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Physiological and Histological Effects Induced by Methotrexate Treatment on the Liver and Kidneys of Female Albino Rats and the Role of Pumpkin Seed Extract Against Them

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Abstract:
The current study sought to ascertain the impact of methotrexate therapy on the kidneys and liver of albino rat fetuses as well as the potential protective effects of pumpkin seed aqueous extract. In this investigation, 25 white mice weighing between 150 and 200 grams were employed. At dusk, they were put in plastic cages with other males of the same breed, and they were watched until mating took place. Evidence of insemination can be seen in vaginal plugs or the presence of sperm in the vaginal fluid. The women who are on their first day of pregnancy are tallied the next morning and split into five groups, with five women in each group. First group: this group was given only distilled water as a control; second group received methotrexate treatment for 7 days at a dose of 1.3 mg/kg; third group received oral methotrexate treatment for 21 days at a dose of 1.3 mg/kg; and fourth group received an extract dose. For 21 days, pumpkin seeds were fed orally through a feeding tube at a dosage of 300 mg/kg once a day. For the fifth group, a weekly dose of 1.3 mg/kg of methotrexate was administered along with a daily oral dose of 300 mg/kg of pumpkin seed aqueous extract for a duration of 21 days. The nutrition given to the animals was typical. Experimental animals were slaughtered on the twentieth day of pregnancy in order to obtain samples and ascertain the pathogenic and protective effects on the groups under study. The outcomes demonstrated that, in comparison to the control group, the group receiving 21 days of methotrexate therapy exhibited a number of alterations. Additionally, the recent investigation demonstrated that the dosed group The natural state was reflected in the aqueous extract of pumpkin seeds, whereas the two treatment groups exhibited phenotypic changes in the aqueous extract of pumpkin seeds as well. However, these changes were not as great as those observed in the groups treated with methotrexate treatment alone in comparison to the control group.

Key words: *methotrexate, rheumatism, liver injury, kidney injury.*

Introduction

An antagonist of folic acid is methotrexate (MTX). MTX is utilized in many different conditions, such as autoimmune inflammatory illnesses, rheumatic disorders, and malignant tumors. 4-Amino-10 methyl folic acid

is its scientific name (NLM, 2005). MTX is used to treat ectopic pregnancies non-surgically and for elective pregnancy termination. It is acknowledged Fetal methotrexate syndrome (FMS) was identified as a result of the teratogenic consequences of MTX therapy during the 1960s, as evidenced by the examination of congenital abnormalities. When taking MTX between four and six weeks following conception, there is often a risk of developing FMS (Nguyen et al., 2002). It was developed to treat some types of cancer and has been used in high doses as a cancer treatment, and since 1990 it has been used in lower doses to treat rheumatic diseases (Benedek, 2010). There is a difference between individuals in the absorption of the drug in the digestive system. MTX is more commonly used to treat rheumatism than as medications Other disease-modifying antirheumatic drugs (DMARDs) control the disease rather than treat it, as the symptoms of the disease improve without any noticeable effects on joint destruction (ACRS. 2002). It is believed that the reason for the clinical improvement caused by MTX is its inhibition of the activity and vitality of activated immune cells For antigen. This drug is considered an immunosuppressive drug by inhibiting cell metabolism, meaning that it makes the body's immune system less effective than normal conditions and thus reduces inflammation (Jarmalaitė et al., 2008).

Recently, medicinal plants have aroused great interest in the therapeutic field thanks to their use as drugs to treat a variety of diseases, making them an important health and preventive means in many countries. Since the mechanism of action of these plants is not fully known, they require in-depth study (Aleksic & Knezevic, 2014). Among these medicinal plants, the pumpkin plant is an important agricultural crop belonging to the Cucurbitaceae family, and has witnessed increased interest recently due to its important nutritional and health benefits (Xanthopoulou). et al., 2009). Pumpkin is considered a rich source of the antioxidant beta-carotene, which plays an important role in strengthening the immune system and reducing the chances of developing diseases such as heart disease and cancer. Pumpkin also has other health benefits, as it contains proteins and sugars that have been shown to be effective in fighting skin cancer. Pumpkin is very popular in traditional medicine, where it is used to treat a variety of diseases such as diabetes, high blood pressure, and tumors, boosting immunity, fighting bacteria, lowering cholesterol levels, fighting intestinal parasites, and alleviating inflammation and pain (Abou-Elella & Mourad, 2015 Sharma et al., 2013)

