

## Ameliorative Effects of *Ziziphus spina-christi* (Sidr) Fruit Extract on 5-Fluorouracil-Induced Nephrotoxicity and Splenic Injury in Male Wistar Rats

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### Abstract:

**Background:** 5-Fluorouracil (5-FU) is a chemotherapeutic agent that induces toxicity in several organs, especially nephrotoxicity and splenic injury. **Aim:** The aim of the study was to evaluate the ameliorative effects of *Ziziphus spina-christi* (ZSC) fruit extract on 5-FU-induced nephrotoxicity and splenic injury in male Wistar rats. **Methodology:** Rats were divided into four groups: control, 5-FU (150 mg/kg), and two ZSC pre-treatment groups (200 and 400 mg/kg/day) followed by 5-FU administration. Renal function parameters and spleen indices, oxidative stress indicators (MDA, SOD, CAT, GSH) as well as histopathology were measured. **Results:** 5-FU elevated serum creatinine ( $1.28 \pm 0.08$  mg/dL) and BUN ( $68.5 \pm 4.2$  mg/dL) significantly, which indicated severe renal dysfunction. It also increased the MDA significantly in the kidneys ( $48.7 \pm 2.5$  nmol/mg protein) and spleen ( $39.1 \pm 2.1$  nmol/mg protein) and seriously reduced the antioxidant enzymes (SOD, CAT) and GSH. There was widespread tubular necrosis in both kidneys and significant lymphoid depletion in spleens identified by histopathology. These adverse effects were mitigated by ZSC pre-treatment on a dose-dependent basis. A 400 mg/kg ZSC dose has almost normalized creatinine ( $0.52 \pm 0.04$  mg/dL) and BUN ( $28.7 \pm 2.0$  mg/dL) levels, ZSC significantly reduced MDA levels (kidney:  $18.1 \pm 1.2$ ; spleen:  $16.9 \pm 1.0$ ), in addition to restoring SOD, CAT, and GSH activities to control levels. Histologically, ZSC played a major role in preserving the renal and splenic architecture and decreased necrosis and lymphoid depletion and tissue destruction. **Conclusion:** *Ziziphus spina-christi* fruit extract is a promising natural agent to counteract 5-FU-induced nephrotoxicity and splenic injury through the processes of improving antioxidant responses and tissue preservation.

**Keywords:** 5-Fluorouracil, Nephrotoxicity, *Ziziphus spina-christi*, MDA, SOD, CAT.

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## Introduction

The administration of chemotherapeutic agents continues to be one of the fundamental pillars in the treatment of all types of malignancies, but the clinical efficacy of these agents is often compromised by the occurrence of severe systemic toxicities. The most commonly used of these agents is 5-Fluorouracil (5-FU) which is an antimetabolite of the pyrimidine family of analogs and has wide usage in cancer treatment of solid tumours such as gastrointestinal malignancies, breast malignancies and head and neck malignancies (Longley et al., 2003). Although 5-FU possesses strong anti-tumor properties, it has been linked with damage to multiple organs which is mainly because this agent lacks specificity against the neoplastic cells. Though its myelosuppressive and gastrointestinal effects are well reported, there are some emerging clinical and experimental findings that define its negative effects on renal and splenic tissues which may result in discontinued use or decreased dosage (Rashid et al., 2013). The 5-FU induces nephrotoxicity in the kidney particularly because it is involved in 5-FU excretion as well as because of its metabolites, which exhibits remarkable changes in functional renal biomarkers, happening as tubular necrosis, and glomerular congestion (Rashid et al., 2014). This renal injury is a complex pathophysiology, which includes the creation of oxidative stress, the activation of the pro-inflammatory pathway, and the initiation of the apoptotic cascade in renal tubular cells (El-Gendy et al., 2024). In particular, 5-FU metabolites develop reactive oxygen species (ROS), which overwhelm the endogenous antioxidant defense system inducing lipid peroxidation and causing oxidative damage to DNA (Rashid et al., 2014).

At the same time, the spleen being one of the key peripheral lymphoid organs will also experience major structural and functional damages after 5-FU administration. The splenic injury is commonly accompanied by decreased weight of the spleen, loss of the splenic white pulp and expansion of spleenocytes apoptosis development (Du et al., 2023). This immunotoxicity does not only suppress the immune surveillance of the host, but also leads to increased systemic inflammatory response that is caused by chemotherapy. Recent evidence has shown significant relationship between the induction of splenic damage by 5-FU and the inhibition of antioxidant enzymes as well as the upregulation of pro-apoptotic proteins like Bax and caspase-3 (Du et al., 2023). Thus, it is seriously unnecessary that the nephroprotective and immunomodulatory approaches be developed to help avoid these side effects, without disrupting the main anti-cancer effect of the drug. Natural commodities and medicinal plants have received significant concern in the recent years as potential adjunct therapy to decrease the toxicity of chemotherapy. *Ziziphus spina-christi* (L.) Desf., also referred to as Sidr, is a type of tree, of the family Rhamnaceae, which has applied usage in the traditional medicine of the Middle East and North Africa over centuries (Al-Azzawi, 2010). The different sections of the plant especially its fruits and leaves are well known because of their variety of pharmacological effects such as anti-inflammatory, antimicrobial, and antioxidant effects of the highest order (Dkhil et al., 2018). A high concentration of bioactive secondary metabolites gives these therapeutic advantages, which include flavonoids, alkaloids, saponins and phenolic acids (Almeer et al., 2018).

