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Effects of Valproat and Clonazepam on Kidney Tissue of Female Rats

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The aim of this study was to investigate the histotoxic effects of sodium valproat and clonazepam on kidney tissue. It was used which weights 270 to 300 g and 7 to 8 months Sprague Dawley female rats. Five groups were prepared for the experimentations and 0.5 mL serum physiologic was given in I Group (control), in the other groups were given 150 mg kg⁻¹ sodium valproat in II Group and 300 mg kg⁻¹ sodium valproat in III Group, 0.25 mg kg⁻¹ clonazepam in IV Group and 0.50 mg kg⁻¹ clonazepam in the V Group, respectively, as intra peritoneal at the same time for each day in the 7 days. In the kidney tissue sections, it was observed the normal structural properties in I and II Groups, distal tubular dilatations in III and IV Groups and the normal histological structures in V Group. In the II Group which was given 150 mg kg⁻¹ of sodium valproat, the structures in kidney were found as the similar to that of the control. The kidney cortex of III Group which was given 300 mg kg⁻¹ sodium valproat has been observed the dilatations in the distal tubules and the some infiltrations have been around of the glomerulus's. The epilepsies are a range of multifaceted disorders that can be affected many aspects of a person's life. No single outcome measure can reflect their complex nature and impact in the individual patient. The aim of drug treatment is the prevention of symptoms with no or tolerable side-effects. While this is possible for the majority of patients, there remains a significant proportion in whom ongoing symptoms and increasing drug burden exact a heavy toll.

Key words: Sodium, valproat, Clonazepam, kidney

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INTRODUCTION

The therapy by antiepileptic drugs has an important in the clinical applications and these have been used for a long time by the physicians. These drugs prevent the epileptic symptoms but they have not been a radical therapy. Sometimes, the epileptic drugs have been as the combinations with other drugs. The symptoms could have been controlled in 50% of the epileptic patients^[1-13]. Sodium valproate is the dipropyl acetate and the inhibitor effect of this; is similar to the GABA (gamma amino butyric acid) and this drug have been used as the commonly^[4,8,14]. The valproic acid is fatty acid and it has contained eight carbon^[5-18]. It's closed formulation is $C_8H_{15}NaO_2$. The effect of valproic acid is the central neuronal system and that is, the brain. Valproic acid has increased the effect of the GABA^[11-15]. Valproic acid is absorbed the rapidly from gastrointestinal system. But, this absorption has been the gently after the eating. Valproic acid has the ability of the binding at higher ratio to the plasma proteins (95%). Valproic acid is metabolized by P-450 system and thus activity of P-450 system is increased. It's half-life is approximately 16 h^[12-19]. The contraindications of valproic acid are the peritoneal cramps, tremor, vomit and the nausea. It has hepatotoxic potential and sometimes is caused to the hepatitis. This substance is inhibiting the beta oxidation of fatty acids by CoA in the liver and therefore it can make the ketoacidosis. Furthermore, it inhibits the urea biosynthesis and thus it is caused hyperammonemia and encephalopathy. On the other hand, it is caused the thrombocytopenia, the inhibition of the trombocyte aggregation, leucopenia and bone-marrow suppression and the pancreatitis^[1-19]. Clonazepam is another antiepileptic which is used the frequently in clinics. This drug is derivative of the benzodiazepam and it's chemical structure is 5-(12-chlorophenyl)-1,3 Dihidro-7-nitro-2 H-benzodiazepine). Clonazepam is increased in the hyperpolarisation by binding to the GABA A/C ionophore complex in post synaptic neurons^[16-19].

When it took orally, it's absorption is speedily from the intestine and it is arrive at the brain in 25 min and it is arrive at the highest concentration in plasma^[12-20]. The binding rate of plasma proteins is 85% and it's the half-life in the plasma is 22 to 33 h. This antiepileptic substance is metabolized in liver and it's metabolites is inactive^[12-20]. The contraindications of clonazepam are hyperkinesias, respiratory depression, ataxia, insensibility and anxiety.

It was met the rarely studies with related to the effects of valproic acid and clonazepam on kidney tissue. Therefore, the present study was tested on rats. The aim of this study was investigate histotoxic effects of sodium valproate and clonazepam on kidney tissue.

MATERIALS AND METHODS

Experimental animals: The present study, 25 female rats (Sprague Dawley) was used who have weights 270 to 300 g and 7 to 8 monthly. Animals were obtained from culture. The rats were placed in the metal cages in 23x32x50 cm measurements. Their ration has 88% of the crude matter, 14% of protein, 11% of cellulose, 10% of the crude ashes, the dissolved ashes in 2% HCl, 2% Ca, 1% P, 0.5-1.0% Na, 1% NaCl and vitamins A, D, and E. All of the animals were also given the tap water. The cage medium was 12 h light and the temperature of the room was approximately 32°C and the moisture rate was 50-60%.

