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# Role of Portulaca Oleracea Extract to Mitigate Histological Alterations in the Liver and Kidneys Induced by the Depakine Drug in Male Rats

Waleed khaled Mohamed[1], Rashid Khamees Shaban[2]

## Abstract

*Depakine (sodium valproate) (VPA) is the most widely used drug in the treatment of emotional and nervous depression, psychological disorders, epilepsy, and bipolar disorder. Liver and kidney toxicity is one of the confirmed side effects of Depakine. This study aims to identify the preventive effect of the aqueous extract of the Portulaca oleracea plant against oxidative damage induced by the Depakine drug. The study included 30 male laboratory rats divided into six groups. The results of the microscopic examination showed no pathological changes in the tissues of the liver and kidneys in the group treated with the preventive aqueous extract of the Portulaca oleracea plant, while the two groups treated with Depakine showed pathological changes represented by damage to the liver cells, Inflammatory cell infiltration, blood congestion, and shrinkage of the renal glomerulus, Degeneration of renal tubule endothelial cells. While it was observed that there was an improvement and repair in the liver and kidney tissues in the two groups treated with the preventive aqueous extract of the Portulaca oleracea plant with Depakine. This improvement is attributed to the fact that the Portulaca oleracea plant is rich in antioxidants such as gallotannin, omega-3 fatty acids, minerals, and vitamins. Antioxidants protect cells from damage by free radicals and oxidants.*

***Keywords:*** *Depakine, sodium valproate, Portulaca oleracea plant, liver, kidney.*

## Introduction

Valproic acid and sodium valproate (VPA), with the trade names depakine and valpakine, are antiepileptic agents that can control bipolar disorder (Mathew et al., 2016). Moreover, VPA is widely used to treat various types of simple seizures, myoclonic seizures, epilepsy, localized seizures, grand mal, and mixed seizures. It is also often used to treat migraines, chronic headaches, bipolar disorder, schizophrenia, and personality disorders (Haddad et al., 2009). Studies have shown that Depakine can cause damage to the liver and kidneys (Chen et al., 2019).

VPA can accelerate the deterioration of liver tissue by changing the levels of liver enzymes, and increasing the level of lipid peroxidation (Tanvir et al., 2015). The main cause of toxicity of Depakine is oxidative stress (Jafarian et al., 2013). Oxidative stress is a physiological condition characterized by excessive production of reactive oxygen species (ROS) and a decreased ability of the body's antioxidant defense system to effectively neutralize them (Birben et al., 2012). reactive oxygen species (ROS) can lead

[1] Department of Biology - college of education for pure science, Tikrit University, Iraq, albilogywww19995@gmail.com
[2] Department of Biology - college of education for pure science, Tikrit University, Iraq, rashid.khamees@tu.edu.iq

to peroxidation of membrane lipids and MDA production in liver and other organs (Tong et al., 2005). Free radicals are highly reactive species capable of damaging DNA, carbohydrates, proteins, and lipids, structurally leading to cell damage or apoptosis (Sheng et al., 2014).

The kidneys are an essential metabolic organ in the human body. The primary function of the kidneys is to produce urine and remove metabolites and wastes. In addition, the kidney can retain water and other important substances, such as glucose, proteins and amino acids, through reabsorption, maintaining water-electrolyte and acid-base balance. Renal damage caused by VPA is associated with increased serum liver enzymes and decreased plasma albumin. In addition, VPA can reduce glomerular filtration rate, which is manifested by increased levels of uric acid and creatinine in the blood, and decreased blood flow (El-Shenawy and Hamza, 2016).

Portulaca oleracea belongs to the Portulacaceae family (Okafor et al., 2014). The antioxidant and anti-inflammatory properties of this plant extract may be due to the large amounts of antioxidant compounds including gallotannin, omega-3 fatty acids, and ascorbic acid (Zhu et al., 2010). ). The aqueous extract of Portulaca oleracea reduces high-fat diet-induced oxidative stress including blood and liver antioxidant enzymatic systems by modulating leptin and liver function (Chen et al., 2012). Therefore, the aim of the study was to investigate the preventive role of the aqueous extract of Portulaca oleracea against the drug Depakine.

## Materials and methods

Animals Used

The experiment was conducted on male laboratory white rats by selecting 30 animals whose weight ranged between 190-210 grams and whose ages ranged between 10-12 weeks. The animals were placed in plastic cages in the animal house at the College of Veterinary Medicine / Tikrit University, and the experiment continued for 30 days under standard laboratory conditions. They were divided into six groups that were treated as follows: The first group was a control group that was dosed with distilled water only.

