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# THE PROTECTIVE ROLE OF COLCHICINE ON DICLOFENAC SODIUM INDUCED HEPATORENAL TOXICITY IN ALBINO RATS MODEL

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**Keywords:**

Colchicine, diclofenac sodium, hepato-renal protective, oxidative stress biomarkers

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**ABSTRACT:** Diclofenac (DIC) is a widely used as an anti-inflammatory drug and it is used as the toxicant in hepatoprotective studies. In the present study, hepato-renal protective activity of Colchicine against DIC induced liver and kidney injury in albino rats was assessed. Animals were injected with Diclofenac sodium at the single dose of 150 mg/ kg body weight daily for 28 days. Oral administration of colchicine at a concentration of 10µg/kg b.w daily for 28 days showed a significant decrease in plasma AST, ALT and creatinine as well as hepatic and renal thiobarbituric acid reactive substances and hydroperoxides. The treatment also resulted in a significant increase in GSH, SOD, CAT and GPx in the liver and kidney of DIC treated rats. The results clearly suggest that the colchicine treated group may effectively normalize the impaired antioxidant status in DIC- induced liver and kidney injury than the vitamin C-treated groups. However, Colchicine rapid protective effects against lipid peroxidation by scavenging of free radicals reduce the risk of oxidative complications.

**INTRODUCTION:** Liver diseases are considered to be serious health disorders. The liver has one of the highest value of importance for the systemic detoxification and deposition of endogenous and exogenous substances. Diclofenac (DIC) is a phenylacetic acid derivative that was developed specifically as a non-steroidal anti- inflammatory (NSAID) drug[1]. DIC causes a rare but potentially severe liver injury in humans [2,3]. The hepatotoxicity of DIC was also documented in experimental animal studies [4-6].

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The mechanism of DIC hepatotoxicity involves covalent protein modification by reactive metabolites [7-9], oxidative stress generation by peroxidase-catalyzed reaction [3, 10] and mitochondrial injury propagation by reactive oxygen species [11, 12].

Liver damage occurs infrequently, and is usually reversible. Hepatitis may occur rarely without any warning symptoms and may be fatal. Patients with osteoarthritis more often develop symptomatic liver disease than patients with rheumatoid arthritis. Liver function should be monitored regularly during long-term treatment. If used for the short-term treatment of pain or fever, diclofenac has not been found to be more hepatotoxic than other NSAIDs [13, 14]. Although several natural products have been shown to protect against chemical-induced liver and renal toxicity, a consensus on the

protective effects of natural products for the treatment of DIC- induced hepato-renal toxicity however has not yet been reached.

Colchicine is an alkaloid drug, chemically known as N-[(7S)-1, 2, 3, 10-tetramethoxy-9-oxo-5, 6, 7, 9-tetrahydrobenzo[a] heptalen-7-yl] acetamide, and widely used for the treatment of gout disease [15].

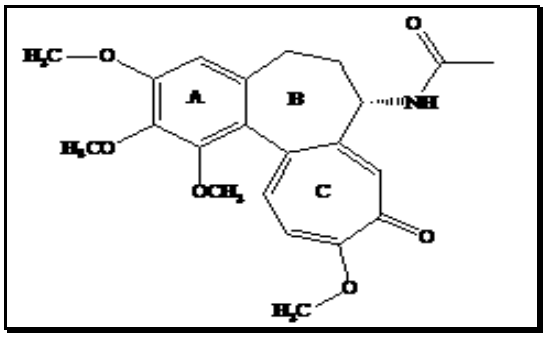



**FIG. 1: STRUCTURE OF COLCHICINE**

Colchicine has a high market value and consistent demand in the field of medicine[16]. The alkaloid, colchicine is the drug of choice to relieve acute attack of gout, familial Mediterranean fever [17] and a cure for cancer related diseases[18, 19]. Also, colchicine, a recognized liver protector which prevents the assembly of cytoplasmatic microtubules, inhibits the transcellular movement of collagen [20, 21], stimulates the production of collagenase in cultures of synovial tissue [22] and exerts a stabilizing effect on the plasma membranes of the hepatocyte [23]. It prevents infiltration reverses $CC1_4$-induced liver cirrhosis in rats [24, 25].

