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# Protective effect of *Ginkgo biloba* extract against diclofenac induced nephrotoxicity

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The objective of the present study was to evaluate the ability to orally administration of *Ginkgo biloba* (G.b) extract against kidney damage induced by diclofenac (DF) in male rats with different doses and times The experimental rats were divided into six groups that further divided to 6 subgroups. The first group was served as control, the second group was given G.b extract orally at a dose of 400 mg/kg b.wt/day using the gastric tube for 7 or 14 days. The third group was injected i.m. once/day DF at a dose of 10 mg/kg b.wt. Then, animals injected i.m DF at a dose of 10 mg/kg b.wt/daily and received orally freshly prepared G.b extract at a dose of, 100 mg/kg, 200 mg/kg, and 400 mg/kg b.wt for 7 or 14 days. Administration of DF induced adverse effects on the kidney functions estimated parameters such as creatinine, urea, and uric acid concentration, disturb oxidative stress and antioxidant markers (MDA, GSH, SOD, CAT, and GPx), as well as abnormal in anti-inflammatory markers, prostaglandin, TNF-a, IL-1 β and IL-6. The administration of G.b extract with different doses (100, 200, 400 mg/kg) for 7 or 14 days suppresses the renal injury induced by DF and keeps 2 the kidney functions nearly within the normal limit. The most effective protective role was recorded at the highest dose and longer time.

**Keywords: Diclofenac, *Ginkgo biloba*, Oxidative stress, anti-inflammatory markers, KidneyDamage.**

## INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are mostly used for relief of muscular pain, due to its anti-inflammatory action as well as their analgesic one, Crofford (2013). Diclofenac sodium (DF) is a phenylacetic acid derivative, which has been indicated for the treatment of pain due to its degenerative post-traumatic fatigue, Hoy (2016). 60-70% of diclofenac and its metabolites are mainly eliminated via urine and bile and 30% is eliminated in the feces, Davies and Anderson (1997).

*Ginkgo biloba* contains high amounts of glycosides, flavonoids, terpenoids, diterpenes, sesquiterpenes, flavanol, and polyphenols which have strong antioxidants effects that directly scavenges ROS, and protect against oxidative cell damage,9 Escárcega-González et al. 2016). Gb contains 24% flavanol glycosides and 6% terpene lactones. The flavonoid fraction is primarily composed of quercetin, kaempferol, and isorhamnetin. The terpenoid fraction primarily contains ginkgolides A, B, C, J, and M, as well as bilobalide, Song et al. (2013).

### Aim of the work:

The purpose of the present study, was to investigate the renal-protective efficacy of a natural plant *Ginkgo* 3 *biloba* extract against chronic kidney injury induced by diclofenac in male rats.

## MATERIALS AND METHODS

### 2.1. Experimental animals:

Seventy-two adult male Wistar rats, weighing 150-180 g were used; they were obtained from Helwan animal Farm, Cairo, Egypt. Rats were housed in stainless steel cages in an artificially illuminated and thermally controlled room (22- 25°C and 12 h light / dark cycle). They were acclimized for one week of pre-experimentation.

### 2.2. Chemicals:

Diclofenac sodium (Voltaren) ampoules were produced by Novartis Pharma Company; each ampoule was diluted in 2 ml saline immediately before intra-muscular (i.m) administration. All other diagnostic kits and chemicals used were of the highest purity and purchased from specific agents.

**2.3. *Ginkgo biloba* L. family (Ginkgoaceae), were purchased from Orchidia Pharmaceutical Company.** The powder of *Ginkgo biloba* leaves was confirmed and analyzed by Orchidia Pharmaceutical Company, Cairo, Egypt**.**

**2.4. Preparation of aqueous extract of *Ginkgo biloba*:** 15 grams of G.b powder were dissolved in 500 ml cold water, and mixed in an electric mixture for 20 minutes. then centrifuged in 750 xg, the supernatant were removed and kept in a refrigerator at 2–8oC as a final extract for experimental treatments, (Al-Attar, 2012).