The second group was dosed with Depakine at a therapeutic dose (22.5 mg/kg), the third group was dosed with Depakine at a double dose (45 mg/kg), the fourth group was dosed with the preventive aqueous extract of the Portulaca oleracea plant only (200mg/kg), and the fifth group was dosed with the preventive aqueous extract of the Portulaca oleracea plant only (200mg/kg). Dosage with Portulaca oleracea extract (200mg/kg) with Depakine at a therapeutic dose of 22.5 mg/kg. The sixth group was dosed with Portulaca oleracea extract (200mg/kg) with Depakine at a double dose (45 mg/kg).

Preparing the Portulaca oleracea plant

The samples were collected on 8/5/2022, which were in the fruiting stage (presence of seeds). After the collection process, the plant was washed with water to get rid of dust and dirt particles, then it was cut into small parts, and the plant was placed on a piece of cloth in a dry, cool place away from sunlight for two weeks As a maximum. The plant material was then ground, after making sure it was completely dry, with an electric machine. The ground material was then stored in a tightly sealed glass bottle, away from light, heat, and moisture until used.

Preparation of aqueous extract of portulaca oleracea

50 g of ground dry plant powder was weighed and placed in a 1000 ml glass beaker. Then 500 ml of distilled water was added, and the content was mixed using a hot plate device and a magnetic stirrer to facilitate the mixing process, and the process continued for 20 minutes at a temperature of 40 degrees Celsius. The mixture was left on the shaker for 24

hours, after which the mixture was filtered using several layers of gauze and the undissolved materials were separated using a centrifuge. The resulting extract was placed in 250 ml glass dishes, 100 ml of the extract was added to it, then it was placed in an electric oven at a temperature of 40 degrees Celsius to obtain the crude aqueous extract. The process was repeated several times to obtain a sufficient amount of extract (El-Demerdash et al., 2005).

Histological study

Liver and kidney tissues were obtained to evaluate whether Depakine and portulaca oleracea extract caused histological changes. Tissues were immediately fixed in 10% formalin for 24 h and then washed with water and dehydrated with a graded series of alcohol concentration, embedded in paraffin and sliced at 5 μm thickness by microtome, and sections stained with hematoxylin and eosin (HE). The slides were observed using an optical microscope.( Bancroft and Cook.,1998).

## Results and discussion

Liver tissue

The current histological study showed, through microscopic examination of the control group and the group dosed with the preventive aqueous extract of the plant Portulaca oleracea, the normal appearance of the hepatocytes, Kupffer cells, blood sinusoids, and central vein, as shown in Figure (1-2). While the two groups treated with the drug Depakine showed histological and pathological changes represented by Blood congestion in the central vein, in addition to thickening of the blood vessel wall, as well as significant degeneration of the hepatic cells, as most of these cells have lost their natural shape, with the loss of the natural arrangement of the hepatic cells, and difficulty distinguishing the blood sinusoids. Some cells show the disintegration of the nuclei, and this indicates the beginning of cell death. Shown in Figure (3-4).

This study is consistent with the study conducted by Al-Hadrawy, (2022) who indicated that treatment with the drug Depakine causes a change in the tissue structure, which included damage to liver cells, infiltration of inflammatory cells, congestion, and central venous bleeding.

As Omidipour et al. (2021) reported, rats treated with Depakine suffered from pathological changes in liver cells, including degeneration, infiltration of hepatocytes, and inflammation of hepatocytes, in addition to Kupffer cell hyperplasia, hepatic necrosis, and nuclear changes. Histopathological examination of VPA-treated rats showed changes in histology. Normal liver, hepatocyte degeneration, and inflammatory cell infiltration. These suggest that VPA-induced oxidative stress lead to a series of inflammation and transcription factors (Abdelkader et al., 2020).

While the aqueous extract of the portulaca oleracea plant led to a significant improvement and repair in liver tissue treated with Depakine, as shown in Figure (5-6). This is due to its important role in suppressing the excess production of reactive oxygen species (ROS) inside the cells caused by TNFα (Swamy et al. ., 2010). Portulaca oleracea demonstrated antioxidant potential using adult male rats and the study also found inhibition of lipid peroxidation and nitric oxide in the liver. All these results together indicate that portulaca oleracea has health-promoting effects and provides protection against free radicals (Mohamed et al., 2011). Antioxidants reduce cellular damage caused by oxidants or inflammatory cytokines ( Sokmen et al., 2012 ).

