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Renoprotective and Antioxidant Effects of Silymarin and Propolis on Diclofenac Sodium - Induced Renal Toxicity in Rats

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ABSTRACT

Diclofenac (DCL) is used to treat painful and inflammatory rheumatic and non-rheumatic conditions. Our aim was to assess the possible protective role of silymarin and propolis against DCL-induced renal damage. DCL sodium (75mg/kg b.wt) was administered orally with or without silymarin (250 mg/kg b.wt) or propolis (8.4 mg/kg b.wt) co-treatment for one month in adult male rats. Serum creatinine, urea, uric acid, renal glutathione, renal glutathione transferase, and lipid peroxidation were assessed. There were significant increases in serum levels of creatinine, urea, and uric acid. Also, renal glutathione and glutathione transferase activities were reduced, but the lipid peroxidation levels in the kidneys increased in DCL group. The administration of silymarin and propolis greatly reduced the adverse changes in the kidney function by raising antioxidant activities and reducing lipid peroxidation. In conclusion, silymarin and propolis may provide natural protection against renal toxicity caused by DCL sodium.

Keywords: *Silymarin, propolis, antioxidant, diclofenac, renal toxicity*

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are a class of analgesic (pain-relieving) medications used in the treatment of acute and chronic pain, inflammation, and illness[1]. DCL (Voltaren) is a NSAID that is widely used to treat a different of rheumatoid illnesses, comprising rheumatoid arthritis, osteoarthritis and acute muscle pain. Analogous to other NSAIDs, DCL sodium can cause renal damage in patients. DCL-induced nephrotoxicity may include the formation of reactive oxygen species (ROS), resulting in oxidative stress[2].

Attention had been given to the protective role of natural compounds in biological systems. Silymarin is a group of plant-derived flavonoids extracted from the seeds & fruits of the milk thistle (*Silybum marianum* L.)[3, 4, 5].

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Silymarin has promising renoprotective effects that have been shown both experimentally and clinically. Silymarin has antioxidant and anti-inflammatory properties that may also play a protective role in nephropathic processes[6]. Thus, silymarin appears to have potential as a renoprotective mediator against nephrotoxic medications because of its antioxidant, anti-inflammatory, and anti-apoptotic properties.

Propolis, a sticky material that honey bees produce thru mixing their saliva and bee′s wax with resins gathered from plants, is used as a sealant and sanitizing agent in honey bee nests[7]. Propolis, which contains a combination of flavonoids, phenolic acids, and additional bioactive component, has antioxidative effects[8,9]. The aim of this study was to evaluate the potential defensive roles of silymarin and propolis against DCL-induced renal damage.

MATERIAL AND METHODS

Experimental animals:

Male Wister albino rats that weigh about 120 – 180g were used as experimental animals in the current work. Rats were brought from the animal house of Research Institute of Ophthalmology, Giza, Egypt. The animals were kept under observation for 2 weeks upon arrival to get rid of any infection. All animals were housed individually in plastic cages at 25 ± 5 °C, in 12 h light/12 h dark cycles, with humidity 40–60%. Furthermore, Rats had free access to water and supplied daily with standard pellet diet *ad libitum.* All procedures were carried out in accordance with the guidelines of Beni Suef University for animal care and use.

Chemicals and drugs:

DCL sodium was purchased from Pharco– company (Egypt), silymarin was purchased from Sedico company (Egypt), propolis was purchased from Sigma pharmaceutical industry (USA), creatinine, urea and uric acid were purchased from BioSystems S.A. Company (Costa Brava 30, Barcelona-Spain), while chemicals used in measurement of antioxidants were purchased at a high purity grade (99%) from Sigma company (Nasr city, Cairo, Egypt).

