

## THE NEPHROTOXIC EFFECT OF CHLOROQUINE, THE OFF-LABEL ANTI COVID 19 AND POSSIBLE PROTECTIVE ROLE OF GINKGO BILOBA EXTRACT IN MALE ALBINO RATS

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### ABSTRACT

**Background:** chloroquine is a historical antimalarial drug with nephrotoxic potential in overdose. Lately, it has been tried for covid 19 therapy. Ginkgo biloba leaves extract is an effective antioxidant and free radical hunter. **Objectives:** this investigation aimed to assess the nephrotoxic effect of chloroquine, and the potential nephroprotective role of ginkgo biloba extract on the kidney of male albino rats. **Methods:** rats were grouped into 4 subgroups. Group i: the control (0.9% saline for 10 days orally). Group ii: (ginkgo biloba leaves extract); (200 mg/kg body weight orally for 10 days). Group iii: (chloroquine) (970 mg/kg body weight chloroquine once orally at the termination of the 9th day). Group iv: (ginkgo biloba leaves + chloroquine); (chloroquine on day seven as mentioned + ginkgo biloba leaves for 10 days). After ten days, rats were anesthetized and sacrificed, and blood samples were collected to measure renal functions, malondialdehyde, and glutathione reductase. Renal tissue catalase enzyme, glutathione peroxidase, glutathione reductase, and malondialdehyde levels were quantified as well. Hematoxylin & eosin-stained renal sections obtained from all groups were examined under a light microscope. **Results:** chloroquine induced significant increases in urea, creatinine, malondialdehyde, and a decrease in other tested antioxidant parameters in the chloroquine group than in the control group. Ginkgo biloba leaves could be of value in chloroquine -intoxicated rats. It significantly recovers renal functions and decreases malondialdehyde, strengthens the antioxidant markers, and improves histopathology. **Conclusions:** ginkgo biloba extract could safeguard renal tissue against chloroquine -induced nephrotoxicity by improving the antioxidant function.

**Keywords:** acute chloroquine toxicity, ginkgo biloba extract, oxidative stress, nephrotoxicity, histopathological changes.

### INTRODUCTION

Chloroquine (4-aminoquinoline) is a famous historical first-choice drug for the therapy and prevention of malaria (Ferner et al., 2020).

Chloroquine is used to treat systemic lupus erythematosus, rheumatoid arthritis, and hepatic amoebic abscess as an anti-inflammatory and immunomodulatory agent as well (White et al., 2020).

In 2019, chloroquine and hydroxychloroquine resurfaced as a potential off-label therapeutic modality for COVID-19 (Ferner & Aronson., 2020).

The anti-inflammatory and antiviral properties of chloroquine inhibit viral proliferation (Ferner et al., 2020). Clinical guidelines in China, Belgium, Italy, France, Iran, India, and South Korea have approved chloroquine and hydroxychloroquine in COVID 19 prevention and treatment in 2020 (FDA., 2020). However, the safety and efficacy of chloroquine and hydroxychloroquine are debatable and have not been thoroughly investigated to date (de Barros et al., 2020).

Following oral ingestion, chloroquine is promptly and completely absorbed, reaching

maximal levels within 1-3 hours of consumption. It has a half-life of around 50 days, and it is primarily metabolized by hepatic cytochrome (CYP) P450. It possesses a large distribution volume and strongly binds to tissue proteins. Since chloroquine has high renal clearance, its bioavailability is an important clinical consideration for patients with renal failure (**Mahmoudi et al., 2021**).

Both chloroquine and hydroxychloroquine are structural analogs of quinine possessing similar pathophysiologic mechanisms of intoxication. Direct cardiovascular toxic effects are produced by sodium and potassium channel blockade. Hypotension is primarily caused by myocardial depression rather than an  $\alpha$ -adrenergic blockade. Hypokalemia is caused by intracellular potassium shift rather than total-body potassium lack (**Kim et al., 2010; Hughes., 2020; Lebin and LeSaint., 2020**).

Chloroquine therapy's most frequent side effects are nausea, appetite loss, diarrhea, abdominal colic, itching, and hair loss. However, cumulative doses surpassing 100 grams can rarely produce visual disturbances, corneal opacities, irreversible retinopathy, and sensorineural deafness (**Plantone et al., 2018**).

Acute chloroquine toxicity is a life-threatening, and rapidly progressive clinical condition. Early severe hypotension that deteriorates to cardiogenic shock is frequent. Ventricular dysrhythmias such as ventricular tachycardia, ventricular fibrillation, pleomorphic ventricular tachycardia, QRS, QT interval prolongation, ST-segment, and T-wave depression are all common. Seizures, respiratory depression, and central nervous system depression were also reported (**Hughes., 2020**).

