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Protective Effect of Curcumin on Diclofenac Sodium-Induced Hepatotoxicity in Male Albino Rats: Evidence of Its Antioxidant and Anti-Inflammatory Properties

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Abstract

This research aims to explore if curcumin (CUR) can protect male albino rats from diclofenac (DIC) sodium-induced hepatotoxicity. The adult male Albino rats were divided into four groups (n=8) in this study. Control group: corn oil (i.p.) was given every day for seven days. CUR-treated group was given CUR (200 mg/kg, i.p.) every day for seven days. DIC-treated group was given corn oil (i.p.) every day for seven days, followed by DIC single dose (150 mg/kg, i.p) 2 hours after last corn oil injection. DIC+CUR-treated group was given CUR (200 mg/kg i.p.) every day for seven days followed by DIC single dose (150 mg/kg, i.p.) two hours following the last CUR injection. Animals were sacrificed twenty-four hours following the last treatment. Blood serum was collected and tested for liver function markers. The liver was removed and used for biochemical and light microscopic examinations. The data showed that serum AST, ALT levels, as well as hepatic MDA, NO, and TNFα levels were significantly higher in the DIC-treated group than in the control group. While, serum total protein and albumin levels, as well as hepatic SOD levels, were significantly reduced. In contrast to the DIC-treated group, pretreatment with CUR resulted in a significant decrease in AST, ALT, MDA, NO, and TNF-α as well as a significant increase in total protein, albumin, and SOD levels. CUR pretreatment also ameliorated the histological alterations. In conclusion, CUR treatment provided excellent protection against DIC-induced liver toxicity and oxidative damage in rats.

Keywords: Diclofenac; Curcumin; Drug-induced hepatotoxicity; Oxidative stress; TNF-α.

1. Introduction

The liver is an important organ that plays a role in the body's overall health. It detoxifies a wide range of harmful compounds, chemicals, and microbiological agents. The morphological changes in the liver have a bent to affect the metabolic events of the whole body, that's often related to dysfunction of the detoxification process **(Li et al., 2019)**. Many etiological variables have been linked to liver disease and are highly reactive oxygen species producers (ROS). One of these causes is medication hepatotoxicity, which is linked to the production of reactive oxygen and nitrogen species (ROS and RNS) **(Videla, 2009)**.

Over-the-counter medicines are considered one of the main reasons implicated in liver damage, hence

threatening human health. Drug-induced liver injury (DILI) is usually divided into two categories: intrinsic and idiosyncratic. Dose-dependent hepatotoxicity is a characteristic of intrinsic hepatotoxic drugs. In contrast, Idiosyncratic DILI isn't clearly linked to drug dose, route, or period of administration **(Iruzubieta et al., 2015)**.

Non-Steroidal anti-inflammatory drugs (NSAIDs) are considered the most widely given treatments. They're commonly employed by over 30% in developed countries **(Manov et al., 2006)**. Diclofenac (DIC) sodium (one of the NSAIDs) is a phenylacetic acid derivative. As a prescription drug or over the counter (OTC) medication, it is used as an analgesic, antipyretic, and in the treatment of inflammatory pains linked to musculoskeletal injuries, rheumatoid arthritis, and osteoarthritis **(Aycan et al., 2018)**. DIC works by inhibiting prostaglandin biogenesis in a non-selective manner (inhibiting cyclooxygenase-1 and cyclooxygenase-2 with relative equipotency). When given at therapeutic dosages, DIC is safe; but greater doses given over time cause hepato-, nephron-, and bone marrow toxicity, as well as enteropathy **(Alabi and Akomolafe, 2020)**. NSAIDs, particularly DIC, are one of the commonest drugs associated with drug-induced hepatic injury with an incidence of 3 and 23 per 100,000 patients **(Laine et al., 2009).**

Diclofenac is biotransformed into 4-hydroxy diclofenac and other hydroxylated forms by liver microsomal enzymes through glucuronidation and sulfation, and subsequently eliminated through urine and bile. Its metabolites cause mitochondrial dysfunction, nicotinamide adenine dinucleotide phosphate (NADPH) oxidation, and the production of ROS, all of which cause hepatotoxicity **(O'Connor et al., 2003)**. The occurrence of mitochondrial damage, oxidative stress, and the interaction of DIC's reactive metabolites with cellular macromolecules, all of which result in a change in protein integrity, have been recognized to be the mechanisms underlying DIC toxicity **(Owumi and Dim, 2019)**.

