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### Effects of silymarin against hepatic and renal toxicity induced by methotrexate in rats.

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#### Abstract

The current study aimed to evaluate silymarin's prophylactic and therapeutic effects on methotrexate-induced nephron-hepatotoxicity in albino rats. **Methods:** Twenty-four rats were housed for 15 days in the animal unit facility at the College of Veterinary Medicine / Tikrit University and separated into six groups: 1st group was a healthy control group, the 2nd was a methotrexate induction group, the 3rd was considered therapeutic groups and received methotrexate single dose for one week then received silymarin at day 8 for one week, the 4th group was prophylactic group and received silymarin for one week then received methotrexate single dose for one week.

**Results:** The nephrotoxicity and hepatotoxicity effects of methotrexate were reported via increasing levels of urea, creatinine, Alanine aminotransferase, aspartate aminotransferase, Alkaline Phosphatase, and decreasing Albumin in comparison to the control group. Therapeutic groups and the prophylactic group achieved a significant decrease in urea and creatinine, Alanine aminotransferase, aspartate aminotransferase, and Alkaline Phosphatase, while there was a significant increase in Albumin in the therapeutic and prophylactic group. **Conclusion:** It was concluded that silymarin had a therapeutic and a prophylactic effect against the nephrotoxicity and hepatotoxicity induced by methotrexate in albino rats.

## تأثير السليمارين ضد السمية الكلوية والكلى الناجمة عن عقار الميثوتريكسات في الجرذان

وطبان عبد الله احمد فؤاد كاظم كاطع بان جمعه قاسم سعد بداي نشتر مهند ياسر رديف

### الخلاصة:

هدفت الدراسة الحالية إلى تقييم التأثيرات الوقائية والعلاجية للسليمارين على السمية الكلوية والكلى التي يسببها الميثوتريكسات MTX في الجرذان البيضاء. الطريقة: تم إيواء أربعة وعشرين جرذاً لمدة 14 يوماً في البيت الحيواني في كلية الطب البيطري / جامعة تكريت وتم تقسيمهم إلى ست مجموعات: المجموعة الأولى كانت مجموعة السيطرة الصحية، المجموعة الثانية المصابة بواسطة عقار الميثوتريكسات، المجموعة الثالثة تعتبر مجموعة علاجية تعطى جرعة واحدة من الميثوتريكسات. لمدة أسبوع واحد ثم تعالج بالسليمارين في اليوم الثامن لمدة أسبوع واحد، تم تعطى المجموعة الوقائية الرابعة سليمارين لمدة أسبوع ثم تناولها بجرعة واحدة من الميثوتريكسات لمدة أسبوع واحد. النتائج: أوضحت تأثير السمية الكلوية والسمية الكلوية للميثوتريكسات عن طريق زيادة مستويات اليوريا، الكرياتينين، ALT، AST، ALP، والتناقص في الألبومين مقارنة بالمجموعة الضابطة. حققت المجموعات العلاجية والمجموعة الوقائية انخفاضاً معنوياً في اليوريا، الكرياتينين، ALT، AST، ALP. الخلاصة: لقد توصلنا إلى أهمية السليمارين ضد السمية الكلوية والتسمم الكلوي الناجم عن الميثوتريكسات في الجرذان البيضاء.

### Introduction

Both the methotrexate (MTX) and the methotrexate-polyglutamate inhibit the enzyme dihydrofolate reductase, which catalyzes the conversion of dihydrofolate into tetrahydrofolate, the active form of folic acid<sup>(1)</sup>. Tetrahydrofolate is necessary for the synthesis of the nucleotides of both DNA and RNA. Methotrexate-polyglutamate further inhibits the de novo purine synthesis of both purine and thymidylate synthase, thereby inhibiting DNA synthesis. This mechanism is utilized in the treatment of cancer because of its cytotoxic effect<sup>(2)</sup>. The mechanism of liver injury with methotrexate is believed to be direct toxicity, through inhibition of RNA and DNA synthesis in the liver and producing cellular arrest. Methotrexate therapy has been shown to increase hepatic stellate cell numbers, but the mechanism by which fibrosis induced has not been clearly elucidated. Concurrent therapy with folate has been shown to reduce the rate of serum enzyme elevations during low-dose methotrexate therapy<sup>(3)</sup>. Since MTX is mainly excreted by the kidneys by glomerular filtration and active transport, nephrotoxicity is a usual adverse effect<sup>(4)</sup>. The mechanism by which MTX induces nephrotoxicity is not clear; however, induction of oxidative stress, suppression of DNA production, inflammatory infiltration, and apoptosis

