

# Date palm pollen and silymarin administration protect splenic structure against diclofenac sodium-induced toxicity in female rat

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**Abstract:** Diclofenac sodium is normally used as anti-inflammatory and pain killer drug. The current study was performed to examine the possible protective roles of the date palm pollen and silymarin against diclofenac sodium (DS)-induced histopathological changes in the spleen of rats. Rats were divided into nine groups. Group1 (control), group2 (DS group) was administrated 15 mg/kg b.wt. of DS, group 3 (Date Palm Pollen ,DPP group; 100 mg/kg b.wt),group 4 (silymarin, Sil. group; 100 mg/kg b.wt), group 5 (DPP+DS group), group 6 (DS+DPP group),group 7 (Sil. +DS group), group 8 (DS+ Sil. group) and group 9 (DPP combined with SIL +DS group). DS resulted in a significant alterations in the splenic architecture indicated by cell necrosis at the white pulp, lymphocytic cell necrosis at the red pulp and depletion of lymphocytes at the white and red pulps. Each date palm pollen and silymarin restored the normal histological structure of the spleen. Also, the administration of the combined date palm pollen and silymarin before diclofenac sodium protected the spleen structure against the toxicity of diclofenac sodium. So, the data from this study suggested that date palm pollen and silymarin (as antioxidants) prevented DS-induced spleen structure toxicity and may be beneficial in protecting the therapeutic index of DS.

**Keywords:** Date palm pollen; silymarin, spleen; diclofenac sodium ;rats

## 1Introduction

Diclofenac is a non-steroidal has been used worldwide as anti-inflammatory and antipyretic drug for the treatment of pain conditions such as musculoskeletal and post-operative pains and acute attacks gout and ureteric coli [1]. Diclofenac is commercially found in sodium or potassium form [2]. Diclofenac sodium (DS) has its action via inhibiting the synthesis of prostaglands from arachidonic acid by inhibiting the activity of cyclo- oxygenase 2[3]. Diclofenac is safe at the therapeutic dose. However, various adverse effects of DS have been stated including hematological injury [4 -5], gastrointestinal tract injury [6] , hepato-renal structure and function [ 7 ] and splenic structure and function [8 ]when used at high dose for longer periods. Nonclinical studies on the experimental animals indicated that the most common adverse side effects of DS found in the hepato-renal and gastrointestinal systems [5 - 6]. These nonclinical data revealed that some toxicological effects observed in humans are observed in animals, also. However,

symptoms such as spleen structure-related problems have not been examined in the experimental animals.

In addition to the inhibition of cyclo-oxygenase 2, DS is known to produce toxicity in the biological systems through increased generation of reactive oxygen species resulting from the metabolism of DS via oxidative hydroxylation by cytochrome P4505 [ 9 ]. So, DS excretes the toxicity on the biological system via inducing oxidative stress which resulted in the damage of the biological tissues. Currently available therapies against DS-splenicpathy include concomitant use of natural products with biologically active dietary nutrients on the promotion of splenic health should be used.

Date palm pollen (DPP) is a fine powder formed by the male reproductive cell of the flower of the date palm tree. It is a useful traditional medicinal one that has been used by the ancient Egyptian and Chinese for treating male and female infertility [10].

DPPs were considered as antioxidant prevent the formation of free radical species (ROS and RNS) due to its composition of flavonoids [11]. DPPs act as anti-inflammatory [12], anti-hepato-renal toxicity [ 13 - 14 ] and anti-neurotoxicity [ 15 ]. Also, silymarin is a natural product derived as an extract from seeds and fruits of *Silybum marianum L.* . It is commonly called milk thistle [16] .Traditionally, the plant has been used for the treatment of many disease in the protection of the biological system as prevention of infectious diseases [17]. Silymarin acts as free radical scavengers, preventing free radical formation and activating several antioxidant enzymes via transcription factor [18] due to its flavonoids components [19]. Silymarin has been used to improve hepato-renal and spleen functions [20]. Also, silymarin exhibits neuroprotective effects via inhibiting oxidative stress and inflammatory mediators such as nitric oxide.

There have been limited studies detailing the protective activity of natural products such as date palm pollen and silymarin against the toxicity of DS on the histological structure of spleen. So, the present study was undertaken to explore the effects of date palm pollen and silymarin administration on DS-induced alterations in the splenic architecture in rats.

