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Evaluation of a Protective Effect of the Water Extract of Thymus Leaves Against Diclofenac Sodium-Induced Renal Toxicity in the Syrian Hamster

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***Abstract*—** Diclofenac sodium is a common analgesic and antipyretic drug which is safe in therapeutic doses but can produce life-threatening renal damages with toxic doses.The current study was designed to investigate the protective effects of aqueous extract of thyme leaves (Thymus Vulgaris) against diclofenac sodium-induced toxicity in Syrian hamsters. A total of 32 hamsters were divided equally into 4 major groups. Group I received distilled water (2 ml) daily, group II received thyme extract at a dose of 300 mg/kg b. wt /day, orally Mediated by tubular feeding for five weeks. group III received diclofenac sodium at a dose of 6 mg /kg b.wt three times a week without extract for five weeks, while group IV received diclofenac sodium plus thyme extract for five weeks. then, hamsters were sacrificed by ether anesthesia and blood samples were collected they were autopsied, and kidney tissue samples were prepared .The obtained results showed that, Administration of sodium diclofenac to hamsters induced marked disturbance of renal functions, characterized by a significant increase in the levels of alkaline phosphatase (ALP) and potassium ($p < 0.001$),while the sodium concentration ($p < 0.001$) decreased in the third group compared to the control and the second groups. Histopathological changes showed that sodium diclofenac caused significant structural damages to kidneys and showed clear signs of acute cellular necrosis of the renal tubules and glomeruli.This was evidenced by the breakdown of cell membranes, dense cellular debris in the lumen of tubes and glomeruli, increased bowman area, and severe blood congestion.On the other hand,Oral co-administration of thyme extract with sodium diclofenac significantly decreased the level of ALP and potassium ($p < 0.001$) and significantly increased the level of sodium in group IV animals compared to that in group III. Moreover,thyme extract also exhibited some improvement in the histological architecture of kidney that looked normal.

***Keywords*—** *Hamster, Kidney, Diclofenac Sodium, nephrotoxicity, thyme leaves extract.*

I. INTRODUCTION

Many drugs or chemicals are used to prevent liver and kidney injury, but the excessive use of synthetic pharmaceutical therapy leads to many unwanted side effects and risks. Medicines are a common source of acute renal injury and cause about 20 percent of acute renal failure [1]. A large number of drugs belonging to the class of non-steroidal anti-inflammatory drugs (NSAIDs) are widely used as antipyretic and anti-inflammatory agents. They have also been shown to be effective and useful agents for a variety of diseases including rheumatic, musculoskeletal and cardiovascular diseases [2]. However, cytotoxicity, including acetaminophen, aspirin, indomethacin [3] and sodium diclofenac may cause [4].

Despite the importance of diclofenac sodium as an anti-steroidal anti-inflammatory drug , but many studies have proved its toxicity at high doses and long periods, causing anemia in rabbits [5] And cellular necrosis in the kidneys [6] and hepatocellular hepatoxicity in mice is characterized by high serum enzymes, especially ALT, AST, and ALP enzymes, as well as an increase in the concentrations of bilirubin and oxidative glutathione [7]. It also has negative side effects on the heart muscle and kidneys [8]. The toxicity of the drugs and medicines used is due to their effectiveness in increasing the formation of oxidative reactive oxygen species (ROS) in the cells of a number of organs, especially the liver, kidney ,and heart, where associated proteins, lipids, and nucleic acids, especially DNA and causing oxidation and destruction and activate cell death mechanisms in necrosis [9] ,[10].

Interest has increased markedly over the past decade with plant-derived drugs, especially those that are antioxidant [11]. The best source to obtain a variety of drugs is medicinal plants, which have been used in many countries as an alternative to synthetic drugs. Traditional medicines that are utilized by 80% of the population in developed countries contain compounds derived from herbal plants [12].

Thymus vulgaris L. is one of the most commercially important species of wild thyme. It belongs to the

Lamiaceae family and local aromatic herbs in the Mediterranean region, thyme has many beneficial effects, for example, antiseptic, antimicrobial and antifungal properties [13] and the bactericidal, anthelmintic and antioxidant properties and it has recently been suggested as a natural alternative to synthetic antioxidants [14]. It is used in the treatment of various diseases such as digestive disorders and bronchopulmonary disorders.