*Ziziphus spina-christi* fruit is the fruit much appreciated because of its rich nutrition and large amounts of antioxidant phytochemicals, such as quercetin, kaempferol, and gallic acid (Aldhanhani et al., 2022). These substances are potent radical scavengers having the ability to counter the ROS and restore cellular redox homeostasis (Abdulrahman et al., 2022). The past evidence has shown that *Ziziphus spina-christi* extracts could prevent organ damage of several types by inhibiting the NF- $\kappa$ B signaling pathway and suppressing the expression of harmful cytokines like TNF- $\alpha$  and IL-1 $\beta$  (Hafiz et al., 2019). Moreover, it has been found to promote the action of endogenous antioxidant enzymes, including superoxide dismutase (SOD) and glutathione peroxidase (GPx) and hence a potential inducer of oxidative damages counteractant (El-Khadrawy et al., 2024). Although the *Ziziphus spina-christi* is known to be beneficial in other models of toxicity, its special role on the protection against 5-FU-induced nephrotoxicity and splenic injury is yet to be clarified. The majority of the available literature has concentrated on the hepatoprotective or antimicrobial properties of the leaves but little is known of the therapeutic potential of the fruit extract in relation to chemotherapy-induced multi-organ damage. Since the kidneys and the spleen play a pivotal role in clearing drugs and the immune system, it is of major clinical interest to test a natural substance that can work to save both organs. Thus, this paper was aimed at testing the ameliorative properties of *Ziziphus spina-christi* fruit extract to 5-FU-induced nephritis and splenic injury on a rat model. Evaluating biochemical indicators of renal activity, oxidative stress indicators, and histological outcomes in the kidney and the spleen will enable the discovery of the protective effect of Sidr fruit in detail in this research.

## Materials and Methods

### Chemicals and Reagents

5-Fluorouracil (5-FU) was acquired at Sigma-Aldrich (St. Louis, MO, USA) whereas high-purity analytical grade methanol, neutral buffered formalin (10%), and biochemical assay kits to determine serum creatinine, blood urea nitrogen (BUN), malondialdehyde (MDA), superoxide dismutase (SOD), and reduced glutathione (GSH) were purchased at established international companies, such as Randox Laboratories, Crumlin, UK. Fresh jujube fruits (*Ziziphus spina-christi*) were harvested in Babylon Governorate, Musayyib City, during the best seed season, and were identified under the supervision of a plant taxonomy specialist in the Department of Biology, College of Science, Babylon University.

### Preparation of Plant Material and Extract

The fruits were thoroughly sprayed with distilled water and dried under shade at room temperature and subsequently the mesocarp was deseeded followed by pulverization of the dried mesocarp using mechanical grinder to fine powder. The preparation of the hydro-methanolic extract was performed as follows: 200g of the fruit powder was cold-macerated in 1000 ml of 70% methanol during 72 hours under constant stirring at 150 rpm and then the macerate was filtered through the double layer of Whatman No. 1 filter paper and then evaporated at reduced pressure through rotary evaporator (Buchi, Switzerland) at 40 °C. Lyophilization was used to obtain a stable dry extract (yield: about 15.5% w/w) which was then stored in airtight amber-coloured glass at -20 °C and

reconstituted in sterile distilled water immediately before oral dose administration.

### Experimental Animals

Wistar rats (10-12) weeks, weight (200±20) g were chosen as adult males and kept in sterilized polycarbonate cages in a controlled setting (23±2 °C and 55% humidity) and a 12 h light/dark cycle with standard laboratory pellet diet and with free access to food and water ad libitum (National Research Council, 2011).

### The Treatment Protocol and Experiment Design

After 7 days of acclimatization 32 rats were randomly divided into four experimental groups (n=8/group) which involved Control group which received vehicle by mouth over 14 days and i.p. injection of 5-FU (150 mg/kg b.w.) on day 10 which induced acute nephrotoxicity and splenic injury (Ali et al., 2022), and the two treatment groups as 200 mg/kg b.w. and 400 mg/kg b.w. (orally).

### Sample Collection and Organ Weight Index

The rats were starved overnight at the end of the 14 days of the experimental procedure and profoundly anesthetized through the use of ketamine (i.p., 80 mg/kg) and xylazine (i.p., 10 mg/kg) after which a blood sample was taken via cardiac puncture into non-heparinized tubes using serum separation upon centrifugation at 3500 x g at 4 °C and then stored at -80 °C until biochemical profiling was carried out (Parasuraman et al., 2010). Euthanasia by cervical dislocation under anesthesia was followed by the rapid removal of kidneys and spleens followed by de-fatting and weighing of the two organs. In order to determine the relative organ weight, the ratio of the weight of the organs to that of the body was determined as follows:

$$\text{Relative Organ Weight (\%)} = \left( \frac{\text{Absolute Organ Weight}}{\text{Final Body weight}} \right) 100$$

### Serum Biochemical Assays

The renal functioning was assessed by measuring serum level of creatinine and blood urea nitrogen (BUN) with the help of automated clinical chemistry analyzer and specific diagnostic kits based on the standard procedures (Burtis et al., 2012). Each assay was done in triplicate to improve technical reproducibility.

