to get 80mg/ml stock solution.

Blood Sample Collection and Serum Separation

Blood samples were collected from the marginal ear vein of rabbit and kept in rabbit holder with their heads-protruding out. The edge of the ear was rubbing with alcohol cotton swab and the blood vessels were dilated which was punctured by means of sharp 23-gauge needle in the direction of venous blood flow. The blood was collected into 2ml narrow ependroff tubes and kept in room temperature for 30 min. The blood sample was centrifuged at 3000 RPM for 15-20 minutes and the transparent supernatant liquid (serum) obtained which was transferred into a clean, dry 2 ml ependroff tubes.

Oral Administration of Divalproex Sodium Drug

In Rabbit

The rabbits were fixed in wooden stalls and an oral gag was placed in between the jaws and held in position by holding upper and lower jaw using the left hand. One end of the feeding tube was moistened with glycerin and introduced into the mouth through the central hole of the gag, pushed slowly such that it enters the esophagus. The other end of the feeding tube was connected to a 2-3 ml syringe of distilled water was administrated initially to ensure that the incubation is in right position. The drug solution was administrated similarly and this was followed by 3ml of distilled water to ensure the administration of correct dose of the drug. The oral feeding tube was then gently removed and the animal was removed from the wooden stall immediately and was tilted with its head down to prevent the entry of any fluid into the respiratory tract. The gag and the oral feeding tubes were cleaned.

In Rat

An 18 gauze needle was suitably rounded with metal ball and was fixed to 1 ml tuberculin syringe. The rat was held firmly in left hand. The needle moistened with glycerin was inserted into the esophagus with gently pressing of plunger for drug administration. This was followed by 0.2ml distilled water to ensure correct administration of dose of the drug.

Estimation of Serum Valproic acid

The amount of Valproic acid (VPA) present in serum was calculated using the following equation:

Amount of VPA (µg/ml) =

$$\frac{\text{Peak area x Final volume made (µl) x Factor}}{\text{Volume of injection (µl) x total serum volume (ml)}}$$

Effect of Glimepiride treatment on the Pharmacokinetic parameters of Divalproex Sodium in healthy albino rabbits

Four male albino rabbits weighing between 2.0 to 2.5 kg were selected and placed in different rabbit cages and fasted for 18 hours before commencing the experiment. During this period, rabbits were allowed to take adequate water.

In the first part, animals were administered with solution of Divalproex sodium (6 mg/kg, *p.o.*) and the time of drug administration was noted. The blood sample was collected at 0 min, 30 min, 1st, 2nd, 4th, 8th and 16th hour after the Divalproex administration 1.5 ml of blood was withdrawn at a time and centrifuged after 30 minutes. Two (2µl) of serum was used for the estimation of serum Valproic acid concentration by using UPLC technique equipped with Photo Diode Array Detector. Separations were carried in IIHR, Hesarghatta main Road, Bangalore, C18, 4.6 X 150 mm, 5µm column using isocratic elution. The flow rate was 1.2 ml/min. UV detection was performed at 254 nm [138]. Peak identity was confirmed by retention time comparison and Mass Spectroscopy (Water) i.e. UPLC coupled to Mass (MSMS). The UPLC was operated at room temperature. Animals were left for a washout period of 15 days.

In the second part, the same animals received Glimepiride (0.30 mg/kg, *p.o.*) once daily for a week. On the 7th day, 6 hours after administration of the drug, the rabbits were fasted for 18 hours. On the 8th day, Divalproex (6 mg/kg, *p.o.*) was administered to the rabbits. Blood samples were collected at 0 min, 30 min, 1st, 2nd, 4th, 8th and 16th hour after the Divalproex administration and 1.5 ml of blood was withdrawn and centrifuged after 30 min. 2µl of serum was used for the estimation of serum Valproic concentration by using UPLC technique.

Effect of Glimepiride treatment on Anticonvulsant activity of Divalproex sodium

The effect of Glimepiride (0.30 and 0.78 mg/kg, *p.o.*) treatment on

anticonvulsant activity of Divalproex sodium with 10mg/kg, *p.o.* by using maximal electroshock induced convulsion and pentylenetetrazole induced convulsion method in healthy albino rats respectively.