Research and studies have shown that Aqueous extract of thyme is an antioxidant and has a sweeping activity of free radicals [15] because it contains a high percentage of thymol and carvacrol [16] and The therapeutic potential of thyme rests on its contents of Flavonoids, thymol, carvacrol, eugenol, aliphatic phenols as well as saponins and lutoline [17], Anti-oxidant, anti-thrombin, and anti-hypertensive by maintaining renal function.

Therefore, our research aims to study the effect of thyme leaves extract on hamsters' kidneys from cellular toxin-induced by sodium diclofenac.

II. MATERIALS AND METHODS:

1. Experimental animals:

The current study was conducted on 32 Syrian hamsters aged 3-4 months and 80-110g medium, obtained from the commercial source - Lattakia Animal Breeding Center. Animals were placed in the laboratory in suitable conditions in terms of temperature (25 °C) and lighting (12 hours lighting / 12 hours darkness), and fed them periodically, and left to acclimate for at least 8 days before applying the pilot plan. The sodium diclofenac drug (100mg) was purchased from local pharmacies in the form of pills that were crushed and dissolved with distilled water to reach the required doses (6mg/kg.b.w) immediately before the experimentation of this drug.

2. Preparation of the aquatic extract of thyme leaves:

Thyme (*Thymus vulgaris L.)* is belonging to the Lamiaceae family, The genus: Thymus sp., specie: Thymus syriacus boiss [18]. Harbon, [19] method was used to prepare the water extract of the thyme leaves collected from the fields of Jableh area on 15/5/2017. The doses were given by tube feeding for 5 weeks.

3. Design Experience:

After their acclimated, experimental animals were randomly distributed in four groups, eight per experimental group, as follows:

Group I: the control group, and was injected only with distilled water (2 ml) daily and provided with appropriate food.

Group II: hamsters received thyme extract (300 mg/kg b. wt /day, orally) Mediated by tubular feeding for five weeks.

Group III: In the same way as previous feeding (oral feed), 2 ml of diclofenac sodium solution at a concentration of (6 mg/kg b. wt /day, orally) three times a week for five weeks to develop hepatic toxicity in the animals of this group.

Group IV: hamsters were simultaneously injected with thyme extract (300 mg/kg) daily and sodium diclofenac solution (6 mg/kg) by an oral feeding tube three times a week for five weeks.

At the end of the experiment, all animals were subsequently anesthetized by chloroform. Blood samples were taken from the heart of each experimental animal directly by a 3 mL syringe needle was introduced by the peak of the heart and placed in 5 ml test tubes. The animals were then sacrificed and Their kidneys were removed and stored independently in 50 mL plastic containers for this purpose and containing a formalin solution at a concentration of 10% until the histological study was performed.

4. Biochemical study:

The concentration of alkaline phosphatase enzymes(ALP) was measured directly and within a period of not more than 2 hours from the collection of samples, as well as the calibration of sodium and potassium concentrations according to the approved methods [20] using biochemical laboratories at Tishreen University Hospital.

5. Histological Study:

The histological study was carried out in collaboration with the Department of Anatomy at Tishreen University Hospital, where the samples of the kidneys of the experimental animals were prepared for the preparation of histological sections, Bypassing the samples in the routine preparation stages, which include processing by commercial alcohol and then absolute alcohol and then Kzailin and then integrated into blocks of paraffin.

The tissue sections were made of 5 microns thickness using the microtome (meditome A 550) and were treated them with alcohol and Kzailin solutions for coloring with hematoxylin and eosin (H&E) according to the approved scientific methods [21]. The tissue was then studied microscopically using a light microscope equipped with a digital camera and connected to a computer to investigate histological changes in the hamster's kidneys Induced by the thyme extract and sodium diclofenac.

6. Statistical Analysis:

The results of the calibration of concentrations of enzymes and other blood parameters adopted in this study were expressed by using measurement averages ± error deviation from average for each standard of blood in eight samples belonging to the animals of each group. One-way analysis of variance (ANOVA) was followed by a multi-way ANOVA test, including a student's-t test in the SPSS Software and The value $p<0.05$ is adopted as a minimum

of moral statistical significance For changes in values of serum criteria.