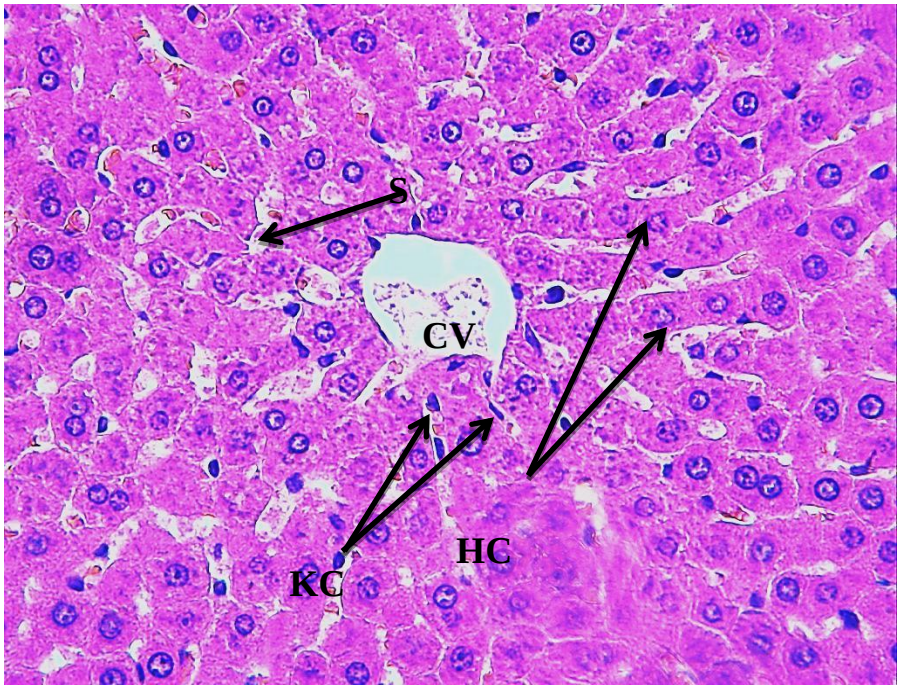



Figure: 1 A section of the liver of the control group showing the central vein (CV), hepatocytes (HC), and blood sinusoids (S). Kupffer cells (KC) can also be observed. H & E× 400,

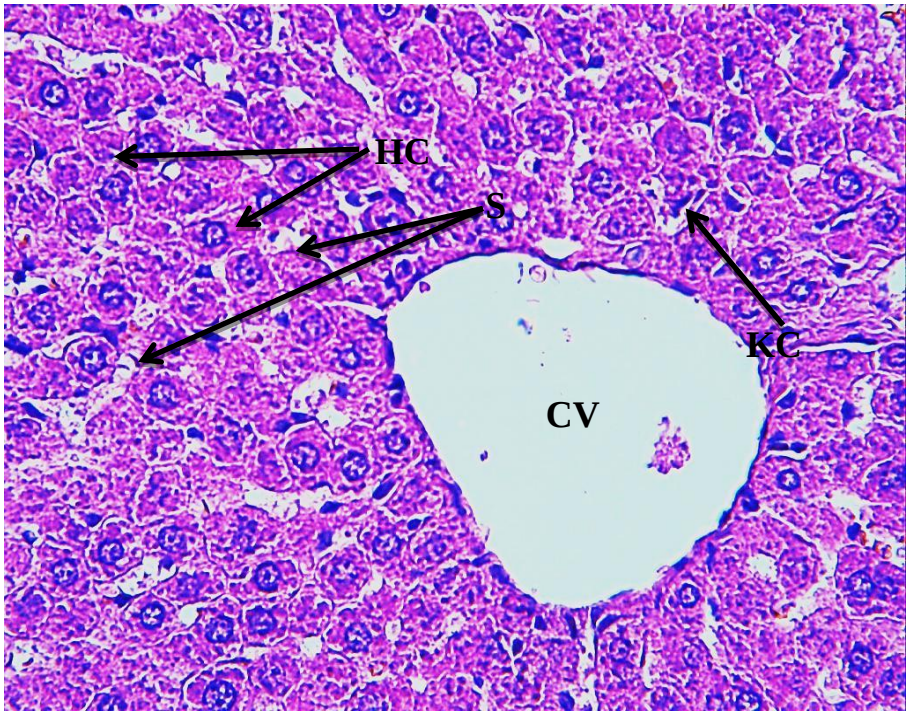



Figure 2: A section of the liver of the group treated with Portulaca oleracea extract showing the central vein (CV), hepatocytes (HC), and blood sinusoids (S). Cover cells (KC) can also be observed in their normal form. H & E 400X,

