Study the effect of a drug depakene (Valproic acid) in the serum levels of immune proteins in albino male rats.

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Abstract
This study aimed to investigate the effect of administration of depakene (valproic acid)(VPA) (15 mg/Kg of body weight, orally) on serum protein levels, including immune proteins, because its functions and the importance of these proteins in the body and they are overlapping, and that was by measured protein levels before and after treatment as concentrations of total protein (Tp), albumin and globulin in normal male rats and in rats that treated with VPA drug along the period of experiment (30) days. The results showed a significant decrease ($P<0.05$) in concentrations of total protein and albumin in comparison with normal control group, whereas did not observe significant differences ($P>0.05$) in globulin concentration compared with normal control group, despite of, there was a little decrease in globulin concentration in rats group that administrated the drug under study but non-significant. It could be concluded of the present study that the VPA drug have an greater side effects on concentration and function of proteins in serum, and subsequently, may extending these effects to comprise its effect on the immunity, osmotic pressure, blood volume, nutrients exchange between the blood and tissues, glomerular infiltration rate, transport many of materials and hormones, and several of physiological and biochemical functions which correlated with the serum proteins.

Key words: Depakene, VPA, Total protein, Albumin, Globulin.

Introduction
Valproic acid (VPA) (2-n-propylpentanoic acid or N-dipropylacetic acid) and brand names (Depakine, Epival, Mevakine, Convulex, Valpo-eastsr, Valkine, Valponex, Valirek, Convep, Valpkine, Encorate, Dekadel , Daviken and Xoplict), molecular formula ($C_8H_{16}O_2$) is an eight carbon branched-chain fatty acid and molecular weight (166.2), it's a coordination compound between sodium VPA and its sodium salt in a 1:1 molar ratio with anticonvulsant properties [1]. VPA was first marketed as (Depakine) in France [2;3].

Ninety percent of VPA in the blood is bound to albumin with a half-life of 9 to 16 hours, and despite its hydrophilic nature enters the CNS by crossing the blood brain barrier via passive diffusion and bidirectional carrier-mediated transport, such as an anion exchanger at the brain capillary endothelium [4;5]. It has been suggested that the acute actions of anti-anticonvulsant effects of VPA involve interference with the GABA system and sodium-channels, VPA also modulates voltage gated sodium channels and voltage dependence of sodium current steady-state inactivation, resulting in reducing cellular excitability and suppressing high-frequency firing of neurons [6;7;8;9].

Valproic acid is an effective anticonvulsant which is relatively free of central nervous system side effects. It is useful in controlling a broad range of clinical seizure disorders, primarily the treatment of absence, tonic-clonic and myoclonic seizures. It is used in the management of grand mal epilepsy and petit mal epilepsy in pediatric patients, often with other adjunctive therapeutic agents. Valproic acid has also been administered under investigational conditions in the treatment of psychiatric and movement disorders, including Huntington's chorea[10]. The measurement of total protein, albumin and other proteins are refer to liver's biosynthetic capacity. The liver is the major source of most the serum proteins. The parenchymal cells are responsible for synthesis of albumin, fibrinogen and other coagulation factors and most of the a and b globulins [11].

Serum proteins are affected by capillary permeability, drugs, impaired liver function, and inflammation and a host of other factors [12;13]. The serum albumin levels tend to be normal in diseases like acute viral hepatitis, drug related hepatotoxicity and obstructive jaundice, nephrotic syndrome and chronic protein losing enteropathies [14;10]. As well as, there are several functions for serum proteins such as in transport (Transferrin transports iron, Ceruloplasmin transports copper,Transcortin transports cortisol and corticosterone, Haptoglobin transports free haemoglobin, Thyroxin binding globulin transports thyroxin and Lipoproteins transport lipids), retinol binding protein transports retinol [15;16;17]. Albumin transports fatty acids, bilirubin calcium, many drugs etc. Osmotic regulation (regulation of colloidal osmotic or oncotic pressure). Catalytic function (enzymes)(e.g lipases for removal of lipids from the blood). Protective functions (immunoglobulins, complement system, enzyme inhibitors remove enzymes, some proteins increase during acute phase and protect the body). Buffering capacity (proteins in plasma help to maintain acid-base balance) [15].