Colchicine was reported to improve survival in a clinical trial for alcoholic liver cirrhosis[26], and is currently under investigation in a long-term Veterans Administration cooperative treatment trial in alcoholic liver disease. Recently, Hussein and Boshra[27] reported the antitumor and structure antioxidant activity relationship of colchicine on Ehrlich Ascites Carcinoma (EAC) in Female Mice. As an extension of our studies on colchicine[27], now we wish to evaluate the hepato-renal protective effect of colchicine against diclofenac sodium induced liver and renal toxicity which may pave the way for possible therapeutic applications.

## MATERIALS AND METHODS:

**Chemicals:** Diclofenac sodium and colchicine were obtained from Merck Ltd., Germany. All the other reagents used were of analytical grade and were obtained commercially.

### Animals

This experiment was conducted in accordance with guidelines established by the Animal Care and use Committee of October 6 University. Adult rats weighing around 180±5gms were purchased from Faculty of Veterinary Medicine, Cairo University. They were individually housed in cages in an air-conditioned room with a temperature of 22 ± 2$^o$C, a relative humidity of 60%, and an 8:00 to 20:00 light cycle. During the acclimatization period, each animal was raised on a regular diet *ad-libitum*.

### Experimental set up:

This experiment was carried out to examine the prophylactic potential of colchicine against diclofenac sodium induced liver and renal toxicity *in- vivo*.

Groups of animals each consisting of 8 rats were treated daily for 28 days as follows.

**Group I:** Normal; was given saline orally for 28 days.

**Group II:** Was treated with colchicine (10µg/kg b.w.) suspended in saline orally in a single daily dose for 28 days[28].

**Group III**: Control; was treated with diclofenac sodium (150mg/kg, i.p.) suspended in saline for 28 days[29].

**Group IV:** Was treated with diclofenac sodium (150mg/ kg, i.p.) + colchicine (10µg /kg b.w. orally) in a single daily dose.

**Group V:** Was treated with diclofenac sodium (150mg/kg, i.p.) + vitamin C (1g/kg b.w. orally) in a single daily dose [30].

Colchicine and vitamin C were suspended in saline and administered orally to its respective group animals for 28 days. At the end of the fourth week, the rats were sacrificed by cervical decapitation and the blood was collected using sodium fluoride as anticoagulant for determination of plasma transaminases; L-alanine (ALT) and L-aspartate (AST) [31] and creatinine [32]. The liver and kidneys were dissected out, washed in ice-cold saline, patted dry, homogenized and used for determination of liver and kidney thiobarbituric

acid reactive substances (TBARS) [33], hydroperoxides (HP) [34], reduced glutathione (GSH) [35], superoxide dismutase (SOD) [36], catalase (CAT) [37], glutathione peroxidase (GPx) [38] and protein content in tissue homogenate [39] were determined.

**Measurement of lipid peroxidation**

A thiobarbituric acid reactive substances (TBARS) assay kit (ZeptoMetrix) was used to measure the lipid peroxidation products, malondialdehyde (MDA) equivalents (33). In brief, liver and renal tissues were homogenized with 0.1 mol/l sodium phosphate buffer (pH 7.4). One hundred microliters of homogenate were mixed with 2.5ml reaction buffer (provided by the kit) and heated at 95 °C for 60 min. After the mixture had cooled, the absorbance of the supernatant was measured at 532 nm using a spectrophotometer. The lipid peroxidation products are expressed in terms of MDA equivalents.

**Measurement of antioxidant enzymes**

Superoxide dismutase (SOD), glutathione peroxidase (GPx), Catalase (CAT) and reduced glutathione (GSH) levels were determined using commercially available assay kits (Biodiagnostic). Briefly, liver, and renal tissues were weighed and homogenized with appropriate buffers (provided by the kits). The homogenates were then determined following the procedures provided by the respective manufacturers. The basis of the GSH determination method is the reaction of Ellman's reagent 5, 5-dithiobis (2-nitrobenzoic acid) (DTNB) with thiol group of GSH at pH 8.0 to give yellow color of 5-thiol-2- nitrobenzoate anion[35].