**2.5. Experimental design:**
Rats were randomly divided into the following groups: Control group (Cont); Animals received basal diet. *Ginkgo biloba* treated group (G.b); Animals received freshly prepared *Ginkgo biloba* extract orally using a gastric tube at a dose of 400 mg/kg b.w/day. Diclofenac treated group (DF); Animals injected i.m daily Diclofenac at a dose of 10 mg/kg b.wt. DF+G.b100 mg/kg treated group; Animals were i.m daily injected DF and in the same time received *Ginkgo biloba* 100 mg/kg. DF+G.b 200 mg/kg treated group; Animals were i.m daily injected DF and received orally freshly prepared *Ginkgo biloba* dose of 200 mg/kg. DF+G.b 400 treated group; Animals i.m daily injected Diclofenac and received *Ginkgo biloba* 400 mg/kg.
All the above-treated groups were divided into two subgroups, the first treated for 7 days and the other treated for 14 days.

**2.6. Blood and tissue samples collection:**
At the end of each experimental period, overnight fasted animals were sacrificed after slight diethyl ether anesthesia by cervical dislocation. Blood samples were collected in a clean centrifuge glass tube. After complete coagulation, tubes were centrifuged for 20 minutes at 860 xg. Sera were separated and immediately frozen at -20°C for further analysis. Then, animals were dissected, the kidney of each rat was carefully removed and cleaned using saline solution, weighed, and known weight of it was homogenized in a 10% w/v distilled water using homogenizer surrounded by ice jacket. These homogenates were kept at -20°C for further biochemical assay.

**2.7. Biochemical parameters:**
Serum urea, creatinine, and uric acid concentration were estimated by the enzymatic colorimetric method as described by Patton and Crouch, (1977); Henry, (1974), and Barham and Trinder, (1972) respectively.

**2.8. Antioxidant enzymatic and non-enzymatic activities, and oxidative stress markers assays:**
The content of renal malondialdehyde (MDA) was evaluated photometrically according to the procedure of Ohkawa et al. (1979). Kidney content of reduced glutathione (GSH) was estimated colorimetrically by the method of Prins and Loose, (1969). The activities of renal superoxide dismutase (SOD) and catalase (CAT) were estimated after Aebi, (1984) and Niskikimi et al. (1972) respectively. Glutathione peroxidase (GPx) activity was determined as described by Paglia and Valentine, (1967).

**2.9. Inflammatory markers:**
For the quantitative determination of serum endogenic rat prostaglandin E2 (PG-E2) concentrations. Using ELISA Kit purchased by cusabio (Catalog Number.CSB-E07967r). The content of interleukin-1 β (IL-1β) and interleukin-6 (IL-6) in renal tissue was determined quantitatively using a rat ELISA kit provided by Ray Biotech (Norcross, Georgia, USA). ELIZA, which is a solid phase enzyme amplified sensitivity immunoassay, is used for the detection of tissue Tumor necrosis factors-α (TNF-α) content.

**3. Statistical analysis**
All data were statistically analyzed by using GraphPad Prism 5.0 software (GraphPad Software Inc., San Diego, California, USA). Results were expressed as means ± standard errors (SE). The values of $p \leq 0.05$ were considered statistically significant, Motulsky, (1999).

**RESULTS**
The administration with *Ginkgo biloba* extract (G.b) alone in the selected time non-significantly effect in the serum urea, uric acid, and creatinine concentration, while animals administered with DF alone showed a significant increase in these parameters when compared to the control group.

**Table 4.1: Serum Urea, Uric acid, and Creatinine concentration in different animal groups.**

| | | Cont. | G.b | DF | DF + G.b 100mg/kg | DF + G.b 200mg/kg | DF + G.b 400mg/kg |
|---|---|---|---|---|---|---|---|
| **Urea (mg/dl)** | 1week | 32.00±2.12 | 30.95±2.95 | 56.00±4.59a | 50.01±1.17$_{a,b}$ | 44.00±3.73$_{a,b}$ | 39.40±4.39$_{a,b}$ |
| | 2weeks | 31.20±2.21 | 30.00±2.34 | 68.60±5.87a | 48.00±2.62$_{a,b}$ | 42.80±3.86$_{a,b}$ | 38.20±2.40$_{a,b}$ |
| **Uric acid (mg/dl)** | 1week | 1.44±0.05 | 1.42±0.09 | 2.15±0.07$_{a}$ | 1.90±0.09$_{a,b}$ | 1.70±0.07$_{a,b}$ | 1.66±0.08$_{a,b}$ |
| | 2weeks | 1.40±0.06 | 1.39±0.07 | 2.93±0.08$_{a}$ | 1.72±0.09$_{a,b}$ | 1.64±0.07$_{a,b}$ | 1.59±0.06$_{a,b}$ |
| **Creatinine (mg/dl)** | 1week | 0.54±0.05 | 0.52±0.04 | 0.91±0.04$_{a}$ | 0.83±0.02$_{a}$ | 0.78±0.04$_{a,b}$ | 0.67±0.03$_{a,b}$ |
| | 2weeks | 0.52±0.06 | 0.50±0.03 | 1.15±0.05$_{a}$ | 0.81±0.05$_{a,b}$ | 0.77±0.03$_{a,b}$ | 0.64±0.04$_{a,b}$ |

