AMELIORATIVE EFFECT OF N ACETYL CYSTEINE AGAINST DICLOFENAC-INDUCED OXIDATIVE STRESS, HEMATOLOGICAL AND SPLENIC TOXICITY IN MALE RATS

BY

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ABSTRACT

Background: Diclofenac sodium is one of the non-steroidal anti-inflammatory drugs that is used as anti-inflammatory, analgesic, and antipyretic. N-acetyl cysteine is an antioxidant and free radical scavenger used to combat oxidative stress-induced damage in various tissues. **Objectives**: The current study was designed to investigate diclofenac sodium toxic effects upon the blood parameters, oxidative stress marker and spleen after repeated oral dose and evaluate the possible protective effect of N- acetyl cysteine when co-administered with it. **Methods:** Forty adult male albino rats were divided equally into four groups. Group I: the rats were fed on basal diet and distilled water. Group II received N-acetyl cysteine orally at the dose of 150 mg /kg /day. Group III: the rats were treated orally with diclofenac sodium at dose 5 mg /kg /day. Group IV received diclofenac sodium and N-acetyl cysteine at the same mentioned dose. After one month, all animals were sacrificed and blood was collected for analysis. The spleen was preserved for histopathological examination. **Results:** The study proved that repeated oral administration of diclofenac sodium induced significant toxic effect on blood parameters, increased serum malondialdhyde level and histopathological changes in spleen. But these toxic effects declined markedly with N- acetyl cysteine co-administration. **Conclusions:** The present study concluded that diclofenac sodium has many toxic effects on blood parameters, induces oxidative stress and histopathological alteration in spleen tissues. Also, the study revealed that N- acetyl cysteine has a potential protective effect against such harmful effects.

Keywords: *Diclofenac sodium; N- acetyl cysteine, blood parameters, MDA, spleen.*

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INTRODUCTION

Diclofenac sodium is one of the non-steroidal anti-inflammatory drugs (NSAIDs). It is a lipophilic, non-steroidal, a phenyl acetic acid compound. It is commonly used as analgesic, antipyretic, anti-inflammatory and anti-rheumatic ***(Ahmed et al., 2017).***

It induces its therapeutic effects by blocking cyclooxygenase (COX) enzymes to prevent prostaglandin synthesis. COX enzymes catalyze the formation of prostaglandins and thromboxane from arachidonic acid. COX enzymes present in two isoforms: COX-1 and COX-2 ***(Arslan et al., 2016).***

The mechanism of toxicity is by damage of mitochondrial trans-membrane due to reduction of glutathione conjugation. This produces the toxic metabolites and decreases antioxidant activity leading to peroxidative damage to cell membranes. This leads to necrosis and diminished ATP production ***(Peter and Prince, 2018).***

Several studies have examined the risk of low versus high doses of NSAIDs. They reported higher risks for patients receiving high doses of NSAIDs in comparison to patients receiving low doses ***(Castellsague et al., 2012; McGettigan and Henry, 2011; Schneider et al., 2006);*** so it is recommended to use NSAIDs at the lowest effective dose for the shortest duration ***(Gomaa, 2017).***

Diclofenac sodium induces various adverse effects on different body systems. There are special concerns to cardiovascular, gastrointestinal and renal toxicity ***(Odom et al., 2014).***

Oxidative stress is a condition that disturbs the oxidant/antioxidant balance as a result of a significant rise in reactive oxygen species (ROS) in cells and a decline in antioxidant

levels. Oxidative damage affects the cellular structural macromolecules such as protein, lipid, carbohydrate and DNA ***(Valko et al., 2006).***

N-acetylcysteine (NAC) is an antioxidant and free radical scavenger. It carries out antioxidant activity directly by reaction with few oxidants such as nitric oxide and hypochlorous acid ***(Soldini et al., 2005).*** Moreover, NAC induces its antioxidant activity by breaking disulfide bridges in different proteins such as mucin ***(Deponte, 2013).***

NAC also act as a precursor of reduced glutathione which acts as antioxidant against various reactive oxygen species (ROS)**.** This reduced glutathione enhances activity of catalase, mitochondrial superoxide dismutase and glutathione peroxidase. These enzymes play an important role in antioxidant system ***(Zhang et al., 2016).***

Oral dose of NAC is totally absorbed, and this gives him advantage in medicament use. Based on the former mentioned characteristics of NAC, it is used as a therapeutic agent in conditions characterized by induction of oxidative stress such as cardiovascular diseases, inflammatory reaction and cancers ***(Kerksick and Willoughby, 2005).***

There are minimal studies on the histopathological changes in the splenic tissues induced by sub-acute diclofenac sodium toxicity. So, this study included a histopathological examination of the spleen of adult male rats exposed to diclofenac toxicity.

The present study aims to investigate diclofenac sodium toxic effects upon the blood parameters, oxidative stress marker (Malondialdhyde) and spleen after repeated oral dose and evaluate the possible protective effect of N- acetyl cysteine when co-administered with it.