### Biomasses of Tissue Homogenization and Oxidative Stress

A motorized homogenizer was used to homogenize portions (10% by weight) of the kidney and spleen into ice cold 50 mM phosphate buffer (pH 7.4). The homogenates were centrifuged at 12,000xg/20 minutes at 4 °C and the supernatants were taken to do the following assays- Lipid peroxidation that was measured as aldehyde malondialdehyde (MDA) content using 5, 5 prime dithiobacillin (2-nitrobenzoic acid) (DTNB) reagent as described by (Ohkawa et al., 1979); Superoxide dismutase (SOD) activity, which was

determined by the inhib The Bradford method was used to measure the total protein in the tube tissue supernatants using the bovine serum albumin as a standard.

### Histopathological and Morphometric Evaluation

Kidney and Spleen Tissue samples were fixed in 10% neutral buffered formalin 48 hours, washed in ascending grades of alcohol, cleared in xylene and embedded in paraffin blocks. Their sections were stained in Hematoxylin and Eosin (H&E) (Fischer et al., 2008) in its sections of 5 µm thickness. The slides were analysed qualitatively and semi-quantitatively under the light microscope (Nikon Eclipse, Japan) by a blinded and an experienced pathologist with regard to the group assignments. Renal sections were assessed on the basis of tubular necrosis, interstitial inflammation, and glomerular change with splenic sections being evaluated in regard to white pulp integrity and apoptotic index.

### Statistical Analysis

Normality tests (Shapiro-Wilk test) were carried out on all the data. The results are in the form of Mean Standard error of the mean (SEM). The one-way analysis of variance (ANOVA) was used to statistically compare the differences between groups and then the post-hoc test (Tukey) was performed to compare the differences between groups. The statistical significance was considered as p<0.05. GraphPad Prism version 9.0 (GraphPad Software, San Diego, CA, USA) was used to do all the calculations and the graphical representations.

## Results

The current work examined the effects of *Ziziphus spina-christi* (ZSC)-fruit extract (to be used as an ameliorant) to treat 5-Fluorouracil (5-FU)-induced nephrotoxicity and splenic injury in male Wistar rats. The results include the observation of the body and organ weight as well as renal function bio-markers, oxidative stress indicators in kidney and other tissues in the spleen and histopathological changes.

### Body and Organ Weights

According to (Table 1), 5-FU administration also led to significant reduction of the body weight of rats in comparison with the control group (p<0.001). But, the ZSC fruit extract at 200 mg/kg and 400 mg/kg dose greatly alleviated this body weight loss (p<0.01 and p<0.001, respectively). No significant difference was observed in kidney weight among groups. On the other hand, the relative weight of the spleen was much lower when the spleen was in the 5-FU-treated group (p<0.001) suggesting that spleen atrophy. Both doses of ZSC extract were successful to counter this decrease in relative spleen weight (p<0.05 and p<0.01, respectively), and it was indicated that ZSC extract could help to protect the mass of the spleen.

**Table 1.** ZSC fruit extract effect on body and relative organ weights in 5-FU-induced nephrotoxicity and splenic injury in rat

| Parameter                  | Control     | 5-FU           | ZSC (200 mg/kg) + 5-FU | ZSC (400 mg/kg) + 5-FU |
|----------------------------|-------------|----------------|------------------------|------------------------|
| Initial Body Weight (g)    | 205.3 ± 4.1 | 206.1 ± 3.9    | 204.8 ± 4.5            | 205.5 ± 4.2            |
| Final Body Weight (g)      | 248.7 ± 5.2 | 192.5 ± 6.8*** | 215.9 ± 7.1**          | 238.2 ± 6.5####        |
| Relative Kidney Weight (%) | 0.38 ± 0.02 | 0.39 ± 0.03    | 0.38 ± 0.02            | 0.37 ± 0.02            |
| Relative Spleen Weight (%) | 0.25 ± 0.01 | 0.15 ± 0.01*** | 0.19 ± 0.01*           | 0.23 ± 0.01##          |

\*Data are expressed as Mean ± SEM (n=8). \*\*\*p<0.001 vs Control; \*\*p<0.01 vs 5-FU; p<0.05 vs 5-FU; ####p<0.001 vs 5-FU; ##p<0.01 vs 5-FU.

**Renal Function Biomarkers**

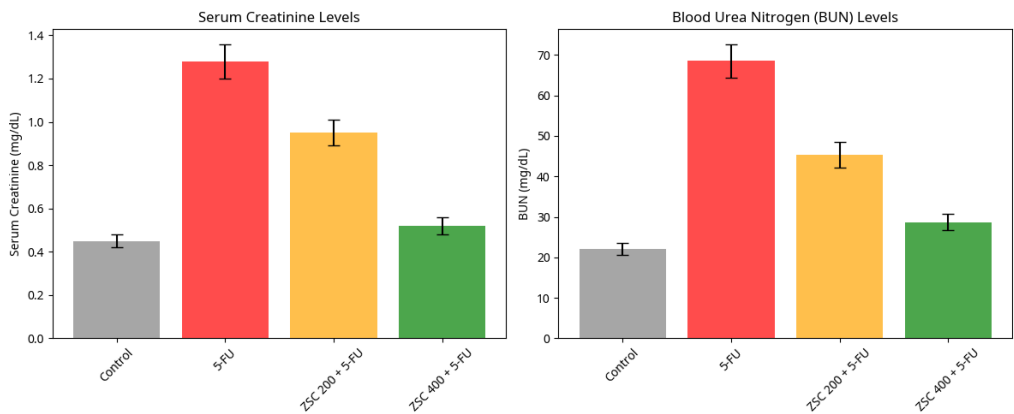
As it could be seen (Fig. 1) and (Table 2), the administration of 5-FU led to the significant increase of serum creatinine and blood urea nitrogen (BUN) levels (p<0.001 in both groups) which are the evidence of severe renal dysfunction. These changes were greatly