Animal grouping and experimental design:

Rats were divided into six groups (six animals/each) designated as follow: *Group I (Normal control group):* Rats of this group were maintained on standard rat chow diet and were given tap water along all the period of the experiment. *Group II (DCL sodium control group):* Rats of this group were administered intraperitoneally DCL sodium at a dose of 75 mg/kg bwt three times a week for four weeks[10]. *Group III (Silymarin control group):* Rats of this group were orally administered silymarin dissolved in distilled water at a dose level of 250 mg/kg bwt three times a week for one month [11]. *Group IV (Propolis control group):* Rats of this group were orally administered propolis dissolved in corn oil at a dose level of 8.4 mg/kg bwt three times a week for four weeks [12]. *Group V (DCL sodium group treated with silymarin):* Rats of this group were administered intraperitoneally DCL sodium at a dose of 75 mg/kg b.wt. then orally treated with silymarin after one hour at a dose level of 250 mg/kg b.wt. three times a week for one month. *Group VI (DCL sodium group treated with propolis):* Rats of this group were administered intraperitoneally DCL sodium at a dose of 75 mg/kg bwt then orally treated with propolis after one hour at a dose level of 8.4 mg/kg bwt three times a week for four weeks.

Preparation of blood and tissue homogenates:

At the end of the experimental times (4 weeks), fasting rats were sacrificed under mild anesthesia using diethyl ether. Samples of blood were collected in dry glass centrifuge tubes and allowed to clot at room temperature. The clear, non-haemolysed supernatant sera were aspirated and stored at -20 ^{o}C for subsequent biochemical analysis. The kidney was rapidly excised, weighed and homogenized in 5 ml of 0.9 % NaCl (10% w/v) by Teflon homogenizer (Glas-Col, Terre Haute, USA). The renal homogenates were centrifuged at 3000 rpm. for 15 minutes and the supernatants were stored at -20^{o}C to assay oxidative stress parameters biochemically.

Assay of kidney functions:

Serum creatinine content and blood urea concentration were determined according to the procedure of Young *et al.*[13] using bio systems automated reagent kits obtained from Costa Brava 30, Chemical Company, Barcelona (Spain). Serum uric acid concentration was determined according to the procedure of Fossati *et al.*[14], Friedman *et al.*[15] using biosystems automated reagent kits obtained from Costa Brava 30, Chemical Company, Barcelona (Spain).

Assay of lipid peroxidation and antioxidant parameters:

Renal oxidative and antioxidant biomarkers were analyzed using chemicals bought at a high purity grade (99%) from Sigma company (Nasr city, Cairo, Egypt). Lipid peroxidation level in homogenates was determined using Jenway Spectrophotometer (Germany), according to the chemical method of Preuss *et al.*[16]. Glutathione-S-transferase (GST) activity in homogenates was measured according to the chemical method of Mannervik & Gutengerg[17]**.** Glutathione level was measured in renal homogenates according to the chemical method of Beutler et al. [18]**.**

Statistical analysis:

Data were analyzed by the method of one-way analysis of variance (ANOVA) followed by Tukey-Kramer procedures for post-hoc analysis. A value of $p < 0.05$ was regarded statistically significant. The statistical analysis were performed by computer programs. Microsoft excel version 10 and SPSS (statistical package for the social science version 20.00) [19]. Data were expressed in figures as mean ±SEM.

RESULTS

Biochemical effects of silymarin and propolis:

The effect of silymarin and propolis on kidney functions is indicated in **Figures (1,2 &3).** The administration of silymarin or propolis alone (G3 & G4) using a dose of 250 mg / Kg bwt for silymarin and 8.4 mg / Kg bwt for propolis three times a week for four weeks showed a non-significant decrease in creatinine and uric acid level (percentage change = -12.16 &-5.41 in creatinine and -7.30 & -11.61in uric acid respectively), as compared to healthy control group (G1), and showed a significant decrease (P < 0.001) in urea level (percentage change = -2.08 & -15.34 respectively) compared to healthy control group (G1).

Injection of DCL sodium (G2) at a dose of 75 mg / Kg bwt three times a week for four weeks induced a significant increase (P < 0.001) in all tested kidney functions; creatinine, urea and uric acid level as compared to healthy control group (G1) (percentage change = 64.86 in creatinine, 49.88 in urea and 60.67 in uric acid respectively).