Data are presented as means ±SE (n=6)
a and b significant changes at p <0.05 compared to control and diclofenac groups respectively

The administration of G.b with different doses (100, 200, and 400 mg/kg) to the rats treated with DF caused a reduction in serum urea, uric acid, and creatinine concentrations when compared to that treated with the DF group. We notice that G.b extract administered at 400 mg/kg after two weeks has the best protective effect against the injured kidney damage.

### 4.2. Renal MDA, GSH content, and SOD, CAT, GPx activities in different animal groups.

The results shown in table 2 indicate that, in both examined times, rats treated with G.b alone showed normal content of kidney MDA, GSH, and SOD, CAT, GPx activities while those administered with DF showed a significant increase in MDA content, associated with a decrease in the GSH content in addition to a decrease in SOD, CAT and GPx activities when compared to the control group. The administration of *Ginkgo biloba* extracts with different doses to the rats treated with DF reduce the renal MDA content and improve in renal content of GSH as well as the activities of SOD, CAT, and GPx when compared to that treated with DF only. The highest dose 400 mg/kg for the longer time 2 weeks showed the highest effective protection.

### 4.3. Serum prostaglandin and renal interleukin-1β, interleukin-6, and tumor necrosis factors- α (TNF- α) concentration in different experimental groups.

Rats treated with G.b alone at the 1st week or 2nd week showed non-significant changes in serum prostaglandin and renal interleukin-1β, interleukin-6, and TNF- α content, while that administered with DF alone produced a highly significant decrease in prostaglandin concentration when compared to the control group, A significant increase in the other estimated parameters. The administration of G.b extract with different doses of DF-treated rats showed a protective role in these parameters.

**Table 4.2: Renal MDA, GSH content, and SOD, CAT, GPx content in different animal groups. Cont**

| | | Cont. | G.b | DF | DF+G.b 100mg/kg | DF+G.b 200mg/kg | DF+G.b 400mg/kg |
|---|---|---|---|---|---|---|---|
| **MDA (nmol/g)** | 1week | 833±18.93 | 828±17.66 | 1210±29.73a | 998±17.15a,b | 950±19.56a,b | 922±15.99a,b |
| | 2weeks | 825±18.22 | 820±16.06 | 1624±46.56a | 972±20.98a,b | 930±13.17a.b | 868±16.39a,b |
| **GSH (mg/g)** | 1week | 4.74±0.13 | 4.84±0.15 | 2.50±0.08**a** | 2.94±0.07**a,b** | 3.30±0.13**a,b** | 3.98±0.05**b** |
| | 2weeks | 4.76±0.11 | 5.01±0.09 | 1.97±0.08a | 3.24±0.14**a,b** | 3.51±0.11**a,b** | 4.06±0.09**a,b** |
| **SOD (U/g)** | 1week | 177±9.50 | 179±6.43 | 134±4.89a | 148±5.17a,b | 152.20±7.32a,b | 157.80±4.88a,b |
| | 2weeks | 176±8.01 | 181±4.83 | 117±5.49a | 150.40±7.59a,b | 157.10±4.11a,b | 160±5.33a,b |
| **CAT (mMg/g)** | 1week | 191.40±7.50 | 194.80±9.26 | 146.40±11.52a | 161.60±11.03**a,b** | 168.80±9.58**a,b** | 171.00±10.01a,b |
| | 2weeks | 195.20±8.69 | 203.20±9.84 | 106.00±14.27a | 163.00±9.23**a,b** | 170.80±8.83**a,b** | 175.60±9.43**a,b** |
| **GPX (nmol/min/ml)** | 1week | 781±8.50 | 791±11.43 | 583.03±12.89a | 640±9.17a,b | 675±9.32a,b | 690±8.88a,b |
| | 2weeks | 782±8.41 | 799±10.83 | 532±13.09a | 670±10.59a,b | 694±9.81a,b | 709±10.33a,b |

Data are presented as means ±SE (n=6).

 a and b significant changes at p <0.05 compared to control and diclofenac groups respectively.