may play important roles<sup>(5)</sup>. Silymarin, an extract from milk thistle seeds, has been used for centuries to treat hepatic conditions. Preclinical data indicate that silymarin can reduce oxidative stress and consequent cytotoxicity, thereby protecting intact liver cells or cells not yet irreversibly damaged. Although the mechanism of action of these compounds is unclear, it has been proven that these compounds have a role in protecting cells, as studies have shown that silymarin has antioxidant properties, and scavenger of free radicals, as well as its role as a regulator of glutathione<sup>(6)</sup>. Silymarin reduces doxorubicin-induced oxidative stress by downregulating Bcl-xL and p53 expression, reducing apoptotic and necrotic cell death in the liver<sup>(7)</sup>. Silybin treatment before or after chemical-induced injury to kidney cells affected by paracetamol, cisplatin, and vincristine has been found to attenuate or avoid nephrotoxic effects<sup>(8)</sup>. Therefore, this study aimed to investigate the effect of Silymarin on therapeutic and prophylactic liver and kidney against toxicity induced by methotrexate.

### Materials and methods

The Protocol of the present study which is a Preclinical experiment Control Randomized study was approved by the Institute Review Board (IRB) of the

College of Medicine / Al-Nahrain University (NO2/3/80 on 10/1/2023, All experiments of the present study were performed from Jan 2022 to May 2023.

#### **Animals**

Twenty-four Albino male rats weighing 200-220 g aged 12-14 weeks were held at the animal house facility, College of Veterinary Medicine /Tikrit University. Rats were housed in plastic cages measuring 46 x 28 x 13 cm. Animals were treated according to standard laboratory conditions such as photoperiod divided into 12 hours of light and 12 hours of darkness, and temperature 25 °C. During the 14-day experiment, animals were fed a conventional laboratory diet consisting of 35% yellow corn, 35% wheat, 20% then administrated on the 8<sup>th</sup> day with methotrexate (50mg/kg/intraperitoneal single dose). The animals were sacrificed on the 15<sup>th</sup> day, The fourth group was therapeutic animals administrated with methotrexate (50mg/kg/ Intra peritoneal single dose) on the 8<sup>th</sup> day and received silymarin 150 mg/ml/day for one week, The animals were sacrificed on the 15<sup>th</sup> day. At the end of the experimental study of each group, blood was taken by heart punctation placed in a test tube free of anticoagulant, incubated at 37 °C for 15 minutes, and then centrifuged at 3000 rpm to obtain serum. Serum was stored at -20 °C before conducting biochemical tests which included: serum creatinine, urea, albumin, ALP, AST, and ALT. The tests were conducted using an analyzing kit provided by (Human Germany).

**Histopathological examination:** Liver and kidney tissues were immediately immersed in formalin 10% for 24 hours before being thoroughly washed and dehydrated by a graded alcohol series of 70%, 80%, 90%, 95%, and 100% before

soybeans, 10% concentrated protein, and 1% dry milk, as well as preservatives and antifungals, and water was freely available.

#### **Experimental protocol**

Rats were distributed into four groups as follows: -

The first group was the healthy control group, the animals were anesthetized by chloroform and sacrificed on day 8<sup>th</sup>

The second group induction was treated with methotrexate (50mg/kg/Intra peritoneal single dose,) The animals were sacrificed on day8<sup>th</sup>

The third group prophylactic group was administrated with silymarin 150 mg/ml/animals/day orally for one week