Chemical substances used in the experiments: Sodium valproate (Depakin, Sanafi Doğu İlaç A.Ş.); Clonazepam (Rivotril, Roche); serum physiologic; Thiopental sodium (Abbott); Nötral formalin (Merck); Ethanol (Merck); Paraffin (Merck), Eozine (Merck); Thymol (Sigma), Heparin (Sigma), Entellan (Merck).

Experimental groups and the dosages applicated: The rats were divided into the five groups and there were five animals in each group. Groups and substances injected were given below:

Groups	Injected substances
I (control)	0.5 mL serum physiologic (0.9%)
II	150 mg kg ⁻¹ day ⁻¹ sodium valproate (as Depakin)
III	300 mg kg ⁻¹ day ⁻¹ sodium valproate ^[1-20]
IV	0.25 mg kg ⁻¹ day ⁻¹ Clonazepam (as Rivotril) ^[12-18]
V	0.50 mg kg ⁻¹ day ⁻¹ Clonazepam (as Rivotril) ^[12-18]

Intraperitoneal (i.p) injections were applicated at 9.30 h a.m. in each day.

Resection of the kidney: Firstly, Thiopental sodium, USP, (Pantothal® Sodium-500 mg, Abbot) was solved in serum physiologic and this anesthetic substance was injected as 0.1 mg kg⁻¹ by intraperitoneal. During anesthesia, their kidneys were resected and they were washed with the serum physiologic. Secondly, each kidney was divided into the small slices and these slices were put into 10% of the neutral formalin. Hereafter, the slices were washed with the water along the night. Thirdly, kidney slices were put into the ethanol series:

1. 30 min in 70% ethanol
2. 30 min in 80% ethanol
3. 30 min in 90% ethanol
4. 20 min in 96% ethanol
5. 15 min in 96% ethanol

After, these slices were put into the xhyllol and the paraffin:

Five each min in the xhylol (I and II series) and 60 each min in the paraffin (I, II and III series), respectively. Five microliter cross sections were prepared from the paraffinised slices by Rotary Microtom (Reichert Jung 820). These cross sections were placed on the sterile glass plate covered the albumin/glycerol and were waited 30 min in 65°C in the etuve and 2 min in the room temperature. Hereafter, the cross slices were waiting for 45 min in the xhylol and were painted with the hematoxylene and the eosin. The slices were waited for 60 min in the xhylol and these were put into the ethanol series:

1. 5 min in 96% ethanol
2. 5 min in 96% ethanol
3. 3 min in 90% ethanol
4. 3 min in 80% ethanol
5. 3 min in 70% ethanol

Other processes:

1. 5 min in distilled water
2. 1 min in the hematoxylene
3. 1 min in tap water
4. 5 min in eosin
5. 1 min in tap water
6. Dehydration in the ethanol series (70, 80 and 90%)
7. Five min in 96% of ethanol
8. 1 h in the xhylol I and II
9. The slices were covered with entallon

All of the slices were investigated by light microscope and their photographs were taken by Olympus PM 10-ADS photomicroscope.

RESULTS AND DISCUSSION

In the present study, the photographs of the kidney slices were shown in Fig. 1-6. In I Group, that is, in I Group (control) which was given serum physiologic, the bowman capsules and the tubular and medullar structures were described (Fig. 1).

In II Group (150 mg kg⁻¹ of sodium valproat), the structures in kidney were found as the similar to that of the control (Fig. 2).

The kidney cortex of III Group (300 mg kg⁻¹ sodium valproat) has observed the dilatations in the distal tubules. In addition, the proximal and the distal epithelial cells were normal structures and however some infiltrations have been observed around of the glomerulus's. The atrophy and the growing have not found in glomerulus's (Fig. 3).

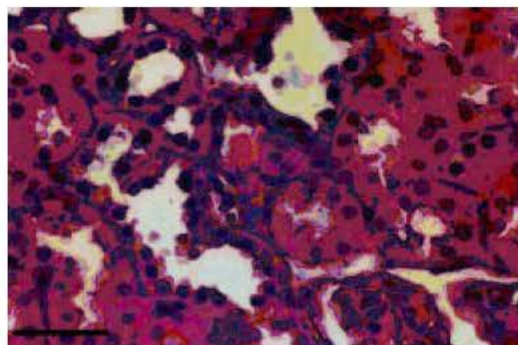


Fig. 1: The photograph to the control group (x40)