Because of the lack of reliable hepatoprotective drugs, herbs play crucial role in ameliorating liver diseases. In recent years, the protective impact of plant products or medicinal plants with antioxidant capabilities, including curcumin (CUR), as a therapy to minimize free radical-induced tissue damage has been focused on **(Elsayed, 2016)**. Because of their wide availability, low toxicity, pharmacological action, chemical variety, and few side effects compared to synthetic medications, herbal medicines are used by around 65 percent of patients in the United States and Europe to treat liver disease **(Zhang et al., 2013)**.

Curcumin is a polyphenol produced from turmeric that has anti-inflammatory and antioxidant properties **(Galaly et al., 2014)**. Turmeric is made up primarily of curcumin, which is found in the rhizome of Curcuma longa. Because of its therapeutic effectiveness and adequate safety specifications, it is frequently used **(Elsayed, 2016)**. CUR decreased oxidative damage and apoptosis in a rat model of gentamicin-induced hepato- and nephrotoxicity. In addition to its potential to reduce oxidative stress and inflammation, CUR has anticancer, anti-atherosclerotic, anti-diabetic, anti-obesity, antihyperlipidemic, and hepatoprotective properties **(Tsuda, 2018; Fu et al., 2021)**.

Based on these findings, the goal of this investigation was to study the capability of CUR to protect male albino rats against diclofenac sodium-induced hepatotoxicity.

2. Materials and methods

2.1. Drugs and Chemicals

Diclofenac sodium was bought from AK Scientific, Inc. (USA). Alpha Global Search (India) provided the CUR.

Kits for tumor necrosis factor-α (TNF-α) assay were obtained from Wuhan EIAab Science Co. Ltd (China) (Cat. No. E0133r). Bio-diagnostic Company in Egypt provided kits for measuring superoxide dismutase enzyme (SOD) (Cat. No. SD 25 21), malondialdehyde (MDA) (Cat. No. MD 25 28), and nitric oxide (NO) (Cat. No. NO 25 32).

Kits for the determination of liver function tests including aspartate aminotransferase (AST) (Cat. No. 260 001), alanine aminotransferase (ALT) (Cat. No. 264 001), total protein (Cat. No. 310 001) and albumin (Cat. No. 210 001) were obtained from Egyptian Company for Biotechnology, Cairo, Egypt. Chemicals were of analytical grade.

2.2. Animals

The experiment was carried out on 32 adult male albino rats (average weight 180-200g) that were obtained from the animal house and housed in an animal facility at Sohag University's Faculty of Medicine in Egypt.

They were housed in standard cages in an air-conditioned room with a temperature of (24 ± 2ºC), a relative humidity of (55% ± 5%) and a 12-hour light/dark cycle.

For acclimatisation, the rats were housed in the same setting for 7 days prior to the experiment. They were fed the same chow diet and given unlimited water.

The experimental methodology was carried out and authorised in accordance with the Institutional Animal Care and Use Committee of Sohag University, Faculty of Medicine, Egypt (Approval No. Sohag-5-5-2022-03).

2.3. Experimental design

The rats were separated into four groups of eight rats each, and were treated every day for seven days as follows:

Control group: Corn oil was given intraperitoneally (i.p.) to rats every day for seven days.

CUR-treated group: CUR suspended in corn oil (200 mg/kg, i.p.) **(Shapiro et al., 2006)** was given to rats every day for seven days.

DIC-treated group: Corn oil (i.p.) was given to rats daily for seven days, followed by a single dosage of DIC (150 mg/kg, i.p.) **(Hamza, 2007)** two hours following the last corn oil injection.

DIC+CUR-treated group: CUR (200 mg/kg i.p.) was given to rats daily for seven days, followed by a single dose of DIC (150 mg/kg i.p.) two hours following the last CUR injection.

2.4. Samples collection

Twenty-four hours after the last treatment, rats of all groups were anesthetized with light ether. Cardiac puncture was used to take blood samples from the heart, which were then centrifuged to extract the serum for the liver function tests. Animals were slaughtered thereafter by cervical dislocation to separate tissue samples.

A portion of liver samples was used to investigate the anti-inflammatory, antioxidant, and oxidative stress parameters. Another portion of the collected samples was kept in 10% formalin for further histopathological examination.

