Al-Kitab Journal for Pure Science
Vol.4 (2), ISSN: 2617-1260 (print), 2617-8141(online)
www.kjps.isnra.org

The Nephroprotective Effect of Zizphus Jujuba Extract Against 5-Flurouracil- Induced Nephropathy

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ABSTRACT

This study assessed the protective effect of Zizphus jujube (ZJ) extract on 5-FU-induced alterations in renal function markers and kidney morphology in Dawley rats. Twenty-four rats were divided randomly into four groups administrated orally with 0.9% normal saline as the control group, 5-FU (40 mg/kg daily for 5 days), ZJ (500 mg/ kg daily for 5 days), and 5-FU+ ZJ (for 6 days). further biochemical experiments carried out on blood collected from the heart. Kidney tissues were obtained for analysis of catalase (Cat), glutathione S-transfers (GST), and lipid peroxide levels as well as histology analysis. 5-FU significantly reduced the enzyme activity of Cat and GST and increased levels of lipid peroxidation and plasma creatinine levels ($P < 0.005$). Histopathological examination showed severe wide ischemia of proximal convoluted tubule (PCT), missing in Bowman's space, and edema in the group treated with 5-FU. In addition, pretreatment with ZJ has significantly improved levels of Cat and GST and reduced lipid peroxidation and plasma creatinine levels ($P < 0.05$). Moreover, the histopathological analysis showed that ZJ relatively prevented the damage in renal tubular cells compared with 5-FU treated group. Supplementation with ZJ may have clinical benefit in nephrotoxicity caused by 5-FU.

Keywords: antioxidant; 5-Flurouracil, ziziphus jujube, nephrotoxicity, glutathione S-transferase, catalase, histological analysis.

DOI: https://doi.org/10.32441/kjps.04.02.p6

Al-Kitab Journal for Pure Science
Vol.4 (2), ISSN: 2617-1260 (print), 2617-8141(online)
www.kjps.isnra.org

تأثير مستخلص نبات الجوجوبا لحماية الكلي ضد المرض الكلوي المستحث بعقار 5- فلورو يوراسيل

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الملخص

تم دراسة تأثير مستخلص نبات الجوجوبا على التسمم المستحث بعقار 5 فلورو يوراسيل وذلك على الكلي الخاصة بالجرذان. تم اختيار 24 حيوان وذلك لإجراء التجربة. تم قياس شكل الكلي والعديد من دلالات التسمم للكلي. تم عمل 4 مجموعات لتلقي المستخلص والمقارنة فيما بنهم. وقد أظهرت نتائج تشريح انسجة الكلي والانابيب الناقلة الي حمايتها من التسمم الحادث من استخدام 5 فلورو يوراسيل وذلك اثر تعاطي مستخلص الجوجوبا مما يؤكد النتائج بضرورة استخدامه لحماية الكلي.

الكلمات الدالة: مضاد الاكسدة, 5 فلورو يوراسيل , مستخلص الجوجوبا .

1. Introduction

5-fluorouracil (5-FU) is a pyrimidine fluorinated analog that is classified as an antimetabolite. It is widely used for the chemotherapeutic treatment of hepatocellular carcinoma. This drug showed activity with many solid tumors, including stomach, breast, pancreas, esophagus, liver, head, neck, and colorectal cancers[1]. 5-FU acts via the incorporation of its metabolites into RNA and DNA, thereby inhibiting thymidylate synthase. Consequently, it causes DNA damage, cell cycle termination, apoptosis, and necrosis of cancer cells[2]. The extended activity of 5-FU on RNA and DNA in normal rapidly dividing cells causing cell damage and death has been associated with its numerous toxic effects[3]. Nephrotoxicity is one of the most disturbing adverse consequences of therapy with 5-FU characterized by arrays of features which include kidney histological changes such as glomerular and tubular degeneration[4]. It is also associated with changes in serum renal biomarkers including electrolytes and acid–base balance and alteration in glomerular filtration rate[4]. Its nephrotoxic effect has been associated with its catalyzed product dihydrouracil which is