Maximal Electroshock (MES) induced convulsion method

In the first part, animals were divided into two groups six animals each, first group received saline orally and served as a control while second group received 10 mg/kg of Divalproex sodium orally. After an hour of treatment, seizures were induced by maximal electroshock in albino rats with the help of electro convulsion-meter by passing current of 150 mA for 0.2 sec using ear clip electrodes. The Divalproex and saline were given orally one hour prior to induction of convulsions. The animals were observed for the extensor phase as well as its duration. The abolition of extensor (tonic phase) in drug treated group was taken as criteria for anticonvulsant activity.

In second part, after a wash out period of 15 days the same groups of animals were administered with Glimepiride (0.6 mg/kg, *p.o.*) once a day for one week. On the 8th day, Divalproex sodium (10 mg/kg, *p.o.*) and Glimepiride (15 mg/kg, *p.o.*) was administered. The test was repeated and the duration of extensor phase was measured.

Pentylenetetrazole induced convulsion method

In the first part of experiment, animals were divided into two groups six animals each, first group which received PTZ (80 mg/kg, *i.p.*), served as a control while second group received 10 mg/kg of Divalproex sodium orally. After one hour of drug treatment, PTZ (80 mg/kg, *i.p.*) animals were observed for clonic convulsions in 30 minutes, duration of convulsions and 24 hour mortality. Absence of clonic convulsions in drug treated groups was taken as criteria for anticonvulsant activity.

In the second part of the experiment, after a wash out period of 15 days the same animals were administered with Glimepiride (0.78 mg/kg, *p.o.*) once daily for a week. On the 8th day, Divalproex sodium (10 mg/kg p.o.) and Glimepiride (0.78 mg/kg, *p.o.*) was administered. The test was repeated and duration of convulsions, onset of clonic convulsion and 24 hour mortality was noted.

Inclusion and Exclusion criteria

Swiss albino rats weighing between 150-200 g were chosen by preliminary screening which showed extension of hind limb and clonic convulsions within 30 minutes by injecting the PTZ in a dose of 80 mg/kg intra-peritoneal were included for the study. Those rats were excluded who didn't show any type of convulsion.

Ethical Consideration

Ethical approval was taken from the office of Institutional Animal Ethical Committee (IAEC), Mallige College of Pharmacy, Banglore, Karnataka, India prior to commencement of the study.

Statistical Analysis

Pharmacokinetic data of Divalproex was measured assuming complete oral absorption. Different pharmacokinetic parameters were determined from the plasma concentration time profiles using a model-independent computer program, Software RAMKIN. All the experimental results were expressed as mean ± SEM and assessed by one way analysis of variance (ANOVA) followed by '*t*' test using parametric statistics. A value of $P < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

Pharmacokinetic drug interaction involves alteration in the absorption, distribution, metabolism or elimination of one drug by other. The most important pharmacokinetic interactions are those involving cytochrome P_{450} isoenzymes in hepatic metabolism. Pharmacodynamic interaction is due to interaction between agonist and antagonist at drug receptor which is seen when two drugs have synergistic or antagonist pharmacological effects. The induction or inhibition of drug metabolizing enzyme is a particularly important cause of clinically significant interactions [20, 21].

This experimental study consists of two period of administration of Divalproex (6 mg/kg, *p.o.*) alone and in combination with Glimepiride (6 mg/ kg, *p.o.*) for 8 days of administration in 4 healthy albino rabbits. Plasma Valproic acid concentrations were analyzed by a validated HPLC method.

Serum concentrations of Divalproex sodium before and after Glimepiride treatment in healthy albino rabbits

Valproic acid pharmacokinetic parameters revealed that its peak effect was at 2^{nd} hour (181.42 ± 0.02714) followed by 1^{st} hour (173.92 ± 0.04265) and $t_{1/2}$ is around 8 hours before Glimepiride treatment in healthy albino rabbits. Similarly, the highest peak effect was also observed in the same time 2^{nd} hour (219.98 ± 0.438) followed by 1^{st} hour (164.30 ± 0.5027) and 4^{th} hour (160.03 ± 0.234) which was found to be increased after Glimepiride treatment in healthy albino rabbits. The results are shown in table 1. The Serum concentration at 30 min, 1^{st}, 2^{nd}, 4^{th}, 8^{th} and 16^{th} hour was estimated to study the effect on absorption, distribution, metabolism and excretion of Valproic acid in the body. Divalproex was well absorbed after oral administration and from visual inspection of experimental data, the maximum plasma concentration (C_{max} = 181.42 µg/ml) and the time reach it (T_{max} = 2hr) were obtained. There was change in the concentration of serum Valproic acid after Glimepiride treatment and increased to 219.98 µg/ml. There was significant change in the concentration of serum Valproic acid after Glimepiride treatment for one week only at 4^{th} hour ($p<0.05$). Effect of Glimepiride treatment on pharmacokinetic parameters of Divalproex sodium in healthy albino rabbits