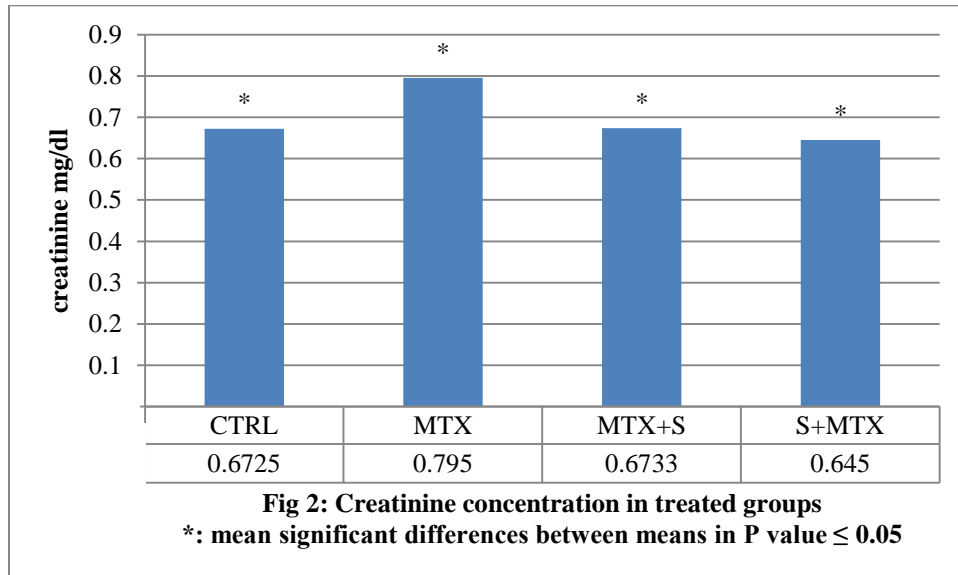
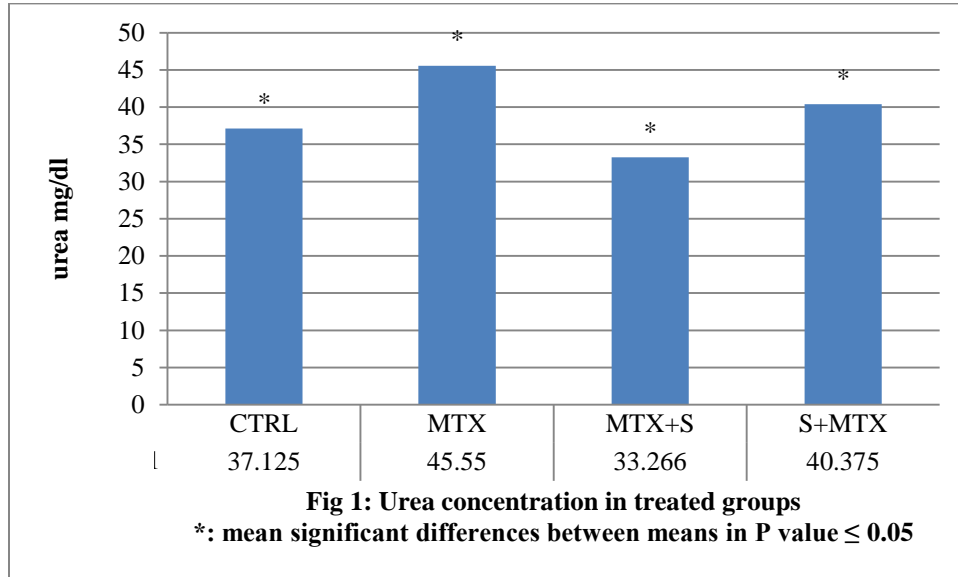
being cleared by xylene and embedded in paraffin. Samples were cut into 5m pieces using a rotary microtome and stained with hematoxylin and eosin (H&E) (9). Slides examination was performed by using light microscopy.

#### **Statistical Analysis**

The data was collected and analyzed using the one-way analysis of variance (ANOVA) test. The Duncan multiple range test was used to assess group differences. The P-value of  $\leq 0.05$  was regarded as a statistically significant value. All statistics were done by using SPSS software version 26.

