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Effects of Valproic acid drug on Hepatic tissues in female mice

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Abstract Valproic acid is one of many prescribed drugs that used to control seizures in people with epilepsy and may cause degrees of hepatic toxicity. This study intended to research the histological changes that may happen in liver in relationship with delayed organization and overdose treatment of valproic acid. 35 female albino mice were used, they were divided into four groups, group (I) included 5 animals and served as control group, group (II) included 10 animals and received gradually doses of valproic acid for ten weeks , starting with (15mg/kg/day) as minimum recommended dose and ending with (60mg/kg/day) as maximum recommended dose, group (III) included 10 animals and received overdose (120mg/kg/day) for one week, group (IV) included 10 animals and treated exactly as group (II) then the animals were left for recovery for three weeks. The animals were sacrificed at the end of each period and the liver was extracted. The valproic acid incited numerous histological changes in liver included inflammatory cells, necrosis, hydropic degenerations, angiectasis, accumulation of kupffer cells and pigments, cytological changes and fibers deposition. It can be concluded that valproic acid causes variable degrees of hepatic injuries and the impact was time related. Incomplete recovery was recorded after stopping the drug administration.
Key words: Valproic acid – Epilepsy – Liver – Mice – Tissue.

تأثيرات عقار حامض الفالبرويك على الانسجة الكبدية في اناث الفئران

الناجي عمر مسعود النقاز و *طارق عبد الله قصيبات و ام العز عبد الجليل الطرشاني و خالدة رشيد السراج و امال ابراهيم الترهوني
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الملخص حامض الفالبرويك يعتبر أحد العقاقير العديدة المستخدمة في علاج نوبات الصرع ومن الممكن ان يسبب تسمم للكبد، هدفت هذه الدراسة لمعرفة مدى تأثير هذا العقار على انسجة الكبد باستخدام الجرعات على المدى البعيد والجرعة القصوى. حيث استعملت في هذه الدراسة 35 من اناث الفئران قسمت الى 4 مجاميع الاولى (الضابطة) واشتملت 5 حيوانات، الثانية (المعاملة) واشتملت على 10 حيوانات في كل منها واعطيت جميعها جرعات تصاعدية يومية من العقار تبدأ ب 15 ملجم/كجم وتنتهي ب 60 ملجم/كجم لمدة 10 اسابيع، بينما المجموعة الثالثة (الجرعة المفرطة) اشتملت 10 حيوانات ايضا واعطيت جرعة قصوى 120ملجم/ كجم لمدة اسبوع، المجموعة الرابعة (التعافي) اشتملت 10 حيوانات وعوملت مثل المجموعه الثانية من حيث الجرعة ثم اوقفت الجرعة وتركت للتعافي مدة 3 اسابيع. مع انتهاء التجريع تم تشريح الفئران واستخرجت الكبد، أحدث العقار العديد من التغيرات النسيجية اشتملت على ظهور الخلايا الالتهابية، تنخر خلوي، تحلل مائي، احتقان وتوسع الاوعية الدموية، تراكم الخلايا البلعمية، البقع الصبغية، التغيرات الخلوية وترسبات الالياف. من ذلك نستنتج ان للعقار تأثيرات متعددة على انسجة الكبد وتكون مرتبطة بمدة التجريع، مع عدم وجود تعافي كامل بعد مرور 3 اسابيع من ايقاف التجريع.
الكلمات المفتاحية: حامض الفالبرويك – الصرع – الكبد – الفئران – النسيج.

1. Introduction:

Valproic acid is one of many prescribed drugs that used to control seizures in people with epilepsy, such as: Phenytoin, Clonazepam and Phenobarbital [1].This drug can be used not only for epilepsy, but also in many other conditions such as: some psychiatric conditions, mood disorders, bipolar depression and migraine [2]. All anticonvulsant medications are associated with adverse effects which may significantly impact on quality of life such as: sedation, tiredness, dizziness, ataxia, tremor, slurred speech, confusion, decreased coordination, dry mouth, nausea and diarrhea [3].