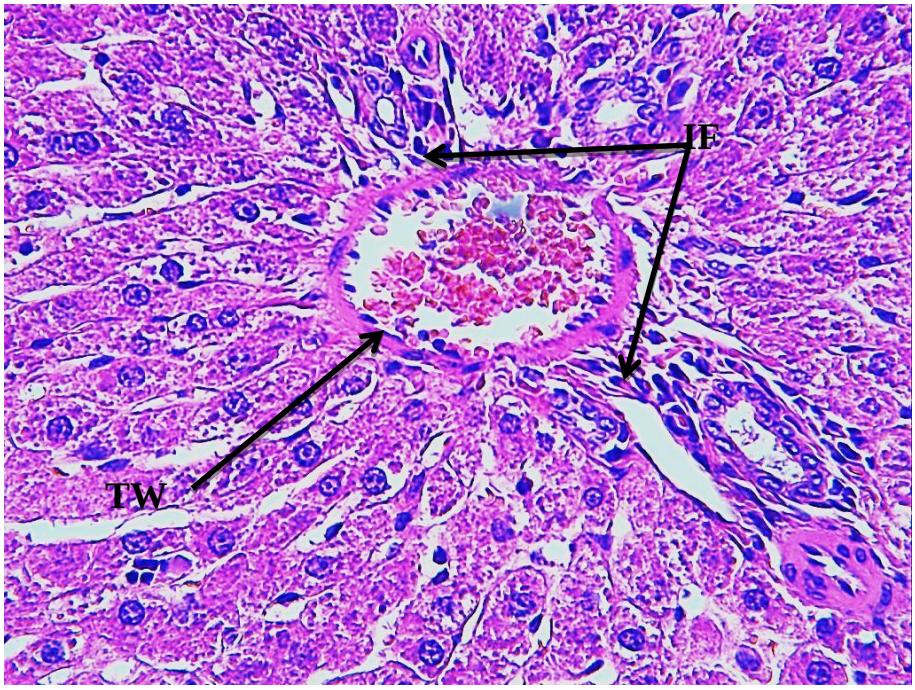
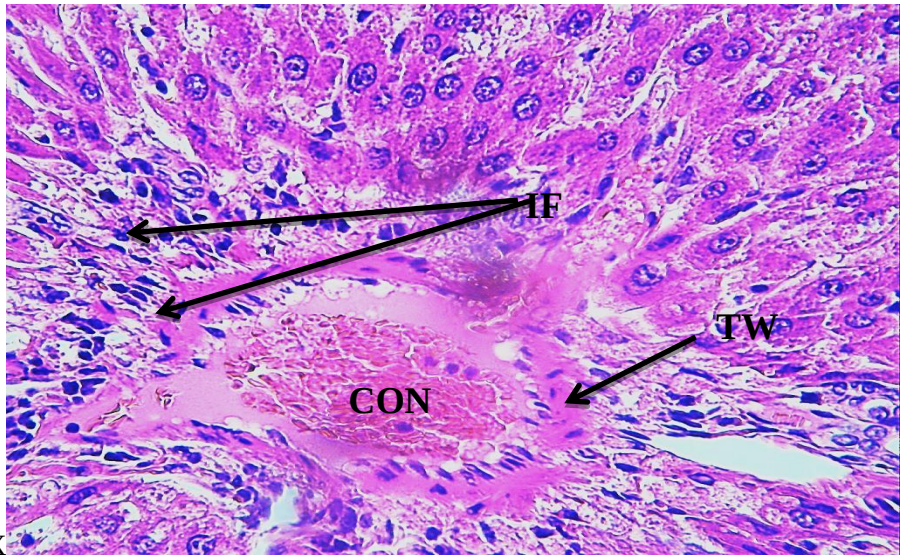



Figure 3: Section of the liver of the group treated with Depakine, a therapeutic dose, showing thickening of the blood vessel wall (TW) and infiltration of inflammatory cells (IF). H & E




400X

Figure 4: Section of the liver of the group treated with double dose Depakine showing thickening of the blood vessel wall (TW), congestion (CON), and infiltration of inflammatory cells (IF). H & E 400X,

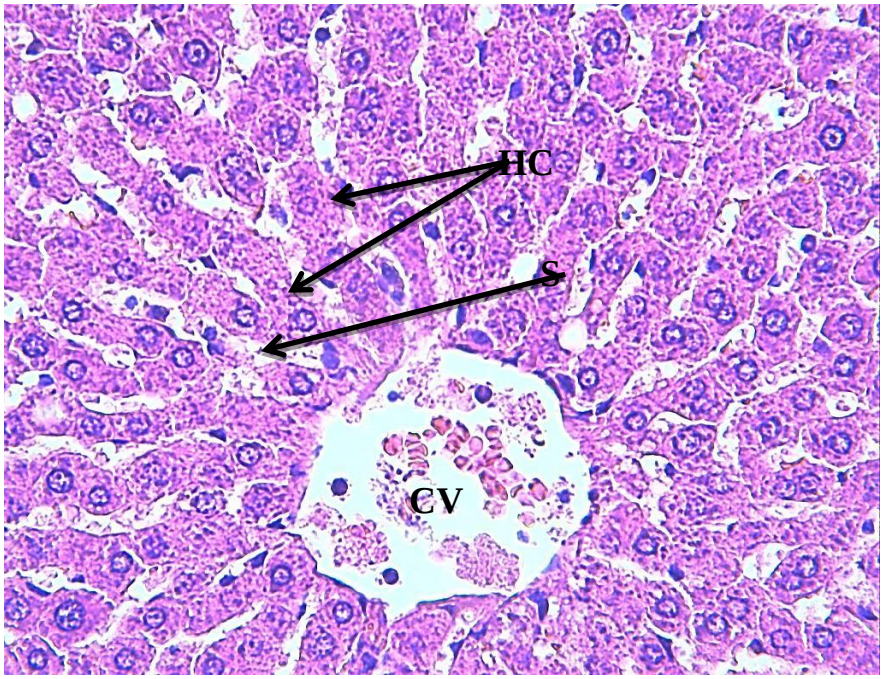



Figure 5: Section of the liver of the group treated with Depakine, a therapeutic dose, with Portulaca oleracea extract, showing the central vein (CV), hepatocytes (HC), and blood sinusoids (S) in their almost normal appearance. H & E 400X