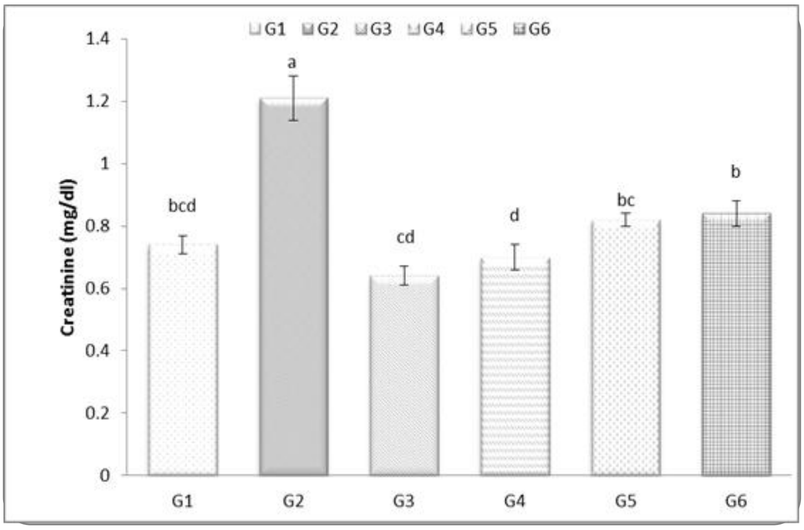
Treatment of DCL sodium intoxicated rats with silymarin and propolis (G5 &G6) using a dose of 250 mg / Kg bwt for silymarin and 8.4 mg / Kg bwt for propolis three times a week for one month after an hour of the DCL sodium administration caused a significant decrease (P < 0.001) in all tested kidney functions; creatinine, urea and uric acid level as compared to DCL sodium injected group (G2) (percentage change = -32.79 & -31.15 in creatinine, -12.71 & -24.76 in urea and -30.30 & -25.99 in uric acid respectively).

Either silymarin or propolis administration (G3 & G4) using a dose of 250 mg / Kg bwt for silymarin and 8.4 mg / Kg bwt for propolis three times a week for one month caused significant increase (P < 0.001) in the level of renal glutathione (GSH) (percentage change = 24.59 & 44.26 respectively), while the activity of renal glutathione transferase (GST) was non-significantly increased (P > 0.001) in silymarin administered group (G3) and propolis administered group (G4) (percentage change = 13.7 & 5.34 respectively), while renal lipid peroxidation content (LPX) was significantly decreased (P < 0.001) (percentage change = -14.57&-11.85 respectively) when compared to healthy control group (G1).

DCL sodium injection (G2) using a dose of mg / Kg bwt for one month (3 times/ week) induced a significant decrease (P < 0.001) in the level of renal GSH and renal glutathione transferase activity (percentage change = -32.79 & -12.29 respectively), while significant elevation ($P < 0.001$) in the level of LPX content (percentage change =119.75) compared to healthy control group (G1) as shown in **Figures (4, 5 &6)**.

Orally treatment of DCL sodium animals with both silymarin and propolis (G5 & G6) using a dose of 250 mg / Kg bwt for silymarin and 8.4 mg / Kg bwt for propolis three times a week for one month after an hour of intraperitoneal injection of DCL sodium caused a significant increase ($P < 0.001$) in the activity of renal GSH (percentage change = 24.39 & 39.02 respectively) and a significant increase in the renal glutathione transferase activity (GST) ($P < 0.001$) for silymarin (G5) and propolis (G6) administered group (percentage change = 15.65 & 6.09 respectively), while renal LPX was significantly decreased ($P < 0.001$) (percentage change = -29.78 & -39.66 respectively) on comparing to healthy control group (G1) as shown in **Figures (4, 5 &6)**.