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Vol.4 (2), ISSN: 2617-1260 (print), 2617-8141(online)
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cleaved to α-fluoro-β-alanine and other by-products in the liver which are injurious to the kidney. In addition, its nephrotoxic effect may involve oxidative stress (OS) through free radical production, inflammation, and the stimulation of apoptic pathways in renal tissues[5].Ziziphus jujube, a species of Ziziphus (L.) in the buckthorn family Rhamnaceae, an fascinating deciduous tree with spiny, twisted branches and an open, irregular form ZJ has been used in folk medicine as demulcent, depurative, bland, emollient, stomachic for toothaches, astringents and as a mouth wash. It has antioxidant activity which occurs through the scavenging and neutralization of free radicals [6] and can increase the expression of enzymes such as superoxide dismutase (SOD) and catalase (CAT) responsible for maintaining oxidation–reduction balance in cells. It can inhibit lipid peroxidation (LPO) by removing lipid peroxides produced in membranes, thereby abrogating the harmful activity of lipid peroxide on biomolecules[7]. ZJ acts as an anti-inflammatory agent by reducing cyclooxygenase 1 enzyme (COX-1). COX-1 catalyzes the first step in the synthesis of prostaglandins which can culminate in reactions facilitating the production of free radicals[8]. Furthermore, it can inhibit the activity of pro-inflammatory cytokines thereby preventing inflammation-mediated damage. ZJ interacts with many receptors, kinases, and other enzymes that could possibly make major contributions to its biological effects[9]. It has shown potential in the treatment of neurodegenerative diseases, diabetes, cancer, and hypertension in animal models[10]. In addition, it has shown protective benefits against several renal injuries caused by toxic insults in animal models[11]. The purpose of this study was carried out to investigate the protective effect of ZJ extract which may weaken this serious toxicity without affecting the efficacy of the drug, in an in vivo model.

2. Materials and Methods

Experimental design

Twenty-four Sprague Dawley rats (male), weighing 160–210 g each and their ages (8- 12 weeks), were obtained from the animal house at JUST, Jordan. They kept at a constant temperature (22±1 ºC) with a regular 12-h light and dark cycle with providing diet and water. rats randomly divided into four groups (6 for each). Drug was administered orally using a ball tipped stainless steel gavage connected to a syringe. An individual body weights were obtained for test animals' prior administration daily. The experimental design included as follows:

- Group 1: saline- control group. Rats will be orally with 0.9% NaCl for 5 days.

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Vol.4 (2), ISSN: 2617-1260 (print), 2617-8141(online)
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- Group 2: ZJ extract group. Rats will be administered orally 500 mg/kg .
- Group 3: IF group. Rats will be administered orally an IF 40mg/kg for 5 days (recommended dose administered for cancer patients).
- Group 4: ZJ extract and IF group. Rats will be administered IF (40 mg/kg) just as the IF group, except that they will be administered with ZJ extract 1 day before and then ZJ extract + 5-FU daily for 5 days.

2.1 Ethical Approval

The study was carried out under veterinary supervision, used in full compliance with local, national, ethical, and regulatory values for animal care and was approved by the Suez Canal University Research Ethics and Animal Care Committee[201703MA2].

2.2 Sample Collection

The animals in all groups were in an ether chamber after 48 h from the last application, after an overnight fast. Blood samples were taken by the intracardiac puncture and collected into the heparin tubes. The samples were centrifuged at 3000 rpm for 10 minutes to separate their plasma and then stored at -18C° until used for determination of biochemical test. The two kidneys were removed for each rat. One kidney was placed in 10% formaldehyde solution for processing to histopathology examination by light microscopy. The other kidney was homogenized with phosphate buffered solution (pH 7.2) to obtain 1: 5 (W/V) homogenate. The latter was analyzed to determine the glutathione reductase activity, glutathione catalase activity, glutathione peroxidase activity, glutathione S-transferase activity and lipid peroxidation.

2.3 Plasma Biochemistry test

Urea and creatinine activity were measured using Biorex Urea and Biorex creatinine kits, respectively (Biorex, diagnostic reagents for laboratories, United Kingdom) according to the manufacturer's instruction.