2.5. Biochemical analysis

2.5.1. Determination of serum biochemical parameters

Total protein, AST, ALT, and albumin (liver function tests). They were spectrophotometrically measured (Jenway 6051 colorimeter spectrophotometer).

2.5.2. Measurements of TNF-α, SOD, NO and the degree of lipid peroxidation

A portion of the liver was weighed and homogenized in phosphate buffered saline (pH 7.4) using a homogenizer after being washed in ice cold saline. The homogenate obtained was centrifuged, the supernatant was collected and held at -80°C until TNF-α, SOD, NO, and the degree of lipid peroxidation (MDA) were determined.

Tumor necrosis factor-α was measured using an enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions. The results were given in pg/g tissue.

Superoxide dismutase was measured using a colorimetric technique, as described by **Nishikimi et al. (1972)**. The results were expressed as U/g tissue.

The colorimetric technique of **Montgomery and Dymock (1961)** was used to assay NO. The data were presented in µmol/g tissue.

The measurement of MDA levels in liver tissue using a colorimetric technique according to **Ohkawa et al. (1979)** was used to determine lipid peroxidation. Data were described as nmol/g tissue.

2.6. Histopathological studies

Liver specimens were processed using the conventional paraffin embedding technique. Light microscopy was used to examine sections (5 µm) stained with haematoxylin and eosin (H & E) to verify histological features.

2.7. Statistical analysis of data

Values were expressed as mean ± SE. The data were analysed using SPSS software (Statistical Package for the Social Sciences, version 20.0, SPSS Inc, Chicago, IL) and a one-way analysis of variance (ANOVA). Tukey's post hoc test was used to compare the groups' mean values. $P<0.05$ was considered statistically significant in all types of statistical tests.

3. Results

3.1. Effect of CUR on serum levels of biomarkers of hepatic function in DIC-induced hepatic damage in rats

When compared to the control normal group, the acquired results in **table (1)** demonstrated a significant increase (P<0.05) in AST and ALT in the DIC-treated group at a single dose of (150 mg/kg ip), as well as a significant drop (P<0.05) in albumin and total protein levels. When compared to the DIC-treated group, pretreatment with CUR (DIC+CUR group) at a dose of (200 mg/kg ip) for 7 days lowered (P<0.05) serum levels of AST and ALT and increased (P<0.05) serum levels of albumin and total protein. In addition, there were non-significant differences in the prior parameters between the CUR and the control groups.

3.2. Effect of CUR on hepatic MDA in DIC-induced hepatic damage in rats

As shown in **figure (1)**, there was a significant increase (P<0.05) in hepatic MDA in the DIC-treated group with a single dose of (150 mg/kg ip) as compared to the control group. When compared to the DIC-treated group, MDA activity in the liver was significantly reduced (P<0.05) in CUR pretreatment (DIC+CUR group) at a dosage of (200 mg/kg ip) for 7 days. There was also a non-significant difference between the CUR and control groups.

Table 1. Effect of curcumin (200 mg/kg/day i.p) on serum levels of biomarkers of hepatic function in diclofenac (150 mg/kg i.p single injection)-induced hepatic damage in rats

Groups	AST (U/L)	ALT (U/L)	Total Proteins (g/dl)	Albumin (g/dl)
Group1 (Control)	38.25±3.51	43.13±3.76	6.55±0.2	4.39±0.19
Group2 (CUR)	41.38±3.75	42.00±3.82	7.15±0.27	4.44±0.2
Group3 (DIC)	166.13±14.18[a]	135.25±10.89[a]	5.37±0.3 [a]	3.36±0.22[a]
Group4 (DIC+CUR)	46.88±4.56[b]	50.63±3.82[b]	7.09±0.3 [b]	4.37±0.23[b]

The data were analysed using one-way ANOVA followed by Tukey post hoc test and reported as mean ± SE (n=8). CUR=Curcumin, DIC=Diclofenac, AST= Aspartate aminotransferase, ALT= Alanine aminotransferase. [a] P<0.05 compared to the control group (Group 1), [b] P<0.05 compared to the DIC- treated group (Group 3).