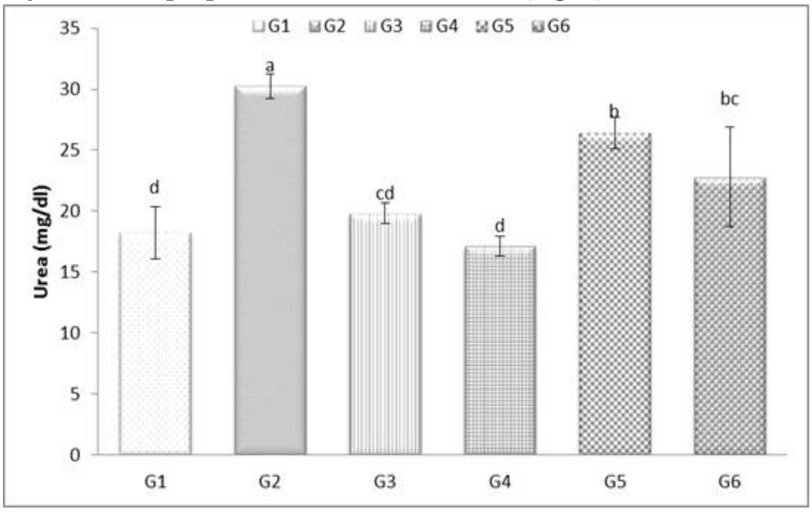
Fig. 1: Effect of silymarin and propolis on serum creatinine concentration (mg/dl) in DCL sodium administered rats



The different letters indicated a significant difference between groups at P> 0.05.

G1=Health group, G2=DCL group, G3=Silymarin group, G4= propolis group, G5=DCL+ silymarin, G6= DCL+propolis

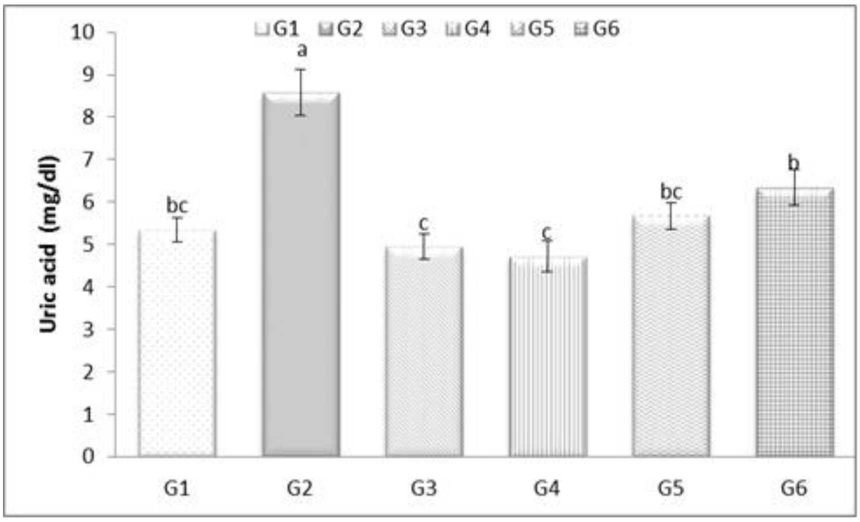
Fig. 2: Effect of silymarin and propolis on urea concentration (mg/dl) in DCL sodium administered rats



The different letters indicated a significant difference between groups at P> 0.05.

G1=Health group, G2=DCL group, G3=Silymarin group, G4= propolis group, G5=DCL+ silymarin, G6= DCL+propolis

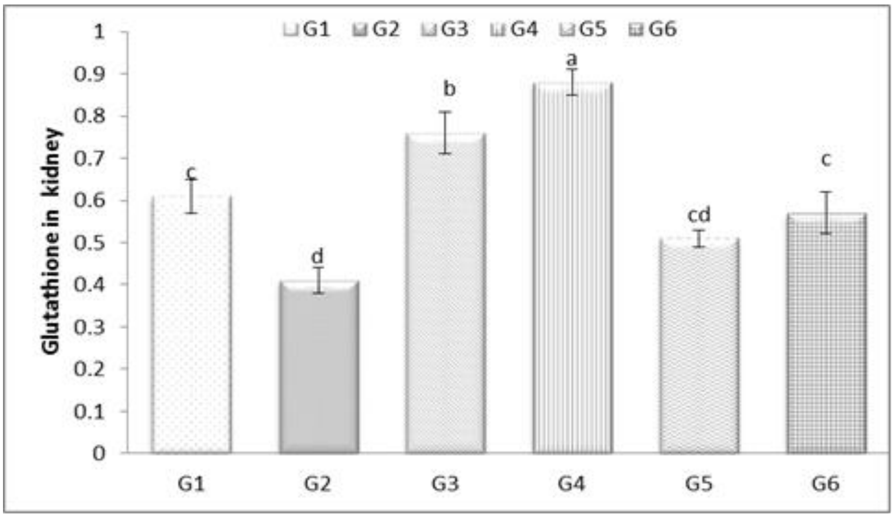
Fig. 3: Effect of silymarin and propolis on serum uric acid concentration (mg/dl) in DCL sodium administered rats



The different letters indicated a significant difference between groups at P> 0.05.