The superoxide dismutase assay kit utilizes a tetrazolium salt for detection of superoxide radicals generated by red formazan dye reduction produced[36]. One unit (U) of SOD activity is defined as the amount of enzyme needed to exhibit 50% dismutation of the superoxide radical. The catalase assay kit utilizes the peroxidative function of CAT for determination of enzyme activity[37]. The method is based on the reaction of the enzyme with methanol in the presence of an optimal concentration of $H_2O_2$. The generated formaldehyde is assayed spectrophotometrically with 4-amino-3-hydrazino-5-mercapto-1, 2, 4-triazole as the chromogen. One unit (U) of CAT activity is defined as the amount of enzyme that will cause the formation of 1.0nmol of formaldehyde per minute at 25°C.

The glutathione peroxidase assay kit measures GPx activity indirectly by a coupled reaction with glutathione reductase (GR) [38]. Oxidized glutathione, produced upon reduction of hydroperoxide by GPx, is recycled to its reduced state by GR and NADPH. The oxidation of NADPH to $NADP^+$ is accompanied by a decrease in absorbance at 340 nm. Under conditions in which the GPx activity is rate limiting, the rate of decrease in the $A_{340}$ is directly proportional to the GPx activity. One unit (U) of GPx activity is defined as the amount of enzyme that will cause the oxidation of 1.0 nmol of NADPH to $NADP^+$ per minute at 25 °C. The specific activities of the various enzymes in the rat liver and renal tissues are expressed in U/mg of the protein with the protein content determined as stated above.

**Statistical analysis**

All data were expressed as mean ± SD. All analyses utilized SPSS 15.0 statistical package for Windows (SPSS Inc., Chicago, IL) [40]. A one-way analysis of variance (ANOVA) was employed for comparisons of means of the different groups. A p-value 0.05 was accepted as statistically significant. Diclofenac sodium control rats were compared with normal control rats as well as Colchicine and vitamin C treated rats were compared with diclofenac sodium control.

**RESULTS:**

**Table 1** shows the levels of plasma ALT, AST and creatinine of control and experimental groups of rats. Diclofenac sodium (DIC) (150mg/ kg, i.p) markedly increased plasma ALT, AST and creatinine levels when compared with the normal group ($p<0.01$). Oral administration of colchicine (10µg /kg b.w.) showed non-significant changes in liver enzymes and creatinine when compared with the normal group. Whereas DIC injected rats-treated with the colchicine (10µg /kg b.w.) and/or vitamin C (1g/kg) ameliorated these increases significantly ($p<0.01$). Amelioration of hepatic marker enzymes was at maximum in colchicine (10µg /kg b.w.) than vitamin C (1g/kg.) when compared with DIC treated rats.

**TABLE 1: ACTIVITY OF ALANINE TRANSAMINASE (ALT), ASPARTATE TRANSAMINASE (AST), CREATININE IN PLASMA OF NORMAL AND EXPERIMENTAL GROUPS OF RATS**

| Groups | ALT (U/L) | AST (U/L) | Creatinine (mg/dl) |
|---|---|---|---|
| Normal A<br>1ml, 0.9 % saline | 37.30 ± 3.95 | 51.8 ± 5.11 | 0.75± 0.14 |
| Normal B<br>Colchicine (10µg /kg b.w.) | 39.52 ± 4.60 | 50.73 ±6.25 | 0.80 ± 0.09 |
| Control<br>DIC (150mg/ kg, i.p) | 95.48 ± 7.13* | 125.70 ± 9.35* | 2.10± 0.18* |
| DIC + Colchicine | 43.18 ± 6.40* | 64.82 ± 9.06* | 1.20± 0.08* |
| DIC + Vitamin C (1g/kg,b.w) | 57.66 ± 4.85* | 79.30 ± 8.04* | 1.64 ± 0.17* |

DIC was given i.p. as a daily dose of 150mg/kg.b.w. for 28 days. It was given to all groups except the normal A and B groups. Colchicine and vitamin C were orally given daily for 28 days as a daily single dose. Values are given as mean ± SD for groups of eight animals each.

* Significantly different from normal group at $p< 0.01$
@ Significantly different from control group at $p< 0.05$.