III. RESULTS

1. **Results of the chemical biochemical study:**

1.1. Effect of water extract of leaves of thyme and diclofenac sodium on ALP:

The results of the biochemical study and the statistical analysis of the serum criteria studied are shown in Table (1), which deals with changes in the average concentrations of alkaline phosphatase enzyme (ALP), by the effect of dosage the animals of the second group with the water extract of Thymus leaves with daily doses of 300 mg/kg for five weeks, No significant increase (P> 0.05) in the mean concentrations of these enzymes in the serum compared to their concentrations in the blood serum of the control group animals.

Table 1. Comparison of the mean changes in the concentrations of ALP enzymes in the serum of the experimental group animals, which injected either with thyme extract (300 mg / kg) or sodium diclofenac (6 mg / kg) or were simultaneously injected with thyme extract and diclofenac sodium Compared to the control group.

Groups	Treatment	ALP (U/L)
Group I	Control	179.71±6.396 U/L
Group II	Thyme Extract	186.29±2,984 U/L
	Value	0.30
Group III	diclofenac sodium	238.57±8.101 U/L
	Value	0.000
Group IV	diclofenac sodium+ Thyme Extract	206.29±6.601 U/L
	Value	0.000

Also, the results showed that the dosage of group 3 animals with oral doses of sodium diclofenac resulted in a significant increase (P <0.001) in the ALP concentrations where the ALP concentration increased by 33% compared with the control group Table (1). As that the concentration of ALP enzyme was still significantly higher (P <0.001) in group IV animals by 15% compared to those in the control group Table (1).

However, the results shown in Table (2) show that the simultaneous dose of the fourth group animals with thyme leaves extract with sodium diclofenac in the same way resulted to a significant decrease (P <0.001) in ALP concentrations by 14% Compared with its concentrations in the serum of the animals of the third group, which dosage only sodium diclofenac, While the results are shown in Table (3) show that the dose of thyme extract did not return concentrations of these enzymes to their normal values in the serum of the animals in group IV, where these concentrations remained high By 11% compared with the second group.

Table 2. Comparison of changes in mean serum concentrations of ALP in Animals of the two experimental groups: III and IV.

Groups	Treatment	ALP (U/L)
Group III	diclofenac sodium	238.57±8.101 U/L
Group IV	diclofenac sodium+ Thyme Extract	206.29±6.601 U/L
	Value	0.000

Table 3. Comparison of changes in the mean concentrations of ALP enzyme in serum group IV (300 mg/kg thyme extract and 6 mg/kg diclofenac sodium) and group II (thyme extract only).

Groups	Treatment	ALP (U/L)
Group II	Thyme Extract	186.29±2,984 U/L
Group IV	diclofenac sodium+ Thyme Extract	206.29±6.601 U/L
	Value	0.000

1.2. Effect of water extract of leaves of thyme and diclofenac sodium on the concentrations of sodium and potassium in the serum of experimental animals.

The results are shown in Table (4) show that the dosage of aqueous extract of the leaves of thyme to the animals of the second group did not lead to significant changes in sodium and potassium concentrations compared to the control group, In addition, diclofenac sodium alone treated third group animals led to Significant increase (P <0.001) in the potassium concentrations by 73% and a significant decrease (p <0.001) in the sodium concentration by 18% compared with the control group, While in group IV animals, the concentration of potassium remained significantly higher by 35%, while the concentration of sodium remained low by 9% compared with the control group. Table (4).

Table 4. Comparison of the mean concentrations of sodium and potassium concentrations in the experimental groups compared to the control group.

Groups	Treatment	Na	K
Group I	Control	132.214±2.12 mEq/l	4.068±0.18 mEq/l
Group II	Thyme Extract	132.843±1.79 mEq/l	4.104±0.28 mEq/l
	Value	0.56	0.783
Group III	diclofenac sodium	108.6±2.268 mEq/l	7.04±0.356 mEq/l
	Value	0.000	0.000
Group IV	diclofenac sodium+ Thyme Extract	120.57±2.858 mEq/l	5.508±0.51 2 mEq/l
	Value	0.000	0.000

But the results are shown in Table (5) show that the simultaneous dosage of thyme extract with diclofenac sodium Leads to significantly reduced in potassium concentrations (p <0.001) by 22% and significantly increased (p <0.001) in sodium concentrations by 11% In blood serum of group IV compared with their concentrations in serum of group III, which dosage only sodium diclofenac.