G1=Health group, G2=DCL group, G3=Silymarin group, G4= propolis group, G5=DCL+ silymarin, G6= DCL+propolis

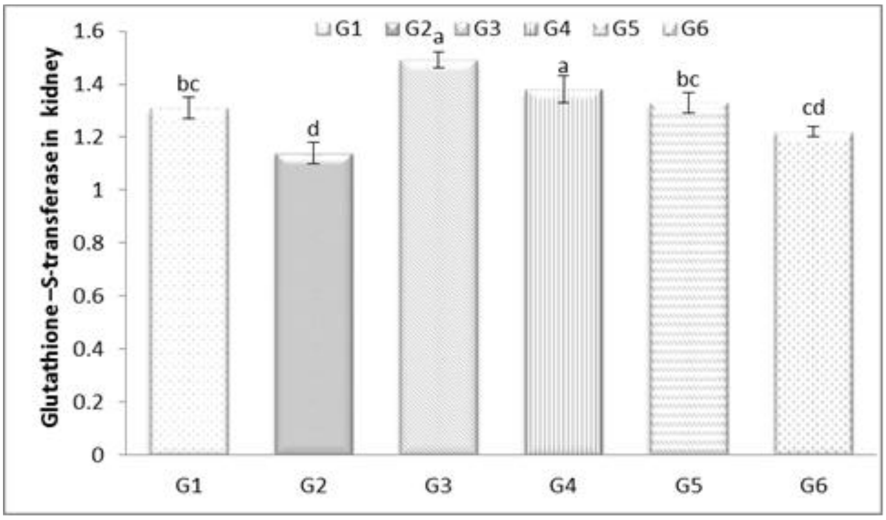
Fig. 4: Effect of silymarin and propolis on serum renal reduced glutathione level (nmol/100mg. tissue) in DCL sodium administered rats



The different letters indicated a significant difference between groups at P> 0.05.

G1=Health group, G2=DCL group, G3=Silymarin group, G4= propolis group, G5=DCL+ silymarin, G6= DCL+propolis

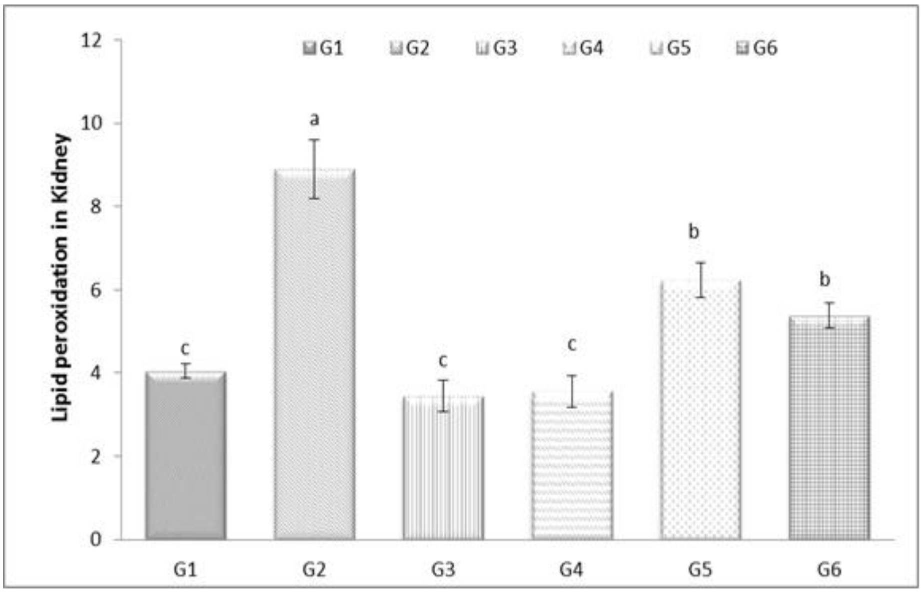
Fig. 5: Effect of silymarin and propolis on serum Renal glutathione transferase activity (µmol/100mg. tissue) in DCL sodium administered rats



The different letters indicated a significant difference between groups at P> 0.05.