**Tables 2 and 3** show liver and renal reduced glutathione (GSH), thiobarbituric acid reactive substances (TBARS) and hydroperoxides (HP) levels of control and experimental groups of rats. Oral administration of colchicine (10µg /kg b.w.) showed non-significant changes in hepatic and renal GSH, TBARs and HP. A significant depletion ($p<0.05$) in the level of hepatic and renal GSH content was noticed in rats treated with DIC compared to normal control rats. Treatment with colchicine (10µg /kg b.w.) significantly ($p<0.05$) restored the level of GSH to near the normal level as compared with DIC treated rats. The levels of TBARS and HP were significantly increased ($p<0.05$) in DIC-treated rats as compared with normal control rats. Oral administration of colchicine (10µg /kg b.w.) as well as vitamin C (1g/kg.) in DIC treated rats significantly lowered the levels of TBARS and HP in liver and kidney compared to DIC-treated rats.

**TABLE 2: LEVELS OF HEPATIC REDUCED GLUTATHIONE (GSH), THIOBARBITURIC ACID REACTIVE SUBSTANCES (TBARS) AND HYDROPEROXIDES (HP) IN NORMAL AND EXPERIMENTAL GROUPS OF RATS**

| Groups | GSH (mg/mg protein) | TBARS (mM/mg protein) | Hydroperoxides (mM/mg protein) |
|---|---|---|---|
| Normal A<br>1ml, 0.9 % saline | 57.22 ± 6.08 | 0.95± 0.20 | 71.85 ± 8.25 |
| Normal B<br>Colchicine (10µg /kg b.w.) | 55.46 ± 8.52 | 0.93±0.18 | 68.42 ±4.69 |
| Control<br>DIC (150mg/ kg, i.p) | 33.25 ± 5.08* | 1.87± 0.07* | 120.16 ± 11.35* |
| DIC + Colchicine | 52.11 ± 4.60* | 1.13± 0.15* | 86.47 ± 5.02* |
| DIC + Vitamin C (1g/kg,b.w) | 44.30±7.10* | 1.54 ± 0.05@ | 97.82±7.64@ |

DIC was given i.p. as a daily dose of 150mg/kg.b.w. for 28 days. It was given to all groups except the normal A and B groups. Colchicine and vitamin C were orally given daily for 28 days as a daily single dose. Values are given as mean ± SD for groups of eight animals each.
* Significantly different from normal group at $p< 0.01$
@ Significantly different from control group at $p< 0.05$.

**TABLE 3: LEVELS OF RENAL REDUCED GLUTATHIONE (GSH), THIOBARBITURIC ACID REACTIVE SUBSTANCES (TBARS) AND HYDROPEROXIDES (HP) IN NORMAL AND EXPERIMENTAL GROUPS OF RATS.**

| Groups | GSH (mg/mg protein) | TBARS (mM/mg protein) | Hydroperoxides (mM/mg protein) |
|---|---|---|---|
| Normal A<br>1ml, 0.9 % saline | 31.50 ± 4.22 | 1.35 ± 0.25 | 45.60 ± 8.25 |
| Normal B<br>Colchicine (10µg /kg b.w.) | 29.76 ± 5.14 | 1.23±0.30 | 47.11 ± 6.08 |
| Control<br>DIC (150mg/ kg, i.p) | 15.73 ± 4.31* | 2.10± 0.22* | 84.36 ± 8.16* |
| DIC + Colchicine | 25.66 ± 3.10* | 1.46± 0.09* | 52.27 ± 6.44* |
| DIC + Vitamin C (1g/kg,b.w) | 20.82 ±6.40@ | 1.70± 0.23@ | 66.30±8.25@ |

DIC was given i.p. as a daily dose of 150mg/kg.b.w. for 28 days. It was given to all groups except the normal A and B groups. Colchicine and vitamin C were orally given daily for 28 days as a daily single dose. Values are given as mean ± SD for groups of eight animals each.
* Significantly different from normal group at $p< 0.01$
@ Significantly different from control group at $p< 0.05$.

**Tables 4 and 5** shows the concentrations of liver and renal superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT) of control and experimental groups of rats. Oral administration of colchicine (10µg /kg b.w.) showed non-significant changes in liver and renal enzymatic antioxidants as compared with the normal group. On the other hand, a significant decrease ($p<0.05$) in the activities of hepatic and renal–enzymatic antioxidants in DIC-treated rats was seen. Treatment with colchicine (10µg /kg b.w.) in DIC treated rats significantly increased the activities of enzymatic antioxidants in liver and kidney as compared with DIC-treated rats.