MATERIAL AND METHODS

- **Material:**

I- Chemicals:

- Diclofenac sodium (Diclo) (each tablet contains 50 mg diclofenac sodium, Novartis Pharma, Cairo, Egypt).
- N-acetyl cysteine (each sachet contains 200 mg acetyl cysteine – SEDICO production, Egypt).
- Solution for complete blood count assay (CyMet NR III) was purchased from Nihon Kohden Company.
- Kits of malondialdhyde (MDA) were purchased from Bio diagnostic company.
- Distilled water from human power one water distiller. Present in Clinical Toxicology Lab - Sohag University Hospital.
- Hematoxylin and eosin stains purchased from ALPHACHEMIKA

II-Apparatuses:

A-CELLTac Mek 6500: configured as a fully automated hematology analyzer. The system provides a complete 19 CBC parameter measurement at a rate of 60 samples per hour. Present in private Clinical Pathology laboratory.

B-Centrifuge, Hettich Zentifugen – model (EBA20), 4000 revolutions per minute (rpm) at Clinical Toxicology lab, Sohag University.

C-UV 2300 spectrophotometer (USA): It is a Split Beam Scanning UV-Vis spectrophotometer which has a wave length range of 190-1100 nm and comes with a programmable 5-turret sample compartment. Present in Faculty of Science, Sohag University.

D-Olympus CX 41 RF Light microscopy. At Pathology Department – Faculty of Medicine - Sohag University.

E-Water distiller: bibby scientific model merit–W400.

III- Animals:

The experimental procedure was conducted in accordance with the guide of the care and use of laboratory animals approved by the Medical Research Ethics Committee of Faculty of Medicine, Sohag University. The study was conducted on 40 adult male albino rats weighing (200 ± 20 gm). They were supplied by Vacsera Vaccination Centers. The animals were housed in animal house, Faculty of Medicine, Sohag University, in metal cages under ambient temperature, 21 ± 3 °C. Animals were fed with standard pellet food and water. They were acclimatized to the laboratory condition for one

week before starting the treatment protocol. The study was performed at October/2019.

- **Experimental design:**

Animals groups: The rats were divided randomly into 4 groups, 10 animals each

Group I (Control group): The rats were fed on basal diet and distilled water.

Group II (NAC group): The rats were treated orally with N-acetyl cysteine (NAC) at dose of 150 mg /kg /day, dissolved in distilled water, for one month ***(Nouri et al., 2017).***

Group III (DF group): The rats were treated orally with diclofenac sodium at dose 5 mg /kg /day (1/10 of LD50), dissolved in distilled water, for one month. The oral LD50 of diclofenac sodium in rats is 55-240 mg\kg ***(Mclean and Khan, 2018; Özgüney, 2011)***

Group IV (DF&NAC group): The rats were treated with NAC at dose 150 mg /kg/day orally followed after 1 hour by diclofenac sodium at dose 5 mg /kg /day for one month.

At the end of the study: Rats were sacrificed by cut throat under light anesthesia. Blood samples were collected after slaughtering for analysis (CBC, MDA). Necropsy was done for all animals; spleen was taken for histopathological examination with light microscopy.

The research was approved by the Medical Research Ethics Committee of Faculty of Medicine, Sohag University

- **Methods**:

i- Blood samples:

- Four ml of blood were drawn from each rat from cervical blood vessels during slaughtering; 2 ml of blood were placed in a tube containing EDTA anticoagulant for CBC. The other 2 ml were placed in a plain tube for MDA.
- Blood smear was prepared for differential leucocyte count.
- The serum was obtained after sampling by centrifugation (4000 rpm for 10 minutes). Then transferred into sterile screw capped polypropylene tubes and analyzed for MDA.

ii- Tissue samples

Necropsy was performed for all groups. The spleens from all animals were fixed in formalin 10% and paraffin embedding. Five µm sections were cut and stained with hematoxylin and eosin then examined by light microscope for the evaluation of any pathological changes and then photographed.

STATISTICAL ANALYSIS

All statistical procedures were computed using Statistical Program for Social Science (SPSS), version 16.0 computer software. SPSS inc. Chicago, USA.

All data were presented as mean ± SD and compared by Student's t-test and one way ANOVA test.

P value < 0.05 was considered as significant.

The data was presented in the form of tables

RESULTS

I-Hematological results, Complete blood count (CBC):

○ **Red blood cells indices: red blood cells (RBCs), hemoglobin (Hb), hematocrite (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC).**

There was no significant statistical difference in the mean values of RBCs, Hb, HCT, MCV, MCH and MCHC in group I (Control group) as compared to group II (NAC group).