alleviated by Pre-treatment with ZSC fruit extract. In particular, ZSC 200 mg/kg lowered serum creatinine (p<0.05) and BUN (p<0.01) levels significantly, whereas the higher dose of 400 mg/kg had a stronger protective effect, but brought both creatinine ( p <0.0001) and BUN ( p <0.0001) near the normal levels of creatinine and BUN.

**Table 2.** Effect of ZSC fruit extract on serum biomarkers of renal function in 5-FU-induced nephrotoxic rats

| Parameter          | Control     | 5-FU           | ZSC (200 mg/kg) + 5-FU | ZSC (400 mg/kg) + 5-FU |
|--------------------|-------------|----------------|------------------------|------------------------|
| Creatinine (mg/dL) | 0.45 ± 0.03 | 1.28 ± 0.08*** | 0.95 ± 0.06*           | 0.52 ± 0.04####        |
| BUN (mg/dL)        | 22.1 ± 1.5  | 68.5 ± 4.2***  | 45.3 ± 3.1**           | 28.7 ± 2.0####         |

\*Data are expressed as Mean ± SEM (n=8). \*\*\*p<0.001 vs Control; \*\*p<0.01 vs 5-FU; p<0.05 vs 5-FU; ####p<0.001 vs 5-FU.



**Fig. 1.** *Ziziphus spina-christi* fruit extract as an effect on serum creatinine, blood urea nitrogen (BUN) in 5-FU-induced nephrotoxic rats

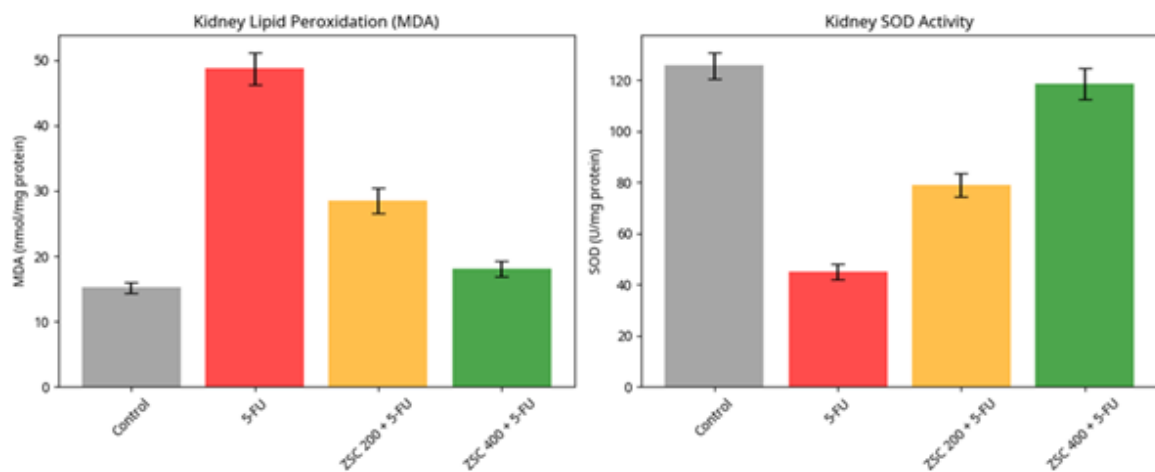
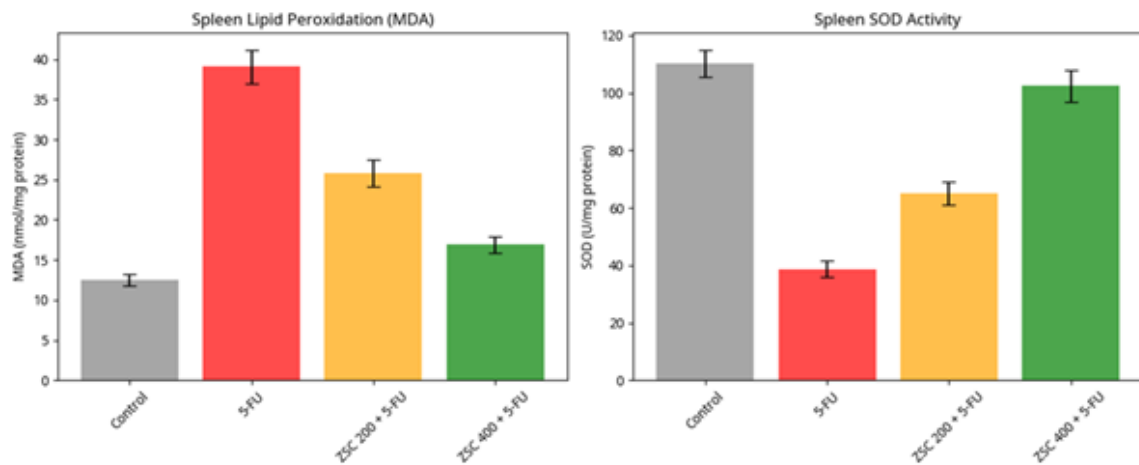
**Oxidative Stress Parameters**