## 2 Material and Methods

**Chemicals:-**Diclofenac sodium and silymarin were purchased from LOBA chemé company (India). Date palm pollen grains, small oval gametocytes with Fine Park were collected from the farm of agriculture faculty, Sohag University, Egypt. The grains were separated from the bark, washed, dried and blended. The powder was kept in the refrigerator until use.

### Experimental animals:-

This study utilized fifty four female albino rats (*Rattus rattus*), three months old, weighing 180-200 gm were obtained from experimental animal house of the faculty of medicine, Assiut University, Assiut, Egypt. The animals were kept in stainless steel cages in a well ventilated room and were allowed to acclimatize for two weeks to the environmental conditions (12:12 h light-dark photoperiod; temperature  $24 \pm 3$  C°). They were led a standard pelleted diet and water *ad Libitum*.

All the experiments were performed in accordance with institutional guidelines for the animal care.

### Preparation of compounds and experimental design:-

DS, DPP and silymarin were suspended in dist. Water for oral administration in accordance with body weights. The treatment groups consisted of nine groups with six rats each as follows:-

- Group 1(C): Served as a control and were administrated food and water only.
- Group 2 (DS group): administrated DS at a dose of 15 mg/kg b. wt.
- Group 3(DPP group): administrated DPP at a dose of 100 mg/kg b. wt.
- Group 4 (Sil. group): administrated Silymarin at a dose of

100 mg/kg b. wt.

Group 5 (Dpp+ DS group): administrated DPP 12 hrs before administration of Ds.

Group 6(DS+DPP group): administrated DS 12 hrs before administration of DPP.

Group 7 (Sil+ DS group): administrated Sil. 12 hrs before administration of DS.

Group 8 (DS +Sil. group): administrated DS 12 hrs before administration of Sil.

Group 9 (Dpp combined with Sil. +DS group): administrated DPP combined with Sil.12 hrs before administration of DS.

### Sample collection and preparations:-

After experimentation period (30 days), rats of each group were autopsied 24 hrs after last treatment and spleen was removed, washed in normal saline (0.9% Na Cl) and blotted on dry filter paper and then fixed in neutral formaldehyde for histological examination. Spleen specimen was prepared for histological investigation and processed for paraffin embedding technique. Embedded tissue was sectioned at 4 um using microtome (Laica, Germany) and stained with hematoxyline and eosin (H&E) according to[21].Light microscope (Zeiss, Germany ) was used for the histological examination of the spleen.

## 3 Results

Over the entire period of the experiment (30 days), there was no mortality and clinical signs due to diclofenac sodium intoxication were observed.

### Histopathological examination-Hematoxylin and eosin (H&E) stain

Normal control group: Microscopically, a splenic tissue is depicting white (WP) and red pulp (RP) with normal population of lymphocytes (L) (Fig. 1 A and B)

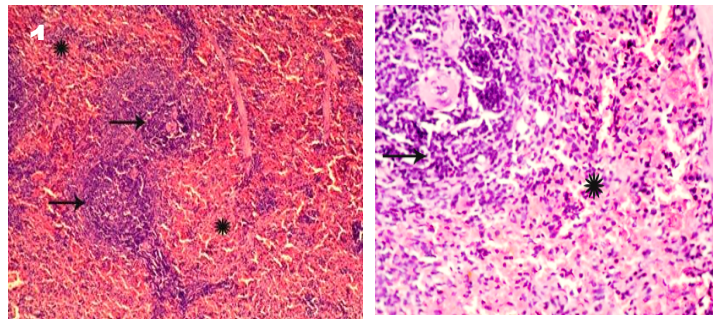


Fig.1A: - Photomicrograph of spleen section of rats from control group showing normal histological structure of both white (WP) and red pulp (RP). H&E.(10x10).

Fig.1B: - Photomicrograph of spleen section of rats from control group showing higher magnification white (WP) and red pulp (RP) with normal population of lymphocytes (L). H&E.(10x40).

Treatment with DS (15 mg/kg b.wt) showed foci of cell necrosis (arrows) at the white pulp (Fig. 2 A); multiple foci of lymphocyte depletion (Star) with lymphocytic cell necrosis (arrows) at the red pulp (Fig. 2 B), and depletion of lymphocytes (star) at the white pulp (Fig. 2 C) of the splenic section.