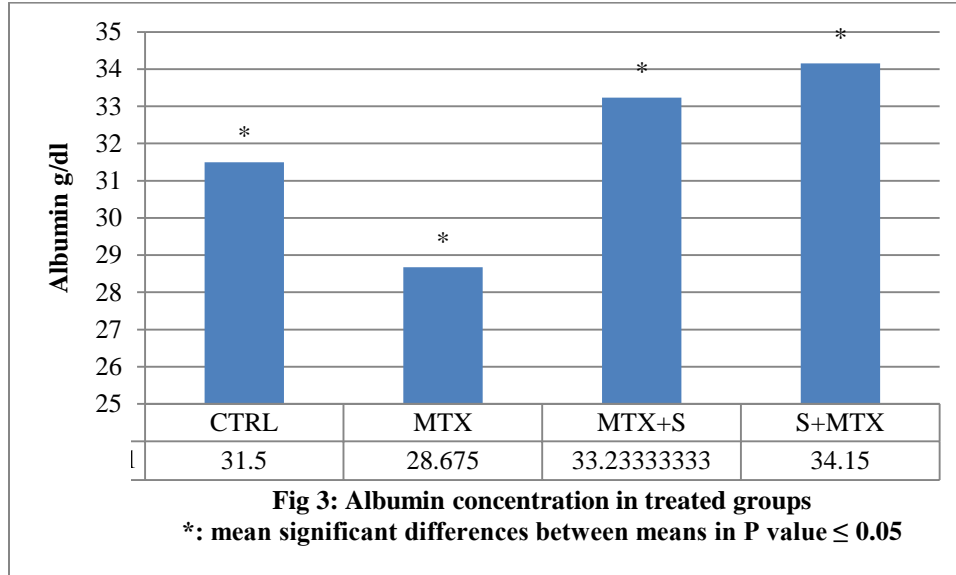
#### **Results**

The results of the study (Fig. 1) exhibited a significant increase in urea in the methotrexate group compared with the control group. The effect in the therapeutic and prophylactic groups presented a significant decrease in the urea level compared to the methotrexate group at a significance value  $P \leq 0.05$ .



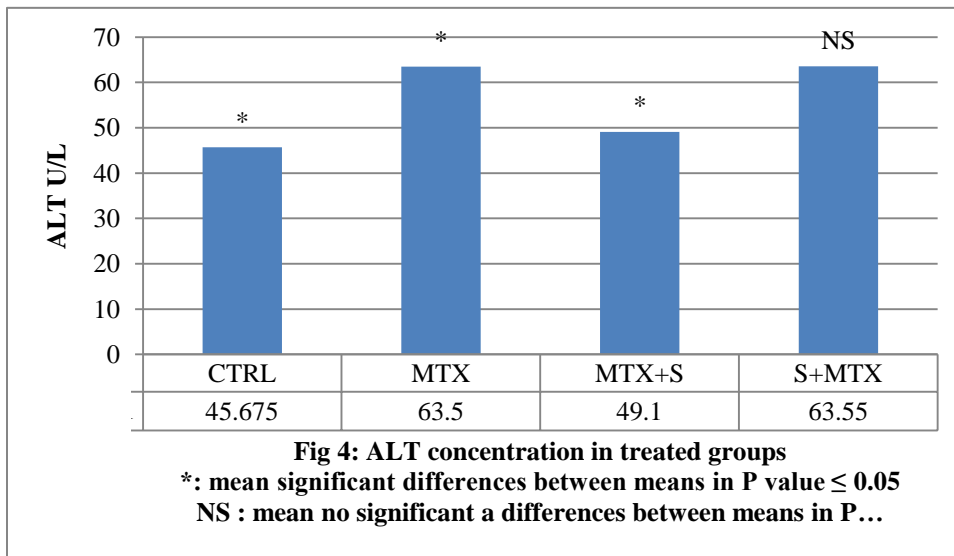
The results (Fig.2) showed a significant rise in the level of creatinine in the methotrexate-treated group compared with the control group. Treatment in both

therapeutic and prophylactic groups showed a significant decrease in creatinine levels compared with the methotrexate induction group at a significance value  $P \leq 0.05$ .