Therapy with valproic acid has been associated with hepatotoxicity, either reversible hepatic dysfunction or irreversible hepatic failure [4]. A short time after the drug was introduced, cases of fulminant liver failure in patients treated with valproic acid were reported [1]. One principal cause of liver failure that is associated with valproic acid therapy is most probably the inhibition of hepatic mitochondrial β-oxidation. Microvesicular steatosis of the liver, one of the most important histological findings in valproic acid-induced liver failure [1,5-7] and may be caused by impaired hepatic β-oxidation [8,9]. In study by scientists [10] have found that, the liver showed focal areas of degeneration and recent liver cell necrosis, with an accompanying inflammatory reaction of adjacent parenchyma. Prominent microvesicular steatosis was present but without specific localization. Since 1978 a

number of reports have indicated a possible association between sodium valproate therapy and the occurrence of hepatitis [11-13].

2. Materials and methods:

2.1. Experimental animals:

35 adult female albino mice species weighing 22-26 g were used in this experiment and were housed under identical conditions, at room temperature.

2.2. Experimental design:

The animals were divided into the following groups:

Group (I) (Control): this group included 5 animals, which were received normal saline orally.

Group (II): included 10 animals and received therapeutic dose of valproic acid (Sanofi Aventis company: France: Sodium valproate syrup). The dose was adjusted for mice according to the formula of [14,15], and was found to be 15 mg/kg/day. The drug was administered orally by using the original gastric tube with a syringe needle head [16]. Animals in this group were given a daily therapeutic dose of valproic acid, starting with 15 mg/kg/day and increased gradually to end with 60 mg/kg /day as following: (15 mg/kg/day for one week – 30 mg/kg/day for one week – 45 mg/kg/day for two weeks – 60 mg/kg/day for six weeks).

Group (III): This group included 10 animals, and received daily overdose of valproic acid (twice the maximum recommended dose) (120 mg/kg/day) for one week.

Group (IV): The animals of this group were treated exactly as group (II), then the drug intake was stopped and the animals were left for recovery for three weeks.

2.3. Histological examination:

Animals of all the above groups were sacrificed following mild diethyl ether anesthesia and the liver tissue was excised (from each animal rapidly) and immediately fixed in 10% neutral phosphate-buffer formalin and processed for paraffin method [17], stained by different stains as following:

I- Hematoxylin and Eosin stains [18,17]: to demonstrate the general histological structure of liver.

II- Masson's Technique [19]: to demonstrate collagen fibers.

III- Silver Nitrate Technique [20]: to demonstrate reticular and collagen fibers.

Chemicals used in this study from BDH Chemical, Ltd., Poole, England.

After staining, the specimens were examined under a CARL ZEISS research microscope and photographed with a digital camera attached.

3. Results:

Group (I) (Control):

Haematoxylin and Eosin stained sections of liver taken from control group, showed normal architecture of the liver tissue with normal appearance of lobules, hepatocytes and central veins (Figs.1&2). Sections stained with Masson's trichrome, showed normal distribution of fine collagen fibers surrounding hepatocytes and central veins (Fig.3). Regarding Silver nitrate stained sections, the liver tissue showed normal distribution of reticular fibers (Fig.4).

Group (II) (10 weeks of treatments):

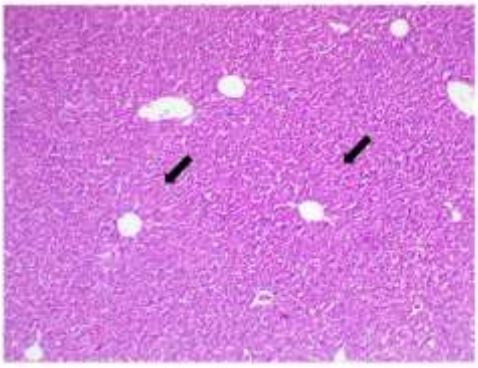
Haematoxylin and Eosin stained sections showed large focal areas of inflammatory cells associated either with central veins which known as pericentrilobular necrosis (Fig.5) or within the parenchymal tissue (Fig.6). In addition, small focal areas of cellular necrosis were also observed in association with some central veins (Fig.7), while other necrotic cells were dispersed throughout the liver sections (Fig.8). More over extensive angiectasis. Congested blood sinusoid were seen in some lobules
(Fig.9), in addition to extensive hydropic degeneration (Figs.10,11) and congested central veins (Fig.12). Sections stained with Masson's trichrome showed high deposition of collagen fibers surrounding many hepatocytes as well as around some central veins (Fig.13). In addition some sections showed collagen deposition extending between two branches of portal vein (Fig.14). Regarding Silver nitrate stain, the liver tissue showed mild to high deposition of collagen and reticular fibers associated with central vein and hepatocytes (Fig.15) and extending between two branches of portal vein (Fig. 16).