**TABLE 4: LEVELS OF HEPATIC SUPEROXIDE DISMUTASE (SOD), GLUTATHIONE PEROXIDASE (GPX) AND CATALASE (CAT) IN NORMAL AND EXPERIMENTAL GROUPS OF RATS.**

| Groups | SOD (U/mg protein) | GPx (U/mg protein) | CAT (U/mg protein) |
|---|---|---|---|
| Normal A 1ml, 0.9 % saline | 6.35 ± 1.78 | 8.10 ± 1.66 | 115.73 ± 6.05 |
| Normal B Colchicine (10µg /kg b.w.) | 6.20 ± 2.50 | 6.80±1.57 | 108.16 ± 5.89 |
| Control DIC (150mg/ kg, i.p) | 4.45 ± 1.09* | 5.26± 0.85* | 85.58 ± 7.13* |
| DIC + Colchicine | 6.25 ± 1.60* | 7.12± 1.20* | 105.83 ± 5.46* |
| DIC + Vitamin C (1g/kg,b.w) | 5.19 ±0.94@ | 6.33± 1.40@ | 94.50±6.79@ |

DIC was given i.p. as a daily dose of 150mg/kg.b.w. for 28 days. It was given to all groups except the normal A and B groups. Colchicine and vitamin C were orally given daily for 28 days as a daily single dose. Values are given as mean ± SD for groups of eight animals each. Activity is expressed as: 50% of inhibition of pyrogallol autooxidation per min for SOD and the obtained values were divided by the protein concentration; GPx: µg of GSH consumed/min mg protein; µmoles of hydrogen peroxide decomposed per min per mg of protein for catalase.

* Significantly different from normal group at *p< 0.01*;
@ Significantly different from control group at *p< 0.05*.

**TABLE 5: LEVELS OF RENAL SUPEROXIDE DISMUTASE (SOD), GLUTATHIONE PEROXIDASE (GPX) AND CATALASE (CAT) IN NORMAL AND EXPERIMENTAL GROUPS OF RATS.**

| Groups | SOD (U/mg protein) | GPx (U/mg protein) | CAT (U/mg protein) |
|---|---|---|---|
| Normal A 1ml, 0.9 % saline | 8.11 ± 2.05 | 10.25 ± 2.40 | 82.45 ± 5.75 |
| Normal B Colchicine (10µg /kg b.w.) | 7.86 ± 1.80 | 10.04±2.30 | 79.56 ± 4.72 |
| Control DIC (150mg/ kg, i.p) | 5.09 ± 0.86* | 6.88± 0.62* | 58.16 ± 3.60* |
| DIC + Colchicine | 7.50 ± 1.30* | 9.15± 1.80* | 75.54 ± 6.28* |
| DIC + Vitamin C (1g/kg,b.w) | 6.83 ±1.75@ | 7.38± 2.64@ | 73.26±8.11@ |

DIC was given i.p. as a daily dose of 150mg/kg.b.w. for 28 days. It was given to all groups except the normal A and B groups. Colchicine and vitamin C were orally given daily for 28 days as a daily single dose. Values are given as mean ± SD for groups of eight animals each. Activity is expressed as: 50% of inhibition of pyrogallol autooxidation per min for SOD and the obtained values were divided by the protein concentration; GPx: µg of GSH consumed/min mg protein; µmoles of hydrogen peroxide decomposed per min per mg of protein for catalase.

* Significantly different from normal group at *p< 0.01*;
@ Significantly different from control group at *p< 0.05*.

**DISCUSSIONS:** Hepatotoxicity from NSAIDs can occur within 28 days of therapy after drug administration[29]. The possible mechanism of Diclofeac induced liver injury is due to hypersensitivity and metabolic aberration which can produce serious liver damage in human and experimental animals with toxic doses[41]. The liver damage causes leaking of cellular enzymes into the plasma due to the disturbance of hepatocytes transport functions. From our findings, it is evident that the colchicine was able to reduce all the elevated biochemical parameters as a result of hepato- and renal toxin challenge, indicating improvement of the functional status of the liver and kidney. Significant changes in classical enzymes such as ALT, AST and creatinine

exclusively, as well as GSH, SOD, GPx and CAT suggest liver and renal impairment since these are reliable indices of liver and kidney toxicity[29]. The protective effects due to treatment with colchicine strongly indicated the possibility of the drug to prevent and/or mitigate any leakages of marker enzymes into circulation, condition the hepatocytes to accelerate regeneration of parenchymal cells, and preserve the integrity of the plasma membranes and hence restore these enzymes levels[27].