Materials and Methods
This study was conducted on uses Wistar albino male rats (*Rattus norvegicus*) of strain Sprague dawely, the weights were in (275-300)g and their ages were ranged (3-4) months. The animals were obtained from the animal house of Veterinary medicine college in Mosul University. The animals were housed according to the institutional guidelines for animal

research in propylene cages and were provided bedding of sawdust. Animal care, handling of cages and alteration of sawdust was done continually each two days. And put under standard laboratory conditions of light a 12 h light and 12 h dark as cycle, and Temperature was maintained at 22±2°C with a relative humidity of 45 ±1% and acclimated to the laboratory environment for two weeks before use. Animals had free access to sterile food (animal chow) (35% wheat, 34% corn, 20% soy-bean, 10% animalistic protein, 1% milk powder and additive 50 gm protective and antifungal substances) [18]. It's given standard food and water *ad libitum* in adequate amounts all through for the experimental period that expanded along between July/September, 2013.

Design of Experiments:

The animals were randomly selected (10) male rats and divided into two experimental groups, each group included (5) rats and take care the converging weights for animals, as follows: The normal control group: gave this group only drinking tap water and food daily for period (30) days, VPA group: this group administered orally valproic acid drug (7.14 mg/kg of body weight) by using a ball tipped stainless steel gavage attached to a syringe, daily for period (30) days.

At the end of experimental period of 4 weeks (30 days), all the animals were fasted for (24) hours, but still allowed free access to water. The animals were anesthetized by chloroform and sacrificed by severance of jugular vein. Then take approximately (3) ml of blood from each animal, put in test tubes devoid of anticoagulant, then it lets in water bath for period (15) minutes at 37°C, after this centrifuged for (15) minutes at 3000 rpm, then separate the serum for measuring the parameters under study.

The biochemical tests:

The biochemical tests included:

Determination of Total protein (Tp)concentration:

The determination of serum total protein (Tp) concentration is by enzymatic method [19], using a (kit) supplied from (BIOLABO SA,France). The final result is Colored Complex blue-violet and read the absorbance at 550 nm against blank, then Calculated the (Tp) concentration depending on the general law for (Tp).

Determination of serum Albumin concentration:

Serum albumin concentration was measured by method of [20], using a kit supplied from (BIOLABO SA, France). The final result colored compound (complex with green color) and read the absorbance at 630 nm against blank, then Calculated the albumin concentration depending on the general law to determine of albumin.

Calculated of serum Globulin concentration:

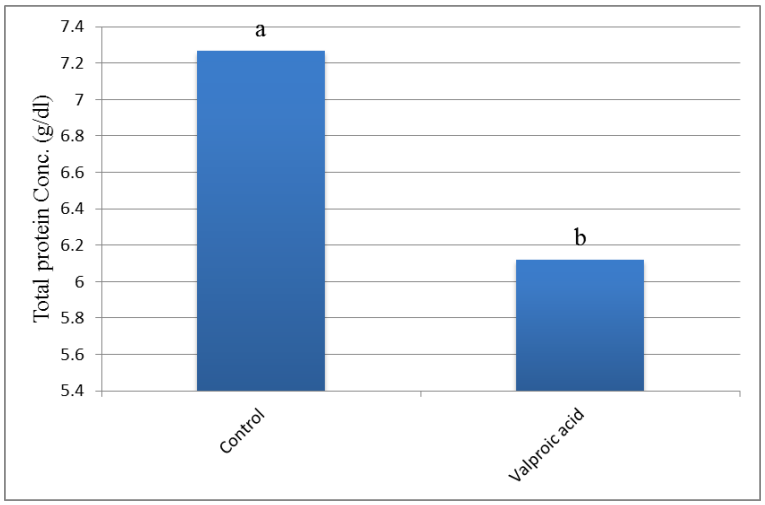
Serum globulin concentration was calculated by the following equation [21]. Concentration of globulin (g/dl) = Total protein Conc. – Albumin Conc.