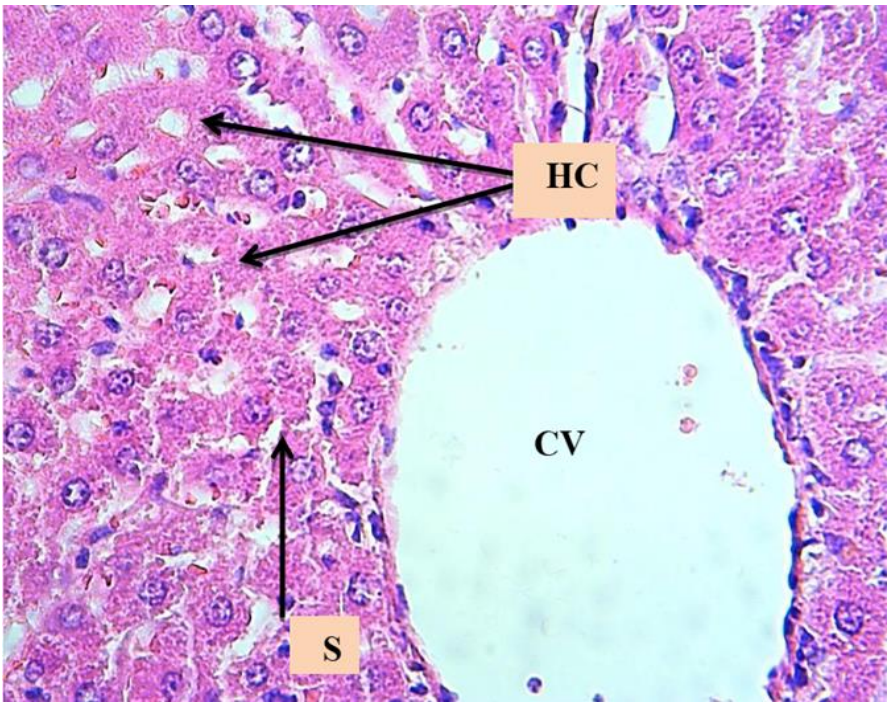



Figure 6: Section of the liver of the group treated with Depakine, a double dose with Portulaca oleracea extract, showing the central vein (CV), hepatocytes (HC), and blood sinusoids (S) in their almost normal appearance. H & E 400X,

kidney tissue

The results of microscopic histological examination of kidney tissue in male rats dosed with the preventive aqueous extract of the Portulaca oleracea plant showed that there were no pathological changes or side effects of the Portulaca oleracea extract compared to the control group, as the shape of the renal glomerulus and the shape of the renal tubules appeared regular, as shown in Figure (7 - 8). While the group treated with Depakine

showed pathological changes and damage to the kidney tissue represented by shrinkage of the renal glomerulus with deterioration in the endothelial cells of the renal tubules, in addition to the occurrence of hemorrhage within the kidney tissue. Blood congestion and swelling of the cells of the lining of the renal tubules appeared, as shown in Figure (9 - 10) One of the side effects of Depakine is Renal function disturbance (Muralidharan et al., 2020). The study conducted by Lee et al. (2023) demonstrated that oxidative stress resulting from VPA causes inflammation in the kidney and reduces Renal function via apoptosis. In addition, the drug Depakine significantly increases caspase 3 mRNA expression, Which participates in apoptosis.

Casp 3 is an enzyme that is activated in the final stage of apoptosis. VPA exposure can cause a urea cycle disorder that prevents the release of ammonia into urea by inactivating the enzyme carbamoyl phosphate synthetase I that involved in the production of carbamoyl phosphate. This process leads to hyperammonemia, which increases blood ammonia concentration, causes nephrotoxicity, or accelerates renal dysfunction (Abbate et al., 2006).

While treatment with the preventive aqueous extract of the Portulaca oleracea plant effectively reduced the harmful effects in the kidneys of rats treated with Depakine, as shown in Figure (11-12). Portulaca oleracea possesses obvious ability to protect the kidneys and has a promising effect in treating acute renal damage caused by nephrotoxins (Uddin et al., 2014). Purslane extract also showed many pharmacological activities, including free radical scavengers ROS (Iranshahy et al., 2017).