The mean pharmacokinetic parameters of Divalproex administered alone and in combination with Glimepiride as well as the statistical significance following their comparisons are tabulated in the Table 2. The pharmacokinetic parameters like AUC, AUMC and MRT were also increased to 1850.32 µg/ml/hr, 16607.56 µg/ml/hr and 15.20 hr respectively. The similar significance was found when comparing AUC, AUMC and MRT parameters after Glimepiride treatment ($p<0.05$). The possible mechanism behind this type of interactions at pharmacokinetic level may be due to common metabolizing enzyme CYP2C9. The pharmacokinetic parameters like Cmax, Tmax and AUC0-∞ were used for the bioequivalence evaluation of Divalproex administered alone (Reference) and in combination with Glimepiride (Test) but no change in Tmax was observed.

Divalproex and Glimepiride has the protein binding capability of 80-90% [22] and 95-99% respectively [23]. But the effect of protein binding displacement would not be expected because Divalproex is bound to albumin and Glimepiride is also bound to albumin but at different sites [22, 23]. Divalproex sodium is substrate for CYP2C9 and inhibits drugs that are metabolized by this enzyme [24] whereas Glimepiride is metabolized by CYP2C9 system [23]. It was observed that co-administration of high doses of Valproic acid with drugs that are primarily metabolized by CYP2C9 may result in significant drug interactions [24]. As free drug it was a major determinant of pharmacological effects, these drug interactions could result in toxicity and/or enhanced efficacy [25].

In addition, extra-pancreatic effects may also play a role in the activity of Sulfonylureas such as Glimepiride. This is supported by both preclinical and clinical studies demonstrating that Glimepiride administration can lead to increased sensitivity of peripheral tissues to insulin. These findings are consistent with the results of a long-term, randomized, placebo-controlled trial in which Glimepiride therapy improved postprandial insulin/C-peptide responses and overall glycemic control without producing clinically meaningful increases in fasting insulin/C-peptide levels [22].

Table 1: Serum concentration of Drug in albino rabbits at different time interval.

Time (hour)	Serum concentration of Drug in µg/m	
	Divalproex sodium (mg/kg,p.o) (Before)	Divalproex sodium (6 mg/kg, *p.o.)* + Glimeperide (10 mg/kg, *p.o.*) (6mg/kg, *p.o.)* + Glimeperide (10 mg/kg, *p.o.*) (After)
0	0	0
0.5	53.42 ± 0.1055	74.65 ± 0.079
1	173.92 ± 0.04265	164.30 ± 0.5027
2	181.42 ± 0.02714	219.98 ± 0.438
4	125.28 ± 0.3981	160.03 ± 0.234
8	79.34 ± 0.4218	94.06 ± 1.23
16	52.91 ± 0.04626	77.65 ± 1.125

Table 2: Mean pharmacokinetic parameters of Divalproex administered alone and in combination with Glimepiride

Parameters	Divalproex Sodium	Divalproex Sodium + Glimepiride 0.30mg/kg
AUC $_{0-t}$ (µg/ml/hr)	1500.778	1850.32
AUMC$_{0-t}$ (µg/ml/hr)	12561.61	16607.65
Tt$_{1/2}$ (hrs)	8.1	10.32
C$_{max}$ (µg/ml)	181.42	219.98
T$_{max}$ (hrs)	2	2
MRT (hrs)	12.84	15.2

(AUC: Area under curve, AUMC: Area under first order moment curve, C$_{max}$: Peak Plasma Concentration, MRT: Mean residential time, Tmax: Time of maximum concentration, Tt$_{1/2}$: Terminal half life)