Table 5. Comparison of changes in the mean concentrations of sodium and potassium in serum group IV animals compared to the third group.

Groups	Treatment	Na	K
Group III	diclofenac sodium	108.6±2.268 mEq/l	7.04±0.356 mEq/l
Group IV	diclofenac sodium+ Thyme Extract	120.57±2.858 mEq/l	5.508±0.512 mEq/l
	Value	0.000	0.000

2. Results of Histological Study (Anatomy):

2.1. Effect of the water extract of Syrian thyme leaves on the structure of the kidney tissue in the hamster:

The results of the histological study of the animals of the second group showed a normal renal tissue structure and the Glomeruli are natural and well Landmarks surrounding the Bowman capsule With a Natural Bowman's space. The renal tubules are normal and their lining cells appear to be normal, cubic-shaped and have clear boundaries with normal circular nuclei. There were no cells that were desquamated and penetrated the tube's lumen, While a little vascular congestion was observed (Fig. 2 A, B). compared with the control group (Fig. 1 A, B).

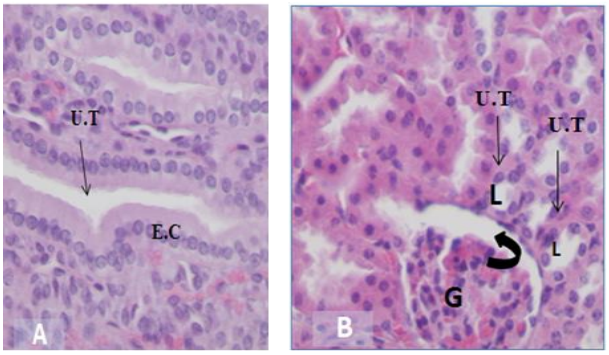

Figure 1. The Kidney histological structure of the control group showing: A- natural Urinary tubes (UT) and epithelial tubular cells (E.C) appear normal, cubic-shaped with natural circular nuclei in the control group (400X). B- A well-structured Bowman's capsule with normal glomerulus (G), and a natural Bowman's space (thick arrows). Urinary tubes (UT) are normal with the normal lumen (L) (400X).

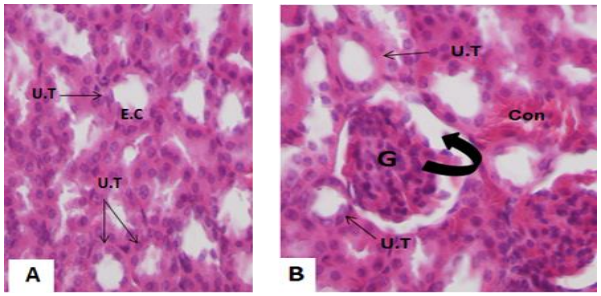

Figure 2. The histological structure of the kidneys of the second group that appear: A- normal Urinary tubes (U.T), the epithelial tubular cells looked normal (E.C). B- natural glomeruli (G), natural Bowman's space (thick arrow), Urinary tubes (U.T), cells lining the tubes and nuclei looked normal, mild vascular congestion(Con).

2.2. Effect of sodium diclofenac dosage on kidney tissue:

Severe necrosis of the lining cells of the tubules was observed and many of them had shrunken nuclei with Condensed chromatin. Many renal tubules were also lost, As for The remaining renal tubules were small in size and suffered from a lack of many cells in the lining of the renal tubules. Tubular obstruction (TO), cellular debris in their lumen and increased hyaline material were observed. (Figure 3. A, B, D). The sections showed severe necrosis of the glomeruli, the debris of their cells was observed, the complete rupture in some cases and significantly increased in Bowman's space, and Severe vascular congestion was observed (Figure 3. C, D).