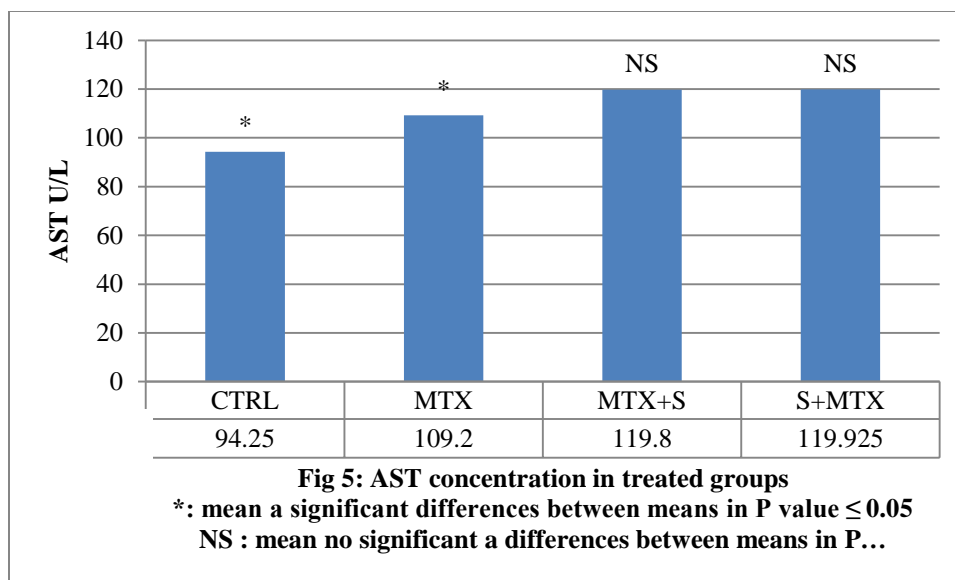
The results (Fig. 3) showed a significant decrease in the level of albumin in the methotrexate-treated group compared to the control group. The treatment in the therapeutic and prophylactic group showed a

significant increase in the level of albumin compared with the methotrexate induction group at a significance value of  $P \leq 0.05$ .



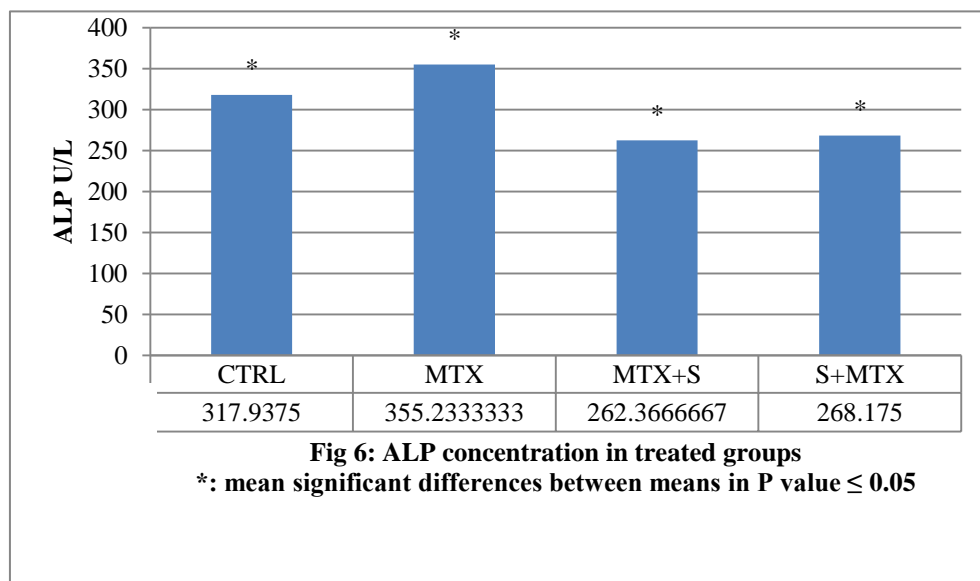
The results of the study (Fig.4) illustrated a significant rise in the ALT level in the methotrexate induction group compared with the healthy control group. Treatments in the therapeutic and prophylactic groups

showed a significant decrease in the ALT level compared with the methotrexate induction group at a significance value of  $P \leq 0.05$ .