Materials and methods: Methotrexate treatment was determined using the effective dose method to calculate the oral dose based on the equivalent human dose, and the dose used for treatment (1.3 mg/kg) according to (Nair & Jacob, 2016). As for the pumpkin plant, it was obtained from farms in Salah al-Din Governorate, where Its seeds were extracted with warm water according to (AboSeda, 2019)

Animals used in the study: 25 white mice were used, their weight ranged between 180-200 grams. These animals were subjected to appropriate laboratory conditions in terms of temperature, taking into account ventilation, and were fed a free diet all the time. The duration of the trial is 19 days. They were placed with males of the same breed inside a plastic cage during the night, and then the next morning the females were examined, where vaginal plugs formed or sperm appeared in the vaginal fluid as evidence that insemination had occurred, and the females were considered to be on day zero of pregnancy (Fox et al., 2006)

Experiment design: The study was designed based on the use of 25 female white mice randomly distributed into five groups according to the following groups:

The first group was dosed with distilled water only

The second group (therapeutic) was dosed with methotrexate (1.3 mg/kg) once a week for 7 days.

The third group (therapeutic) was dosed with methotrexate (1.3 mg/kg) once a week for 21 days.

A dosage of 300 mg/kg of pumpkin seed aqueous extract was given once a day to the fourth group. The fifth group received a weekly dosage of the therapeutic dose of methotrexate together with a daily dose of pumpkin seed aqueous extract for a duration of 21 days.

Anesthesia and dissection: The animals were fasted for 12 hours before the dissection, and then they were anesthetized with chloroform. The females were placed in a glass anesthesia box, inside which was a piece of cotton, and the anesthetic was placed on it. The females were left for 1-2 minutes, then blood samples were drawn from the heart using a cardiac stab. In test tubes to conduct biochemical tests, which are to estimate the concentrations of urea, creatine, and liver enzymes Alkaline phosphatase activity (ALP), Aspartate Aminotransferase activity (AST), and Aminotransferase Activity (ALT). Then the pregnant females were dissected on the 20th day of pregnancy, after which the abdomen was incised and the liver was taken. The kidneys were cut into two or more parts as appropriate with a sharp blade

Results: The results of laboratory tests for liver enzymes and kidney functions showed a significant difference ($P \leq 0.05$), as an increase in liver enzymes (), as well as urea and creatine, was observed in the groups treated with methotrexate treatment. The aqueous extract of pumpkin seeds also showed its protective role in improving the levels of liver enzymes and kidney functions as well. In Figure (1-1), (1-2), (1-3), (1-4), (1-5).

Microscopic examination of the liver also showed infiltration of inflammatory cells, blood vessel congestion, dilatation of blood sinusoids, and rupture of the lining of the central vein in the groups treated with methotrexate treatment, as in pictures (A) and (B), and the results of the group treated with the treatment for 7 days were similar to normal, as in picture (C). As for the group treated with pumpkin seed extract for 21 days, the central vein, cells, and blood sinuses can be observed. Cover cells similar to the normal condition can be observed, as in the picture (D). Microscopic examination showed congestion of the blood vessel, necrosis around the vessel, and infiltration of inflammatory cells in the group treated with methotrexate treatment and seed extract. Pumpkin as in pictures (E) and (F). The results of our current study also showed the effect of methotrexate on the kidneys physiologically and histologically, as treatment for 21 days led to histological changes in the kidneys represented by thickening of the blood vessel wall and the appearance of fibrosis in the kidney tissue and congestion of the blood vessel, as revealed. For bleeding within the kidney tissue, images (G) and (H). The examination also showed that the group treated with methotrexate for 7 days was similar to normal, as in picture (I). As for the group treated with the aqueous extract of pumpkin seeds for 21 days, the kidney tubules and glomeruli appeared similar to normal, as in picture (J). Microscopic examination also showed that the group treated with the treatment and the extract for 21 days showed physiological and histological changes represented by the presence of fibrosis in the glomerulus, bleeding within the kidney tissue, and the presence of Bloody infiltration as in pictures (K) and (L).