Group (III) (Overdose treatment):

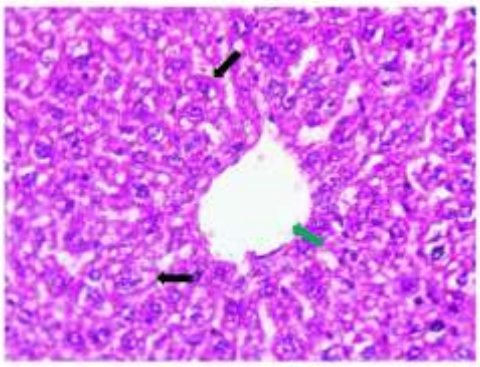
The results of this group almost similar to that observed in group II, but much more extensive (Figs.17 & 18) in addition there were a prominent large necrotic areas of hepatocytes that have been seen at the periphery of one side or more of the tissue section, with both Haematoxylin and Eosin and Silver nitrate stains (Figs. 19&20). High number of kupffer cells which were filled with golden brown pigments were also prominent throughout the parenchymal tissue (Fig.21), in addition to nuclear changes i.e. pleomorphism (Fig.22). High collagen deposition was observed extending between two branches of portal vein with both Silver nitrate and Masson's trichrome stains (Figs.23&24).

Group (IV) (Recovery):

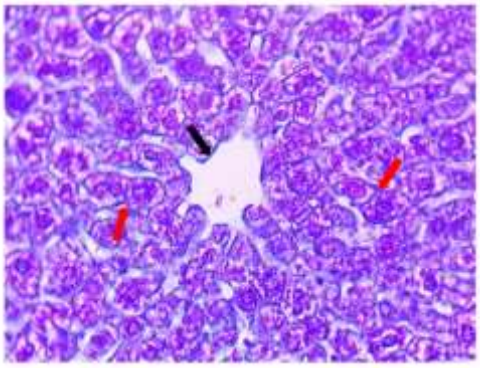
Haematoxylin and Eosin stained sections showed small focal areas of inflammatory cells either associated with the central veins and within the parenchyma (Fig.25). While cytoplasmic and nuclear changes still persist but much less than group II (Figs.26&27). Sections stained with Masson's trichrome, still showed mild deposition of collagen fibers around some central veins and between some hepatocytes (Fig.28).



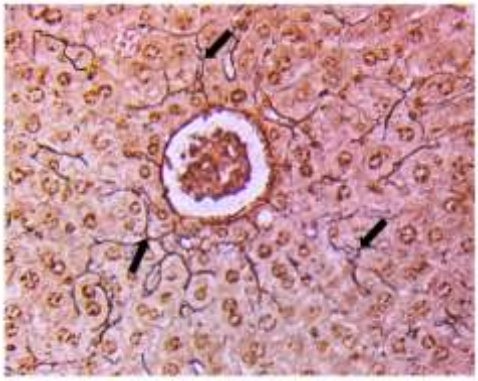
(Fig 1): Photograph of liver tissue (control group) showing normal appearance of lobules (arrows) (H & E X 100).



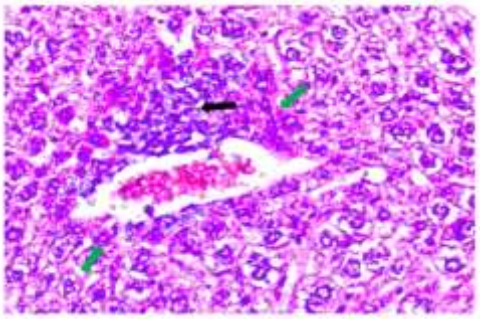
(Fig 2): photograph of liver tissue (control group) showing hepatocytes (black) & central vein (green) (H&E X400).