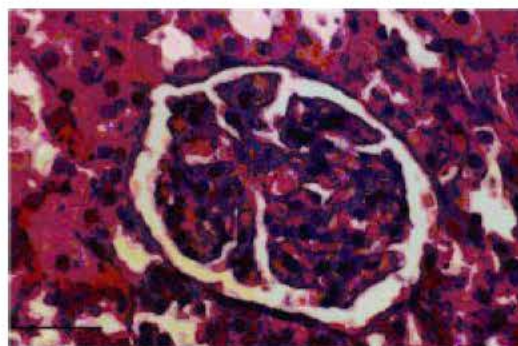


Fig. 2: The photograph of II Group given 150 mg kg⁻¹ of sodium valproat (x 40)

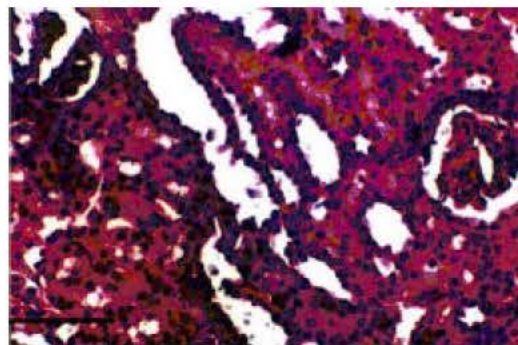


Fig. 3: Histological structure of the kidney of the rats in III Group (x20)

In the present study, the injection of 150 mg kg⁻¹ of sodium valproate has not caused whatever alteration in kidney tissues (Fig. 2). However, it was shown the minimal dilatations in the distal tubules of the kidney cortex (Fig. 3). Furthermore, the damages have not been in the proximal and the distal tubules (Fig. 4). In this area, there are a few studies and therefore amount of the references couldn't meet. In the some investigations with related to the epileptic patients, it has been found the decreasing in the kidney functions^[2-17]. In an another

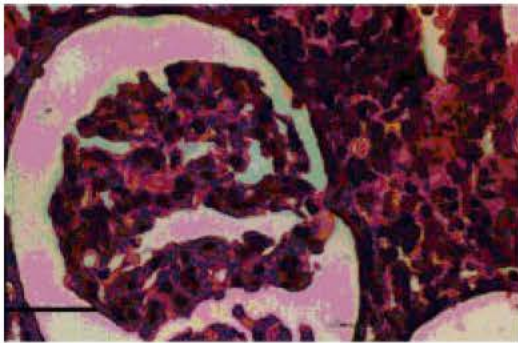


Fig. 4: The infiltration in the kidney slices of III Group (x 40)

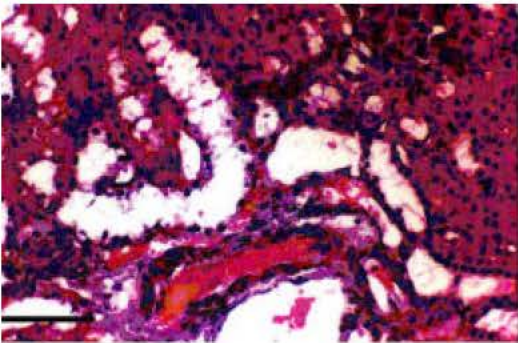


Fig. 5: The dilations in distal tubules in IV Group given 0.25 mg kg⁻¹ of clonazepam (x 20)

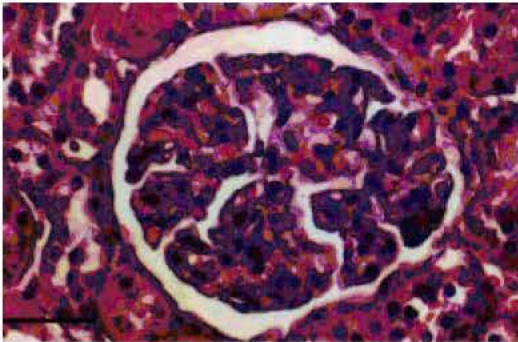


Fig. 6: The normal histological structure of V Group (x40)

study, the interstitial nephritis has recorded in the epileptic patients^[3,17,19,20]. In this study, we couldn't make the nephritis. But, the some infiltrations have been met in slices. These data can help identify patients who are likely to enter remission and those who have a more progressive symptom disorder. These studies require substantial resources and do not usually attract the same level of commercial or grant funding as regulatory or comparative trials. The basic requirement for any long-term outcome

study is that it follows routine clinical practice as closely as possible. Exclusion criteria should be kept to a minimum. Patients with newly diagnosed epilepsy differ from those with difficult-to-control seizures in terms of expected outcomes, side-effect profiles and quality of life issues. These groups should be studied separately. Patient care should not vary from normal except for closer follow up and more objective assessment of efficacy and tolerability^[7,19].

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