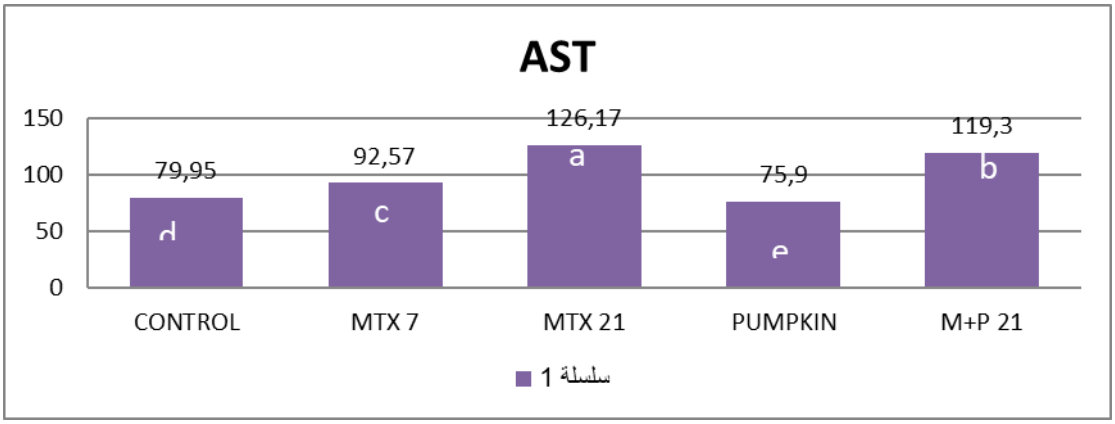


Figure (1_1) The effect of treatment with methotrexate treatment for two different periods, 7 days and 21 days at a concentration of 1.3 mg/kg, and treatment with pumpkin seed extract at a concentration of 300 mg/kg for 21 days, and the effect of treatment with the treatment and the extract for 21 days on the level of the enzyme (AST) in the serum of pregnant white female rats

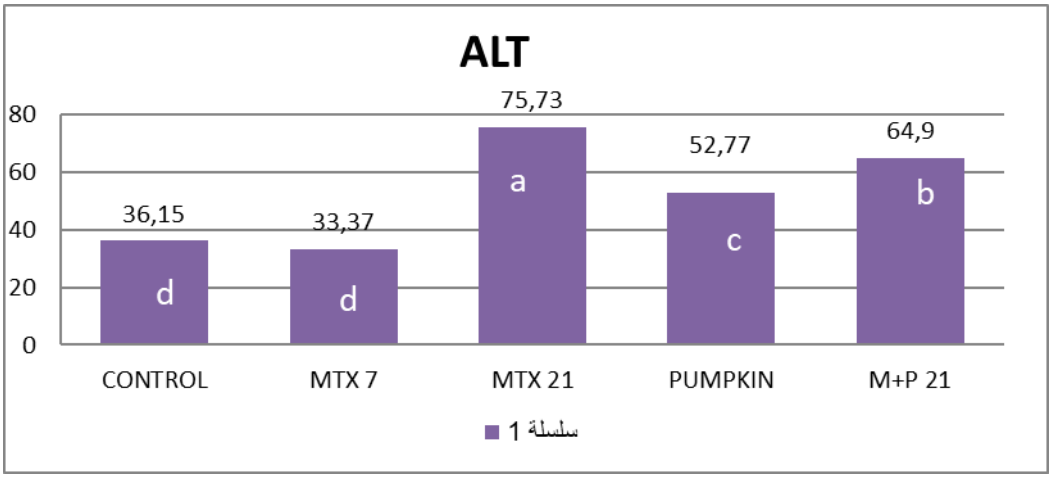


Figure (1_2) The effect of treatment with methotrexate treatment for two different periods, 7 days and 21 days, at a concentration of 1.3 mg/kg, and treatment with pumpkin seed extract, at a concentration of 300 mg/kg for 21 days, and the effect of treatment with the treatment and the extract for 21 days on the level of the enzyme (ALT) in the serum of pregnant white female rats