Effect of Glimepiride treatment on Anticonvolusant activity of Divalproex sodium by Maximal Electroshock (MES) induced convulsion method

There was a synergistic interaction between Glimepiride and Divalproex. The duration of Hind Limb Tonic Extensor (HLTE) in rats treated with Divalproex sodium alone was 4.540 ± 0.1360 seconds. The group of animals treated with Divalproex sodium and Glimepiride exhibits duration of HLTE to 4.278 ± 0.1242 seconds. These results indicated that Glimepiride slightly enhance anticonvulsant activity of Divalproex sodium in rats i.e. Glimepiride treatment for one week alter the HLTE duration in healthy albino rats significantly at the level of p<0.0016. The incidence of convulsion in Divalproex administered rat was 69.92% which decreased to 63.45% in rats treated with Divalproex and Glimepiride. The results are shown in table 3. The slight increase in anticonvulsant activity of Divalproex sodium by Glimepiride treatment for one week may be due to plasma protein binding to albumin which resulted in slight increase in plasma concentration of Divalpoex sodium due to competitive albumin binding between Divalproex sodium and Glimepiride [26, 27].

Table 3: Effect of Glimepiride in healthy albino rats by Maximal Electroshock (MES) induced convulsion method.

Drug Treatment	Duration of Extension Tonus (sec)	Duration of Clonus (sec)	Duration of Stupor (sec)	Death/ Recovery	% Incidence of convulsions
Control	5.322 ± 0.2300	19.68± 0.29	93.4 ± 2.2	100%	100%
Divalproex sodium (10 mg/kg, *p.o.*)	4.540 ± 0.1360**	12.32 ± 0.33	73.2 ± 1.02	100%	69.92%
Glimepiride 0.6 mg/kg, *p.o.*) + Divalproex sodium (10 mg/kg, *p.o.*)	4.278 ± 0.1242*	10.38 ± 0.44	56.6 ± 2.16	100%	63.45%

Number of animals used = 6, All data are expressed in mean ± SEM, Statistical significance at ***P<0.0016 compared to control only

Effect of Glimepiride on anticonvolusant activity of Divalproex sodium by PTZ induced convulsion method

In this study, each rat under the test received a test drug intra-peritoneal 30 minutes before administration of sub-convulsive dose of PTZ (80 mg/kg *i.p.*) [28]. Rats administered with Divalproex sodium (13 mg/kg, *p.o.*) alone exhibits latency period of clonic convulsion of 339.2±16.04 seconds. The latency period of Glimepiride (0.78 mg/kg, *p.o.*) administered for a week followed Divalproex (10 mg/kg, *p.o.*) was 289.8 ± 15.41 seconds. The nature of clonic convulsion was less jerky as compared with control. The percentage prolongation of onset of clonic convulsion was enhanced to 57.18%. The nature of convulsion was jerky movements with lack of straub's tail. These results confirm its anticonvulsant activity in this model. The number of convulsion comparing Divalproex (15 mg/kg, *p.o.*) alone to Divalproex (15 mg/kg, *p.o.*) with Glimepiride (0.78 mg/kg, *p.o.*) was found to be significantly decreased in combination treatment (p<0.05). The results are shown in table 4.

Pentylenetetrazole (PTZ) is a GABA-antagonist [29]. It induces the bilaterally synchronous spike wave discharges that typify absence seizures in rodent [30]. Anti- epileptic drugs which increase the threshold for the onset of myo-clonic jerks and tonic extensor are known to prevent seizure generation and propagation respectively [31].

A role for $GABA_B$-related mechanisms is suggested for the pathogenesis of generalized absence seizures [30]. Pretreatment with $GABA_B$-receptor agonists resulted in generalized absence status epilepticus lasting for hours. These data confirm the concept that specific $GABA_B$- receptor antagonist activity confers anti-absence seizure activity [31]. A study by De Sarro *et al* revealed that $GABA_B$ receptors are able to affect the development of the epileptic kindling state induced by Pentylenetetrazole [32]. In mice, chronic (but not acute) Glimepiride and electroconvulsive therapy increases the function of the Gamma-aminobutyric acid ($GABA_B$) receptor in the frontal cortex modulating 5-HT release [33]. VPA affects GABAergic neurotransmission by acting on GABA receptors too, enhancing responses of both $GABA_A$ and $GABA_B$ receptors [34, 35].