G1=Health group, G2=DCL group, G3=Silymarin group, G4= propolis group, G5=DCL+ silymarin, G6= DCL+propolis

Fig. 6: Silymarin and propolis effect on serum renal lipid peroxidation level (nmol/g. tissue) in DCL sodium administered rats



The different letters indicated a significant difference between groups at P> 0.05.

G1=Health group, G2=DCL group, G3=Silymarin group, G4= propolis group, G5=DCL+ silymarin, G6= DCL+propolis

DISCUSSION

The current work was directed to evaluate and compare the effects of silymarin and propolis against DCL sodium-induced renal toxicity. DCL is a NSAID used in the treatment of rheumatic disorders, comprising ankylosing spondylitis and rheumatoid arthritis [20]. Our results proved that DCL sodium injections in rats cause a significant increase in renal function parameters such as serum creatinine, urea, and uric acid, which indicates that damage to renal tissues has occurred, possibly leading to nephrotoxicity.

The present study shows significant renal decreases in the antioxidant enzyme activities (GST) in the DCL compared to the normal control group, except for the peroxidation content, which indicated a significant increase in the kidney homogenate. The current study indicated that the DCL sodium treated group showed a significant decrease in GSH level and glutathione transferase (GST) activity in the kidney compared to the normal control group and a significant rise in LPX in the kidney homogenate (thiobarbituric acid reactive species, TBARs). These results are in agreement with those recorded by Yogesh *et al.* [21], who stated that the GSH depleted when animals were injected with DCL.

DCL sodium-induced nephrotoxicity was manifested by elevation in the serum levels of creatinine, urea, and uric acid, and was confirmed through oxidative stress disturbances that were previously reported by many authors [22,24]. Creatinine is an anhydride of creatine and is formed by spontaneous and irreversible reaction during skeletal muscle metabolism. Serum creatinine is one of the kidney related variables that indicate renal toxicity[25]. Creatinine may be indicative of kidney-specific physiological disorders[26]. An increase in serum creatinine is a biomarker for renal damage.

Urea is formed by the liver and considered the main end product of protein catabolism in carnivorous and omnivorous species [27]. Plasma urea levels can be a reliable indication of renal function as a significant decrease in plasma urea is observed in severe liver disease due to diminished urea synthesis activity, while a decrease in the rate of excretion of urea produces an increase in the concentration of plasma urea[28, 29].

Uric acid is produced by the breakdown of purines and by straight synthesis from 5-phosphoribosyl pyrophosphate (5-PRPP) and glutamine. Uric acid is passed in the urine in humans, but in other mammals, uric acid is further oxidized to allantoin before excretion[30].

Another explanation of the elevated serum uric acid level in DCL group is the defense mechanisms against free radical-created oxidative damage causes an increase in the concentration of uric acid (electron donors) in order to reduce free radicals[31]. This effect may aggravated the condition of renal damage resulted from uric acid. LPX is a chemical system capable of disrupting the configuration and function of the biological cell membranes as a result of free radical action on lipids[32]. The balance between LPX levels is a result of ROS attack on polyunsaturated fatty acids, proteins, and genetic material, and antioxidant factors affect the level of tissue damage[33]. These mechanisms explain the role of damaging effect of DCL on the body.

GSH is a very efficient intracellular defense against oxidative stress and it performs as a non-enzymatic antioxidant that removes hydroperoxides, H_2O_2, (ROOH) and xenobiotic toxicity[34,39]. The chief detoxifying agent for peroxides are GSH and catalase[40]. By taking part in the glutathione redox-cycle, GSH plus GPx change H_2O_2 and lipid peroxides to harmless products. Glutathione S-transferase (GST) is an antioxidant that catalyzes the conjugation reactions of the molecules having electrophilic sites with reduced glutathione. The general role of GST is to alter reactive lipophilic molecules into water soluble, non-reactive conjugates that may be easily extracted [41]. Decreased activity of one or more of the antioxidant systems due to the direct toxic effect of DCL sodium leads to increased LPX and nephrotoxicity. Oxidative damage plays a vital role in DCL-induced nephrotoxicity[2]. Acute renal failure appears to result from the bio-accumulation of DCL in the kidneys[42].