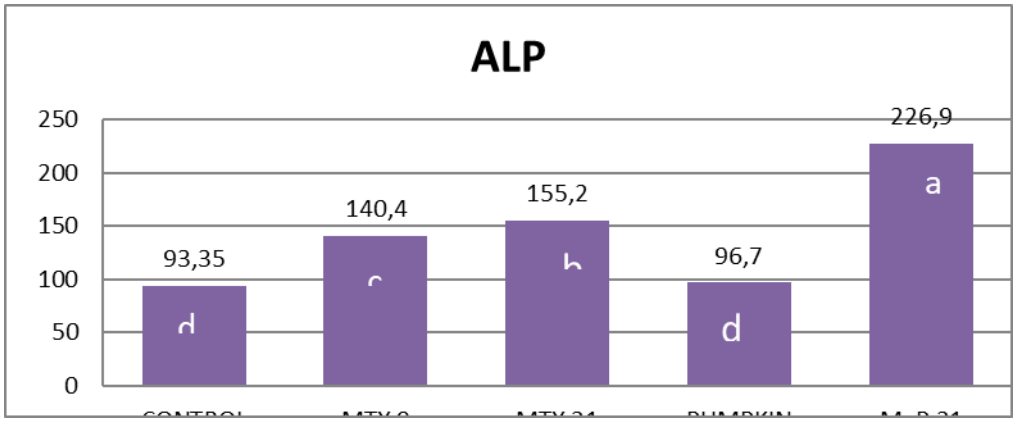


Figure (1_3) The effect of treatment with methotrexate treatment for two different periods, 7 days and 21 days, at a concentration of 1.3 mg/kg, and treatment with pumpkin seed extract, at a concentration of 300 mg/kg for 21 days, and the effect of treatment with the treatment and extract for 21 days on the level of the enzyme (ALP) in the serum of pregnant white female rats.

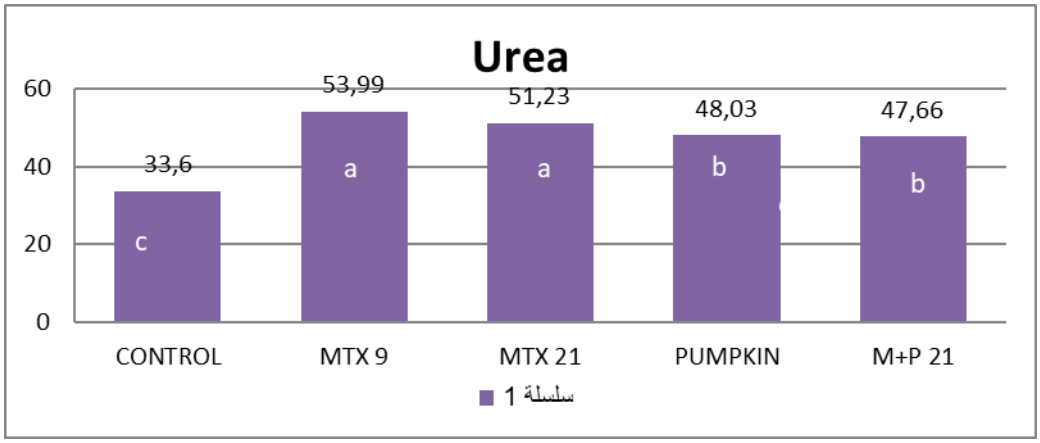


Figure (1_4) The effect of treatment with methotrexate treatment for two different periods, 7 days and 21 days, at a concentration of 1.3 mg/kg, and treatment with pumpkin seed extract, at a concentration of 300 mg/kg for 21 days, and the effect of treatment with the treatment and extract for 21 days on the level of the enzyme (Urea) in the serum of pregnant white female rats.