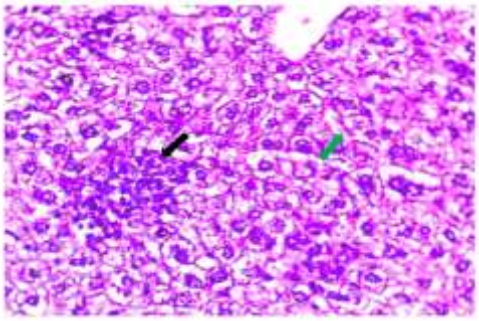
(Fig 3) : Photograph of liver tissue (control group) showing fine collagen fibers surrounding hepatocytes (red) & central vein (black) (Masson's trichrome X 400).



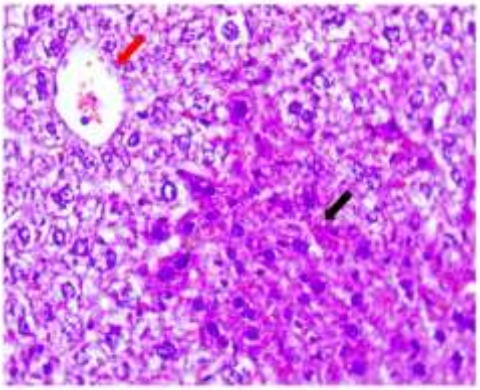
(Fig 4) : Photograph of liver tissue (control group) showing normal distribution of reticular fibers (arrows) (Silver nitrate X 400).



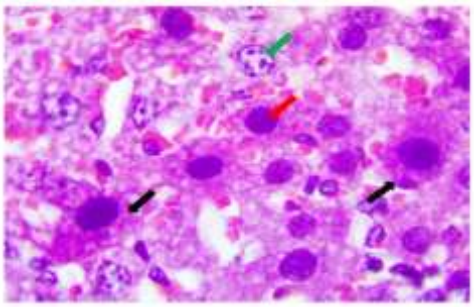
(Fig 5) : Photograph of liver tissue (10 weeks of treatment) showing large focal area of inflammatory cells (black) & pericentrilobular necrotic hepatocytes (green) (H & E X 400).



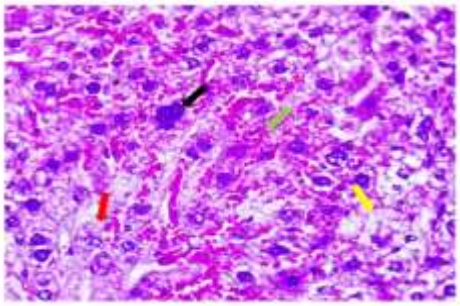
(Fig 6) : Photograph of liver tissue (10 weeks of treatment) showing large focal area of inflammatory cells (black) within parenchymal tissue (green) (H&E X 400).



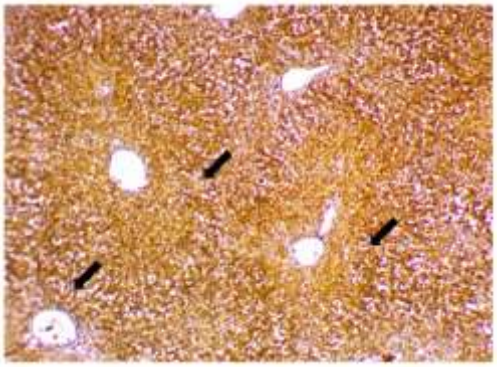
(Fig 7) : Photograph of liver tissue (10 weeks of treatment) showing eosinophilic area of cellular necrosis (black) nearby the central vein (red) (H & E X 400).



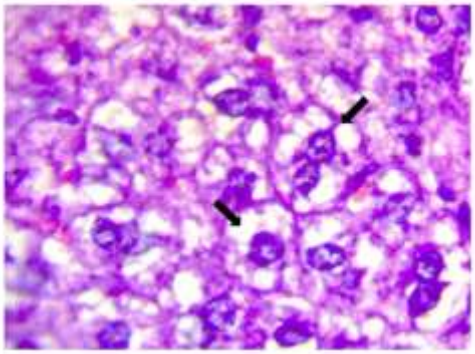
(Fig 8): Higher magnification of liver tissue (10 weeks of treatment) showing necrotic cells dispersed through the parenchyma (black) with pyknotic nuclei (red) & normal cells (green) (H&EX1000).



