Research Article

Effect of Felodipine against Pilocarpine induced Seizures in Rats

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ABSTRACT

Epilepsy is a standout amongst the most well-known genuine cerebrum issue, can happen at all ages. The examination was performed to investigate the conceivable antiepileptic impact of Felodipine against pilocarpine prompted seizure in male rats. The investigation did on forty male Wister rats similarly assigned to four gathering: (1) typical gathering (not got any medication). Gathering (2) negative control gathering (got just pilocarpine amid acceptance of seizure. Gathering (3) positive control gathering (Valproic corrosive gathering got 20 mg/kg orally twice every day). Gathering (4) Felodipine gathering (1 mg/kg got orally once every day). Rats of each gathering (aside from typical gathering) were infused intraperitoneal with pilocarpine hydrochloride (400 mg/kg) following 21 long stretches of tried medications organization orally. The mean beginning and term of seizure were resolved to assess the viability of tried medications and to contrast these impact and that of typical gathering and Valproic corrosive gathering. Additionally, neuroprotective impact (Neu N), NMDA receptor, Sodium diverts were estimated in all gatherings. Felodipine had a preventive and anticonvulsant impact against pilocarpine actuated seizure in rats.

Keywords: Epilepsy, Felodipine, Neuroprotective effect and Pilocarpine hydrochloride.

INTRODUCTION

Epilepsy and seizures issue influence 50 million individuals around the globe and add to dismalness and mortality[1]. The utilization of antiepileptic drugs is constrained because of the huge swath of unfavorable impacts. For example, psychological debilitation, powerful clutters and repeating seizures[2]. Henceforth, there is a requirement for the improvement of new antiepileptic drugs with less unfavorable impacts and high efficacy. Felodipine is a calcium channel blocker follow up on various calcium diverts are available in vascular and cardiovascular tissue[3], Felodipine has also been found to go about as an adversary of the mineralocorticoid receptor, or as an antimineralocorticoid[4]. This investigation meant to assess the conceivable antiseizure impact of Felodipine in avoidance of pilocarpine instigated seizure in rats and to investigate conceivable system of activity of medications in immunofluorescent recoloring of NeuN, Nav1.6 and NMDA receptor in rodent cerebrum tissue segments.

MATERIALS AND METHODS

Animals

40 male Wister rats (200– 300 g) were acquired from the Animal house at the College of Medicine/AL-Nahrain University in Baghdad. The creatures were housed under temperature, mugginess and light-controlled conditions. Every creature convention were affirmed by the Institutional Review Board at the College of Medicine/AL-Nahrain University. In addition, they were nourished standard oxide sense of taste with water not indispensable.

Pilocarpine-induced status epilepticus

The Pilocarpine-prompted status epilepticus methods were executed by Glien *et al* method [5]. Status epilepticus was characterized as a period of constant seizures that went on for no less than 5 min or seizures that repeated at short interims (<1 min) building up a persevering epileptiform condition[6]. Every single consequent analysis were performed in the intense period of pilocarpine-instigated epilepsy. Seizures (summed up limbic seizures with status epilepticus) were prompted by a solitary intraperitoneal organization of pilocarpine hydrochloride 4% (400 mg/kg) [7].

Pretreatment experiments

Four gatherings of rats were utilized for the pretreatment tries, each gathering contain 10 rats were taken standard and Felodipine for 21 days orally.

Gathering 1

(Normal gathering): This gathering not got any medication was filled in as typical control gathering to identify the ordinary qualities.

Gathering 2

(Pilocarpine initiated epilepsy aggregate just): were taken pilocarpine infusion intraperitoneal (400mg/kg) considered as epileptic control.

Gathering 3 (Positive group)

(Valproic gathering): They were taken 20 mg/kg twice day by day of sodium valproate orally before pilocarpine infusion. This gathering filled in as positive control to think about tried gatherings.

Gathering 4

(Felodipine gathering): They were taken Felodipine 1 mg/kg/day orally before pilocarpine infusion

Parameters

In the wake of giving the pilocarpine, each rodent was precisely assessed by distinguishing the beginning of the principal seizure, length of seizure, repetitive of seizures and passing, recorded by stripped eyes ,different parameters incorporate the sodium current through sodium channels, movement of NMDA receptors, Neuro N (measure of neural cell demise).