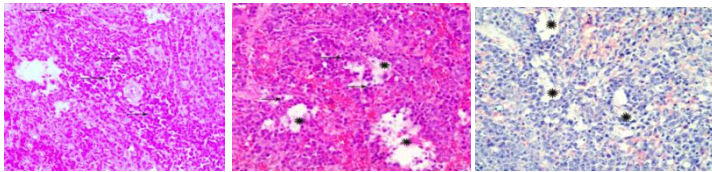


Fig.2A: - Photomicrograph of spleen section of rats from diclofenac sodium-treated groups showing foci of cell necrosis (arrows) at white pulp. H&E. (10 $\times$ 40)

Fig.2B: - Photomicrograph of spleen section of rats from diclofenac sodium-treated group showing multiple foci of lymphocytic depletion (star) with lymphocytic cell necrosis (arrows) at the red pulp. H&E. (10 $\times$ 40)

Fig.2 C: Photomicrograph of spleen section of rats from diclofenac sodium-treated group showing depletion of lymphocytes at the white pulp (stars). H&E. (10 $\times$ 40).

Administration of DPP (100 mg/kg b.wt) showed normal histological structure of both white (Star) and red pulp (Star) of the spleen (Fig 3 A). Also, at higher magnification (10 $\times$ 40), treatment with DPP showed white (Stars) and red pulp (arrow heads) with normal population of lymphocytes (L) (Fig. 3 B) of the splenic section.

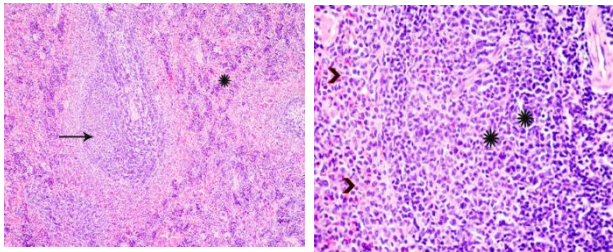


Fig.3 A: - Photomicrograph of spleen section of rats from date palm pollen-treated group showing normal histological structure of both white (arrow) and red pulp (star). H&E.(10 $\times$ 10).

Fig.3 B: - Photomicrograph of spleen section of rats from date palm pollen-treated group showing higher magnification white (stars) and red pulp (arrowheads) with normal population of lymphocytes (L). H&E.(10 $\times$ 40).

Administration of silymarin (100 mg/kg b.wt.) showed normal histological structure of both white pulp (Star) and red pulp (arrow heads) (Fig.4 A ). Also, the spleen section at higher magnification (10 $\times$ 40) showed white (Star) and red pulp (arrow heads) with normal population of lymphocytes (Fig. 4 B).

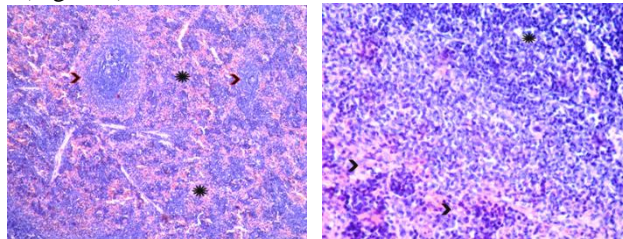


Fig.4 A: - Photomicrograph of spleen section of rats from silymarin-treated group showing normal histological structure of both white (star) and red pulp (arrowheads). H&E.(10 $\times$ 10).

Fig.4 B: - Photomicrograph of spleen section of rats from silymarin-treated group showing higher magnification white (star) and red pulp (arrowheads) with normal population of lymphocytes. H&E 10 $\times$ 40.

Photomicrograph of spleen section from rats which administrated with DPP 12 hrs before DS showed normal histological structure of white pulp (arrow heads) and red pulp (Star) (Fig.5 A). Photomicrograph of the same group showed higher magnification (10 $\times$ 40) white pulp (WP) and

red pulp (RP) with normal population of lymphocytes (Fig. 5B).