It has been often stated that seizures induced by PTZ can be blocked by drugs that reduce T-type Ca^{2+} currents such as ethosuximide [36] and drugs that enhance gamma aminobutyric acid type A($GABA_A$) receptor-mediated inhibitory neuro-transmission such as benzodiazepines and Phenobarbital [37, 38]. Moreover, activation of N-methyl-D- aspartate (NMDA) receptor appear to be involved in the initiation and generalization of the PTZ-induced seizures [39, 40] whereas Valproic acid has broad action that acts on all the receptors [41].

Table 4. Effect of Glimepiride in healthy albino rats by PTZ induced convulsion method

Drug treatment	Onset of clonic convulsion (sec)	% prolongation of onset of clonic convulsion (sec)	No. of convulsion	Nature and severity	Recovery
PTZ (80 mg/kg, *i.p.*)	237.5 ± 4.21	-	3.57 ± 0.007	Straub's tail, continuous jerky movements	0%
Divalproex sodium (13 mg/kg, *p.o.*) + PTZ (80 mg/kg, *i.p.*)	339.2±16.04 ***	57.18%	5.39 ± 0.027	Interrupted Jerky movements	100%
Glimepiride 0.78 mg/kg, *p.o.*) + Divalproex sodium (13 mg.kg, *p.o.*) + PTZ (80 mg/kg, *i.p.*)	289.8 ±15.41**	49.6%	5.57 ± 0.026	Less Jerky movements	100%

n= number of animal per group is 6, All data are expressed in mean ± SEM, Statistical significance at ***P<0.0001† 0.01 compared to Divalproex sodium only

CONCLUSION

Both epilepsy and diabetes are managed clinically by administering numbers of drugs for long duration. Hence, polypharmacy are of wide concern in drug-drug interaction which are important cause of adverse drug reaction and may lead to increased risk of hospitalization and higher increase care cost. Cytochrome P_{450} enzymes are inhibited or induced by many drugs, resulting in clinically significant drug-drug interactions that can cause unanticipated adverse reactions or therapeutic failures. Divalproex sodium is substrate for CYP2C9 and inhibits drugs that are metabolized by this enzyme [24]. Glimepiride is metabolized by CYP2C9 enzyme system [23]. It was observed that co-administration of high doses of Valproic acid with drugs that are primarily metabolized by CYP2C9 may result in significant drug interactions [24].

The present study concluded that Glimepiride treatment for 7 days showed significant change in the pharmacokinetic parameters of Divalproex sodium like AUC, AUMC, $t_{1/2}$, C_{max}, T_{max} and MRT in healthy albino rabbits. Glimepiride treatment showed significant decrease in duration of hind limb extensor time of Divalproex sodium tested by MES induced test and increase in latency period of chronic convulsion in PTZ induced convulsion test in healthy albino rat. Glimepiride has significantly enhanced the anticonvulsant activity of Divalproex sodium in healthy rabbit.

Clinicians should be aware of the potential interactions and get familiar with the substrate, inhibitors, and inducers of the common enzymatic pathways responsible for the drug metabolism. Hence, it is suggested that the duration and frequency of Divalproex sodium has to be readjusted when both drugs are used together.

LIMITATIONS

This experimental research was only conducted in small number of animals but not in the healthy volunteers who has suffered from the epilepsy and diabetes. So, more precise information can be gathered in clinical studies to confirm drug-drug interaction, should be done in diseased patients.

RECOMMENDATION

The research into the metabolic process that leads to many of the clinically relevant drug interaction should be continued.

The study of drug interactions should be screened in vitro.

The role of genetic polymorphism of the drug metabolizing enzymes should be determined that how individuals respond to certain drug combinations.

COMPETING INTEREST

The authors declared that they have no competing interests concerning the work reported in this paper.

AUTHORS CONTRIBUTION

SS- Designed and performed experimental procedure, data collection and involved in writing the first draft of the manuscript.

SP- Designed the concept of study, involved in writing and reviewing the first draft of the manuscript as well as decisive revision of the final manuscript.

RKY- Involved in scripting the first draft of manuscript.

KY- Involved in inscription of the first draft of the manuscript and data analysis.

NCN- Designed the core concept of the study and revision of the manuscript.

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