Statistical analysis:

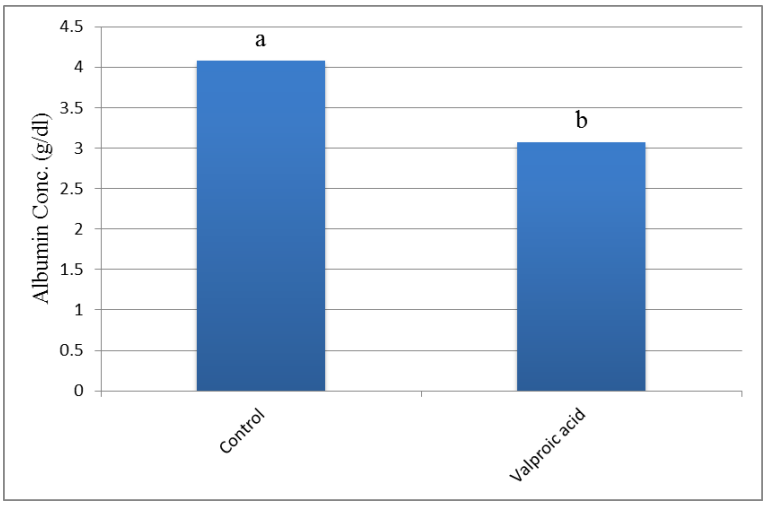
Finally, the statistical analysis was carried out by using statistical program (SAS, 2001) and Comparison between groups were made by using one-way analysis of variance (ANOVA), and tried out the arithmetic means for parameters by using test of duncun multiple range to delimitating significantly differents especially between groups. The level of statistical significance was taken at ($P<0.05$). All data are expressed as mean± standard error (M±S.D) and put above it duncun value (letters).

The Results

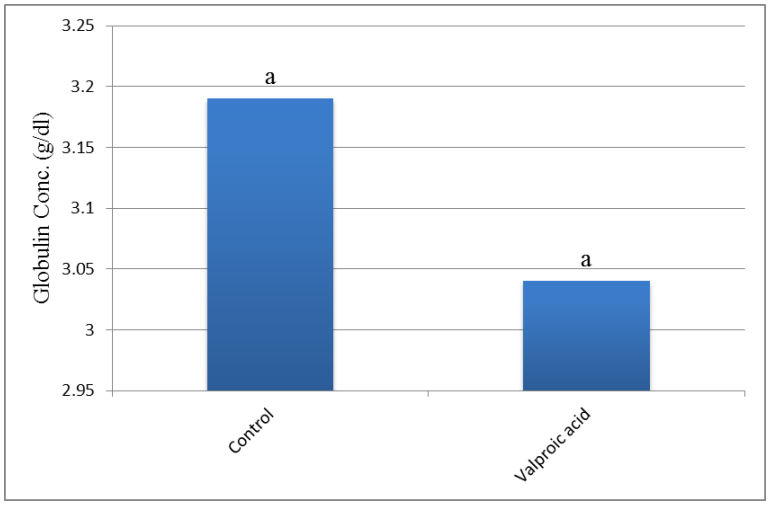
Administrated of valproic acid)(VPA) drug (15 mg/Kg of body weight) to animals group showed a significant decrease ($P<0.05$) in concentrations of total protein and albumin in comparison with normal control group, whereas did not observe significant differences ($P>0.05$) in globulin concentration compared with normal control group, or there was a little decrease in globulin concentration in rats group that administrated the drug under study but non-significant, figures (1,2,3).



Figure(1): effect of administerated Valproic acid (VPA) (7.14 mg/kg of body weight) on total protein concentration in albino male rats for (30) days.



Figure(2): effect of administerated Valproic acid (VPA) (7.14 mg/kg of body weight) on albumin concentration in albino male rats for (30) days.



Figure(3): effect of administerated Valproic acid (VPA) (7.14 mg/kg of body weight) on globulin concentration in albino male rats for (30) days.