Immunohistochemical recoloring of NeuN, NMDA and Nav1.6

Paraffin inserted areas were cut into 5µm thickness on decidedly charged slides. Subsequent to dewaxing and rehydration of tissue segment achieved through inundating of slides in consecutive weakening's of ethanol took after by refined water for 5 minutes. Blocking step performed by including Peroxidase Square at that point washed and includes protein hindering of Non-particular official of essential counter acting agent. One hundred microliter of weakened essential immunizer (table 1) added to each segment independently and brooded in damp chamber for an hour.

Table 1: Antibodies used in the study

No.	Item name	Manufacturer company	Quantity	Catalogue No.	Host	Target	Clonality
1	**NeuN antibody**	Biorbyt (UK)	100 µg	**orb11112**	Rabbit	Rat	Polyclonal
2	**Nav1.6 antibody**	Biorbyt (UK)	100 µg	**orb101715**	Rabbit	Rat	Polyclonal
3	**NMDAR2B**	Biorbyt (UK)	100 µg	**orb6540**	Rabbit	Rat	Polyclonal
4	**Super Sensitive IHC Detection System Kit (Mouse/Rabbit)**	**Biorbyt (UK)**	**1 kit**	**orb219874**	-	**Rabbit and mouse**	-

Subsequent to washing with PBS, Fifty µl of the counter acting agent speaker (optional immune response) connected onto the areas at that point brooded at 37ºC for 30 minutes. In the wake of washing, fifty µl of HRP polymer was set onto the tissue segment and hatched for 30 minutes at 37˚C in muggy chamber. Fifty µl of the DAB-substrate chromogen was put onto the tissue area and hatched for 5 minutes at 37˚C in muggy chamber, and afterward slides were flushed in refined water, depleted and blotched delicately. Slide counterstained with Mayer's Hematoxylin for 1 minute. At that point, washed three times in refined water, 1 minute every; at that point depleted and smudged delicately. Slides were dried out by putting them in Ethanol and Xylene then a drop of mounting media put onto segment and the tissue area was immediately secured with cover slip and slides were left to dry. Negative control was incorporated for each kept running of immunohistochemistry. The slide was not permitted to dry in any progression of the immunostaining.

Assessment of the Immunostaining

Assessment of IHC comes about for performed by light magnifying lens (Genex 20, America) at 40X target focal point with add up to intensity of amplification 400X. The commonplace aftereffects of immunochemical recoloring found in entorhinal cortex (EC). All outcomes considered a relative level of positive cells recolored with dim darker shading out of aggregate check of positive and negative cells (9).

Measurable examination

The measurable examinations were performed with GraphPad Prism® 7.0e (USA). The qualities are introduced as the mean ± standard deviation of the mean. The information were examined utilizing one-route examination of change (ANOVA) trailed by a LSD Post Hoc to recognize noteworthy contrasts between tried medication gather with every typical control, negative control and positive control gathering. The extents of recurrence of seizure and death rate were portrayed as check and rate. Factual hugeness was characterized as $P \leq 0.05$.

RESULTS

1-onset of seizure

After pilocarpine injection, the mean onset of convulsion of Felodipine group was (22.9±5.72) minutes.

Table 2: Effect of Felodipine on Onset of Seizure compared with Negative control and Valproic acid in Pilocarpine induced Seizure in rats (n= 10/group).

	Negative	Positive	Felodipine
Onset of seizure	9.6±2.12	27±8.11	22.9±5.72
Negative		<0.001**	<0.001**
Positive			0.641^{NS}

Data presented as Mean±SD, NS: None statistical significant difference (p>0.05); *: Statistical significant difference (p≤0.05); **: Highly statistical significant difference (p≤0.001).

According onset of seizure in present study, Felodipine has highly significant difference (p≤0.001) when compared with negative control whereas none significant difference (p= 0.641) and nearly comparable when compared with valproic acid.