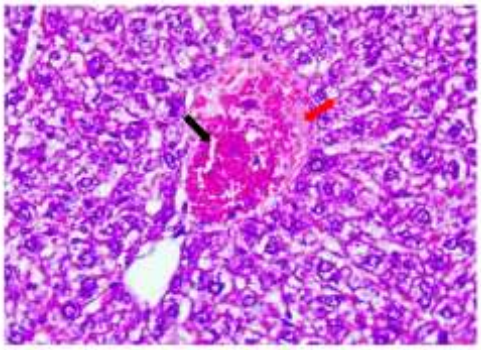
(Fig 9) : Photograph of liver tissue after (10 weeks of treatment) showing congested blood sinusoid (green), nuclear pleomorphism (black), necrotic hepatocyte (red) & hydropic degeneration (yellow) (H & E X 400).



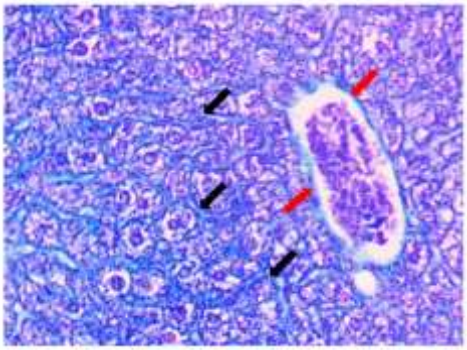
(Fig 10) : Photograph of liver tissue after (10 of treatment) showing extensive hydropic degeneration at the peripheral part of each lobule (black) (Silver nitrate X100).



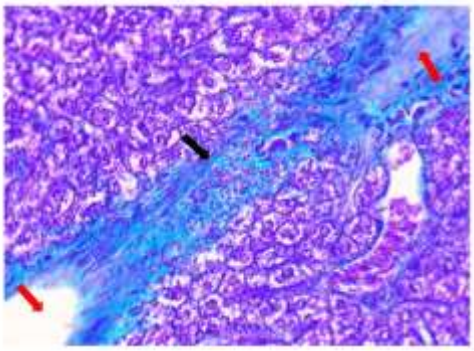
(Fig 11): Higher magnification of liver tissue (10 weeks of treatment) showing extensive hydropic degeneration (black) (H & E X 1000).



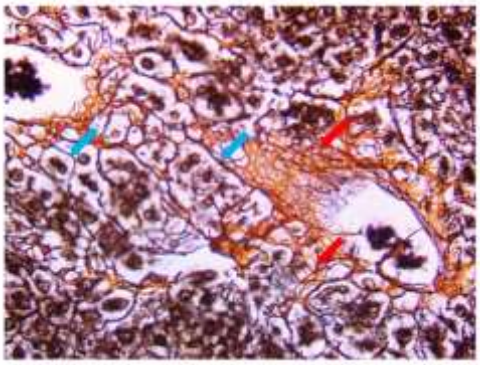
(Fig 12): Photograph of liver tissue after (10 weeks of treatment) Showing congested central vein (black) & hemolysis (red), (H&E X 400).



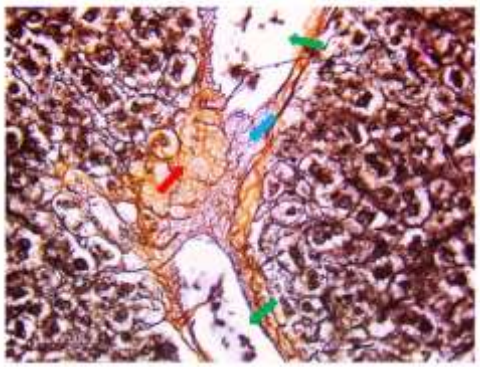
(Fig 13) : Photograph of liver tissue (10 weeks of treatment) showing high deposition of collagen fibers surrounding many hepatocytes (black) & around central vein (red) (Masson's trichrome X 400).