The (Table 3) and (Fig. 2 & 3) illustrate the ZSC effects on the oxidative stress markers in a nutshell. The levels of MDA were found to increase significantly the kidney and spleen tissues in the 5-FU-treated (p<0.001 in both cases), showing the presence of increased lipid peroxidation. At the same time, the antioxidant enzyme (SOD and CAT) activity and the GSH concentration level

was much lower in both organs (p<0.001 in all cases) than the control levels did. These adverse changes were reversed greatly by ZSC pre-treatment. Both doses of ZSC considerably reduced the MDA levels of kidney (p <0.01 0.001) and spleen (p <0.05 0.001). In addition, ZSC has remarkably recovered SOD activity (kidney: p<0.01, p<0.001; spleen: p<0.05, p<0.001), CAT activity (kidney: p<0.01, p<0.001; p<0.05, p<0.001) and GSH content (kidney: p<0.01, p<0.001; p<0.05, p<0.001) in

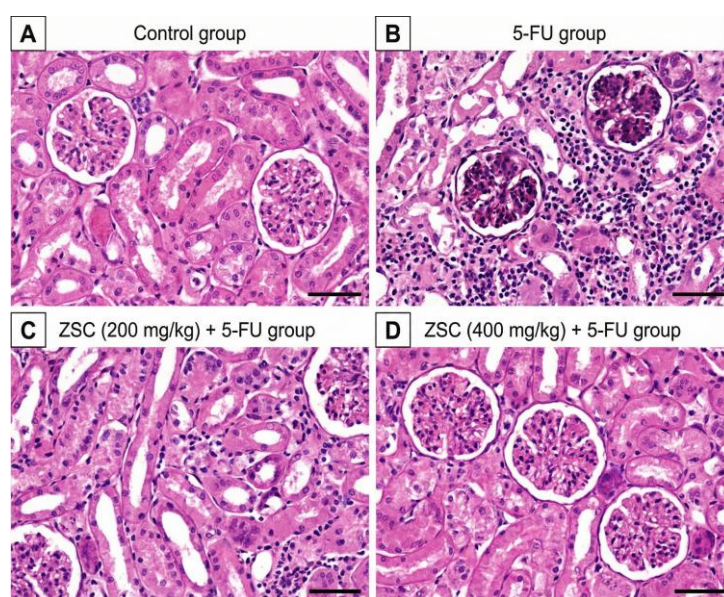
**Table 3.** ZSC fruit extract on the effect of ZSC fruit extract on oxidative stress parameters in kidney and spleen tissues of 5-FU induced nephrotoxicity and splenic injury in rats

| Parameter             | Organ  | Control     | 5-FU           | ZSC (200 mg/kg) + 5-FU | ZSC (400 mg/kg) + 5-FU |
|-----------------------|--------|-------------|----------------|------------------------|------------------------|
| MDA (nmol/mg protein) | Kidney | 15.2 ± 0.8  | 48.7 ± 2.5***  | 28.5 ± 1.9**           | 18.1 ± 1.2###          |
|                       | Spleen | 12.5 ± 0.7  | 39.1 ± 2.1***  | 25.8 ± 1.7*            | 16.9 ± 1.0###          |
| SOD (U/mg protein)    | Kidney | 125.6 ± 5.3 | 45.2 ± 3.1***  | 78.9 ± 4.5**           | 118.5 ± 6.2###         |
|                       | Spleen | 110.3 ± 4.8 | 38.7 ± 2.8***  | 65.1 ± 3.9*            | 102.4 ± 5.5###         |
| CAT (U/mg protein)    | Kidney | 8.9 ± 0.4   | 3.1 ± 0.2***   | 5.8 ± 0.3**            | 8.2 ± 0.4###           |
|                       | Spleen | 7.5 ± 0.3   | 2.5 ± 0.2***   | 4.9 ± 0.3*             | 6.8 ± 0.3##            |
| GSH (μmol/mg protein) | Kidney | 1.85 ± 0.09 | 0.62 ± 0.04*** | 1.15 ± 0.07**          | 1.78 ± 0.08###         |
|                       | Spleen | 1.60 ± 0.08 | 0.50 ± 0.03*** | 0.98 ± 0.06*           | 1.51 ± 0.07###         |

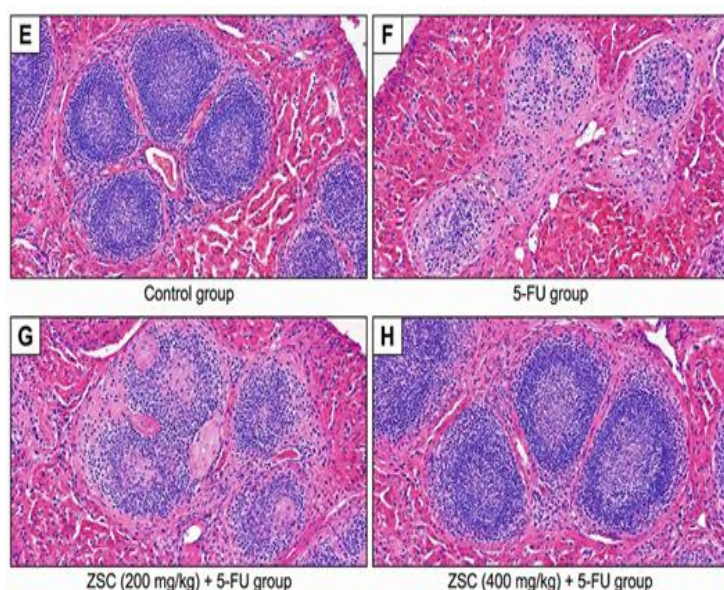
**Fig. 2.** Effect of *Ziziphus spina-christi* fruit extract on oxidative stress markers (MDA and SOD) in kidney tissue of 5-FU-induced nephrotoxicity in rats**Fig. 3.** The action of *Ziziphus spina-christi* fruit extract on the oxidative stress indicators (MDA and SOD) of spleen tissue of 5-FU induced splenic injury in rats.