2.4 Histological analyses

The left kidneys of each rat were removed and fixed immediately in 10% formaldehyde, and then the fixed samples were washed in 70% ethanol. Dehydration was performed by passing the materials in elevated ethanol concentrations as follows: 80%, 90%, 95% and absolute (two hours in each change) using the processor, and then cleared in xylene for 20 minutes. Infiltration was carried out using paraffin wax (melting point 60°C), samples were Embedded with pure melted paraffin wax and poured in a cassettes. Thereafter Blocks were trimmed.

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Then, serially sections of 5 µm thickness were done using a Wild microtome. The serial sections were put on glass slides and then immerged in hot water (45-50°C) for few second. Serial sections that stained by using Ehrlich hematoxylin and eosin Y stains (H&E), were mounted with dibex and examined on Leitz Laborlux 11 microscope and photographed.

2.5 Determination of enzymes activities

Glutathione S-transferase activity was measured spectrophotometrically at 340 nm for the rate of conjugation of 1-chloro-2,4-dinitrobenzene (CDNB) with GSH as a function of time[12]. The assay mixture contained the following: 2900 µl of 0.1 M potassium phosphate buffer pH 6.5, 50 µl of 0.1 M GSH, 30 µl of 0.1 M CDNB dissolved in minimum volume of absolute ethanol and 20 µl of the crude. The specific activity of the enzyme was expressed as units per mg protein. Using an extinction coefficient of 9.6 mM^{-1} cm^{-1}. Catalase activity was measured spectrophotometrically according to Aebi (1984) [13] as the following: To a three ml reaction, one ml of 30 mM H2O2 and 1.980 ml of 50 mM phosphate buffer (pH 7.00) were applied into a quartz cuvette, the reaction was initiated by the addition of appropriate volume of the crude. The rate of hydrogen peroxide decomposition was monitored at 240 nm and Specific activity was expressed in units per mg protein[13]. Level of lipid peroxidation was determined by method described earlier[14]. The final volume of the reaction mixture was 4 ml. The following were sequentially added: 1.5 ml of 20 % acetic acid, 0.2 ml of 8.1 % SDS, 1.5 ml of 0.8 % TBA, 0.77 ml distilled water and 30 µl of crude. The reaction was incubated at 95°C for one hour, after cooling the reactions were centrifuged at 2500 rpm for 8 minutes. The optical density of the chromogen malondialdehyde (MDA) was estimated versus blank at 532 nm. Finally, Total protein concentration was assessed according to the method of Bradford, using bovine serum albumin as standard[15].

2.6 Statistical analysis

The results were analyzed using the SPSS software version 20. Results were stated as a mean ± S.D. The data was analyzed using one-way analysis of variance (ANOVA) followed by LSD multiple range test for the statistical comparison between groups; A P-value of less than 0.05 was considered statistically significant.

3. Results

3.1 Body weight changes

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Within 48 hours of the last oral administration, no mortality was seen in any of the control, 5-FU, ZJ, and ZJ+5-FU group. For the duration of the experiment (5 days) was shown an increase of body weight gain in control, ZJ, and ZJ+5-FU groups, while it decreased significantly in 5-FU group. Table (1) shows the mean body weight difference expressed as percentage (%).

Group that given ZJ (500 mg/70 kg) has shown the enhancement in body weight, while group administrated 5-FU (40 mg/ kg) alone has shown significantly reduced in body weight when compared with others groups. We can see that the group which administrated ZJ alone increases in body weight as compared with ZJ+5-FU group.

Table 1: The mean body weight difference expressed as percentage (%) for each group.