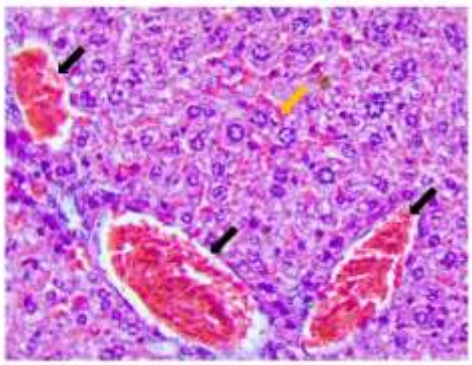
(Fig 14): Photograph of liver tissue (10 weeks of treatment) showing high deposition of collagen fibers between branches of portal vein (black) & branches of portal vein (red) (Masson's trichrome X 400).



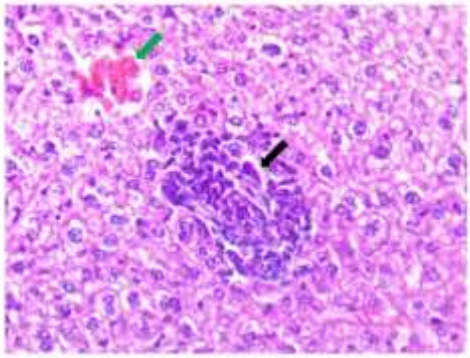
(Fig 15): Photograph of liver tissue after (10 weeks of treatment) showing mild-high deposition of collagen fibers (red) & normal distribution of reticular fibers (blue) (Silver nitrate X 400).



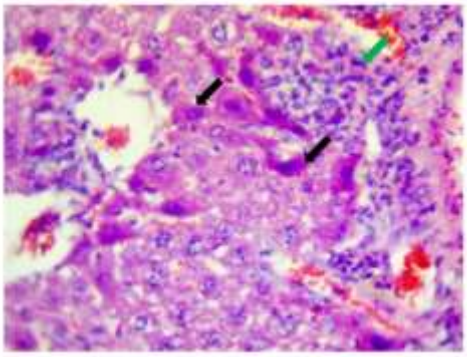
(Fig 16) : Photograph of liver tissue after (10 weeks of treatment) showing high deposition of collagen (red) & reticular fibers (blue) between two branches of portal vein (green) (Silver nitrate X 400).



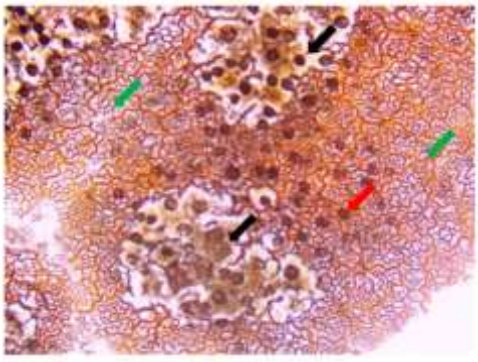
(Fig 17): Photograph of liver tissue after (overdose treatment) showing extensive congested branches of portal vein (black) & congested blood sinusoid (yellow) (H & E X 400).



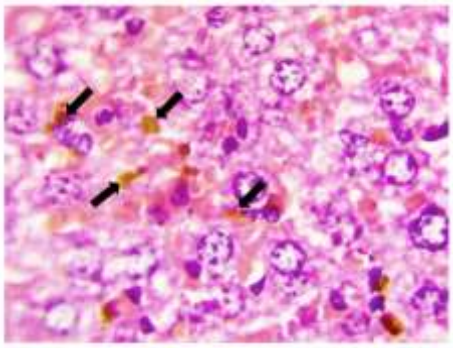
(Fig 18): Photograph of liver tissue after (overdose treatment) showing large focal area of inflammatory cells (black) nearby central vein (green) (H&E X 400).



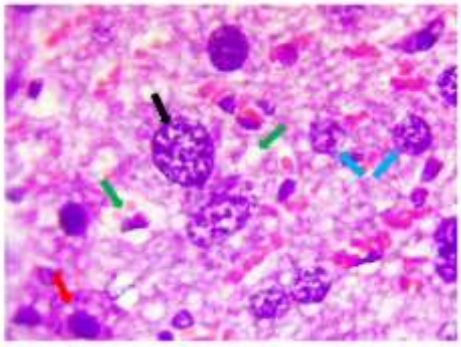
(Fig 19): photograph of liver tissue after (overdose treatment) showing necrotic cells (black) with inflammatory cellular infiltration (green (H & E X 400).