There was a significant statistical decrease in the mean values of RBCs, Hb, HCT, MCV, MCH and MCHC in group III (DF group) as compared to control group (0.001, < 0.001, 0.003, 0.02, 0.01, 0.002 respectively)**(Table 1).**

There was a significant statistical decrease in the mean value of RBCs in group IV (DF&NAC group) as compared to control group (0.006). While, there was no significant statistical difference in the mean values of Hb, HCT, MCV, MCH and MCHC in group IV as compared to control group **(Table 1).**

There was a significant statistical increase in the mean values of RBCs, Hb, HCT and MCHC in group IV (DF&NAC group) as compared to group III, DF group (0.001, < 0.001, 0.01, 0.001 respectively). While, there was no significant

statistical difference in the mean values of MCV and MCH in group IV (DF&NAC group) as compared to group III (DF group) **(Table 1).**

Table (1): Statistical analysis of red blood cells indices in the different study groups

RBCs indices Mean ±SD	Group I, Control group (n = 10)	Group II, NAC group (n = 10)	Group III, DF group (n = 10)	Group IV, DF&NAC group (n = 10)	*P value by t- test
RBCs 10^6 /μL	9.7 ± 1.15	8.7 ± 1.01	5.4 ± 0.55	7.6 ± 0.40	P1=0.05 P2=0.001* P3=0.006* P4=0.001*
Hb gm/dl	14.7 ±0.70	14.9 ± 1.04	9.8 ± 0.26	14.1 ± 0.37	P1=0.8 P2< 0.001* P3=0.08 P4 <0.001*
HCT %	45.6 ±2.46	45.3 ± 3.89	40.2 ±2.36	44.2 ± 2.39	P1=0.87 P2=0.003* P3=0.33 P4=0.01*
MCV fL	61.6 ±3.95	60.9 ± 4.08	56.3 ± 3.09	58.5 ± 3.47	P1=0.75 P2=0.02* P3=0.22 P4=0.21
MCH Pg	20.4 ±1.16	19.9 ± 1.10	18.6 ± 0.94	18.9 ± 1.35	P1=0.50 P2=0.01* P3=0.06 P4=0.66
MCHC gm/dl	32.4 ± 0.45	32.9 ± 0.62	28.3 ± 1.86	32.8 ± 1.13	P1=0.13 P2=0.002* P3=0.45 P4=0.001*

SD: Standard Deviation, RBCs: red blood cells, Hb: hemoglobin, HCT: hematocrit, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin and MCHC: mean corpuscular hemoglobin concentration.
**Significant if P ≤ 0.05. P1: comparison between group II and group I, P2: comparison between group III and group I, P3: comparison between group IV and group I, P4: comparison between group III and group IV*

- **Platelets:**

There was no significant statistical difference in the mean values of platelets in group I (Control group) as compared to group II(NAC group)..

There was a significant statistical increase in the mean value of platelets in group III (DF group) as compared to control group (0.02). Also, a significant statistical increase was noticed in the mean value of platelets in group IV (DF&NAC group) as compared to control group (0.01). While, there was no significant statistical difference in the mean value of platelets in group IV (DF&NAC group) as compared to group III, DF group **(Table 2).**

- **White Blood Cell indices (WBCs, Neutrophils, lymphocyte, monocyte, basophil):**

There was no significant statistical difference in the mean values of WBCs, lymphocyte, neutrophil, monocyte and basophil in group I (Control group) as compared to group II (NAC group).

Significant statistical increase was noticed in the mean value of WBCs and lymphocyte in group III (DF group) as compared to control group (0.03, 0.04 respectively). While, there was a significant statistical decrease in the mean value of neutrophil in group III (DF group) as compared to control group (0.03).

There was no significant statistical difference in the mean value of monocyte and basophil in

group III (DF group) as compared to control group **(Table 3).**

No significant statistical difference was observed in the mean value of WBCs, lymphocyte, neutrophil, monocyte and basophil in group IV (DF&NAC group) as compared to control group.

A significant statistical decrease was observed in the mean value of WBCs, lymphocyte in group IV (DF&NAC group) as compared to group III, DF group (0.02, 0.03 respectively). While, a significant statistical increase in the mean value of neutrophil was noticed in group IV (DF&NAC group) as compared to group III, DF group (0.01)

No significant statistical difference was noticed in the mean value of monocyte and basophil in group IV (DF&NAC group) as compared to group III, DF group **(Table 3).**

Table (2): Statistical analysis of platelet count (× 10³/µL) in the different study groups

Mean ±SD	Group I, Control group (n = 10)	Group II, NAC group (n = 10)	Group III, DF group (n = 10)	Group IV, DF&NAC group (n = 10)	*P value by t-test*
platelet count	591.3 ± 52.51	610.5 ± 92.30	922.0 ± 262.92	849.6±173.75	P1=0.67 P2=0.02* P3=0.01* P4=0.58

SD: Standard Deviation * Significant if P ≤ 0.05. P1:comparison between group II and group I, P2: comparison between group III and group I, P3: comparison between group IV and group I, P4: comparison between group III and group IV.