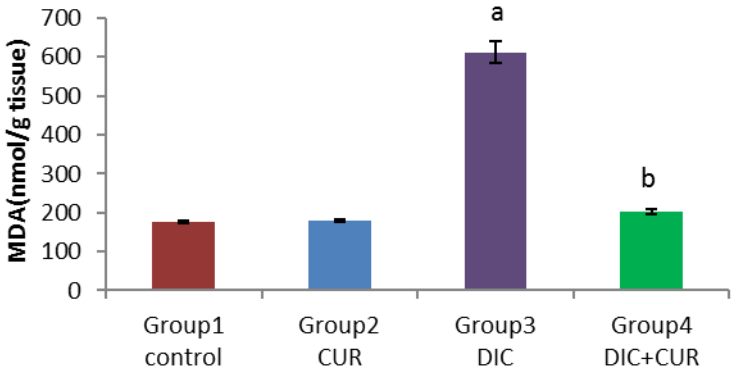

Figure 1. Effect of curcumin (200 mg/kg/day i.p) on hepatic MDA in diclofenac (150 mg/kg i.p single injection)-induced hepatic damage in rats. The data were analysed using one-way ANOVA followed by Tukey post hoc test and reported as mean ± SE (n=8). CUR=Curcumin, DIC=Diclofenac, MDA=Malondialdehyde. [a] P<0.05 compared to the control group (Group 1), [b] P<0.05 compared to the DIC- treated group (Group 3).

3.3. Effect of CUR on hepatic NO in DIC-induced hepatic damage in rats

As shown in **figure (2)**, there was a significant increase in hepatic NO (P<0.05) in the DIC-treated group with a single dose of (150 mg/kg ip) as compared to the control group. There was a significant reduction (P<0.05) in NO activity in the liver after 7 days of CUR pretreatment (DIC+CUR group) at a dosage of (200 mg/kg ip) when compared with the DIC-treated group. A non-significant difference existed between the CUR and control groups as well.

3.4. Effect of CUR on hepatic SOD in DIC-induced hepatic damage in rats

Figure (3) indicates that the injection of a single dose of DIC (150 mg/kg ip) in the DIC-treated group led to a significant decrease (P<0.05) in hepatic SOD activity relative to the control animals. However, compared to the DIC-treated group, previous treatment with CUR (DIC+CUR group) for 7 days at a dosage of (200 mg/kg. ip) increased (P<0.05) liver SOD activity.

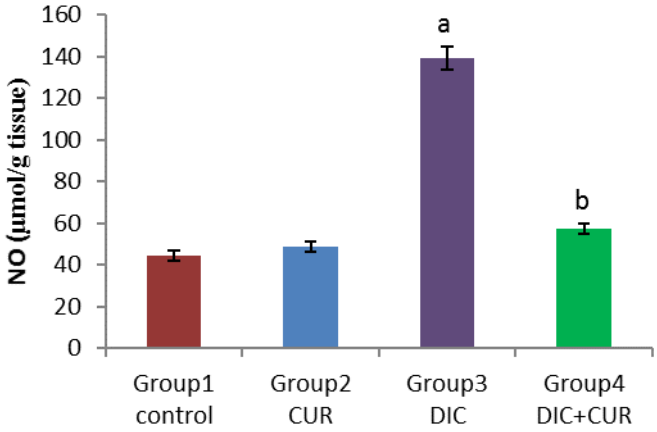

Figure 2. Effect of curcumin (200 mg/kg/day i.p) on hepatic NO in diclofenac (150 mg/kg i.p single injection)-induced hepatic damage in rats. The data were analysed using one-way ANOVA followed by Tukey post hoc test and reported as mean ± SE (n=8). CUR=Curcumin, DIC=Diclofenac, NO=Nitric oxide. [(a)] P<0.05 compared to the control group (Group 1), [(b)] P<0.05 compared to the DIC- treated group (Group 3).