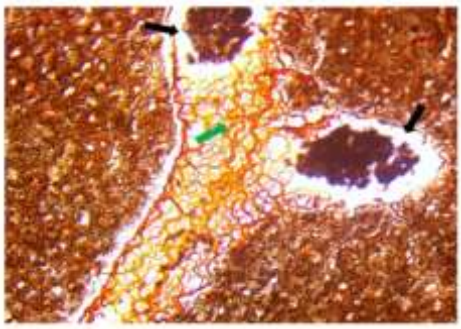
(Fig 20): photograph of liver tissue after (overdose treatment) showing large necrotic area of hepatocytes (black), pyknotic nuclei (red) , collagen & reticular fibers (green) (Silver nitrate X 400).



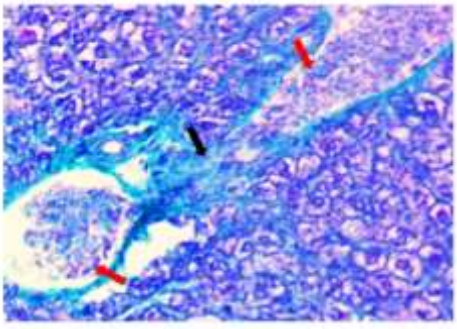
(Fig 21): Higher magnification of liver tissue after (Overdose treatment) showing many macrophages (kupffer cells) within the hepatocytes which were filled with golden brown pigment (black) (H & E X 1000).



(Fig 22): Higher magnification of liver tissue after (Overdose treatment) showing necrotic cells (red), hydropic degeneration (green) , normal nuclei (blue) & karyomegaly (black) (H&E X1000).

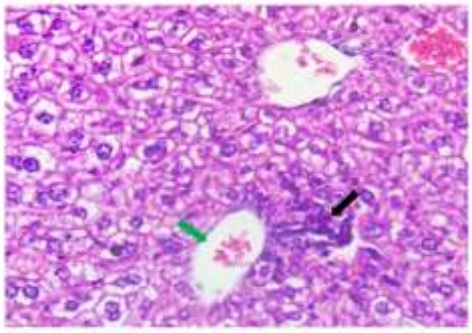


(Fig 23): Photograph of liver tissue after (overdose treatment) showing high collagen deposition (green) between two branches of portal vein (black) (Silver nitrate X 400).

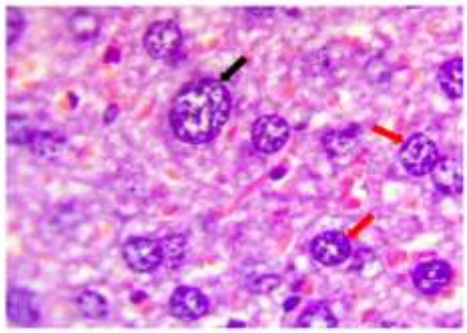


(Fig 24): Photograph of liver tissue after (overdose treatment) showing high collagen deposition

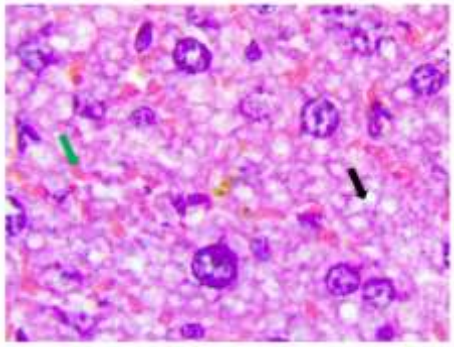
(black) between two branches of portal vein (red) (Masson's trichrome X 400).



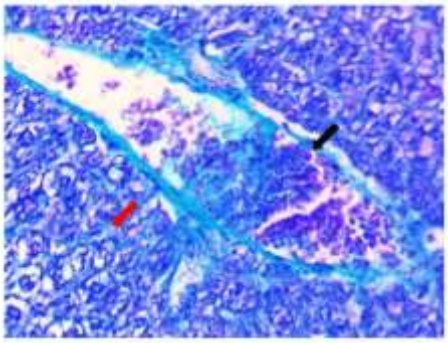
(Fig 25): Photograph of liver tissue (recovery group) showing small area of inflammatory cells (black) associated with central vein (green) (H & E X 400).