Table (3): Statistical analysis of white blood cells (WBCs) indices in the different study groups:

Mean ± SD	Group I, Control group (n = 10)	Group II, NAC group (n = 10)	Group III, DF group (n = 10)	Group IV, DF&NAC group (n = 10)	*P value by t- test*
WBCs (×10³/µL)	12.9 ± 2.15	11.8 ± 1.47	17.4 ± 3.61	12.5 ± 2.53	P1=0.35 P2=0.03* P3=0.80 P4=0.02*
Lymphocyte%	64.60 ± 13.05	67.60 ± 7.46	78.60 ± 5.85	69.50 ± 7.20	P1=0.63 P2=0.04* P3=0.45 P4=0.03*
Neutrophil%	31.60±13.81	29.30 ± 7.45	15.8 ± 5.68	26.00 ± 6.84	P1=0.72 P2=0.03* P3=0.39 P4=0.01*
Monocyte%	2.50 ± 0.84	1.60 ± 0.52	2.10 ± 0.41	2.00 ± 0.63	P1=0.07 P2=0.40 P3=0.27 P4=0.60
Basophil%	1.10 ± 0.41	1.00 ± 0.01	1.30 ± 0.52	1.60 ± 0.82	P1=0.36 P2=0.55 P3=0.22 P4=0.42

*SD: Standard Deviation. * Significant if P ≤ 0.05. P1: comparison between group II and group I, P2: comparison between group III and group I, P3:comparison between group IV and group I, P4: comparison between group III and group IV.*

II- Oxidative stress marker, Malondialdhyde (MDA):

There was no significant statistical difference in the mean values of serum MDA level in group I (Control group) as compared to group II (NAC group).

Significant statistical increase was noticed in the mean value of serum MDA level in group III (DF group) and group IV (DF&NAC group) as compared to control groups (<0.001, 0.006 respectively). While, a significant statistical decrease was observed in the mean value of serum MDA level in group IV (DF&NAC group) as compared to group III, DF group (0.002) **(Table 4).**

Table (4): Statistical analysis of serum malondialdhyde (MDA) in the different study groups

Mean± SD	Group I, Control group (n = 10)	Group II, NAC group (n = 10)	Group III, DF group (n = 10)	Group IV, DF&NAC group (n = 10)	*P value by t- test
Serum MDA (mg/dl)	2.19 ±0.15	2.13 ± 0.20	2.72± 0.11	2.46± 0.08	P1=0.5 P2<0.001* P3=0.006* P4=0.002*

*SD: Standard Deviation .*Significant if P ≤ 0.05. P1: comparison between group II and group I, P2: comparison between group III and group I, P3: comparison between group IV and group I, P4: comparison between group III and group IV*

III- Histopathological results of the study groups:

Group I (Control group): Hematoxylin and eosin (H&E) examination of a section of a spleen of group I revealed normal histological appearance of the splenic architecture. Normal red pulp, white pulp, marginal zone and central arterioles were noticed. Red pulp and white pulp separated by marginal zone. **(Figure 1 A&B).**

Group II (NAC group): H&E examination of a section of a spleen of group II revealed normal histological appearance of the splenic architecture. There were normal red pulp, white pulp, marginal zone and central arteriole with normal splenocytes radiating from it **(Figure 2).**

Group III (DF group): Histopathological examination of a section of a spleen of group III revealed marked congestion of red pulp with marked atrophy in lymphoid follicle in white pulp **(Figure 3 A & B).**

Group IV (DF&NAC group): Stained slides of section of spleen of group IV revealed mild congestion of red pulp (as a sign of improvement) with normal central arteriole and trabeculaes **(Figure 4).**

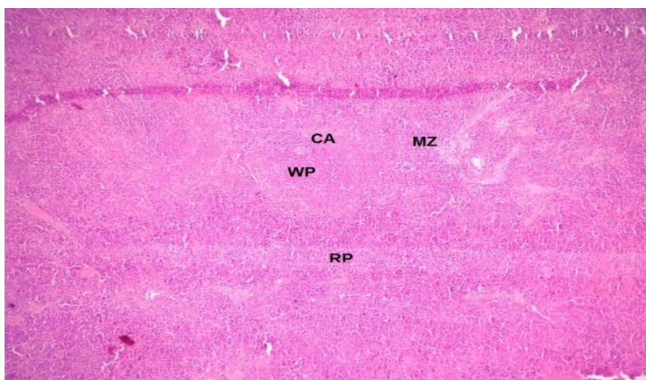

Figure (1 A): A photomicrograph of spleen section of group I (Control group) showing normal splenic architecture. Normal red pulp (RP), white pulp (WP), marginal zone (MZ) and central arterioles (CA). H& E staining x100.

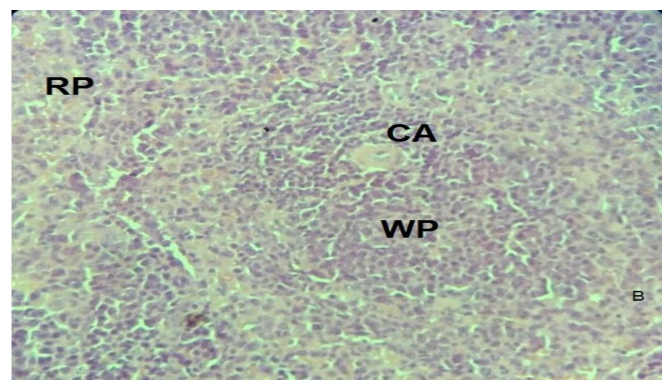

Figure (1B): A photomicrograph of spleen section of group I (Control group) showing normal splenic architecture. Normal red pulp (RP), white pulp (WP) and central arterioles (CA). H& E staining x 400.