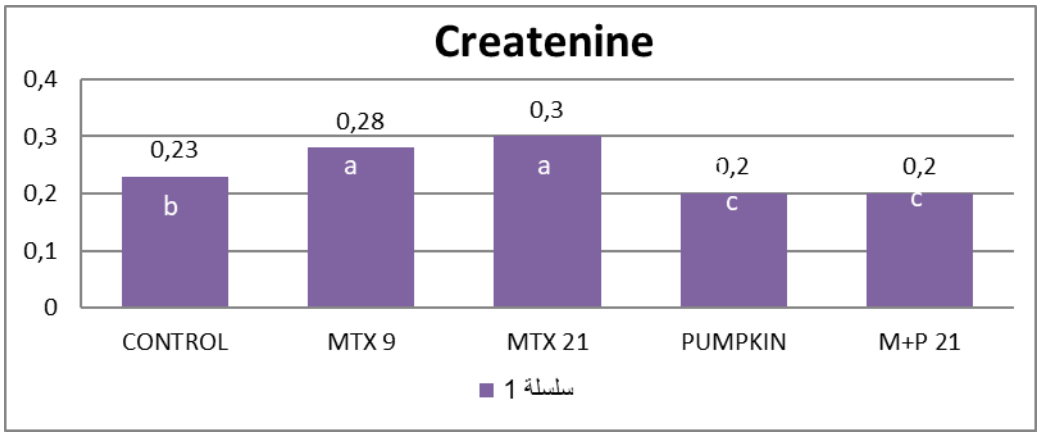
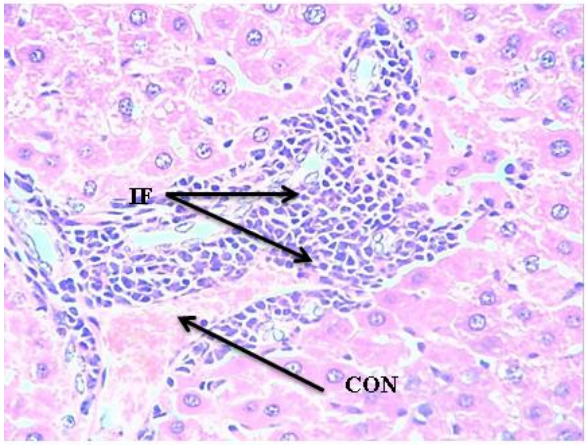
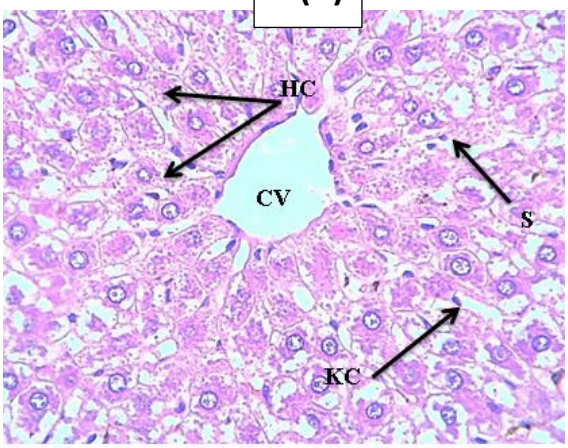


Figure (1_5) The effect of treatment with methotrexate treatment for two different periods, 7 days and 21 days, at a concentration of 1.3 mg/kg, and treatment with pumpkin seed extract, at a concentration of 300 mg/kg for 21 days, and the effect of treatment with the treatment and extract for 21 days on the level of the enzyme (Createnine) in the serum of pregnant white female rats.

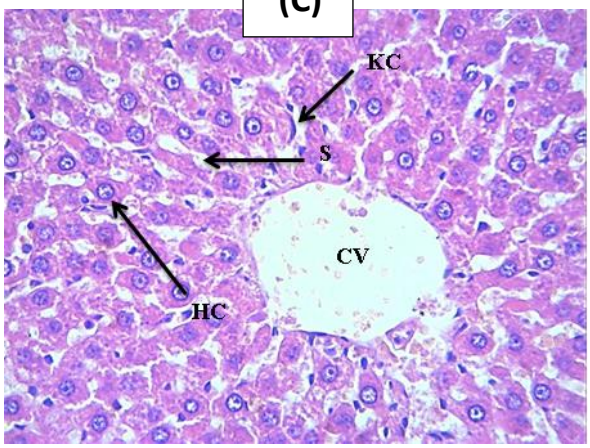
(A)



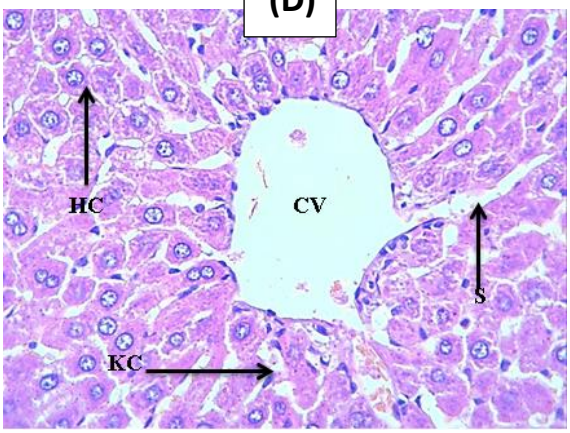
(B)