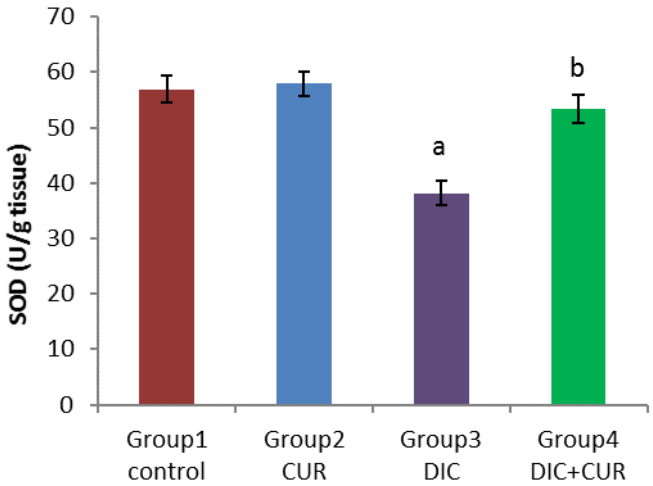

Figure 3. Effect of curcumin (200 mg/kg/day i.p) on hepatic SOD in diclofenac (150 mg/kg i.p single injection)-induced hepatic damage in rats. The data were analysed using one-way ANOVA followed by Tukey post hoc test and reported as mean ± SE (n=8). CUR=Curcumin, DIC=Diclofenac, SOD= Superoxide dismutase. [(a)] P<0.05 compared to the control group (Group 1), [(b)] P<0.05 compared to the DIC- treated group (Group 3).

3.5. Effect of CUR on hepatic TNFα in DIC-induced hepatic damage in rats

Figure (4) shows a significant (P<0.05) rise in TNFα in the DIC-treated group with a single dose of (150 mg/kg ip) when compared to the control animals. In contrast to the DIC-treated group, CUR at a dosage of (200 mg/kg, ip) for 7 days prior to DIC induced a significant decrease (P<0.05) in TNF-α. In addition, there was no statistically significant difference between the CUR and control groups.

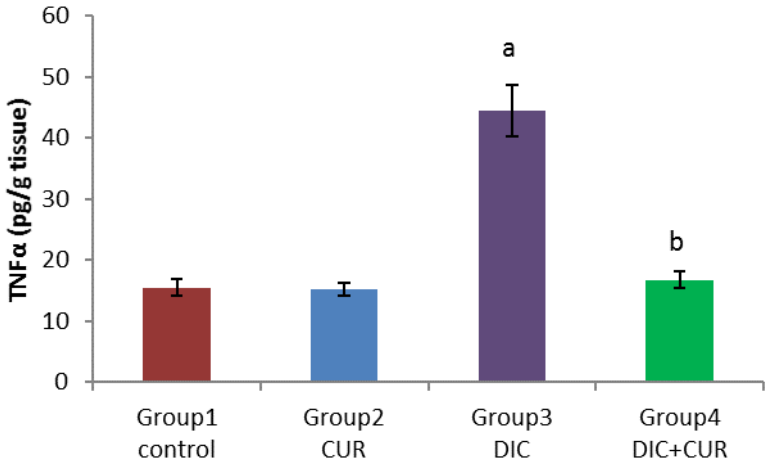

Figure 4. Effect of curcumin (200 mg/kg/day i.p) on hepatic TNFα in DIC (150 mg/kg i.p single injection)-induced hepatic damage in rats. The data were analysed using one-way ANOVA followed by Tukey post hoc test and reported as mean ± SE (n=8). CUR=Curcumin, DIC=Diclofenac, TNF-α=Tumor necrosis factor-α. [(a)] P<0.05 compared to the control group (Group 1), [(b)] P<0.05 compared to the DIC- treated group (Group 3).

3.6. Histopathological study

The architecture of the livers of control and CUR-treated rats was examined microscopically and indicated normal architecture **(Figure 5A-C)**.

When compared to the normal control group, the liver tissue of DIC-treated rats exhibited extensive histopathological hepatic injuries characterized by dilated congested central vein, deeply stained nuclei with eosinophilic cytoplasm (Councilman bodies) and congested sinusoids, proliferating bile ductules, degenerated hepatocytes and lymphocytic infiltrate with numerous apoptosis, and apoptotic bodies, and hydropic degeneration (cytoplasmic vacuolations) **(Figure 5D- G)**.

Histopathological lesions caused by DIC treatment, on the other hand, were reduced in rats given both DIC and CUR (DIC+CUR-treated group). Liver sections showed normal architecture and apparently normal-looking hepatocytes with central vesicular nuclei, kupffer cell hyperplasia, and slightly dilated veins **(Figure 5H & I)**.

The results of serum biomarkers of hepatic function, hepatic oxidative stress and cytokine levels corroborated these histological findings.

4. Discussion

In the EU and the United States, drug-induced liver injury (DILI) is the chief cause of acute liver failure, with a high death rate and few effective therapeutic options **(Habib and Shaikh, 2017)**. Hepatotoxicity is already a class warning for NSAIDs, and hepatic injury has been recorded with almost all commercially marketed NSAIDs occasionally **(Higushi et al., 2007)**. Because of its broad therapeutic use, DIC's toxicity has great interest.