**Table 4.3: Serum prostaglandin and renal interleukin-1β, interleukin-6, and tumor necrosis factors- α (TNF- α) concentration in different experimental groups**

| | | Cont. | G.b | DF | DF + G.b 100mg/kg | DF + G.b 200mg/kg | DF + G.b 400mg/kg |
|---|---|---|---|---|---|---|---|
| **prostaglandin (pg/ml)** | **1week** | 16.00±0.16 | 16.50±0.19 | 3.60±0.04a | 5.32±0.08a,b | 6.71±0.35a,b | 7.30±0.50a,b |
| | 2week | 16.40±0.10 | 16.90±0.12 | 2.40±0.07a | 6.82±0.24a,b | 7.90±0.32a,b | 9.00±0.25a |
| **Renal IL-1β (Pg/mg)** | 1week | 105.00±9.80 | 101.00±9.00 | 232.80±11.90a | 198.20±10.50a,b | 167.00±9.90a,b | 135.40±10.00a,b |
| | 2week | 106.00±8.80 | 100.98±8.90 | 292.00±10.90a | 170.60±9.30a,b | 150.00±9.10a,b | 128.00±8.90a,b |
| **Renal IL-6 (Pg/mg)** | 1week | 3.90±0.07 | 3.81±0.04 | 8.22±0.41a | 6.23±0.21a,b | 5.91±0.16a,b | 5.03±0.18b |
| | 2week | 3.74±0.03 | 3.59±0.06 | 10.05±0.20a | 5.13±0.09a,b | 4.97±0.15a,b | 4.15±0.18a.b |
| **Renal TNF-α (Pg/mg)** | 1week | 15.33±1.47 | 14.45±1.32 | 40.00±3.48a | 35.60±1.16a | 28.40±2.89**a,b** | 21.60±1.54**a,b** |
| | 2week | 15.00±0.47 | 14.07±0.28 | 51.20±1.27a | 33.20±1.59**a,b** | 22.40±1.63**a,b** | 17.00±0.84**a,b** |

 Data are presented as means ±SE (n=6).

 a and b significant changes at p <0.05 compared to control and diclofenac groups respectively.

## DISCUSSION

Diclofenac is safe at therapeutic doses and has proven to cause kidney papillary necrosis and renal failure in long-term drug administration in Wister rats, Alabi *et al.* (2020). Toxicities of DF may be through its reactive metabolites 4\(OH). DF and 5\-(OH) DF and the highly reactive benzoquinone imines, conjugate with reduced GSH as reported by Boerma *et al.* (2012).

In this study, diclofenac (DF) treatment caused an alteration in the antioxidant and oxidative stress status, lipid peroxidation and significantly increases the concentration of urea, uric acid, creatinine, plasma prostaglandin synthesis, and pro-inflammatory response. However, *Ginkgo biloba* (G.b) extract pretreatment reduces these toxic effects of DF.

Urea, uric acid, and creatinine concentration are used as markers of kidney function, but the creatinine test is more sensitive than urea, Vasudevan and Sreekumari, (2007). Exceed urea, uric acid, and creatinine may indicate kidney injury, with resultant reduced glomerular filtration. Creatinine is also removed from the blood by glomerular ultrafiltration of the kidneys, Famurewa et al. (2020).

Daily administration of diclofenac induced nephrotoxicity that was characterized by markedly exceed in the serum concentration of creatinine, urea, and uric acid. The results of this study agree with the findings of Syed et al. (2012) who reported diclofenac sodium impairment renal function, as this drug increase serum urea and creatinine concentration in animal groups. These results may be due to the nephrotoxic effect of the drug, leading to reduced renal function, as recorded by, Famurewa et al. (2020).

Diclofenac could change kidney functions by its role in prostaglandin synthesis, leading to decrease GFR and accumulation of both creatinine and urea in blood, an explanation which in accordance with Orinya *et al.* (2016). Diclofenac administration decreased the glomerular filtration 10

rate, resulting in decreased excretion of urea, leading to increase in the concentration of the blood urea, Alabi and Akomolafe (2020).

The obtained increase in serum urea, uric acid, and creatinine concentration in DF treated rats compared to normal rats are in agreement with Mostafa et al. (2020). The disadvantage of the glomerular filtration rate of the kidneys may be due to the role of diclofenac to inhibit cyclooxygenases, thereby suppressing the production of prostaglandins, Dhanvijay et al. (2013). The obtained exceeded uric acid concentration in DF treated group, may be explained by the production of uric acid through the breakdown of purine and synthesis of glutamine, in addition, this increase may be a defense mechanism against the free radical groups created oxidative stress, as uric acid may act as an electron donor which reduce the free radicals, Sivaraj and Umarani (2003).