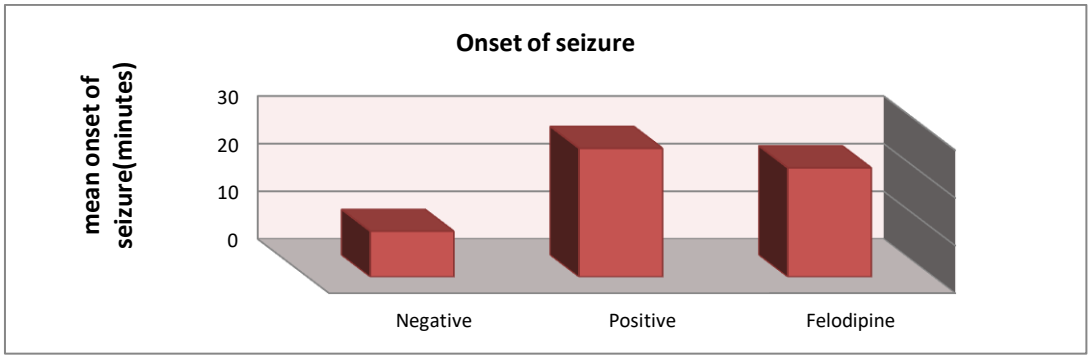


Figure 1: Effect of Felodipine on Onset of Seizure compared with Negative control and Valproic acid in Pilocarpine induced Seizure in rats (n= 10/group).

2-Duration of seizure

After pilocarpine injection, the mean duration of convulsion of Felodipine group was (8.4±1.43) seconds.

Table 3: Effect of Felodipine on duration of Seizure compared with Negative control and Valproic acid in Pilocarpine induced Seizure in rats (n= 10/group).

	Negative	Positive	Felodipine
Duration of seizure	26.5±3.5	9.3±2.21	8.4±1.43
Negative		<0.001**	<0.001**
Positive			0.948 ^{NS}

Data presented as Mean±SD, NS: None statistical significant difference (p>0.05); *: Statistical significant difference (p≤0.05); **: Highly statistical significant difference (p≤0.001).

According duration of seizure in our study, Felodipine has highly significant difference (p≤0.001) when compared with negative control whereas none significant difference (p>0.05) when compared with valproic acid.

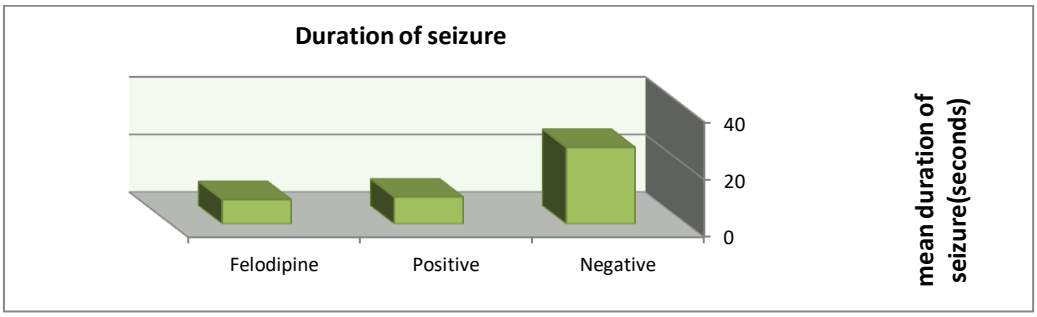


Figure 2: Effect of Felodipine on duration of Seizure compared with Negative control and Valproic acid in Pilocarpine induced Seizure in rats (n= 10/group).

3-Neu N:

After pilocarpine injection, the mean of Neu N of Felodipine group after convulsion was (69.6±5.1%).

Table 4: Effect of Felodipine on neuron cell compared with normal control, Negative control and Valproic acid in Pilocarpine induced Seizure in rats (n= 10/group).

	Normal	Negative	Positive	Felodipine
Neu N	100±0	62.1±8.54	83.5±6.64	69.6±5.1
Normal		<0.001**	<0.001**	<0.001**
Negative			<0.001**	0.099 ^{NS}
Positive				<0.001**

Data presented as Mean±SD, NS: None statistical significant difference (p>0.05); *: Statistical significant difference (p≤0.05); **: Highly statistical significant difference (p≤0.001).