In the present investigation, our findings demonstrated that giving normal rats a single dosage of DIC (150 mg/kg, i.p) resulted in a significant impairment in liver functions evidenced by a major increase in the levels of AST and ALT when compared with control group, this can be attributable to diclofenac-induced liver cell destruction, demonstrating DIC's hepatotoxic potential. Additionally, total proteins and albumin levels were significantly decreased compared to their corresponding values in control group. Our results are in accordance with prior researches that showed an elevation in AST and ALT, as well as a decrease in total protein and albumin levels in DIC-treated rats **(Baravalia et al., 2011; Ahmed et al.,**

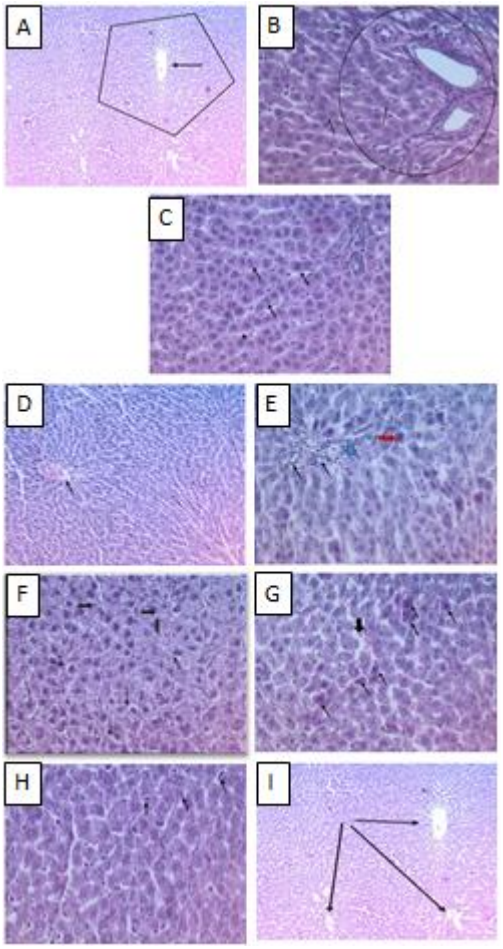

Figure 5. Photomicrographs of Liver sections of the Control group (A, B), the CUR group (C), DIC-treated group (D, E, F, G), and DIC and CUR-treated group (H, I).

A) Normal architecture, well-formed lobules. The center of the lobule is the central vein (Arrow). At the periphery of the lobule are portal triads (H&E, x100).
B) Normal line cell plates (Arrows) with a normal portal area (circle) (H&E, x400).
C) The hepatocytes are arranged in one cell layer thick plates (Arrows), separated by sinusoids (Arrowhead), (H&E, x 200).
D) Dilated congested central vein (Arrow), (H&E, x100).
E) Proliferating bile ductules (Thin Arrows), degenerated hepatocytes (Red Arrow) lymphocytic infiltrate (Blue Arrow), (H&E, 200x).
F) Numerous apoptosis and apoptotic bodies (Thick Arrows) with hydropic degeneration (cytoplasmic vacuolations) (Thin Arrows) (H&E, x400).
G) Deeply stained nuclei with eosinophilic cytoplasm (Councilman bodies) (Thin Arrows) and congested sinusoids (Thick Arrow) (H&E, x400).
H) Normal architecture and apparently normal-looking hepatocytes with central vesicular nuclei, with kupffer cell hyperplasia (Arrows) (H&E, x400).
I) Normal architecture, well-formed lobules with slightly dilated veins (Arrows) (H&E, x100).

2012; Taha et al., 2015). In addition, **Ramezannezhad et al., (2019)**, **Esmaeilzadeh et al., (2020)** and **Hassan et al., (2021)** reported remarkably increased AST and ALT levels in DIC-treated group.

One of the primary indicators of liver damage is the presence of specific enzymes, such as AST and ALT, which are released into the bloodstream when hepatocyte transport mechanisms are disrupted. This results in higher enzyme levels in serum **(Sadasivan et al., 2006)**. On the other hand, hypoalbuminemia occurred in acute hepatotoxicity may be attributed to inflammation, which increases capillary permeability and escape of serum albumin, causing expansion of interstitial space and increasing the distribution volume of albumin. Albumin's half-life has been observed to shorten, resulting in a decrease in total albumin mass and

hence, total proteins **(Soeters et al., 2019)**.