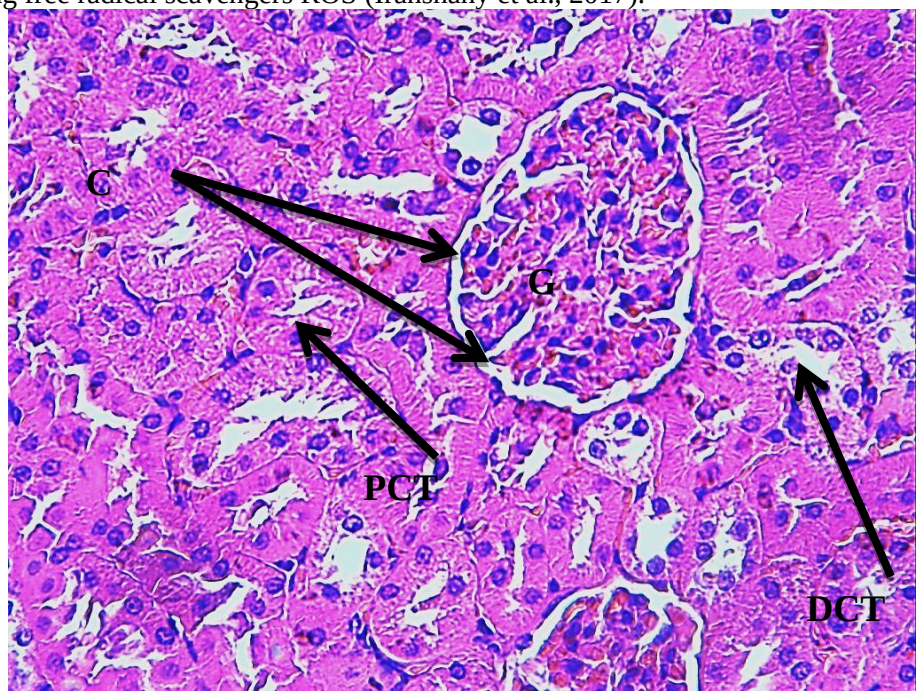



Figure 7: A section of the kidney of the control group showing the renal glomerulus (G), the proximal convoluted tubule (PCT), the distal convoluted tubule (DCT), and the capsular space (C). H & E 400X

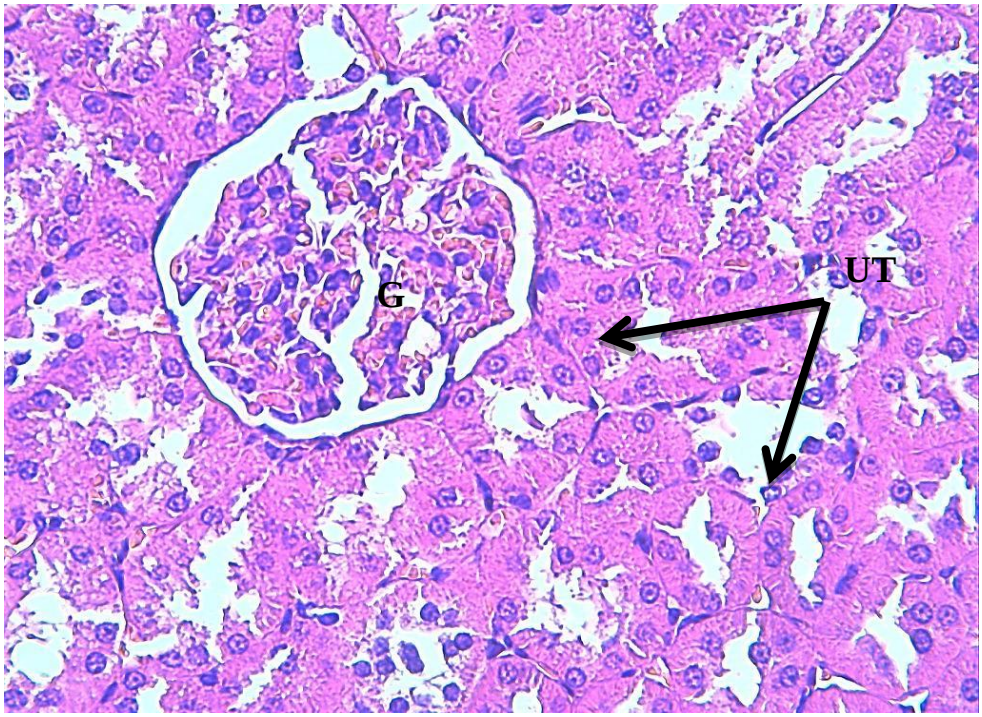



Figure 8: Section of the kidney of the group treated with Portulaca oleracea extract showing the renal glomerulus (G) and urinary tubules (UT) in an almost normal appearance. H & E 400X,