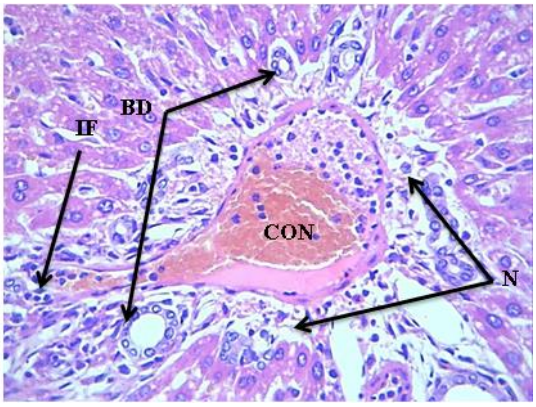


(C)

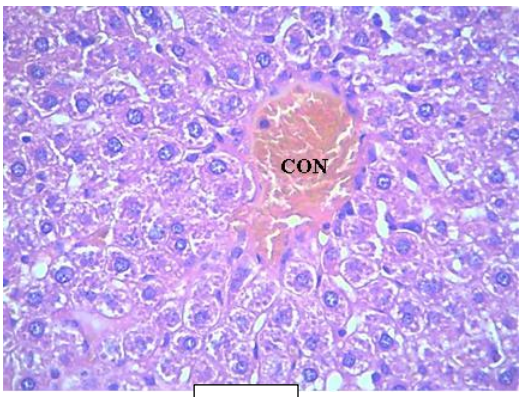


(D)

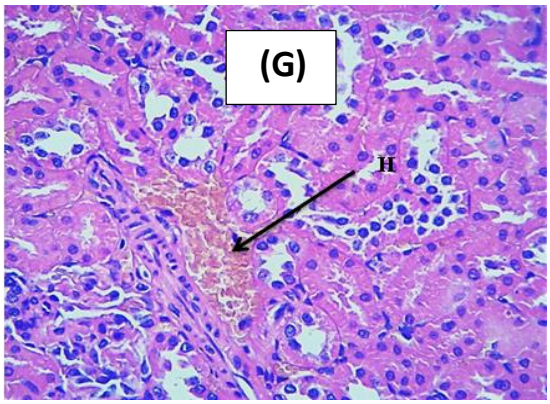


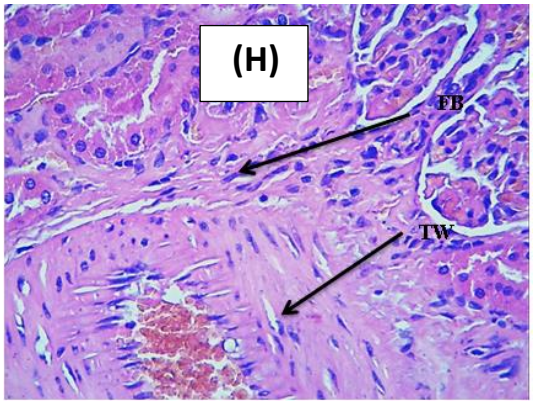


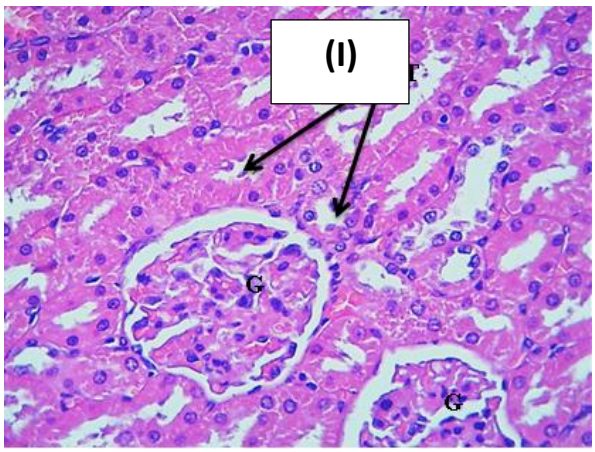
(E)