According Neuron antigen in our study, Felodipine has highly significant difference (p≤0.001) when compared with normal control and positive control whereas none significant difference (p>0.05) when compared with negative control.

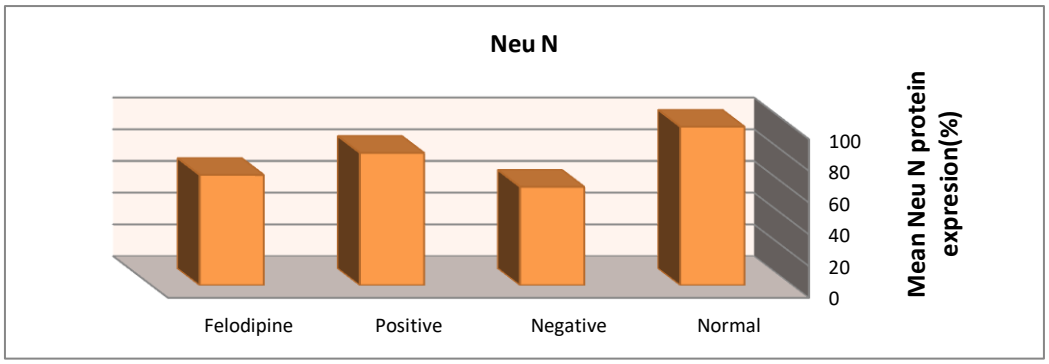
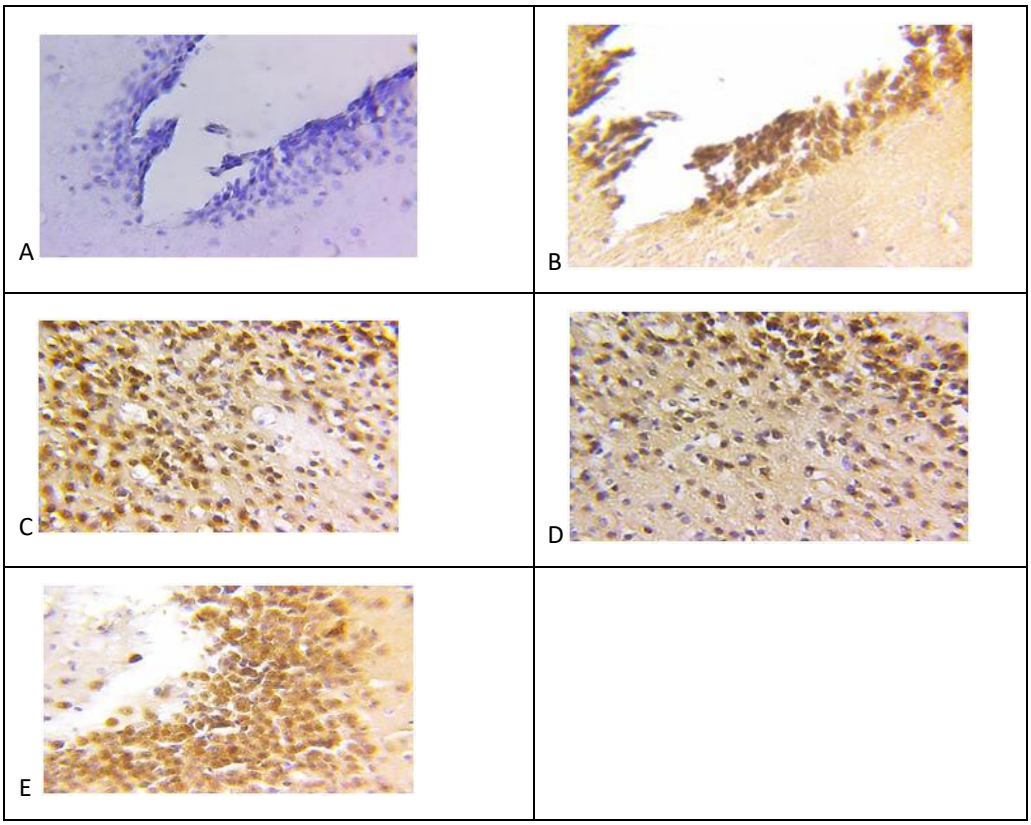


Figure 3: Effect of Felodipine on neuron cell compared with normal control, Negative control and Valproic acid in Pilocarpine induced Seizure in rats (n= 10/group).