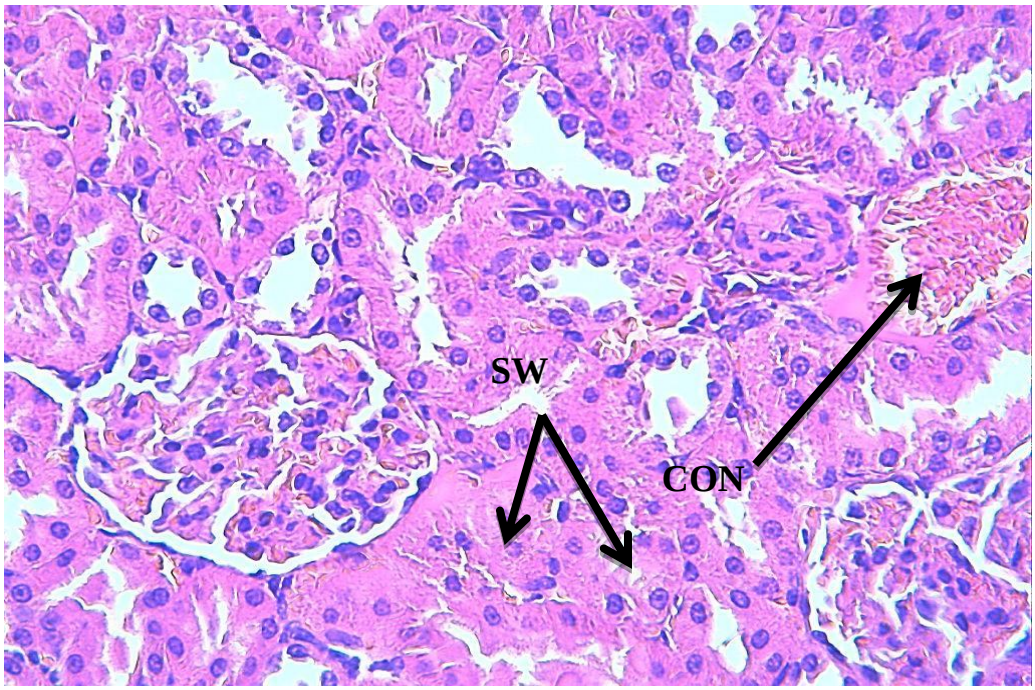



Figure 9: Section of the kidney of the group treated with Depakine, a therapeutic dose, showing blood congestion (CON) and swelling of renal tubule lining cells (SW). H & E 400X,

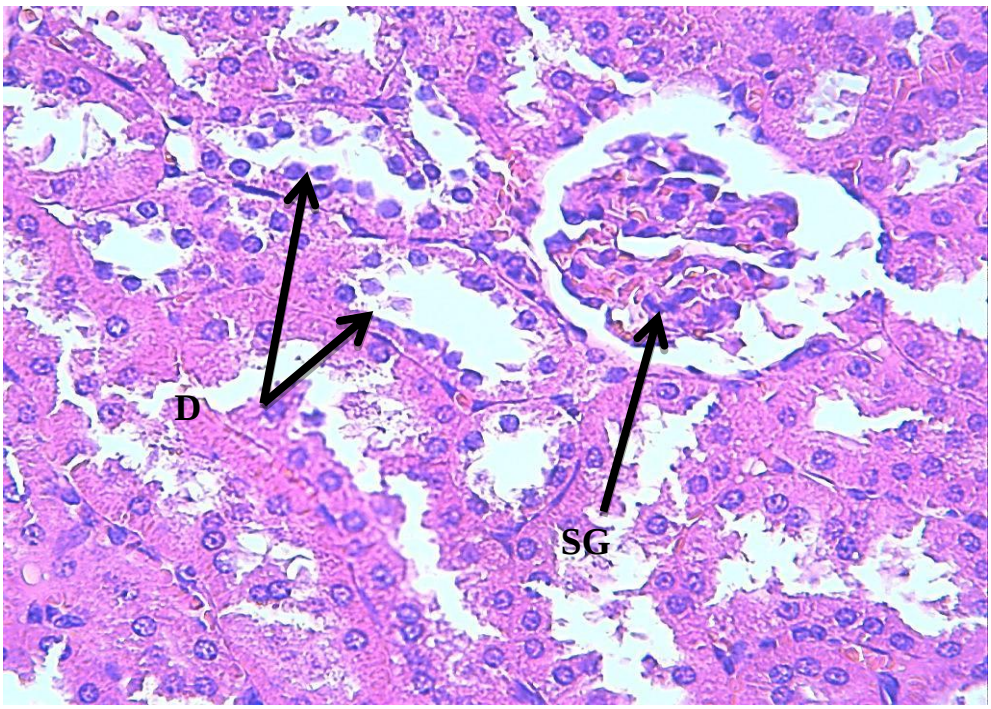



Figure 10: A section of the kidney of the group treated with Depakine, a double dose, showing shrinkage of the renal glomerulus (SG) and degeneration of the endothelial cells of the renal tubules (D). H & E 400X,