## Histopathological Findings

Morphological analysis of tissues of kidneys and spleen gave morphological confirmation to the biochemical data, as shown in (Fig. 4 & 5)



**Fig. 4.** Representative photomicrographs of sections of the kidneys stained with Hematoxylin and Eosin (H&E) (400x magnification). **Control Group (A):** Healthy renal structure, normal renal architecture, and inflammatory infiltration. **5-FU Group (B):** 5-FU Group, severe tubular necrosis, glomerular congestion, and inflammatory infiltration. **ZSC (200 mg/kg) + 5-FU Group (C):** Intermediate lessening of tubular devastation and lympho-inflammation. **ZSC (400 mg/kg) + 5-FU Group (D):** High-order protection of the renal structure, which is similar to control



**Fig. 5.** Hematoxylin and Eosin (H&E) representative photomicrographs of splenic sections of the spleen (400x magnification). **Control Group (E):** White Pulp (architecture) is if normal red and white pulp. **5-FU Group (F):** EVID: Pulp lymphoid depletion and disorganization. **The group with ZSC (200 mg/kg) + 5-FU (G):** Lymphoid cellularity partially restored. **ZSC (400mg/kg) + 5-FU Group (H):** There was significant white pulp preservation and normal cellularity

## Discussion

The present study will provide evidence about the protective action of *Ziziphus spina-christi* (ZSC) fruit extract against the multi-organ toxicity of 5-Fluorouracil (5-FU) which targets the renal and the splenic system since the use of this agent is limited by the fact that it is non-selectively cytotoxic to healthy tissues. The results of our study prove that 5-FU results in body weight and splenic mass

losses, accompanied by increases in renal biomarkers and oxidant-antioxidant homeostasis. On the other hand, these pathological alterations were counteracted by the pre-treatment of ZSC fruit extract which was probably due to the phytochemical content present in the extract and the capacity to regulate oxidative stress. A decrease in both the body weight and the relative weight of the spleen after 5-FU is one of the manifestations of systemic toxicity. The cause of weight loss could be increased due to decreased feed

consumption, gastrointestinal mucositis, and metabolic derangements by catabolic metabolites (Zhang et al., 2008). Moreover, myelosuppressive action of the drug is seen in splenic atrophy. The spleen is also very prone to the DNA-damaging effects of 5-FU which blocks thymidylate synthase and incorporated into RNA and DNA resulting in the apoptosis of rapidly dividing lymphocytes (Vodenkova et al., 2020). ZSC has an immunoprotective effect as witnessed by its capacity to maintain relative weight of the spleen.

Kidney failure is the severe side effect of 5-FU, in which the filtration and tubular damage are observed. Acute nephrotoxicity has been proved in our study by the change in serum creatinine and blood urea nitrogen (BUN) in the 5-FU group, where the values increased. These indicators indicate the reduction of the glomerular filtration rate. Mechanism of 5-FU-induced renal injury Genetic effects of Metabolites of 5-FU on the tubular lining and vascular congestion (Adikwu & Biradee, 2019). These biochemical observations are supported by our histopathological results (tubular necrosis) of the 5-FU group. Interestingly, ZSC pre-treatment led to a dose-dependent decrease in creatinine and BUN parameters and the 400 mg/kg dose almost normalized the parameters. This protective effect on the kidney by this extract can be associated with the potential of the extract to protect the integrity of the basement membrane and the clearance of the kidney. Oxidative stress, on a molecular level, is the major cause of organ damage caused by 5-FU. The 5-FU metabolism results in overproduction of reactive oxygen species (ROS) which overwhelm antioxidant defense systems. It results in lipid peroxidation as the level of malondialdehyde (MDA) in the tissues of the kidneys and spleen increases in the 5-FU group. MDA is an indicator of oxidative damage on membranes (Ayala et al., 2014). Coincidental occurrence of reduced glutathione (GSH) depletion and inhibition of superoxide dismutase (SOD) and catalase (CAT) activity further increase cell damage. GSH is an essential non-enzymatic antioxidant, which cancels the effect of ROS and SOD and CAT are the initial enzymatic lines of defense (Halliwell & Gutteridge, 2015). The fact that the quantity of these antioxidants decreases in the 5-FU group shows a condition of extremity of oxidative exhaustion.