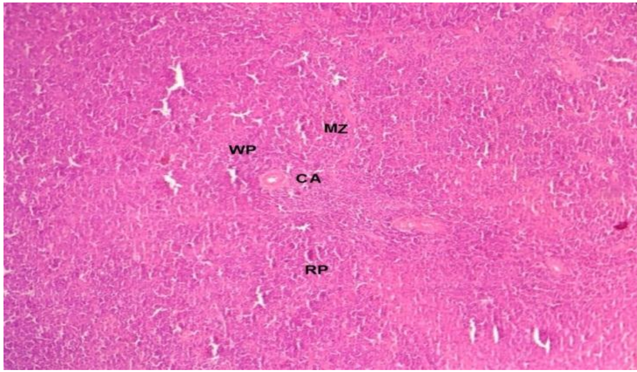

Figure (2): A photomicrograph of spleen section of group II (NAC group) showing normal splenic architecture. Normal red pulp (RP), white pulp (WP), marginal zone (MZ) and central arteriole (CA) with normal splenocytes radiating from it. H& E staining x100.

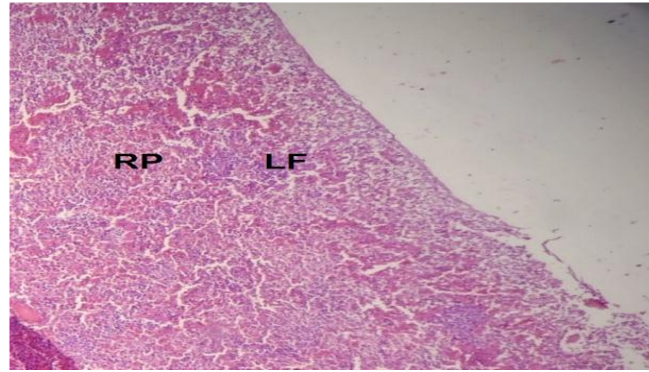

Figure (3 A): A photomicrograph of a section in the spleen of group III (DF group) showing marked congestion of red pulp (RP) and atrophy of lymphoid follicle (LF) .H&E staining x100.

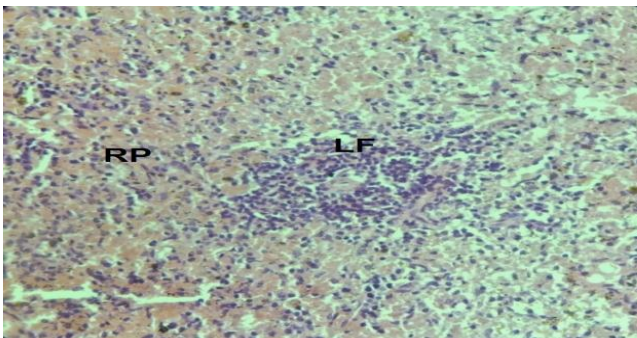

Figure (3 B): A photomicrograph of a section in the spleen of group III (DF group) showing marked congestion of red pulp (RP) and atrophy of lymphoid follicle (LF). H&E staining x 400.

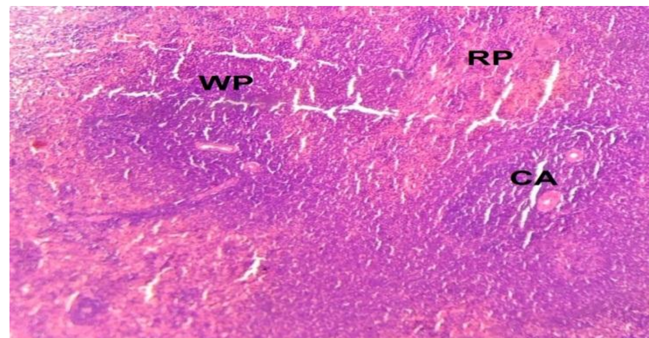

Figure (4): A photomicrograph of a section in the spleen of group IV (DF&NAC group) showing mild congestion of red pulp (RP) with normal central arteriole(CA). H&E staining x100.

DISCUSSION

Diclofenac sodium (DF) is one of the widely used non-steroidal anti-inflammatory drugs. It is an antipyretic, pain relieving, and anti-inflammatory drug with inhibitory effect on prostaglandin biosynthesis ***(Hesham et al., 2019).***

It is reported that DF is hydroxylated through CYP2C11 to 4´-hydroxydiclofenac and 5´-hydroxydiclofenac which are oxidized to electrophilic benzoquinones ***(Grillo et al., 2003).*** These compounds induce oxidative stress and attach to sulfhydryl groups, membrane proteins containing these groups, and cellular enzymatic antioxidant systems ***(Yapar et al., 2008.)***

In the current study, there was a significant statistical decrease in the mean values of RBCs indices (RBCs, Hb, HCT, MCV, MCH and MCHC) in group III (DF group) as compared to control group.