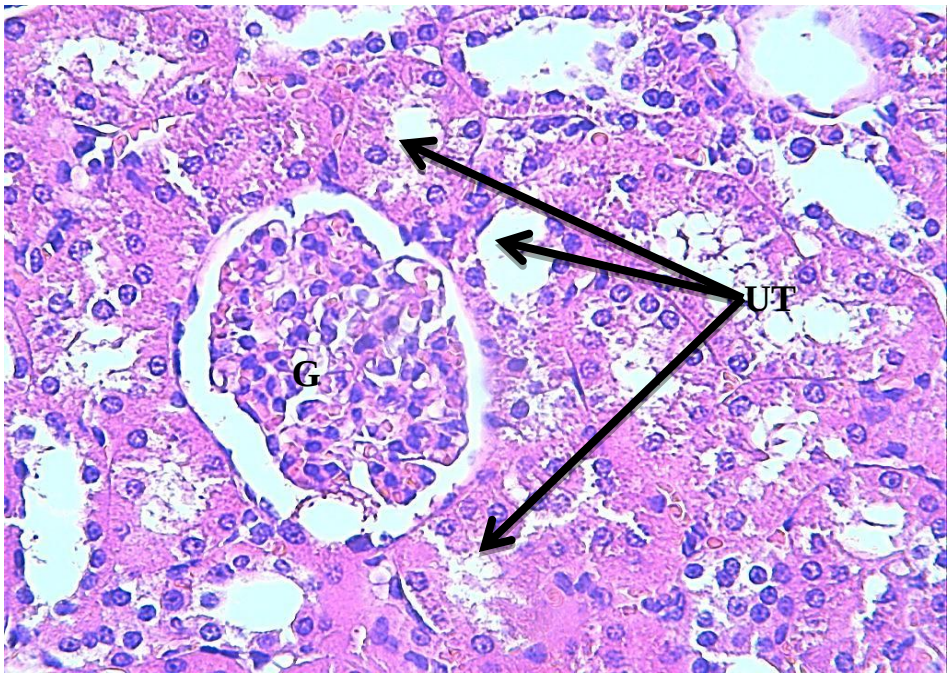



Figure 11: Section of the kidney of the group treated with Depakine, a therapeutic dose, with Portulaca oleracea extract. The renal glomerulus (G) and urinary tubules (UT) appear almost normal. H & E 400X,

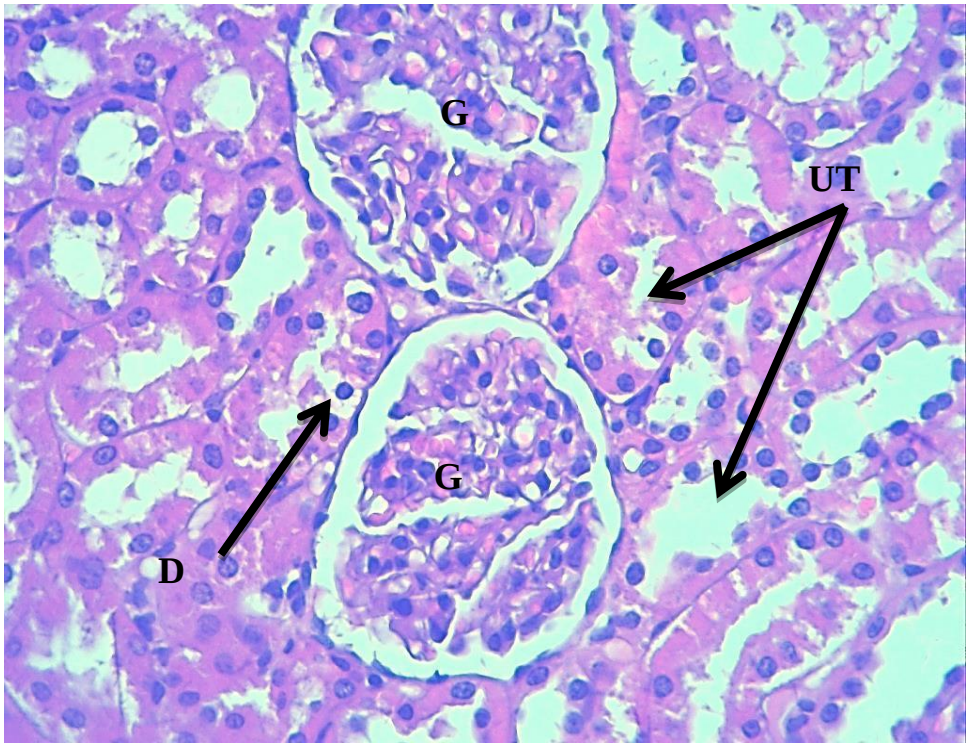



Figure 12: Section of the kidney of the group treated with Depakine, a double dose with Portulaca oleracea extract, showing the renal glomeruli (G) and urinary tubules (UT) normal, with degeneration in the cells lining some of the tubules (D). H & E 400X

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