Discussion

The present study showed a significant decrease in concentrations of total protein and albumin in the group which administration of VPA drug in comparison with normal control group. These may be refer to VPA may interfere with carnitine, thereby, lead to carnitine deficiency or abnormalities in the carnitine acyltransferase systems result in a reduced β-oxidation of fatty acids and therefore, reduce production of energy (ATP) which may using for construction of proteins [22;23], thereby, this influence may lead to decrease of total protein.

In addition to, this may be refer to administrated VPA and LEV may lead to secondary oxidation of proteins in physiological systems occurs by spontaneous autoxidation of cysteinyl thiols in proteins structure, interaction of proteins with reactive oxidizing intermediates [24].

Also, the cause for this decrease in the total protein concentration in the serum may due to that giving present medications cause some complications, including a defect in renal glomeruli and therefore a disorder in ultra-filtration of the kidney, which results in increasing the size of molecules that passing through the renal glomeruli and this leads to a loss of proteins from blood during kidney filtration process through the urine. On the other hand, oxidative stress is caused mainly by increasing lipid peroxidation and depletion of glutathione, which, in turn, induces apoptosis of renal proximal tubule cells and consequent kidney dysfunction [25], this may lead to increase elimination of proteins.

Or this may be due to the VPA and LEV may lead to disorder in liver function, any disorder in the liver leads to a defect in synthesis of total protein, albumin and other proteins that are produce in the liver because it is considered an indicator of the biosynthesis ability of the liver. Moreover, VPA probably competes with FFA for albumin binding [26], this effect lead to increase of FFA in blood and decreasing of albumin concentration. Furthermore, albumin acts as an antioxidant (0.5 and 1%) as it specifically reacts against ROS such as peroxyl radical and prevents the propagation of peroxidtive

damage in cells [27;28], this process may be cause to decrease of albumin concentration. In support of this exegesis, an decrease in albumin along with elevated liver enzymes is a more specific marker of liver dysfunction [29]. In the same situation, negative acute-phase proteins such as albumin inversely respond: their circulating levels decrease in response to injury and inflammation and increase during the recovery phase [30;31].

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دراسة تأثير عقار الديباكين Valproic acid في مستويات المصل من البروتينات المناعية في ذكور الجرذان البيض

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الملخص

استهدفت هذه الدراسة التحري ومعرفة تأثير إعطاء الديباكين (Valproic acid)(VPA) بجرعة (7.14 ملغم/كغم من وزن الجسم) عن طريق التغذية الانبوبية على مستويات البروتين في مصل الدم بضمنها البروتينات المناعية، وذلك لما يوجد من وظائف واهمية لتلك البروتينات في الجسم وبصورة متداخلة، وذلك تم من خلال قياس مستويات البروتينات قبل وبعد المعاملة كتركيز البروتين الكلي (Total protein)، الالبومين (Albumin) والكلوبيولين (Globulin) في مصل دم ذكور الجرذان البيض السليمة والمعاملة بعقار (VPA) وطيلة فترة التجربة البالغة (30) يوماً. النتائج اظهرت وجود انخفاض معنوي ($P<0.05$) في تركيزي البروتين الكلي والالبومين مقارنة مع مجموعة السيطرة السليمة. في حين لم يلاحظ فرق معنوي ($P>0.05$) في تركيز الكلوبيولين مقارنة مع مجموعة السيطرة السليمة، بالرغم ان لوحظ انخفاض قليل في تركيز الكلوبين في مجموعة الجرذان التي اعطيت العقار قيد الدراسة لكن غير معنوي. وقد استنتج من هذه الدراسة ان لعقار (VPA) تاثيرات جانبية كبيرة على تركيز ووظيفة البروتينات في مصل الدم وبالتالي قد تمتد هذه التاثيرات ليشمل تاثيرها على المناعة والضغط الازموزي وحجم الدم وتبادل المواد الغذائية بين الدم والانسجة ومعدل الترشيح الكلوي، نقل العديد من المواد والهرمونات في الدم والعديد من الوظائف الفسلجية والكيموحيوية المرتبطة بالبروتينات في مصل الدم.

الكلمات المفتاحية: الديباكين، البروتين الكلي، الالبومين، الكلوبيولين.