These effects could be explained that diclofenac toxicity may depress the activities of endogenous enzymatic and non-enzymatic antioxidants, and increase lipids peroxidation in the membrane of RBCs. This process induces rapid RBCs destruction due to altered RBCs membrane permeability, increased RBCs mechanical fragility ***(Alabi and Akomolafe, 2020).***

Also, the decrease of HB, RBCs, and HCT in diclofenac treated rats may be linked to up regulation of pro-inflammatory cytokines, including IL-6. It is believed that the rise in synthesis of IL-6 stimulates the development of anemia by induction of hepcidin ***(Nemeth et al., 2004).***

Abnormal increased hepcidin prevent iron recycles from old RBCs and/or inhibit iron export from the gut enterocytes leading to significant

reduction in serum iron and eventually leads to anemia ***(Knutson et al., 2005).***

Another explanation is that gastrointestinal bleeding induced by diclofenac sodium toxicity may lead to anemia ***(Gomaa, 2018).*** Also, it is believed that prolonged use of DF causes suppression of renal prostaglandins, so reduces the amount of erythropoietin. ***(Bahmut et al., 2018).***

These findings are in agreement with previous studies recorded by ***Alabi and Akomolafe, (2020)*** who reported that administration of diclofenac at dose 10 mg/kg intramuscularly daily for 7 days in rats led to significant decrease in RBCs, HB, and HCT.

Additionally, ***Gomaa, 2018*** mentioned that intraperitonial administration of diclofenac at dose (14.8 mg/kg) for 28 days in mice led to decrease in RBCs count, HB and HCT.

On the other hand, ***Shafi et al, 2015*** noticed that there was no significant difference in the hematological observations of the treated groups compared to the control. Diclofenac was administered at dose of 10, 20 and 30 mg/kg b. wt. for 5 days in chicken. This difference may be due to different species, dose or duration of administration.

In the present study, there was a significant statistical increase in the mean values of RBCs, HB, HCT and MCHC in group IV (DF&NAC group) as compared to group III (DF group). There was no significant statistical difference in the mean values of HB, HCT, MCV, MCH and MCHC in group IV (DF&NAC group) as compared to control group.

This means that combination of antioxidant (NAC) with toxic dose of diclofenac showed protection of blood parameters as compared with control groups.

This can be explained that NAC is an artificial precursor of GSH. It increases GSH levels and acts as a powerful scavenger of oxygen free radical, forming NAC-disulfide end products**.** ***(El-Sayed et al., 2010).***

In agreement with the present results, ***Alabi and Akomolafe (2020)*** noticed that the antioxidant capacity of kolaverin is responsible for the significant improvement in RBC, HB, and HCT count in the treated groups with diclofenac. This confirms the role of antioxidant.

There was a significant statistical increase in the mean value of platelets (PLT) in group III (DF group) and group IV (DF&NAC group) as compared to control group.

The effect of diclofenac on PLT can be explained by tissue injury and inflammatory response caused by DF toxic metabolites ***(Basavraj et al., 2012).***

Similarly, ***Alabi and Akomolafe, (2020)*** found that a significant increase in PLT count was noted in the DF group, compared to control. Also, ***Gomaa (2018)*** reported that mice showed increase PLT count in comparison with control group after administration of diclofenac at dose of (7.4 mg/kg and 14.8 mg/kg) for 28 days

On the other hand, ***Osojnik and Kamenik (2020)*** found that the PLT count showed no statistically significant differences between human administrated diclofenac after surgery and human group that administrated saline.

Also, ***Alsaady et al. (2011)*** noticed non-significant difference in PLT count of treated group as compared with control group after administration of diclofenac at 0.3 mg / kg IM for 60 days in rabbits. This difference may be due to different species or route of administration.

Co-administration of NAC with toxic dose of diclofenac could not prevent the increase of platelet count but the rise was less severe.

This may be attributed to NAC not used for pretreatment of treated group. It was found that pretreatment of animals with NAC for 5 days ameliorated the hematological, apoptotic, oxidant and antioxidant alterations ***(El-Sayed et al., 2010).***

In the current study, a significant statistical increase was noticed in the mean value of WBCs and lymphocyte. While, there was decrease in the mean value of neutrophil with no significant statistical difference in the mean value of monocyte and basophil in group III (DF group) as compared to control group.

It is known that diclofenac has direct toxicity to myeloid cells, particularly neutrophils. The toxicity may be due to either the parent drug or a toxic metabolite. The severity of neutropenia

increases with higher dose of the drug ***(Pontikoglou and Papadaki, 2010).***

Also, neutropenia may be due to DF induced antibodies against peripheral neutrophils or their bone marrow precursor which is hard to be identified *(**Al-Saady et al., 2011).***

In agreement with the present results, ***Owumi and Dim (2019)*** noticed that DF administration at dose of 10 mg/kg for 7 days in rats induced an increase in WBC and lymphocyte count but decreased neutrophils and eosinophils' level.