Parameter	Control (NS)	ZJ	5-FU	ZJ + 5-FU
Body weight difference (%)	16 %	7.5 %	-12.5 %	0 %

3.2 Histological analysis

Haematoxylin and eosin staining (H & E) of the kidney showed mass edema and less number of tubules and blood congestion in N.S as a control group (Figure 1). The 80mg/kg 5-FU dose produced significant changes compared to the control group as it was shown in figure (2a+b) it caused severe wide ischemia of PCT, missing in Bowman's space. There was also a significant amount of cell death, hemorrhage, edema and congestion. The rat group that had been administrated ZJ (500 mg/ kg) showed good structure of glomeruli and tubule and increase in number of cells and less blood congestion and less edema (Figure 3a). The group of ZJ (500 mg/kg) with 5-FU (40 mg/kg) showed mild ischemia of PCT and the nephrons were near of normal (Figure 3b).

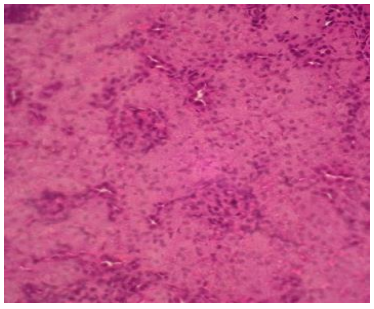

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Figure 1: N.S group: Backed glomeruli, less number of tubules, mass edema, water inside Obliteration of Bow man's spaces, Magnification of X400 for better visualization.

A **b**

Figure 2a: 5-FU 40 mg/kg group: a lot of congestion glomeruli, mass edema, less number of tubules ,increases of cell Obliteration of Bow man's spaces, a lot of blood congestion. **2b:** 5-FU 40 mg/kg group, Magnification of X400 for better visualization.

A **b**

Figure 3a: ZJ group: good structure of glomeruli and tubules, increase of cells, Obliteration of Bow man's spaces, blood congestion. **3b:** ZJ+ 5-FU group: Good structures, less glomerular congestion, increase of cells, less edema congestion, increase of cell Obliteration of Bow man's spaces. Magnification of X400 for better visualization.

3.3 Plasma biochemistry test

Plasma creatinine level was significantly increased in rats that received 5-FU (40 mg/kg) alone when compared with control group. Administration of ZJ (500mg/ kg) + 5-FU reduced creatinine level with significant. While Groups that received ZJ alone showed that the creatinine level lower than those in control group. ZJ alone prevented significantly the elevation of creatinine level when compared with 5-FU group (table 2). ZJ have no clear effect on plasma urea level.

Table 2: The effects of each experimental group on plasma biochemical parameters in rats

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Parameters	Control N.S	ZJ	5-FU	ZJ+5-FU	P-value
Creatinine (mmol/l)	16.0± 4.166 [b]	12.01± 4.166 [b]	43.30± 6.250 [a]	27.10± 7.511 [b]	0.001*
Urea (mmol/l)	20.3± 1.18	28.16± 7.57	19.90± 3.33	20.59± 4.44	0.032

Abbreviations, *: Significant at α=0.005; a: Significant when compared with N.S control group; b: Compared with 5-FU group. 5-FU; 5-fluorouracil, ZJ; ziziphus jujube. Data expressed as mean± S.D.

3.4 Activities of antioxidant enzymes

The activities of three different antioxidant enzymes have been measured in all individuals within the Control group 5-FU, ZJ+5-FU, and ZJ, were in the rats treated with 40 mg/kg 5-FU, GST activity was lower than that in the group treated with N.S, ZJ+5-FU and ZJ but not significantly (Table 3), while the activity of CAT in kidney homogenate was significantly reduced in rats that received 5-FU alone as compared with control group. Group that received ZJ+5-FU showed an elevation in Cat activity without significant as compared to IF group.

Table 3: The average mean values of the specific activities of the three enzymes for each experimental group

parameters	Control N.S	ZJ	5-FU	ZJ+5-FU	P-value
Cat mmol/l	864.53± 113.5 [b]	846.77± 102.1	514.93± 141.6 [a]	603.87 ±183.7	0.03*
GST mmol/l	0.5611± 0.335	0.6121± 0.155	0.337± 0.147	0.5481± 0.097	0.06*

Abbreviations, *: Significant at α=0.05; a: Significant when compared with N.S control group; b: Compared with 5-FU group; N.S: Normal saline; 5-FU: 5-fluorouracil; ZJ: Ziziphus jujube; Cat: catalase; GST: glutathione S-transferase. Data expressed as mean± S.D: standard deviation.