The decrease in serum urea, uric acid and creatinine are in *Ginkgo biloba* extract treated group pre-DF treatment may be due to the role of G.b extract to scavenge reactive oxygen species (ROS) Lu et al. (2007), and / or by decreasing oxidative stress and nitric oxide concentration in renal tissues, Abd-Ellah et al. (2007). Treatment with *Ginkgo biloba* extracts resulted in a significant decrease in serum concentration of urea, uric acid, and creatinine relative to that treated with DF indicating the suppression of kidney structure and functions abnormalities, especially in the highest concentration after 14 days.

Administration of diclofenac induced a significant increase in lipid peroxidation product malondialdehyde (MDA) in kidney tissues these results are in accordance with Galati *et al.* (2002), and a remarkable reduction in the GSH content when compared to that of the control as a result of down-regulation of the antioxidant system which may be attributed to the generation of ROS, hence increase oxidative stress, reduce the antioxidant system, and body defense mechanism to scavenge the free radicals, an explanation which in parallel with Alabi and Akomolafe (2020). The obtained increase in MDA in DF treated group may be partially attributed to biological cell damage caused by peroxidation of PUFA degradation leading to cross-linking of proteins, that provoked the generation of MDA. Oxidation damage can also produce disulphide bonded polymers from protein thiol oxidation, an explanation which is in accordance with Basu (2003).

The obtained depletion of non-enzymatic antioxidant GSH content in renal tissues of DF treated rats indicates GSH utilization which subsequently leads to renal toxicity, a result which is in accordance with Kumar *et al.* (2007). The decline in GSH content may confirm an impaired antioxidant defense and thus increase the susceptibility of the kidney to oxidative stress. Induced tissue toxicity by DF may be by inhibition of cyclooxygenase-catalyzed prostaglandin production and impair renal function by decreasing the synthesis of vasodilator prostaglandins from arachidonic acid, an explanation which run parallel with Brater (2002).

The endogenous antioxidant enzymes such as SOD, CAT, and GPx activities decrease in the kidney of DF-treated rats compared to the control, these data are in agreement with Peter et al. (2017). The antioxidant activities of SOD and CAT decrease in rat kidney daily administered with DF after two weeks, a result which agrees with Hickey et al. (2001) The decrease in the GPx activities in the renal tissues of DF-treated rats are in 12

agreement with Hickey et al. (2001). Reduction in these antioxidants may result from the accumulation of cytotoxic metabolites in the renal tissues of rats after DF treatment, an explanation which agrees with Owumi and Dim (2019). The resulted depletion in oxidative stress markers in DF-treated rats may be explained by its role in mitochondrial damage, as reported by Masubuch *et al.* (2002). The adverse effect in DF treated group may be through the toxic effect of accumulated superoxide anions in the cells, Al-Attar (2012). The noticed alteration in estimated oxidative stress and antioxidant parameters MDA, GSH, SOD, CAT, and GPx in DF-treated rats may be due to the enhancement of ROS production leading to renal oxidative stress, Obajimi et al. (2007).

*Ginkgo biloba* extract administered groups in concomitant to DF treatment nearly keep normal kidney antioxidant SOD and CAT which stabilize non-enzymatic antioxidant GSH concentration, DeFeudis et al. (2003). The suppressive effect of G.b extract may be explained by its ability to neutralize ferryl ion-induced peroxidation, an explanation which is in accordance with Bridi et al. (2001). *Ginkgo biloba* has the capability of inactivating oxo-ferryl radical species, which are more efficient oxidative agents than classical hydroxyl radicals, Schindowski et al. (2001).

The features of the natural antioxidant G.b bring many beneficial effects against free radical injuries that prevent the harmful effect in the elimination of ROS or other reactive by-products generated by DF toxic metabolites in the kidney tissues, an explanation which coincides with that of Song et al. (2013). However, the treatments of the DF-intoxicated rats with G.b extract (100, 200, and 400) mg/kg decrease the oxidative stress marker where the activities of SOD and GPx have significantly increased these findings are in agreement with DeFeudis et al. (2003).