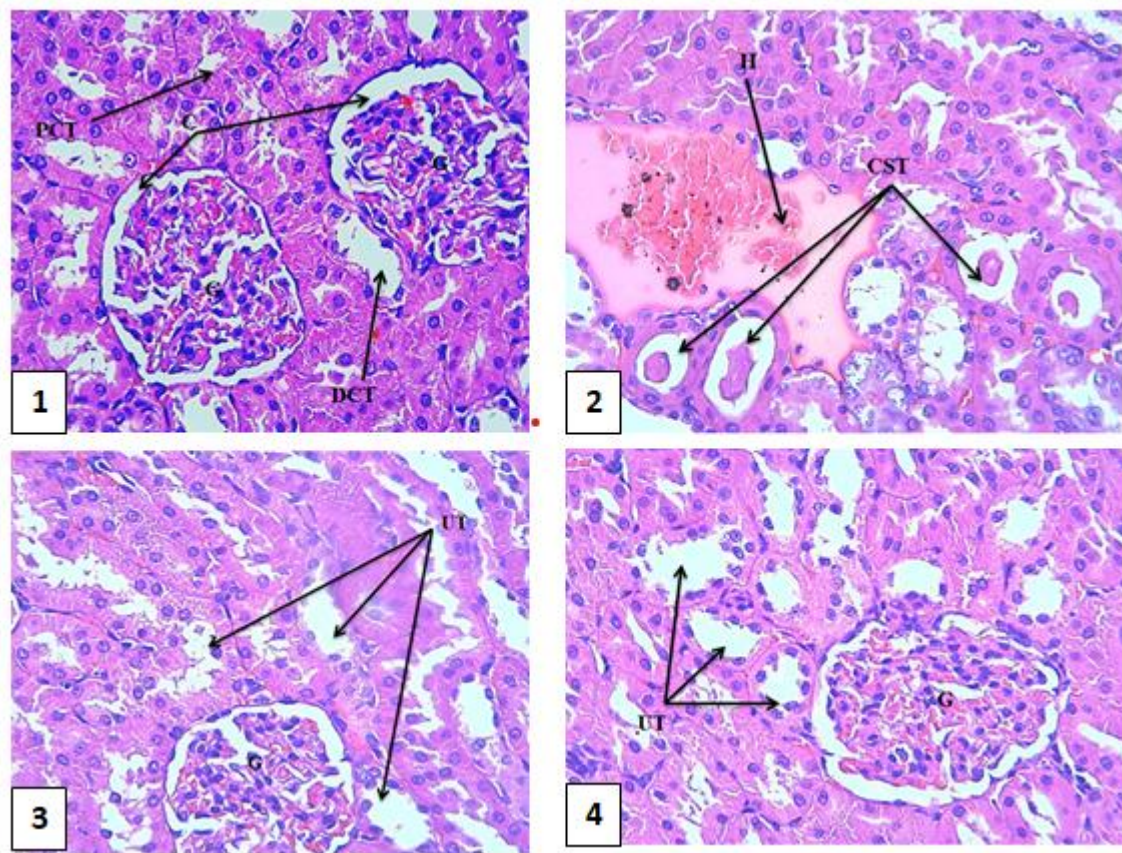
The results of this study (Fig. 5) showed a significant rise in the AST level in the methotrexate induction group compared with the healthy control group. Treatment in

the therapeutic and prophylactic groups showed no significant difference compared to the methotrexate group at a significance value  $P \leq 0.05$