Kidney plays a key role to remove the metabolic wastes such as creatinine from body, thereby helping to maintain body homeostasis. The persistent oxidative stress biomarkers changes within the kidney tissue and free radical generation mediated stress in DIC treated rats producing renal dysfunction resulting in elevation of creatinine levels in blood[29]. In the present study, elevated levels of ALT, AST and creatinine suggested the occurrence of liver and kidney damages after the administration of DIC to the rats compared to normal rats (table 1). Administration of colchicine and/or vitamin C to the DIC-treated rats significantly reduced the ALT, AST and creatinine levels representing the preventive action against DIC toxicity on liver and kidney damages.

Free radicals may also be formed *via* the auto-oxidation of unsaturated lipids in plasma and membrane lipids. The free radical produced may react with polyunsaturated fatty acids in cell membranes leading to lipid peroxidation. Lipid peroxidation will in turn results in an elevated production of free radicals[42].

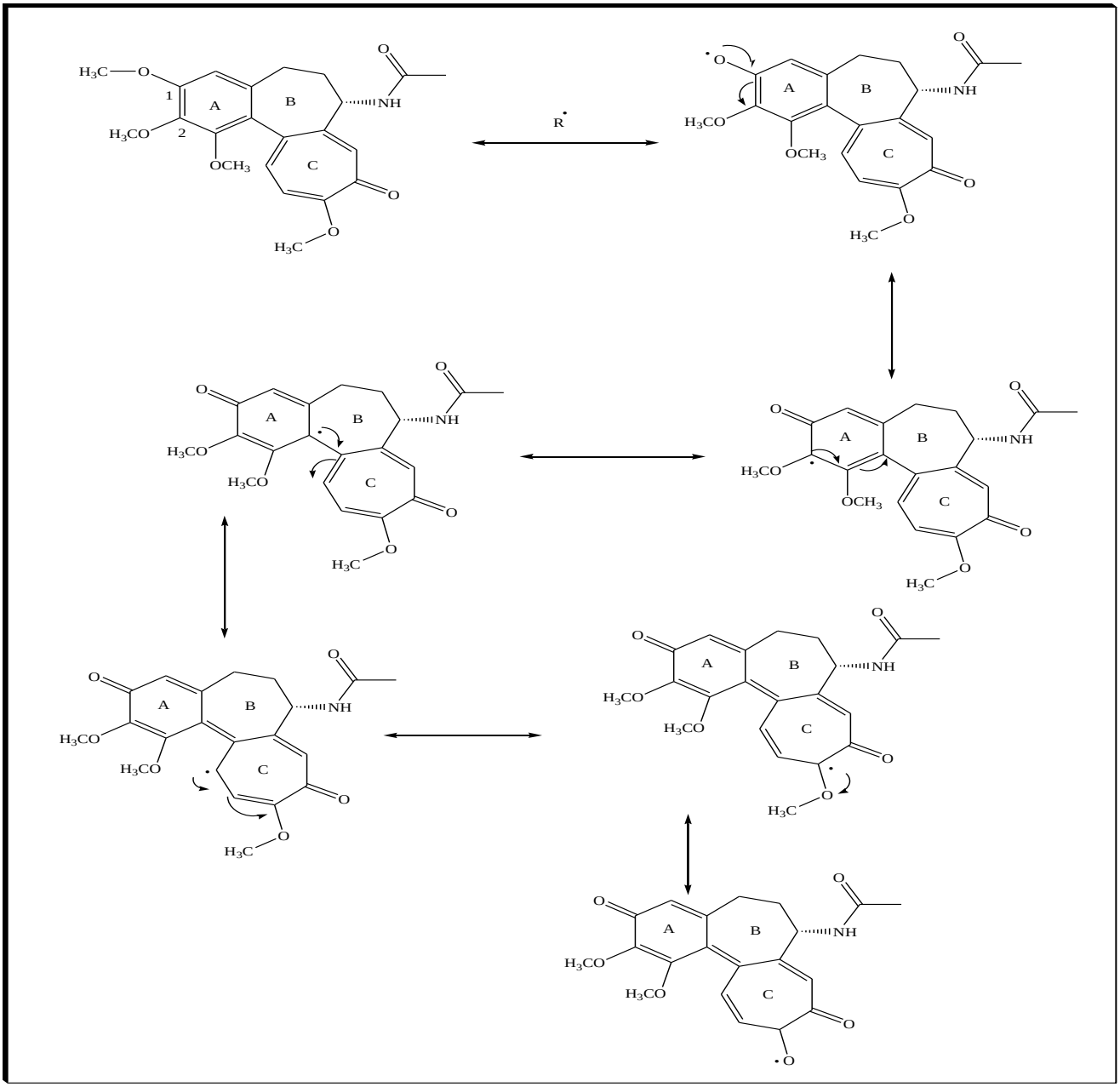
Lipid peroxide mediated tissue damage has been observed in DIC treated rats. It has been observed that DIC administration is associated with lipoxygenase-derived peroxides[43]. The increased lipid peroxidation in the DIC-treated animals may be due to the observed remarkable increase in the concentration of TBARS and HP (lipid peroxidative markers) in the liver and kidney[6]. Hussein[6] has reported that the concentration of lipid peroxides increases in the tissues of DIC-treated rats. In the present study, TBARS level in liver and kidney were significantly lower in the colchicine –treated groups compared to the DIC-treated control group[44] (tables 2 and 3). The above result suggests that the colchicine may exert antioxidant activities and protect the tissues from lipid peroxidation. GSH has a multifactorial role in antioxidant defense. It is a direct scavenger of free radicals as well as a co-substrate for peroxide detoxification by glutathione peroxidases. Hussien and Gobba[45] suggested that the decrease in tissue GSH could be the result of decreased synthesis or increased degradation of GSH by oxidative stress. Increased oxidative stress, resulting from significant increase in aldehydic products of lipid peroxidation has probably decreased hepatic GSH content. In the present study, the elevation of GSH levels in liver and kidney were observed in the colchicine-treated rats.