Oxidative crisis was reversed effectively by the administration of ZSC fruit extract. The extract reduced the levels of MDA and replaced the functions of the SOD, CAT, and GSH in both organs. Bioactive secondary metabolites such as flavonoids, phenolic acids, and saponins have been identified as a cause of such an antioxidant activity. *Ziziphus spina-christi* contains ample polyphenols such as quercetin and gallic acid which is reported to have radical scavenging ability (Michel et al., 2011). These substances stabilize the free radicals inhibiting lipid peroxidation. Furthermore, ZSC contains saponins which have been identified to activate antioxidant genes, which might increase the production of antioxidant enzymes such as SOD and CAT that are produced endogenously (Michel et al., 2011). This two-step, which is simultaneous scavenging and enhancement of cellular antioxidants, is the basis of organ protection by the extract.

The findings were visualized by histopathological assessment. Extensive tubular necrosis and inflammatory cell infiltration of the kidney and lymphoid depletion of the spleen were found in the 5-FU group. These changes correspond with great MDA and low antioxidant conditions. Oxidative stress causes an inflammatory

response, indicating that the inflammatory infiltrates are instigated by NF- $\kappa$ B signaling, which controls the pro-inflammatory cytokines (Adikwu & Deo, 2019). The dose-dependent effect improvement of tissue architecture on ZSC-treated groups is impressive. The dose of 400mg/kg dose did not cause any significant morphological deviations in both organs. This has been preserved perhaps due to the potential of the extract to prevent lipid peroxidation, as well as, inhibiting the cascade of inflammatory processes that lead to cell death. Attention should be paid to the immunomodulatory effect of ZSC in the spleen. The spleen is a crucial component of the immune system, its impairment may result in the predisposition to infections. The damages caused by 5-FU are associated with the release of the intrinsic apoptotic pathway due to the up-regulation of pro-apoptotic proteins such as Bax (Ghafouri et al, 2020). ZSC has phytochemicals which can disrupt this apoptotic signaling. Since ZSC can preserve the level of white pulp in spleen and lymphocytes, it can be used to reduce immune incompetence caused by chemotherapy and this is highly clinical.

As compared to other natural products, the *Ziziphus spina-christi* fruit extract efficacy seems to be competitive. Numerous researches have been done on the use of plant-based compounds to reduce the toxicity of 5-FU, yet ZSC presents a different approach in terms of balancing nutritional specifics and prescription opportunities. Its fruits contain antioxidants and are important providers of vital vitamins in physiological healing (Abdallah, 2017). In our work, we point out that a hydro-methanolic extract of ZSC fruits is especially efficient due to the fact that the given solvent system extracts polar and semi-polar molecules of bioactive properties. This study is limited though it gives promising results. Studies on the prophylactic effect were studied and further conclusions ought to consider the therapeutic potential once the toxicity has developed. Further bioassay-directed fractionation is also required to identify particular molecules that mediate the renoprotective and splenoprotective effects. Mechanism ZSC would be investigated to investigate the role of ZSC in the expression of molecular targets such as Nrf2.

## Conclusion

*Ziziphus spina-christi* fruit extract exhibits strong ameliorative effects against nephrotoxicity and necrosis of spleen induced by 5-FU. ZSC is a potent natural mechanism to reduce side effects of 5-FU chemotherapy by inhibiting oxidative stress, improving the antioxidant defense mechanism, and maintaining structural integrity. The findings provide an indication that ZSC may be formulated as an useful adjunctive therapy in order to enhance the safety and tolerability of 5-FU-based treatment in clinical oncology.

## Ethical Approval

This study was conducted in accordance with the ethical approval granted by the Institutional Ethics Committee of Al-Furat Al-Awsat University, under Protocol No. 73, dated April 8, 2025.

## Conflict of Interest

There are no conflicts of interest in this article.