Picture 1: Showing Neu N staining using Rabbit polyclonal anti- Neu N diluted as 10pg/ml visualized by peroxidase conjugate enzyme. (A). IHC quality control (staining without adding primary antibody) showing negative results. (B). Normal rat (without treatment or induction) showing staining of all neuronal cells with intense dark brown color. (C). Negative control (induction only), (D). Positive control (treated with valproic acid), (E). Felodipine treated.

4-NMDA receptors

After pilocarpine injection, the mean of NMDA of Felodipine group after convulsion was (7.1±2.51%).

Table 5: Effect of Felodipine on NMDA compared with normal control, Negative control and Valproic acid in Pilocarpine induced Seizure in rats (n= 10/group).

	Normal	Negative	Positive	Felodipine
NMDAR	3.9±1.2	33.4±9.5	9.8±4.69	7.1±2.51
Normal		<0.001**	0.132^{NS}	0.783^{NS}
Negative			<0.001**	<0.001**
Positive				0.870^{NS}

Data presented as Mean±SD, NS: None statistical significant difference (p>0.05).

*: Statistical significant difference (p≤0.05).

**: Highly statistical significant difference (p≤0.001).

According NMDA in present study, Felodipine has none significant difference (p≤0.001) when compared with normal control and valproic acid whereas highly significant difference (p>0.05) when compared with negative control.

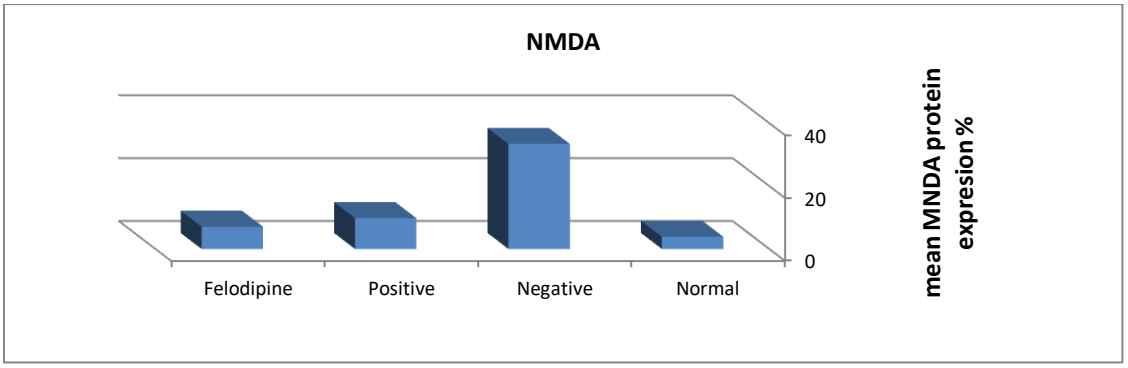
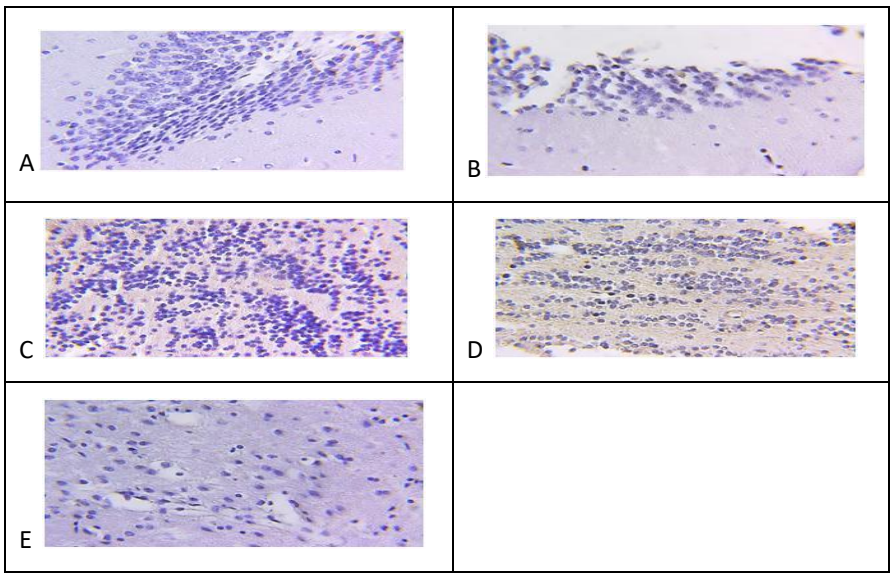