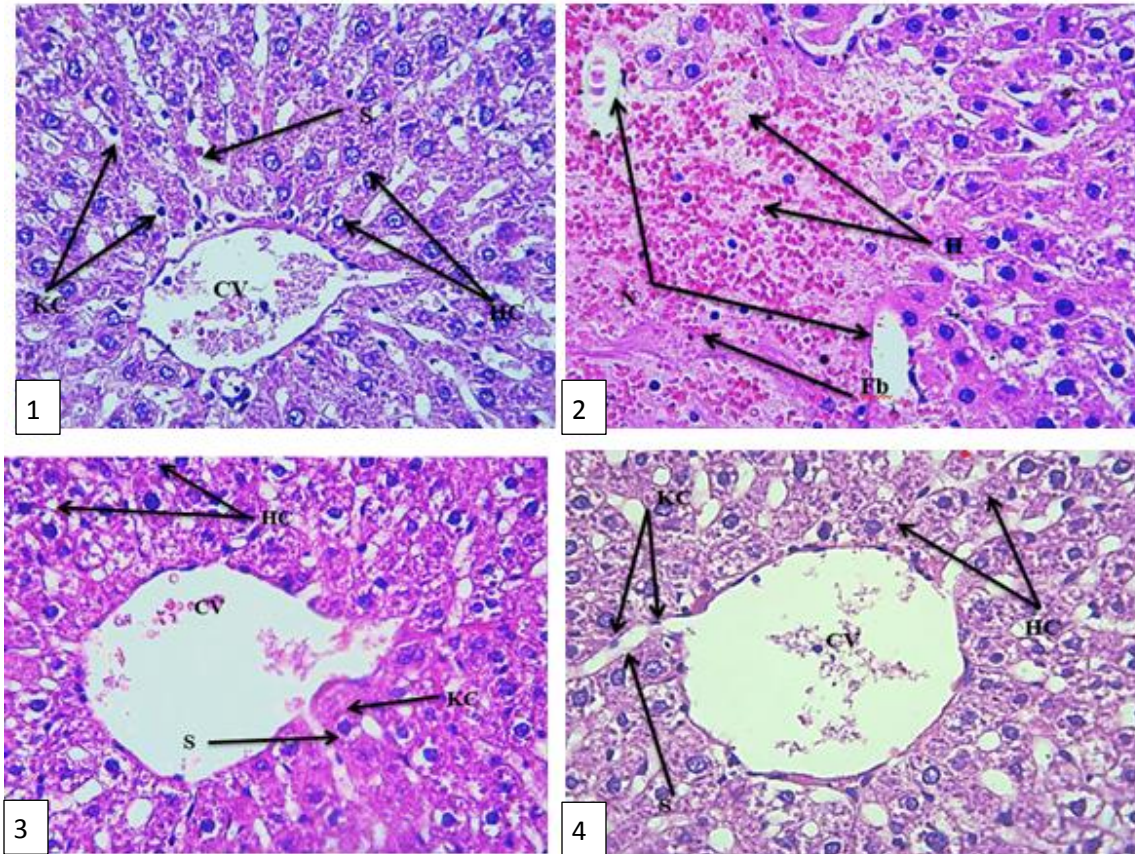


The results of the study (Fig. 6) showed a significant rise in the level of ALP in the methotrexate induction group compared with the healthy control group. Treatments in the therapeutic and prophylactic groups

showed a significant decrease in the level of ALP in all groups compared to the methotrexate at a significance value of  $P \leq 0.05$ .



**Figure (7): Sections of the kidneys in treated groups. (1) Control group showing renal glomerulus (G), the proximal convoluted tubule (PCT) distal convoluted tubule (PCT) distal convoluted tubule (DCT) the space around the glomerulus (C). (2) The Methotrexate group shows the presence of hemorrhage (H) and the crystal (CST) inside renal tubules. (3) Section of the kidney of the group treated with methotrexate and prophylactic silymarin showing normal renal glomerulus (G) and the urinary tubules (UT). (H&E, 40X) (4) Section of the kidney of the group treated with methotrexate and therapeutic silymarin showing normal renal glomerulus (G) and the urinary tubules (UT). (H&E, 40X)**



**Figure (8):** Sections of liver tissue in treated groups. The control group (1) Shows the central vein (CV), hepatocytes (HC), blood sinusoids (S), and Kupffer cells (KC). Methotrexate group (2) Showing severe hemorrhage (H) Necrosis (N), and the onset of fibrosis (Fb). Methotrexate and Silymarin (protective) group (3) Section of the liver of the group treated with methotrexate and prophylactic silymarin, showing focal mild hepatocytes (HC) degeneration, and normal central vein (CV), blood sinusoids (S), and kupffer cells (KC). (H&E, 40X) (4) Section of the liver of the group treated with methotrexate and therapeutic silymarin, showing focal mild hepatocytes (HC) degeneration, and normal central vein (CV), blood sinusoids (S), and kupffer cells (KC). (H&E, 40X)