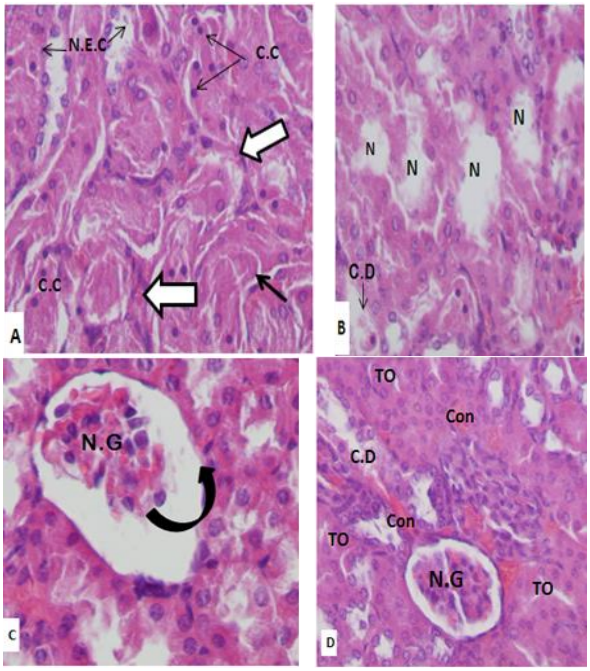

Figure 3. A microscopic image of a section of tissue in the kidneys of the third group Which dosage sodium diclofenac is shown: A- Severe necrosis in cells lining the renal tubules (N.E.C), The nuclei are necrosis and the chromatin condenses in them(C.C), increasing the hyaline material (white arrows), loss of many tubes (thin arrows). B- severe necrosis in the renal tubules (N), and many cells in the lining of the renal tubules were lost, cellular debris (C.D) into the tube's lumen. C- severe necrosis in the glomerulus (N.G) and significantly increased in Bowman's space (thick arrows). D - severe necrosis in the glomerulus (N.G), cellular debris (C.D) into the tube's lumen and Tubular obstruction (TO), and vascular congestion (Con).

2.3. the effect of the simultaneous dosage of thyme extract with sodium diclofenac on the kidney tissue:

The results of the histological study of the animals of the fourth group showed: the structure of the kidney closer to the natural tissue, Normal renal tubules and lining cells are mostly normal and have normal nuclei. No cellular necrosis or inflammatory infiltration is observed, but some epithelial tubular cells haven't clear boundaries, And mild vascular congestion, compared to the third group (Figure

4.A). The glomeruli also look almost normal with a naturally Bowman's space (Figure 4.B).

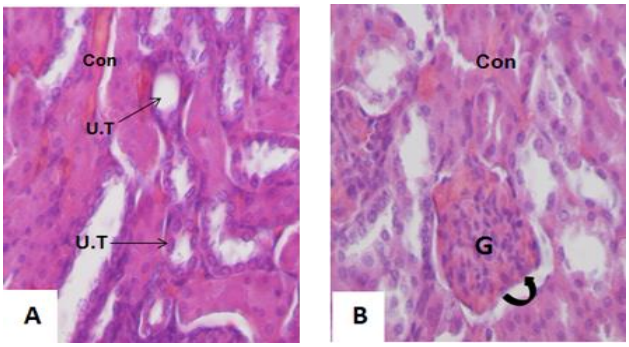

Figure 4. A microscopic image of a histological section in the kidneys of animals of the fourth group, which is dosed with thyme extract and diclofenac sodium together is shown: A- normal Urinary tubules (U.T) with normal cells, natural nuclei, mild vascular congestion(Con). B- Glomeruli are almost normal (G), natural Bowman's space (thick arrow), mild vascular congestion(Con).

IV Discussion:

The histological study of the kidneys of animals of the third group showed that is dosed with diclofenac sodium alone led to pathological changes in the kidney tissue, which led to damage to the renal tubules, renal cell damage, Cell death by necrosis and kidney tissue damage, These results confirmed with previous findings of Hickey et al.[22], who reported that sodium diclofenac-induced nephrotoxicity is due to the production of reactive oxygen species that lead to oxidative stress and DNA fragmentation, and may eventually lead to programmed cell death of kidney cells, These results are in agreement with previous findings of El- Maddawy and El-Ashmawy [20], where the Kidneys being organs of excretion are affected by the toxic metabolites of the drugs.