Figure 4: Effect of Felodipine on NMDA compared with normal control, Negative control and Valproic acid in Pilocarpine induced Seizure in rats (n= 10/group).



Picture 2: Showing NMDA receptor staining using Rabbit polyclonal anti- NMDA antibody diluted as 10pg/ml visualized by peroxidase conjugate enzyme. (A). IHC quality control (staining without adding primary antibody) showing negative results. (B). Normal rat (without treatment or induction) showing staining of all neuronal cells with intense dark brown color. (C). Negative control (induction only), (D). Positive control (treated with valproic acid), (E). Felodipine treated.

5-Nav 1.6

After pilocarpine injection, the mean of Nav1.6 of Felodipine group after convulsion was (11±4.4%)

Table 6: Effect of Felodipine on Nav1.6 compared with normal control, Negative control and Valproic acid in Pilocarpine induced Seizure in rats (n= 10/group).

	Normal	Negative	Positive	Felodipine
Nav1.6	2.1±1.29	28.5±7.25	10.4±4.12	11±4.4
Normal		<0.001**	0.003*	<0.001**
Negative			<0.001**	<0.001**
Positive				0.999 ^{NS}

Data presented as Mean±SD, NS: None statistical significant difference (p>0.05).

*: Statistical significant difference (p≤0.05).

**: Highly statistical significant difference (p≤0.001).

According Nav1.6 in present study, Felodipine has none significant difference (p≤0.001) when compared with valproic acid whereas highly significant difference (p>0.05) when compared with negative control and normal control.

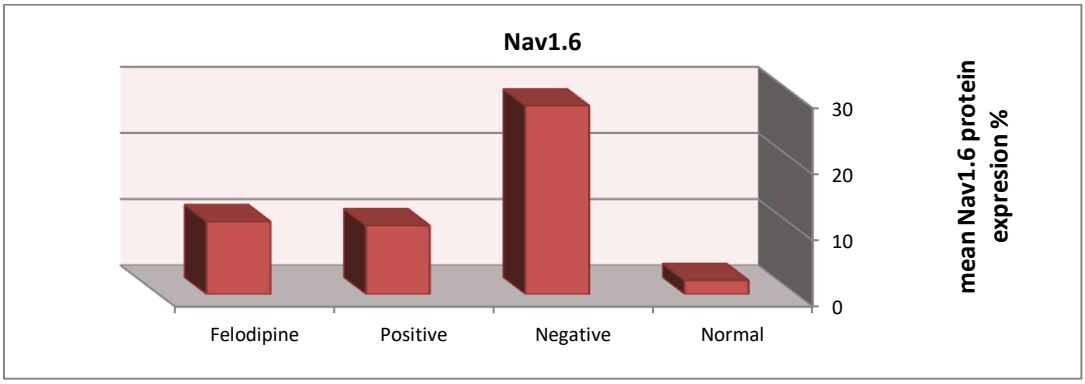
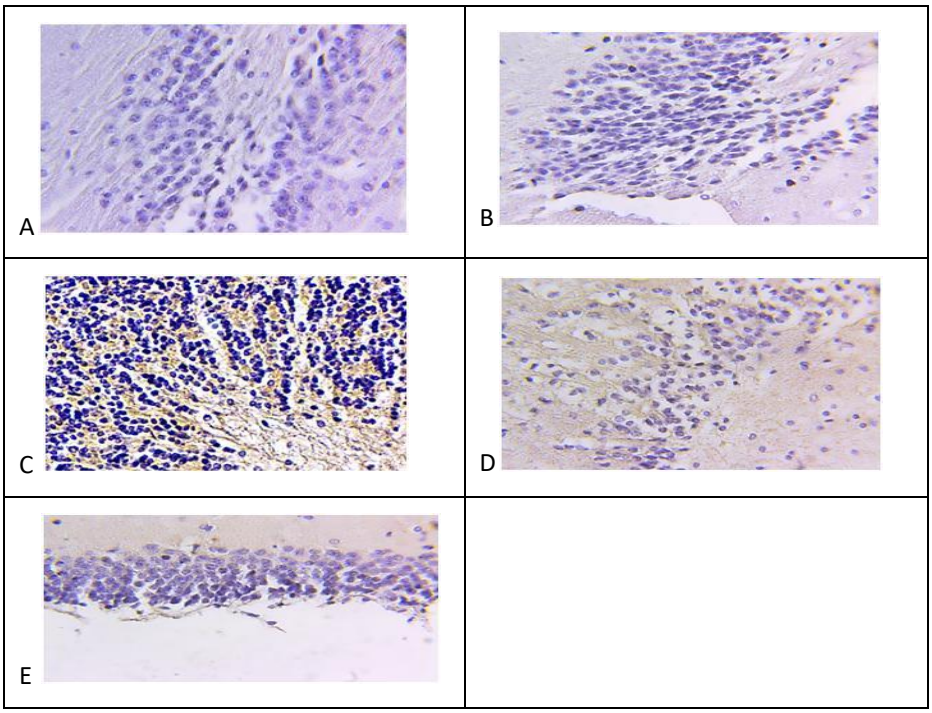