Also, ***Adeyemi and Olayaki (2018)*** documented that administration of diclofenac in rats at dose of 10 mg/kg/day (IM) for 7 days led to increase in total WBC count. However, significant rise in neutrophils count and significant decline in lymphocyte count was found.

Opposite results were reported *by **Alabi and Akomolafe (2020).*** They found that administration of diclofenac for 7 days to rats at dose of 10 mg/kg IM led to decrease in WBC, lymphocyte count and increase in neutrophil. The decline in WBC count may be due to different dose, duration or route of administration.

Also, ***Soussi et al (2019)*** found that intraperitoneal injection of DF at a dose of 2.37 mg\kg. For 5 days in mice led to significant drop in WBC count. This can be explained that DF decreased the heart rate, and so, the oxygen carrying capacity of the blood decreased leading to decrease of the RBC, WBC, HB, and HCT levels**.**

There was a significant statistical decrease in the mean value of WBC, lymphocyte, while increase in the mean value of neutrophil and no significant statistical difference in the mean value of monocyte and basophil in group IV (DF&NAC group) as compared to group III (DF group).

There was no significant statistical difference in the mean value of WBC lymphocyte, neutrophil, monocyte and basophil in group IV (DF&NAC group) as compared to control group. This means that administration of NAC improved the toxic effect of diclofenac.

This coincides with ***Dhouib et al (2015),*** who reported that NAC has a great role in improvement of hematological parameters that affected by carbosulfan. The improvement with NAC could be explained by the presence of acetyl and sulfhydryl groups make NAC a potent inhibitor of lipid peroxidation. NAC is a source of sulfhydryl groups in cells and scavenger of free radicals as it interacts with ROS such as OH and H2O2.

Lipid peroxidation is conventionally considered as a common marker to confirm oxidative stress. MDA serves as a reliable marker of lipid peroxidation and protein oxidation ***(Heidarian et al., 2017).***

In the present study, there was a significant statistical increase in the mean value of MDA in group III (DF group) and group IV (DF&NAC group) as compared to control group.

This reflects lipid peroxidation that is indicative of oxidative deterioration of polyunsaturated lipid induced by generated reactive oxygen ***(Owumi and Dim, 2019).***

Diclofenac has been found to be the most potent among the NSAIDs in inhibiting mitochondrial electron transport chain complex-I, thereby leading to electron leakage from the respiratory chain ***(Sandoval-Acuña et. al., 2012).*** The leaked electron causes partial reduction of molecular oxygen to form superoxide, O_2^-. Intra-mitochondrial O_2^- which is membrane impermeable is instantly converted to H_2O_2 by the mitochondrial superoxide dismutase, as a protective response against oxidative stress. However, being membrane permeable, H_2O_2 escapes the mitochondria causing oxidative damage to cellular macromolecules including DNA, protein, lipids and carbohydrates, and so increases MDA level ***(Mazumder et. al., 2016).***

*Similar results were obtained by **Elshopakey and Elazab, (2021)**.* They found that administration of diclofenac sodium at dose 100 mg /kg orally in rat caused increase in the level of MDA. They reported that diclofenac toxicity causes depression of the antioxidant activities of SOD, CAT, GST, and GSH, enhancing ROS production and deteriorating lipid peroxidation.

Additionally, ***Esmaeilzadeh et al, (2020)*** found that administration of diclofenac sodium at dose of 50 mg/kg I.P for 5 days in rats led to increase in level of MDA.

Co-administration of NAC with diclofenac resulted in a significant statistical decrease in the mean value of MDA in group IV (DF&NAC group) as compared to group III (DF group).

The ameliorative effect of NAC may be largely attributed to its ability to eradicate free radicals. Evidence supports the potent radical scavenging activity of NAC as being a sulfhydryl donor that can directly react with free radicals; so the protective effect of NAC is mostly mediated through its antioxidant activity ***(Abdel-Wahab and Moussa, 2019)***.

This coincides with ***Nouri and Heidarian (2019)*** who reported that co-administration of NAC (100 mg/kg bw/day, orally) with DF (50 mg/kg bw, i.p) for 5 days led to a remarkable decline in serum MDA levels, relative to the group received DF only.

Also**, *Nouri et al, (2017)*** noticed that co-administration of NAC (150 mg\kg orally) with DF (50 mg\kg ip) for 4 days led to decrease in level of MDA.

In the present study, no abnormal finding was detected in the spleen of control group and NAC group (groups I&II). While, there were marked congestion of red pulp, marked atrophy in lymphoid follicle in white pulp and fibrosed capsule in DF group (group III).

These results could be explained by production of ROS by the mitochondrial respiratory chain and increased toxicity of DF due to intracellular metabolism by cytochrome P450 ***(Van Leeuwen et al., 2012).***

Diclofenac has electrophilic metabolites, they form stable covalent protein adducts. Also, the reactive metabolites can react with nucleophilic residues in the enzyme's active site leading to enzyme suppression **(Eno and Cameron, 2015).**

Co-adminstration of NAC in group (IV) decreased the severity of damage to spleen tissue that caused by DF in the form of mild congestion of red pulp with normal central arteriole and trabeculae.