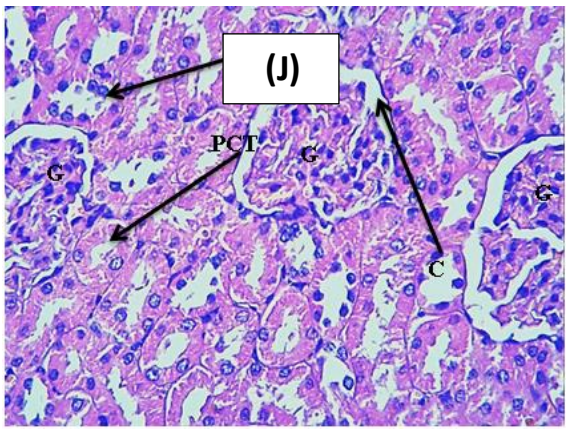


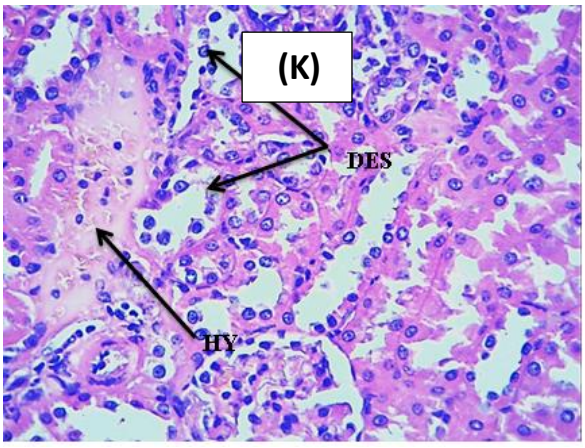
(F)

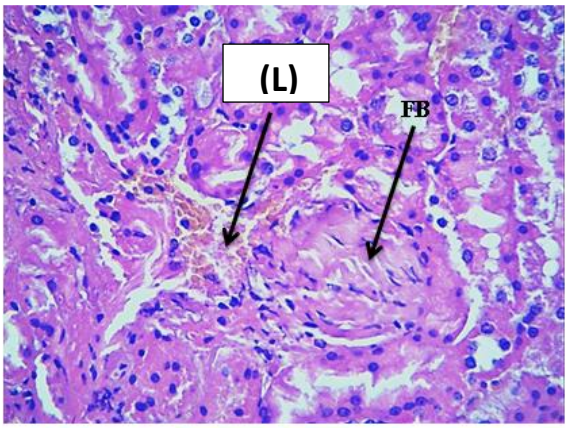












Discussion: Treatment with methotrexate treatment led to biochemical changes in the levels of liver enzymes, which were indicated by the results of the current study. A significant increase in the liver enzymes AST-ALT-ALP when compared with the control group. The reason for this is the ability of the treatment to cause hepatotoxicity as a result of fibrosis and degeneration in liver cells. Accompanying liver dysfunction, the

histological effects of methotrexate on the liver were also shown, represented by inflammatory cell infiltration, blood vessel congestion, central vein rupture, and sinusoidal dilatation.

Studies' findings show that MTX damages oxidative tissue by raising lipid peroxidation in liver tissue and lowering antioxidant enzyme levels. This view is further supported by elevated AST and ALT readings, biochemical markers of liver injury, and histological results. Additionally, it has been demonstrated that administering beta-carotene 21 days before to MTX administration offers a notable buffer against hepatotoxicity caused by MTX (Vardi et al., 2010).

Our current study's findings demonstrated a substantial rise in urea and creatine levels in the blood serum of groups treated with methotrexate therapy in pregnant female rats, at the probability level ($p \leq 0.05$). The histological analysis of the kidneys revealed that tissue damage and malfunction in the kidney nephrons are the primary causes of this rise.

The kidneys' glomeruli filter metabolic waste products such urea and creatine (Gaspari et al., 1998). When renal cells malfunction or the kidneys' glomerular filtration rate decreases, blood serum concentrations of urea and creatine increase (Mouton & Holder, 2006).

It was found that there was a significant difference in the effect of aqueous pumpkin seed extract, as it showed its effective preventive role in reducing the negative effects of methotrexate treatment, as it contributed to reducing the level of liver enzymes, urea, and creatine in the group treated with pumpkin seed extract, as well as the group treated with the treatment and the extract for 21 days in a row. The reason is due to the preventive role. Pumpkin seed extract because of its effective natural substances such as phenols and flavonoids (Dawood and Ahmed, 2021)

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