Malondialdehyde level in the 5-FU-treated animals were higher than those of the control groups with significant (Table 4). The combined treatment of ZJ plus 5-FU and ZJ were reduced the elevations of MDA level.

Table 4: The average mean values of the specific activities of lipid peroxidation (MDA) for each experimental group

	Control (N.S)	**ZJ**	**5-FU**	**ZJ+5-FU**

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MDA (mmol/mg protein)	0.0063± 0.0032	0.0076± 0.0055	0.0138± 0.0022 [a]	0.0084± 0.0010

Abbreviations: *: Significant at α=0.05 when compared with IF group. N.S: Normal saline; IF: Ifosphamide; ZJ: Ziziphus jujube; MDA: Malondialdehyde.

4. Discussion

Nephrotoxicity is a major therapy- limiting side effect of 5-FU. It is thought to be induced by OS[16]. The present study demonstrates the renal toxicity of the 5-FU in an animal model and supports previous evidence of nephrotoxicity [4]. A clinically relevant dosage of 5-FU (80mg/kg) resulted significantly in altered kidney functions as shown by elevations of blood creatinine. This result is like that recorded by previous studies [17-19]. At the same time, it produced remarkable oxidative damage as shown by the elevated lipid peroxide levels and decreased the activity of glutathione S-Transferase in the kidney tissue homogenate. Similar results were also observed by other studies [1,2].

These observations are correlated well with the renal histological findings in the current study, the administration of 5-FU (40 mg/kg) induced damage to renal tubules. Similar changes were also reported by previous studies[20,21].

ZJ, belongs to the family rhamnaceous plant, that used in treatment of various diseases such as digestive disorders, weakness, liver complaints, obesity, urinary troubles, diabetes, skin infections, loss of appetite, fever, pharyngitis, bronchitis, anemia, diarrhea, and insomnia[22,23]. In our study, obtained results showed that ZJ was able to minimize the elevated levels of serum creatinine. Critically, ZJ reduced the severity of 5-FU-induced renal toxicity, as evidenced by almost normal morphology of the tubules and glomeruli of animals that received combined treatment with ZJ and 5-FU. Lipid peroxidation becomes more likely in cell membranes because of impaired antioxidant defense mechanisms. The measurement of MDA, product of lipid peroxidation, has been used as an indicator of lipid peroxidation level[24]. As shown in our findings, the levels of MDA in the IF treated group were increased when compared with those of the control groups. The impaired renal function was accompanied by increasing MDA concentrations in kidney tissue. Combined treatment with ZJ and 5-FU attenuates the elevation of lipid peroxidation level. Glutathione S-transferase is an enzyme that facilitates the conjugation of GSH with reactive metabolites leading to the

formation of a thioether bond making less reactive conjugate than the parental compound. There is some evidence that ZJ can enhance GST activity, and we observed, in the present study, a non-significant trend towards increased GST activity when ZJ was administered alone. More importantly, ZJ prevented the decrease of GST activity when it was administered together with the 40mg/kg concentration of 5-FU. Animals in the 5-FU group showed a significant loss in body weights. This reduction in body weight is attributed to reduced food intake and inhibition of protein synthesis due to 5-FU. This is a well-described side effect in patients treated with anticancer drugs[25] and was also reported in 5-FU-treated rats[4,21]. According to our biochemical findings, this was supported by histopathological evidence, administration of ZJ minimized some nephrotoxic effects of 5-FU in an in vivo model. These findings indicate that ZJ supplementation can reduce 5-FU-induced nephrotoxicity.

5. Conclusion

Our study revealed that ZJ provided a significant nephroprotective effect against 5-FU-induced nephrotoxicity. Thus, ZJ can be considered a potential candidate to minimize nephrotoxicity that induced by 5-FU which is a major clinical problem.

6. Funding

No financial support was provided for this study.

7. Conflict of interest

The authors declare no conflicts of interest or financial interest in any product or service mentioned in this article.

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