The current study found that giving CUR (200 mg/kg i.p.) for seven successive days before DIC administration elicited a significant reduction in liver enzymes levels (AST and ALT). These findings are consistent with prior reports; **Lin et al. (2012)** stated that treatment with CUR before ischemia-reperfusion–induced liver injury significantly attenuated AST and ALT levels, **Soliman et al. (2015)** who showed significant decrease in serum ALT and AST with concurrent use of CUR with lead (Pb), also, **Alhusain et al. (2022)** showed significant reduction in serum AST and ALT after curcumin treatment. These findings suggested that CUR protects hepatocytes from DIC-induced hepatotoxicity, possibly due to its capacity to prevent cellular outflow and the loss of the cell membrane's functional integrity. Also, administration of CUR before DIC returned the decreasing values of total proteins and albumin levels to normal levels. This result is in accordance with **Sayed and Elkordy (2014)** who showed significantly increased plasma total proteins and albumin levels when CUR is administered with paracetamol in rabbits.

Production of ROS and reactive metabolites is the primary cause of DIC-induced hepatotoxicity. The oxidation of DIC by cytochrome P450 produces 2,5-quinone imines, as well as 4′ - and 5-hydroxy-diclofenac **(Al-Dossari et al., 2020)**. To explain the allergic and intrinsic hepatotoxicity of the medicine, both the production of a toxic metabolite and covalent binding of the drug to hepatic proteins were postulated **(Pumford et al., 1993)**. DIC hepatotoxicity is also hypothesized to be caused by mitochondrial damage and NADPH shortage **(El-Maddawy and El-Ashmawy, 2013)**. As a result, it's logical to predict that substantial antioxidant levels would be required to reduce DIC-induced toxicity.

The levels of MDA, a lipid peroxidation marker (LPO), in the DIC-treated rats were significantly greater than in the control group, according to this study, which agreed with **Owumi and Dim. (2019)**, **Esmaeilzadeh et al. (2020)** and **Varışlı et al. (2022)** who revealed that DIC increases hepatic MDA levels. In comparison to the DIC-treated group, CUR given before DIC resulted in a decrease in MDA levels in hepatic tissues. Similarly, CUR has been reported to inhibit hepatic MDA elevation in the previous reports; **Samarghandian et al. (2017)** showed that CUR administered intraperitoneally substantially reduced MDA in immobilization-induced oxidative stress in rat liver, **Al-Dossari et al. (2020)** reported that CUR prevented liver injury and oxidative stress in lipopolysaccharide (LPS)/DIC-induced rats. Furthermore, **Alhusain et al. (2022)** reported significant reduction in MDA level after curcumin treatment.

The elevation of the pro-oxidant enzyme inducible nitric oxide synthase (iNOS), which produces NO, has been linked to hepatic damage **(Navarro-Antolín et al., 2007)**. In comparison to the control group, the DIC-treated group had a considerable rise in hepatic NO, according to our findings. These findings are consistent with prior researches that found elevated NO levels in DIC-treated rats **(Ahmed et al., 2012; Taha et al., 2015; Owumi and Dim, 2019; Al-Dossari et al., 2020)**. On the other hand, CUR pretreatment decreases NO. This result is in harmony with **Tokaç et al. (2013)** who showed that CUR significantly reduced nitric oxide levels thus protecting against oxidative stress induced by bile duct ligation, and **Al-Dossari et al. (2020)** who found that NO was significantly reduced in the liver of LPS/DCL-administered rats treated with CUR. In addition, **Alhusain et al. (2022)** mentioned that curcumin treatment caused significant reduction in NO level. CUR therapy lowered MDA and NO levels in hepatic tissue and increased the activity of antioxidant defense system enzymes, indicating its antioxidant potential.

An essential antioxidant defense enzyme is superoxide dismutase. This study revealed that the injection of DIC led to a significant decline in hepatic SOD activity in the DIC-treated group compared to the control animals. DIC has been found to impair the activity of antioxidant enzymes in the liver in various investigations **(Owumi and Dim., 2019; Esmaeilzadeh et al., 2020; Varışlı et al., 2022)**. Increased oxidative stress was a result of decreased SOD activity caused by DIC injection. In the DIC and CUR treated group, there was a rise in SOD level. This result is in consistence with **Cao et al. (2015)** who found that curcumin promoted the restoration of SOD in carbon tetrachloride (CCl_4)-induced liver damage. **Samarghandian et al. (2017)**, also stated that administration of CUR significantly increased antioxidant defense enzyme activity (SOD) in rat liver tissue.