Serum prostaglandin concentration showed significant decrease in DF treated rats if compared with that of control, this result may be due to inflammatory effects of DF on the rat's kidney, as shown by Alabi and Akomolafe (2020) where COX-2 is over-expressed due to pro-inflammatory cytokines induction, which triggers prostaglandins released at the site of inflammation, Fonseca et al. (2010). Diclofenac has a selective inhibitory effect on COX-2 than COX-1 as showed by Erdal and Sefa (2017). Diclofenac treated rats significantly reduced COX-2 protein expression, as well as renal PGE2 concentration as there are correlations between them, Gan (2010). This result is in agreement with Yasmeen et al. (2007); and Ratliff et al. (2016) who stated that, inhibition of prostaglandin production results in disruption of glomerular filtration rate as well as the destruction of renal tubular function and renal metabolism.

The obtained anti-inflammatory effects of G.b against DF-induced rat,s renal inflammation is in accordance with El-Ghazaly *et al.* (2015). The action of *Ginkgo biloba* extract to reduce the synthesis of COX-2 mediated PGE2 release is the inhibition of COX-2 activity as being the major target of the non-steroidal anti-inflammatory drugs (NSAIDs) such as diclofenac, ibuprofen, and many others. COX-2 activity is non significantly affected by G.b, whereas DF significantly inhibited COX-2 activity, Gargouri *et al.* (2018). Administration of G.b show a significant increase in renal COX-2 protein expression and PGE-2 renal content leading eventually to significant protection against DF toxicity. This data implicates that G.b reduced renal toxicity which may be through inhibiting the inflammatory and pro-14

inflammatory responses. *Ginkgo biloba* extract administration prevented alteration caused by DF in renal PGE2 synthesis, as G.b extract increase the GSH content in the biological system, hence facilitating the renal PGE2 synthesis, Gargouri et al. (2018).

The pro-inflammatory cytokines are produced predominantly by activated macrophages and are involved in the up-regulation of inflammatory reactions such as IL-1β, IL-6, and TNF- α, Zhang and An (2007). The obtained increase in IL-1β, IL-6, and TNF- α in DF treated group are in agreement with Baldwin (2001) and may be by excessive production of free radicals and oxidative stress stimulates the synthesis of nuclear factor-kappa B (NF-kB) and other intracellular signaling cascades which in turn stimulate the expression of the pro-inflammatory gene as IL-1β, IL-6, TNF- α, and COX-2 which induces cell damage. Obtained increase in IL-1β, IL-6, and TNF- α may be also due to increase of ROS and the impairment of antioxidant defense mechanisms in DF treated group, Alabi and Akomolafe (2020). These results after DF treatment may be attributed to increased myeloperoxidase (MPO), a critical effector of tissue inflammation, that generates H2O2, NO, deleterious hypochlorite levels, and pro-inflammatory cytokines as TNF-α during the inflammation process, increase in renal TNF-α content in rats Nouri and Heidarian (2019). In addition to increase of pro-inflammatory cytokines including IL-1β, IL-6, and TNF-α may be due to the role of DF which induce circulation of macrophages and monocytes, as shown by Peter et al. (2017).

The anti-inflammatory effect of G.b may be due to its various active components such as terpenoids, flavonoids, bi-flavonoids, and organic acids that have been potent anti-inflammatory properties, Chan et al. (2007) that 15

playing role in scavenging reactive oxygen species and ferryl ion species, DeFeudis et al. (2003). In addition, G.b showed a significant improvement in the expression content of pro-inflammatory cytokines, such as IL-1, IL-6, and TNF-α, Kaur et al. (2017). *Ginkgo biloba* extract plays a role as a strong suppressor of IL-6 promoter activity; which has shown universal inhibitory effects on the production of other pro-inflammatory mediators to different extents in macrophages, Gargouri *et al.* (2018). The anti-inflammatory property of G.b extract could be also related to its ability to down-regulate the production of (IL-1, IL-6, and TNF- α) concentration as shown by Kaur *et al.* (2017).

CONCLUSION

In conclusion: It seem that Diclofenac may seriously injured kidney and the pretreatment with *Ginkgo biloba* extract suppress to some extent, these adverse effect especially at dose 2 weeks in male rats.

## CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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## AUTHOR CONTRIBUTIONS

Gamal M. F. Edrees: Suggested the work protocol, Analyzed & interpreted the data, Revised the manuscript.
Dr. Hanaa M. Serag: Conceived & designed the

experiments, contributed materials, analysis tools or data, Analyzed & interpreted the data
Sally M. E. Ramadan: Performed the laboratory experiments, data, Analyzed, Wrote the paper.



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