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## References

- [1] Abdallah, E. M. (2017). Antibacterial Activity of Fruit Methanol Extract of *Ziziphus spina-christi* from Sudan. *International Journal of Current Microbiology and Applied Sciences*, 6(5), 1111-1117.
- [2] Abdulrahman, M. D., Ali, A. M., & Hamad, M. N. M. (2022). Ethnopharmacology, Biological Evaluation, and Chemical Composition of *Ziziphus spina-christi* (L.) Desf.: A Review. *Evidence-Based Complementary and Alternative Medicine*, 2022, 4495688.
- [3] Adikwu, E., & Biradei, I. (2019). Therapeutic benefit of resveratrol in 5-fluorouracil-induced nephrotoxicity in rats. *Biomedical Research Journal*, 6(2), 45-52.
- [4] Adikwu, E., & Deo, O. (2019). Ameliorative effect of resveratrol on 5-fluorouracil-induced nephrotoxicity in rats. *Biomedicine & Pharmacotherapy*, 111, 1252-1259.
- [5] Al-Azzawi, M. A. (2010). Effect of Sidr (*Ziziphus spina-christi*) fruit extract on the central nervous system in male albino rats. *Iraqi Journal of Veterinary Sciences*, 24(2), 123-131.
- [6] Aldhanhani, A. R. H., Al-Dhaheri, A. S., & Ibrahim, W. H. (2022). Maturity stage at harvest influences antioxidant phytochemicals and antibacterial activity of jujube fruit (*Ziziphus mauritiana* Lamk. and *Ziziphus spina-christi* L. Desf.). *LWT - Food Science and Technology*, 161, 113333.
- [7] Ali, H. H., Ibrahim, O. M., & Al-Shinnawy, A. S. (2022). Effect of Telmisartan and Quercetin in 5 Fluorouracil-Induced Renal Toxicity in Rats. *Iraqi Journal of Pharmaceutical Sciences*, 31(1), 153-161.
- [8] Almeer, R. S., El-Khadragy, M. F., Abdelhabib, S., & Moneim, A. E. A. (2018). *Ziziphus spina-christi* leaf extract ameliorates schistosomiasis liver granuloma, fibrosis, and oxidative stress through downregulation of fibrinogenic signaling in mice. *PLOS One*, 13(10), e0204923.
- [9] Ayala, A., Muñoz, M. F., & Argüelles, S. (2014). Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. *Oxidative Medicine and Cellular Longevity*, 2014, 360438.
- [10] Burtis, C. A., Ashwood, E. R., & Bruns, D. E. (2012). *Tietz Textbook of Clinical Chemistry and Molecular Diagnostics* (5th ed.). Saunders.
- [11] Dkhil, M. A., Al-Quraishy, S., & Moneim, A. E. A. (2018). *Ziziphus spina-christi* leaf extract pretreatment inhibits liver and spleen injury in a mouse model of sepsis via anti-oxidant and anti-inflammatory effects. *Inflammopharmacology*, 26(3), 779-791.
- [12] Du, K., Wang, L., Wang, Z., Xiao, H., Hou, J., Hu, L., Fan, F., & Wang, Y. (2023). *Angelica Sinensis* polysaccharide antagonizes 5-Fluorouracil-induced spleen injury and dysfunction by suppressing oxidative stress and apoptosis. *Biomedicine & Pharmacotherapy*, 162, 114631.
- [13] El-Gendy, H. F., Al-Shorbagy, M. Y., El-Ghazaly, M. A., & Abd El-Hameed, A. K. (2024). Unraveling the Potential of *Saccharum officinarum* and *Chlorella vulgaris* in Mitigating 5-Fluorouracil-Induced Nephrotoxicity and Splenic Injury in Rats. *Pharmaceuticals*, 17(7), 885.
- [14] El-Khadrawy, O. H., El-Khadrawy, H. H., & Al-Ghazaly, M. A. (2024). Protective effects of *Ziziphus spina-christi* on cadmium-induced nephrotoxicity in rats. *Indian Journal of Animal Research*, 58(2), 245-252.
- [15] Fischer, A. H., Jacobson, K. A., Rose, J., & Zeller, R. (2008). Hematoxylin and eosin staining of tissue and cell sections. *Cold Spring Harbor Protocols*, 2008, pdb.prot4986.
- [16] Ghafouri-Fard, S., Shoorei, H., & Taheri, M. (2020). Non-coding RNAs in the pathogenesis of 5-fluorouracil resistance. *Frontiers in Oncology*, 10, 1368.
- [17] Hafiz, T. A., Mubarak, M. A., Diab, M. S. M., Dkhil, M. A., & Al-Quraishy, S. (2019). Ameliorative role of *Ziziphus spina-christi* leaf extracts against hepatic injury induced by *Plasmodium chabaudi* infected erythrocytes. *Saudi Journal of Biological Sciences*, 26(7), 1531-1535.
- [18] Halliwell, B., & Gutteridge, J. M. (2015). *Free Radicals in Biology and Medicine* (5th ed.). Oxford University Press.
- [19] Longley, D. B., Harkin, D. P., & Johnston, P. G. (2003). 5-fluorouracil: mechanisms of action and clinical strategies. *Nature Reviews Cancer*, 3(5), 330-338.
- [20] Michel, C. G., El-Sayed, N. S., Moustafa, S. F., & Ezzat, S. M. (2011). Phytochemical and biological investigation of *Ziziphus spina-christi* leaves. *Journal of Pharmacy and Pharmacology*, 63(1), 114-120.
- [21] National Research Council. (2011). *Guide for the Care and Use of Laboratory Animals* (8th ed.). The National Academies Press.
- [22] Ohkawa, H., Ohishi, N., & Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry*, 95(2), 351-358.
- [23] Parasuraman, S., Raveendran, R., & Kesavan, R. (2010). Blood sample collection in small laboratory animals. *Journal of Pharmacology and Pharmacotherapeutics*, 1(2), 87-93.
- [24] Rashid, S., Ali, N., Nafees, S., Ahmad, S. T., Arjumand, W., & Hasan, S. K. (2013). Abrogation of 5-fluorouracil induced renal toxicity by bee propolis via targeting oxidative stress and inflammation in Wistar rats. *Journal of Pharmaceutical Research International*, 7(2), 156-164.
- [25] Rashid, S., Ali, N., Nafees, S., Ahmad, S. T., Arjumand, W., & Sultana, S. (2014). Mitigation of 5-Fluorouracil induced renal toxicity by chrysin via targeting oxidative stress and apoptosis in wistar rats. *Food and Chemical Toxicology*, 66, 184-193.
- [26] Vodenkova, S., Buchler, T., Cervena, K., Veskrnova, V., Vodicka, P., & Vymetalkova, V. (2020). 5-Fluorouracil and other fluoropyrimidines in colorectal cancer: past, present and future. *Pharmacology & Therapeutics*, 206, 107447.
- [27] Zhang, N., Yin, Y., Xu, S. J., & Chen, W. S. (2008). 5-Fluorouracil: mechanisms of resistance and reversal strategies. *Molecules*, 13(8), 1551-1569.