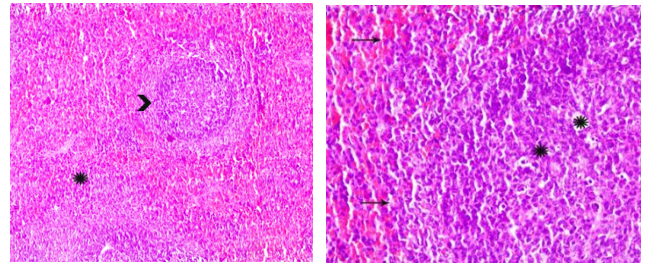


Fig.5 A: - Photomicrograph of spleen section of rats from date palm pollen plus diclofenac sodium-treated group showing normal histological structure of both white (arrowhead) and red pulp (star). H&E.(10 $\times$ 10).

Fig5 B: - Photomicrograph of spleen section of rats from date palm pollen plus diclofenac sodium-treated group showing higher magnification white (WP) and red pulp (RP) with normal population of lymphocytes . H&E.(10 $\times$ 40).

Photomicrograph of spleen section of rats from DS treatment 12 hrs before DPP showed normal white pulp (Star) and red pulp (arrow) (Fig.6 A). Also, Photomicrograph of spleen section of the same group showed higher magnification (10 $\times$ 40) showed normal white pulp (Star) and minimal necrosis at the red pulp (arrow heads) (Fig. 6B).

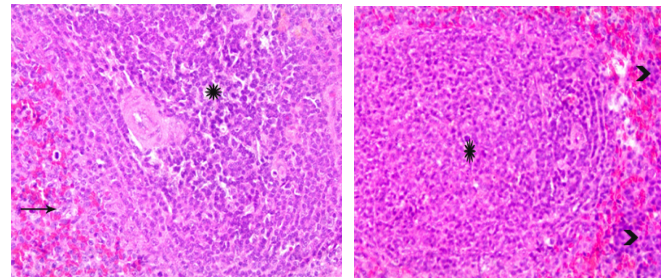


Fig.6A- Photomicrograph of spleen section of rats from diclofenac sodium plus date palm pollen-treated group showing normal white (star) and red pulp (arrow) . H&E.(10 $\times$ 40)

Fig.6 B: - Photomicrograph of spleen section of rats from diclofenac sodium plus date palm pollen-treated group showing normal white (star) with minimal necrosis at the red pulp (arrowhead).H&E.(10 $\times$ 40).

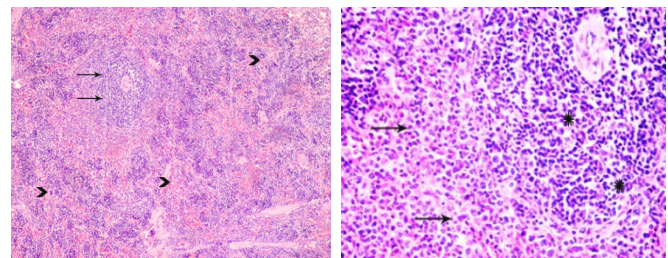


Fig.7 A: - Photomicrograph of spleen section of rats from silymarin plus diclofenac sodium-treated group showing normal histological structure of both white (arrows) and red pulp (arrowheads). H&E. (10 $\times$ 10).

Fig. 7B: - Photomicrograph of spleen section of rats from silymarin plus diclofenac sodium-treated group showing higher magnification white (star) and red pulp (arrows) with normal population of lymphocytes. H&E.(10 $\times$ 40).

Photomicrograph of spleen section of rats-treated with silymarin 12 hrs before DS showed normal histological structure of both white (arrows) and red pulp (arrow heads) (Fig.7 A). Also, Photomicrograph of the same group showed higher magnification of white (Star) and red pulps (arrows) with normal population of lymphocytes (Fig. 7B).

Photomicrograph of spleen section of rats-treated with DS 12 hrs before silymarin showed normal histological structure of both white pulp(Star) and red pulps(arrow heads) (Fig.8 A). Also, Photomicrograph of the same group showed higher magnification (10×40) of white pulp (WP) and red pulp with normal population of lymphocytes.

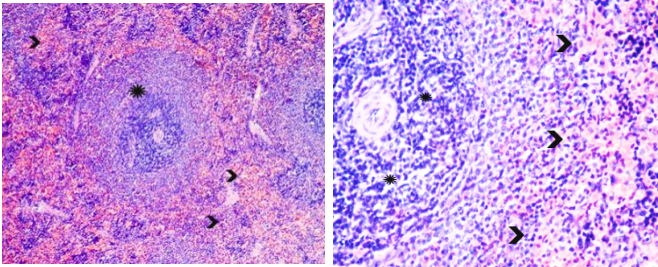


Fig.8 A: - Photomicrograph of spleen section of rats from diclofenac sodium plus silymarin-treated group showing normal histological structure of both white (star) and red pulp (arrowheads). H&E.(10×10).