N-acetyl-cysteine has antioxidant activity in different tissue by stimulation of protein phosphatase activity and increase the intracellular level of reduced glutathione. This reduced glutathione enhances the activity of catalase, mitochondrial superoxide dismutase and glutathione peroxidase. These enzymes play a significant role in antioxidant system ***(Al-hazmi, 2020).***

Similar results were obtained by ***Taib and Jarrar (2006),*** who reported that exposure of rabbit to a daily dose of diclofenac sodium (1.5 mg/kg) I.P for 6 weeks led to considerable increase in the number of resident macrophages in splenic cords and more prussian blue positive granules were observed in the cytoplasm of the macrophages.

Similar results were observed by ***[Nouri and Heidarian (2019) and Nouri et. al.,(2017)]***. They reported improvement of renal and hepatic histopathological changes in rats received NAC with DF in comparison to groups received DF only.

Also, ***Satvati et al, 2022*** reported that liver histopathological changes were improved by co-administration of NAC with ibuprofen (NSAIDs) in comparison to groups received ibuprofen only.

CONCLUSION

It has been found that repeated oral administration of diclofenac sodium leads to significant changes in CBC induces oxidative stress and histopathological changes of spleen. N-Acetyl-cysteine co administration provides potential protection against these harmful effects.

RECOMMENDATIONS

- It is advisable to restrict prolonged use of diclofenac to avoid its toxic effect on blood and spleen.
- It is recommended that physicians prescribe N-acetyl cysteine when prolonged use of diclofenac is indicated as in cases of osteoarthritis.
- Other experimental studies on the efficacy of NAC in diclofenac toxicity should be performed to confirm the results of the present study.
- Clinical trials on the efficacy of NAC on sub-acute toxicity of diclofenac should be conducted.
- Further studies should be performed on other antioxidants and evaluate its effect on diclofenac toxicity.
- Increase the administration of fruits and vegetables as natural antioxidants.
- Further studies to detect mechanism of diclofenac toxicity on spleen.

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التأثير الوقائي لـلاسيتيل سيستيين علي الإجهاد التأكسدي وسمية الدم والطحال الناجمة عن الديكلوفيناك في ذكور الجرذان

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المقدمه: يعتبر مركب الديكلوفناك من أشهر مضادات الالتهاب غير الستيرودية الشائعة التي يتم استخدامها كمضاد للالتهاب مسكن و وخافض للحرارة.. ان اسيتيل سيستيين هومضاد للاكسدة ومزيل للجزئيات الحرة. يستخدم في مكافحة الأضرار الناجمة عن الإجهاد التأكسدي في الأنسجة المختلفة. **الهدف من الدراسة :** تهدف هذه الدراسة إلي دراسه سمية عقار ديكلوفناك صوديوم علي الدم (صورة دم) ، دليل الاجهاد التأكسدي (MDA) والطحال وكذلك دراسة التأثير الوقائي المحتمل لمركب إن اسيتيل سيستيين. **طريقة البحث:** تم إجراء البحث على عدد ٤٠ من ذكور الجرذان البيضاء البالغة. تم تقسيم الجرذان لأربع مجموعات متساويه. المجموعة الاولي تم امدادها بالغذاء والماء المقطر يوميا .المجموعة الثانية تم اعطائها إن اسيتيل سيستيين عن طريق الفم بجرعة ١٥٠ مجم /كجم يوميا. المجموعة الثالثة: تم اعطائها ديكلوفيناك صوديوم عن طريق الفم بجرعة ٥ مجم /كجم يوميا.المجموعة الرابعة تم اعطائها ديكلوفيناك صوديوم و إن اسيتيل سيستيين بنفس الجرعات السابقة يوميا. بعد مرور شهر تم ذبح الجرذان و جمع عينات الدم لفحصها وتشريح الجثة لجميع الحيوانات واستخراج الطحال لفحص الأنسجة .**النتائج:** كان هناك تغير في صورة الدم للمجموعة الثالثة التي تم اعطاءها الديكلوفيناك لمدة شهر. هذه التغيرات كانت أقل شدة في المجموعة الرابعة التي تم اعطائها الاسيتيل سيستيين مع الديكلوفيناك. كذلك لوحظ زيادة في دليل الاجهاد التأكسدي (MDA) في المجموعة الثالثة كانت اقل في المجموعة الرابعة. لوحظ بعض التغيرات الباثولوجية في الطحال في المجموعة المعالجة بالديكلوفيناك. كانت هذه التغيرات اقل عند إعطاء الاسيتيل سيتيين في المجموعة الرابعة. **الخلاصة:** التناول المتكرر للديكلوفيناك يسبب تأثيرات سمية علي مؤشرات الدم، دليل الاجهاد التأكسدي (MDA) و تغيرات باثولوجية في الطحال. تبين ان تناول الاسيتيل سيستيين يقلل من هذه التأثير السمية.