A previous study also demonstrated that DCL was toxic to renal mitochondria in rat[43]. DCL-induced renal damage has not only been reported in both animal models and human clinical settings[44]. Mechanisms of DCL-related renal injury could be referred to the inhibition of prostaglandins secretion[45] and mitochondrial targeting has also been suggested[43,46]. Previous findings indicated that ROS production seems to be involved in DCL-induced renal damage as TBARs in the kidney homogenates increased[2].

The nephrotoxicity of these medication is strictly linked to the action of ROS; that includes superoxide anion, hydrogen radicals, nacent oxygen, and nitrite. These ROS directly involved in the oxidative injury of lipids, proteins and nucleic acids as cellular macromolecules in the tissues[47].

Removal of DCL after high doses, may be decreased, producing exhaustion of GSH, resulting in numerous unfavorable reactions of the presumed toxic metabolite and falling cellular protection against oxidative stress [48, 49]. Moreover, DCL is metabolized through peroxidases, composing various radicals that consumed GSH and can interrupt the mitochondrial transmembrane potential, so reducing ATP formation, consequently causing apoptosis or necrosis[20,48,49]. In summary rises in urea, creatinine, uric acid and LPX while, decrease in antioxidant renal GSH resulted in renal damage due to DCL administration therefore, our trial for treating these pathological effects of DCL through using sylimarine and or propolis as a potential antioxidant and safe agent.

Our results revealed that treatment with DCL+silymarin showed a significant decrease and restoration in renal function tests for creatinine, urea, and uric acid when compared to the DCL sodium-treated group. These data were associated with a significant increase in renal GSH activity and renal GST, while the level of LPX was significantly decreased. This result is in agreement with Karimi *et al.*[50] who stated that silymarin pretreatment protected against nephrotoxicity. Silymarin may inhibit LPX by scavenging free radicals and increasing the intracellular concentration of GSH [51]. Moreover, silymarin suspension improved the low level of GSH and high level of TBARS induced by paracetamol [52] and DCL in our work.

Treatment with propolis (DCL+ Propolis) resulted in a significant reduction in serum creatinine, urea, and uric acid and its restoration to normal value. This result showed significant rise in renal GSH and a non-significant increase in renal GST activity, while the level of LPX significantly decreased compared to the DCL sodium group. This result is in agreement with[53], who stated that propolis improved the renal injuries caused by cobalt oral administration.

The improved effect of propolis extract on kidney might be because of its several bioactive constituent such as esters and flavonoids. These compounds inhibit membrane fragility and subsequently decrease the release of biomarker enzymes into the blood, thus enhanced regeneration of renal parenchymal cells. The bioactive properties of propolis extracts are definitely associated with its chemical structure and its polyphenolic composites are responsible for these properties[54,55&56]. Moreover, various flavonoids, detected in propolis, improved the expression of gamma-glutamyl cysteine synthase and the synthesis of glutathione generally. These phenolic and flavonoids compounds present in propolis had been described by some authors to display antiradical action[12,57&58].

Administration of propolis was related to increase in the antioxidant enzymes activity and the concentration of renal GSH. These results agree with that of another researcher [53], who found that propolis in the food of rats treated with cobalt improved the antioxidant and histological aspects of the kidney tissue. This role of propolis might be due to its ability to decrease the accumulation of free-radical production during DCL-induced LPX and exhaustion of antioxidant system**.** Furthermore administration of DCL caused abnormal renal functions by altering serum creatinine, blood urea and uric acid levels which were significantly increased as compared to normal control value. Our data show the beneficial effects of silymarin and propolis on the prevention or reduction of renal function induced by DCL.

In conclusion, silymarin and propolis are suggestive promising positive effects on DCL-induced nephropathy. Because of the high problem that DCL places with regard to patient morbidity, mortality and health-related costs, silymarin and propolis may be recommended as a renoprotective agents to attenuate toxicity of DCL that currently have a high probability of inducing nephrotoxicity.

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