Fig. 8B: - Photomicrograph of spleen section of rats from diclofenac sodium plus silymarin-treated group showing higher magnification white (WP) and red (RP) pulp with normal population of lymphocytes . H&E.(10×40).

Photomicrograph of spleen section of rats-treated with combined DPP and silymarin 12 hrs before DS showed normal histological structure of both white pulp(WP) and red pulps(RP) (Fig.9 A). Also, Photomicrograph from the spleen section of the same group showed higher magnification (10×40) of white pulp (Star) and red pulp (arrow heads) with normal population of lymphocytes.(Fig.9 B).

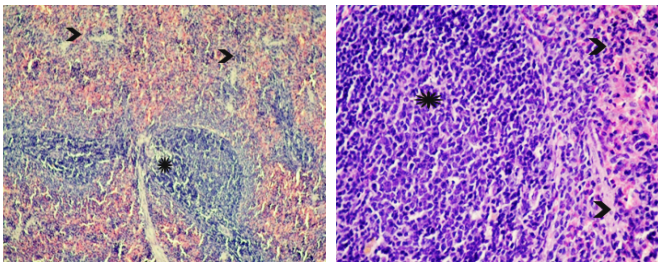


Fig.9 A: - Photomicrograph of spleen section of rats from date palm pollen combined with silymarin plus diclofenac sodium-treated group showing normal histological structure of both white (WP) and red pulp (RP). H&E.(10×10).

Fig.9 B: - Photomicrograph of spleen section of rats from date palm pollen combined with silymarin plus diclofenac sodium-treated group showing white (star) and red pulp (arrowheads) with normal population of lymphocytes. H&E.(10×40).

#### 4 Discussion

Splenic damage produced by non-steroidal drugs, such as DS is still a significant clinical problem. The central mechanisms which are responsible for splenic damage are believed to be the inhibition of cyclogenase and oxidative

stress excreted by this drug [22 -23 ] .There is evidence supporting the protective effect of natural products against drug-induced histopathological alteration in the biological system of the experimental animals [20 ] .Therefore, the current study was conducted to evaluate the potential properties of DPP and silymarin against DS-induced histopathological alterations in the spleen of female rats.

In the present study, DS induced histopathological changes in the spleen indicated by forced cell necrosis and depletion of lymphocyte at the white pulp; and multiple foci of lymphocyte depletion and lymphocytic cell necrosis at the red pulp. These findings are consistent with the studies which reported that DS administration resulted in degenerative lymphoid follicles with multiple foci and severe hemorrhage in splenic parenchyma [24] ;depletion of lymphoid in the malphigian corpuscles of the spleen, moderate rare action of periarteriolar lymphoid sheath of the spleen[25]; marked congestion of red pulp with marked atrophy in lymphoid follicle of white pulp [22]. Based on these results, it is evident that DS is a potent toxicant inducing histopathological changes in the splenic structure. This may be attributed to the effect of DS on the hematopoiesis, probably in terms of increased destruction of erythrocytes [26] or may be due to oxidative stress excreted by DS on the splenic structure causing an increase in LPO [27].

In the present study, oral administration of each DPP and silymarin alone did not change the normal histological structure of the spleen. However, oral administration of DPP and silymarin 12 hrs before and after DS restored completely the normal histological structure of the spleen. Also, the administration of combined DPP with silymarin 12 hrs before DS improved completely the splenic structure.

It means that the histological structure of the spleen become the same like that of the control. So, it can be stated that DPP and silymarin as photochemical antioxidant have an ameliorative and protective role against the toxicity of DS on the splenic architecture.

In supporting of this suggestion, it was found that DPP and silymarin induced a marked decrease in the LPO activity and a marked increase in SOD and NO activities (Preliminary results,( El-Sayed *et al.*, 2022 ,unpublished data).

#### 5 Conclusion

It was concluded that DS had a toxic effect on the splenic structure of the female rats. However, administration of DPP and silymarin before and after DS restored the normal histological structure of the spleen. Therefore, DPP and silymarin (as exogenous antioxidants) hold protective effects as adjuvant supplements for management of DS-induced spleen toxicities.

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