This indicates that colchicine can either increase the biosynthesis of GSH or reduce the oxidative stress leading to less degradation of GSH, or have both effects. SOD has been postulated as one of the most important enzymes in the enzymatic antioxidant defense system which catalyses the dismutation of superoxide radicals to produce $H_2O_2$ and molecular oxygen[45], hence diminishing the toxic effects caused by their radical. The observed decrease in SOD activity could result from inactivation by $H_2O_2$.

The superoxide anion has been known to inactivate CAT, which involved in the detoxification of hydrogen peroxide[46]. Catalase (CAT) is a heme protein which catalyses the reduction of hydrogen peroxides and protects the tissues from highly reactive hydroxyl radicals[47]. GPx plays a primary role in minimizing oxidative damage. Glutathione peroxidase (GPx), an enzyme with selenium and Glutathione-s-transferase (GST) works together with glutathione in the decomposition of $H_2O_2$ or other organic hydroperoxides to non-toxic products at the expense of reduced glutathione[48].

In the present study, increased liver and renal SOD, GPx, CAT and GSH levels (tables 4 and 5) as well as reduced TBARS and HP levels were noticed in DIC-treated rats after the administration of colchicine and or vitamin C. The above action represents the antioxidant property of colchicine in DIC-treated animals due its structure property. The structural requirement considered essential for effective radical

scavenging by colchicine is the presence of *P*-dimethoxy groups at carbon number 1 and 2 in a ring and conjugated double bond. The presence of double bond in A ring makes the electrons more delocalized to form quinone structure which possesses electron donating properties and is a radical target[27] **(Scheme 1)**. This important property may be responsible for its antioxidant and hepato-renal protective activity against DIC-induced toxicity.



**SCHEME 1: PROPOSAL MECHANISM OF COLCHICINE ANTIOXIDANT ACTIVITY[27]**

Amelioration of colchicine against DIC-induced hepato-renal toxicity has not been reported earlier to our knowledge, and this study is perhaps the first observation of its kind.

**CONCLUSIONS:** The present study showed that colchicine possesses potent antioxidant activity and has an ability to prevent DIC-induced tissue injury. Further studies are in progress to give scientific evidence to the medical use of colchicine in the treatment of other oxidative stress induced complication models.

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