Figure 5: Effect of Felodipine on Nav1.6 compared with normal control, Negative control and Valproic acid in Pilocarpine induced Seizure in rats (n= 10/group).



Picture 3: Showing Nav 1.6 receptor staining using Rabbit polyclonal anti- Nav 1.6 antibody diluted as 10pg/ml visualized by peroxidase conjugate enzyme. (A). IHC quality control (staining without adding primary antibody) showing negative results. (B). Normal rat (without treatment or induction) showing staining of all neuronal cells with intense dark brown color. (C). Negative control (induction only), (D). Positive control (treated with valproic acid) (F). Felodipine treated.

DISCUSSION

Epilepsy is a standout amongst the most widely recognized neurological issues everywhere throughout the world, being related with paroxysmal release of cerebral neurons and is portrayed by a few side effects including modifications of practices and cognizance managed change in cerebrum work[10]. The pilocarpine gives a helpful creature model to considering instruments and remedial ways to deal with epilepsy. In this model, exorbitant and maintained incitement of cholinergic receptors can prompt seizure-related cerebrum harm in rodents[11]. In the present examination, Felodipine showed anticonvulsant movement as confirm by its capacity to drag out the beginning and decline the seriousness of seizures delivered by pilocarpine in rats by affecting the Na diverts and NMDA receptor what's more of the calcium channels blockage by utilizing histoimmunochemistry strategies, this information concur with Sai-roughage Yiu, *et al* [12] announced that Felodipine gave security against maximal electroshock-incited seizures in mice. They trusted that the Felodipine was add to the direct anticonvulsant action. Information announced by Umukoro *et al*[13] demonstrated that the Ca channels blockers showed anticonvulsant movement as prove by its capacity to delay the beginning of seizures created by strychnine in mice by hindering of the calcium channels to keep the expansion in intracellular calcium which assume an imperative part in frequency of specific sorts of seizures. Also, information detailed by El-Azab and Moustafa [14] demonstrated that calcium channel adversary improved the anticonvulsive movement of valproate in pentylenetetrazole-ignited mice. Asadi-Pooya *et al*[15]demonstrated that calcium channel rival can be utilized as aide in treatment of antiepileptic medicate (AED) - safe patients since it is a known inhibitor of P-glycoprotein and may capacity to square P-glycoprotein–regulated efflux of antiepileptic sedates in the mind, along these lines raising the intracellular centralization of antiepileptic drugs and eventually diminishing seizure trouble in patients with stubborn epilepsy.

CONCLUSION

Felodipine has a substantial preventive impact against seizures incited by Pilocarpine in rodent which were practically identical to that of valproate corrosive.

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