Because of the β-diketone group in its structure, CUR is considered an antioxidant. CUR promotes

most of its effects by inhibiting superoxide radicals, hydrogen peroxide, and nitric oxide radicals, according to **Joe and Lokesh (1994)**. Antioxidant enzymes like catalase and SOD have been shown to be enhanced by CUR **(Reddy and Lokesh, 1994)**. These activities help to minimize lipid peroxidation and liver damage. In addition, CUR reduces mitochondrial ROS, improves mitochondrial activity, and lowers TNF-α level, **Wang et al. (2015)** verified all these findings in a rat model. Curcumin may accelerate the detoxification process of DIC, which may contribute to its hepatoprotective impact, in addition to its well-known antioxidant action **(Iqbal et al., 2003)**.

Our study indicated that, in comparison to the control animals, the DIC-treated group had a significant rise in hepatic TNF-α. Our results agree with **Hassan et al. (2021)** who showed significant elevation in hepatic TNF-α in DIC-injected rats. DIC has also been found to cause macrophages and monocytes to circulate, thus TNF-α, as well as other pro-inflammatory cytokines, are produced and released as a result **(Esmaeilzadeh et al., 2020)**. Through nuclear factor kappa-B (NF-κB) signalling, DIC increases inducible nitric oxide and NO production, promoting cell damage **(Kakita et al., 2009)**. Increases in hepatic MDA, NO, and TNF-α levels in rats treated with DIC alone may contribute to hepatic injury by triggering inflammatory and nitrosative stress responses.

Curcumin treatment before DIC, on the other hand, resulted in a decrease in TNFα levels compared to the DIC-treated group. This finding is consistent with **Cao et al. (2015)**, and **Al-Dossari et al. (2020)** who found that treatment with CUR significantly reduced inflammatory mediators including TNF-α, indicating CUR's anti-inflammatory effect. CUR could alleviate liver injury by decreasing the inflammatory response by inhibiting nuclear factor κB-mediated production of inflammatory cytokines, according to **Gonzales and Orlando (2008)**. CUR inhibits TNF production and TNF-mediated cell signalling in a range of cells, suppressing pro-inflammatory pathways connected to the majority of chronic illnesses. CUR has been shown in vitro and in vivo to be a TNF blocker by directly binding to TNF **(Gupta et al., 2014)**.

Our biochemical findings point to alterations in the livers of treated rats, which were confirmed by histological testing. Our findings revealed that liver tissue of rats of the DIC-treated group showed extensive histopathological hepatic changes. These changes are in agreement with the results of other researchers who reported that DIC in high doses induced hepatotoxic and hepatocellular necrosis in the liver **(Tolman, 1998; Baravalia et al., 2011; Owumi and Dim., 2019)**.

On the other hand, liver sections of rats treated with DIC, and CUR showed markedly alleviated histopathological lesions compared to the DIC-treated rats. Liver sections also showed Kupffer cell hyperplasia. The liver phagocytic system and Kupffer cells release a multitude of mediators that protect the body against xenobiotic chemicals and materials **(Sadauskas et al., 2009)**. This coincides with other investigators **(Somanawat et al., 2013; Sayed and El-Kordy, 2014)** who reported that liver sections of rats and rabbits treated with curcumin revealed marked regeneration and improvement in hepatic cells.

As demonstrated in the present results, CUR's antioxidant and anti-inflammatory properties are thought to be responsible for its improvement of DIC sodium-induced hepatic damage. It reduces the inflammatory response by suppressing NF-κB-mediated production of inflammatory cytokines and neutralizes free radicals, which are highly unstable chemicals that can harm cellular structures through aberrant oxidative processes.

5. Conclusion

In conclusion, CUR administration protected rats from DIC-induced hepatic toxicity and oxidative damage, as these natural antioxidants can improve serum biomarkers of hepatic function, enzymatic antioxidant defense system, prevent lipid peroxidation and oxidative stress, reduce pro-inflammatory cytokines, and improve histopathological alterations in hepatic tissues.

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Conflict of Interest

There are no potential conflicts of interest.

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