Some of the adverse side effects associated with diclofenac therapy, such as nephropathy, are also thought to be directly related to inhibition of prostaglandin synthesis which renal vasodilatory, whereas the inhibition of cyclooxygenase enzyme by Diclofenac sodium results in diminished prostaglandins synthesis resulting in severe renal vasoconstriction which ultimately leads to renal failure([23], [24]) Leading to a significant increase ($P<0.001$) in the concentration of ALP enzymes in the blood serum of the animals of the third group compared with the control group([25] , [26]).

Alkaline phosphatases are also membrane proteins which are anchored to lipid bilayers in cell membranes. therefore, Any damage to the cell membranes of kidney cells will affect their serum concentration [27] these findings are consistent with the results of previous studies that showed a high ALP rate due to a number of cellular stress factors, particularly methotrexate [28], diclofenac sodium[29], cadmium [30] and carbon tetrachloride [31].

In addition, the dosage of diclofenac sodium caused a significant increase ($P <0.001$) in potassium concentration and a significant decrease ($P> 0.001$) in sodium concentration compared to the control group, It is believed that this is due to necrosis of the lining cells of the renal tubules, which affects their ability to reabsorb sodium and subtract potassium and thus cause a high concentration of potassium and a decrease in sodium, this is consistent with the study of Mohammed Ali and Saeed [32] conducted in mice, which showed a significant increase in potassium values and a significant decrease in sodium due to induced renal poisoning with gentamicin at a dose (100 mg /kg) for eight days, as agreed with the study Khattab et al.[33], who came to the same conclusion.

On the contrary, treatment of the fourth group animals with thyme aqueous extract in conjunction with sodium diclofenac resulted in a significant reduction ($P <0.001$) of the enzyme concentrations (ALP), a significant decrease ($P < 0.001$) of potassium values and a significant increase ($P< 0.001$) of values sodium in the serum of the fourth group animals, compared with the third group dosage only diclofenac sodium, Also, the results of the histological study showed normal areas of renal cells in kidney tissue and also significantly reduced all manifestations of damage and tissue cellular necrosis, and vascular congestion compared with the third group dosage diclofenac sodium, and this indicates the ability of thyme to alleviate the manifestations of oxidative stress-induced diclofenac Sodium and the effectiveness of wild thyme components in inhibiting the degenerative action of diclofenac sodium, protecting kidney cells and improving renal function, This is because thyme extract contains natural antioxidants such as phenols, beta-carotene, flavonoids, Tannins, thymol, and terpenine, which protect kidneys, liver and other tissues from the effects of oxidative stress [34] , [35].

This is consistent with the results of other studies that have shown the protective role of thyme extracts in reducing the concentrations of ALT, AST, and ALP enzymes in serum of experimental mice and in protecting kidney and liver cells from induced cellular necrosis, both cisplatin [36] , Aflatoxicosis [37], paracetamol [38],or alcohol [39].

It should be noted that although the simultaneous dosing of thyme leaf extract with diclofenac sodium significantly reduced ($P< 0.001$) the induced effect of diclofenac sodium, the concentrations of the enzyme ALP and potassium remained significantly elevated ($P< 0.001$) and the sodium concentrations It was still significantly lower ($P< 0.001$) in the fourth group animals compared to those in the control group. Table (1, 4).

Also, ALP concentrations remained significantly higher and statistically significant ($P <0.001$) in the fourth group compared with the concentrations in the second group, it can be assumed that this is because the antioxidant effect of thyme aqueous extract was not enough to reverse kidney damage and return norms to their normal, A higher dose is likely to be required to reduce free radicals, protect cells from oxidation and prevent kidney damage with diclofenac sodium. . This was confirmed by the histological study of

the kidneys of animals of the fourth group, which showed some effects of oxidative stress, such as signs of mild vascular congestion, unclear boundaries of renal cells and increased hyaline material.

On the other hand, the results of the biochemical study of thyme alone treated second group animals showed no significant differences in the concentrations of ALP, Na, K compared to the control group. Table (1,4) However, the histological study showed minor changes in renal tissue that could disappear by reducing the dose used from the extract.

V. CONCLUSION

Finally it may be concluded that diclofenac sodium at the dose of 6 mg/kg body weight causes damage to the kidney, Results obtained in this study demonstrated that high doses of diclofenac causes alterations in biochemical parameters and histopathological changes in the Kidney due to oxidative stress and the use of thyme extract had improved the toxic effect of diclofenac.

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