(Fig 26): Photograph of liver tissue (recovery group) showing hepatocyte with karyomegaly (black) & normal cell with normal nuclei (red) (H&E X 1000).



(Fig 27): Photograph of liver tissue (recovery Group) showing mild congested blood sinusoid (Green) & little hydropic degeneration (black) (H & E X 1000).



(Fig 28): Photograph of liver tissue (recovery Group) showing mild deposition of collagen fibers Around central vein (black) & between some hepatocytes (red) (Masson's trichrome X 400).

4. Discussion:

Since 1978 a number of reports have indicated a possible association between sodium valproate therapy and the occurrence of hepatitis [10-13]. In the present study the animals that received normal saline were used as a control for the experiment and showed a typical morphological and histological picture of normal liver [21,22]. The result of our study showed that the hepatocytes, central veins, portal veins and blood sinusoids, exhibit the most important changes compared with control group. The cellular changes that have been seen in our results were characterized by cellular enlargement, nuclear changes (i.e. pleomorphism) and cytoplasmic changes (i.e. hydropic degeneration). Researchers [23] have shown that the oral administration of diclofenac and valdecoxib result in enlarged hepatocytes with some vacuolation in the cytoplasm.

Hydropic changes occurs whenever the cell is unable to maintain ionic and fluid homeostasis. It reflects an excessive accumulation of water in the cells cytoplasm and it is commonly used to refer to advanced stages of cellular swelling [24]. Hydropic changes is an early manifestation of cellular illness whereas fatty changes represent a more severely ill cell [25]. Authors [26] have shown that the administration of large single intraperitoneal doses of sodium valproate into the rats can produce a dose dependent microvesicular hepatic steatosis.

The most prominent nuclear changes that was associated with valproic acid treatment are frequent polyploidy hepatocytes and nuclear pleomorphism. Our results are paralled to those found by [27], who demonstrated that methotrexate can induce a variety of histologic changes including steatosis, anisonucleosis (i.e. nuclei of varying sizes and fibrosis). Anisonucleosis (polyploidy) is known to occur as an age-related phenomenon, the nuclear and cellular changes can also be induced by xenobiotics [28]. So most probably that the valproic acid in our study play a role in developing nuclear changes.

Many large focal areas were found within the parenchymal tissue as well as associated with central veins after ten weeks and overdose treatment. It is formed as a result of an inflammatory reaction in body tissue, and consisting of mononuclear inflammatory cells. Such changes were found to be associated with many drugs such as acetaminophen, aspirin and certain toxins [29].

Numerous kupffer cells (Macrophages of the liver) with golden brown pigment were noticed lining blood sinusoids throughout the liver tissue section, after overdose treatment. Such brown granules were described by [30] to be hemosiderin pigments which may indicate recent parenchymal damage.

Different morphological changes of hepatocytes were seen including hypertrophic hepatocytes and atrophic hepatocytes with pyknotic nuclei, such hepatocyte changes were descriped by many pathologists [30,31] to be hydropic (ballooning)

degeneration, acidophilic degeneration or coagulative necrosis.

Congestion of central veins as well as angiectasis were prominent in liver sections taken after ten weeks and overdose treatment. Congestion represent the increase of blood in an area, due to dilatation of small vessels. While angiectasis, is an incidental finding, occasionally noted in aging mice. The above changes are associated with many drugs and certain toxins such as acetaminophen [32] and nitrosamines [33].

The marked deposition of collagen fibers which was detected after overdose treatment around the central veins and between hepatocytes (i.e. in sub-endothelium of blood sinusoids) may lead to sinusoidal obstruction. The same findings were also observed with carbon tetrachloride-induced acute liver injury [34] and following intake of certain drugs such as methotrexate [35].

5. Conclusion:

As a result of our study it can be concluded that long-term treatment of therapeutic dose and overdose of valproic acid cause variable degrees of hepatic injuries in mice as far as the histological studies concerned. The study assisted with demonstrating valproic corrosive reactions on liver, and because of this examination alert ought to be taken when valproic acid administration, particularly in patients with medical issues. Discontinuation of the valproic acid administration for three weeks results an incomplete recovery in hepatic tissues.

6. References :

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