## **Discussion**

Silymarin has liver-protective characteristics and is used in the therapy of various hepatic disorders<sup>(10)</sup>. Study suggest Silymarin has stronger antioxidant activity<sup>(11)</sup> and indicates beneficial effects against liver toxicity caused by a large several of agents from inhibition of lipid peroxidation<sup>(6,12)</sup>. The impacts of silymarin in plasmatic ALT, AST, ALP, as well as glutathione, are investigated after APAP administered rats. The info did not indicate a significant difference in plasmatic ALT levels between Animals normotensive and hypertensive. But a significant rise (72% and 71%, resp.) in ALT levels after acetaminophen therapy, in each group, was checked, while silymarin therapy was reinstated (64% and 58%) towards normal levels<sup>(13)</sup>. Similar studies have been made in this area, including the research of Banaee et al. (2015), who evaluated the preventive effects of silymarin in the liver toxicity in animal samples. They discovered that silymarin dramatically lowered the level of liver enzymes. They reported silymarin may affect liver enzyme decrease in hepatotoxicity<sup>(14)</sup>. According to Solhi (2014), silymarin can be useful in lowering liver enzymes in people who have nonalcoholic fatty liver disease<sup>(15)</sup>. Their findings were congruent with ours since, in our investigation, ingesting silymarin on the third day dramatically reduced hepatic enzyme levels.<sup>(15)</sup>. According to Taghvaei (2013), silymarin reduces liver enzymes in people with alcoholic fatty liver disease and is beneficial in treating it Their findings were consistent with ours because the level of gamma-glutamyl transferase and liver enzymes decreased with silymarin usage in our investigation<sup>(16)</sup>. In Urea and Creatinine, The results of the study (Fig. 1) exhibited a significant increase in urea in the methotrexate group compared with the control group. The effects in the therapeutic and prophylactic groups presented a significant decrease in the urea level compared with the methotrexate induction group. The results (Fig.2)

showed a significant rise in the level of creatinine in the methotrexate-treated group compared with the control group. Treatment in both therapeutic and prophylactic groups showed a significant decrease in creatinine levels compared to the methotrexate group. In another study, serum ALT, AST, AGT, ALP enzyme activity and urea, uric acid, and creatinine, tissues of the kidney levels of Malondialdehyde (MDA) considerably rose, tubular blockage occurred, and epithelium distributed across the tubules lumen were observed in the tetrachloromethane CCl<sub>4</sub> induction group. They attributed this to the anatomy of the liver and kidney, as well as the impairment of their activities. They discovered that when rats were given honey and silymarin, their activity and levels dropped, as did tubular occlusion, epithelium distributed over, and tubulus lumen. They claimed that this was due to the presence of flavonoid and phenolic chemicals in honey and silymarin, which have antioxidant characteristics.<sup>(17)</sup> According to Study findings, silymarin may reduce the negative effects of flunixin on both the kidneys and the liver. Because of its low toxicity, powerful antioxidant, and regenerating potential, silymarin has received interest for medical use<sup>(18)</sup>. Several molecular mechanisms for silymarin's antioxidant properties have been determined, including (I) preventing the formation of free radicals by blocking enzymes that produce specific reactive oxygen species (ROS) or improving mitochondrial integrity under stress (II). reducing inflammatory reactions from preventing nuclear factor B (NF-B)-dependent processes, and (III) maintaining an ideal redox balance between enzymes and nonenzymatic antioxidants, primarily from stimulating nuclear factor-erythroid two-related factor in the cell by activating a variety of antioxidants<sup>(19)</sup> Albumin may reduce the level of free radicals in the body. Research indicates that the high concentration of albumin is the result of the protective effect of the albumin molecule because it contains the ionic and

hydrophobic character. In the occurrence of oxidation in its various reactions when it's in free form, and because it is widespread within the blood plasma that is subject to continuous oxidative stress, the quantitative effect of the albumin molecule may play its role in being antioxidant<sup>(20)</sup>.

### **Limitation**

Difficulty in obtaining rats that have not been subject to previous experiments due to their lack of availability within the province and the delay in the arrival of the kits for the chemical analysis .

### **Conclusion**

This study concluded that Methotrexate has a toxic effect on kidneys and liver functions due to tissue damage. silymarin has a significant role in attenuating the tissue damage and oxidative effect of methotrexate in most parameters.

### **Consent for Publication**

We declare that there is no conflict of interest to disclose and no financial support was submitted for this work

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