Chronic chloroquine administration was linked to agranulocytosis, aplastic anemia, hypersensitivity response, hepatitis, myopathy, and neuropathy. Cardiomyopathy, as well as devastating life-threatening toxic nephritis, are common with chronic use. Hemolysis may develop in G6PD deficiency individuals due to an oxidative stress state (**Luzzi et al., 1993**).

Because of the generation of extremely toxic radicals, toxic concentrations of chloroquine cause direct or indirect oxidative stress-mediated cellular damage to different organs, including the liver, kidneys, and cardiomyocytes. It affects the anatomy of the kidney and impairs renal functions

by retaining salts in the renal tubules and producing alterations in active renal hormones (**Akuodor et al., 2018**).

It causes increased cell membrane lipid peroxidation and shrinks antioxidant activities in rat liver and kidney (**Klouda et al., 2020; Gregório et al., 2021**).

Numerous hemorrhagic and necrotic spots with renal tubular cloudy swelling were detected histopathologically in rats' kidneys pre-treated with chloroquine (**Wang et al., 2020**).

Ginkgo biloba leaves extract is a widespread traditional herbal remedy that contains high quantities of medicinal nutritional glycosides. The extract possesses anti-inflammatory and apoptotic and antioxidant qualities; hence it is extensively utilized to treat Alzheimer's disease (**Guan et al., 2014; Fattiny et al., 2019; Tousson et al., 2019**).

The extract of Ginkgo biloba leaves has the potential to scavenge free radicals, and several studies have suggested that it can help prevent oxidative stress-induced illness (**Brkić et al., 2022**). It could recover brain death associated with renal damage (**Li et al., 2017**).

Preceding studies have evaluated the nephroprotective role of Ginkgo biloba leaves extract against various drug-induced renal insult as methotrexate, gentamicin, vancomycin, amiodarone and cisplatin (**Öztürk et al., 2004; Celik et al., 2005 Fattiny et al., 2019; Al Kury et al., 2020**).

To the author's knowledge, no available investigations focused on the potential role of Ginkgo biloba leaves on chloroquine-induced nephrotoxicity. Thus, the current study aimed to assess the nephrotoxic effect of chloroquine and its potential nephroprotective role of ginkgo biloba leaves extract on the kidney of male albino rats.

#### **MATERIALS & METHODS:**

**Type of study:** Experimental animal study

**Plant material:** Ginkgo biloba leaves extract tablets were obtained from Gardenia Pharmacy, Egypt.

**Experimental rats and Diet.**

Forty male adult albino rats with a weight range from (150 to 200 grams) were brought from the faculty of medicine Tanta University's house of animals. The rats under study were

accustomed to the ideal laboratory environment in wire mesh cages for one week before the start of the experiment with free access to water and standard nourishment (Reeves et al., 1993). Animals were exposed to room temperature ranging from 22-24 °C and light/ dark cycles (12:12 hours). Animal food was withdrawn for one day before the experiment to ensure an unfilled stomach (only water was allowed).

#### **Ethical consideration:**

The study was carried out according to the faculty of medicine Tanta University ethical research committee rules for experimental animals (Permission number: 34464/2/21). The minimum calculated research animal sample was used to obtain reliable results.

#### **Chemicals, kits, and drugs**

Chloroquine tablets were obtained from a nearby pharmacy (Egypt). Analytical chemicals and kits of high diagnostic value were bought from the Biodiagnostic Company (Cairo, Egypt).

#### **Induction of nephrotoxicity**

The experimental rats were given 970 mg/kg body weight (BW) chloroquine suspended in 1 mL saline once via oral cannula to induce nephrotoxicity (Pari and Murugan, 2006).

#### **Experimental design**

Rats were randomly distributed and categorized into four subgroups (10 rats in either group).

**Group I (Control):** ten rats have administrated 1 ml of 0.9% saline (dissolving agent) via an oral cannula for 10 uninterrupted days.

**Group II (Ginkgo biloba leaves):** ten rats have administrated ginkgo biloba extract tablets (200 mg/kg body weight) (Song et al., 2013) for 10 uninterrupted days via an oral cannula.

**Group III (chloroquine):** ten rats received a dose of CQ once (970 mg/kg body weight, dissolved in 1 mL saline) (Pari and Murugan, 2006) via an oral cannula at the end of the 9<sup>th</sup> day.

**Group IV:** (Ginkgo biloba leaves + chloroquine) ten rats have administrated Ginkgo biloba leaves extract (200 mg/kg body weight) (Song et al., 2013) via oral cannula for 10 consecutive days, and a single oral dose of chloroquine on the 7<sup>th</sup> day, 970 mg/kg body weight dissolved in 1 mL saline. Ginkgo biloba leaves extract was continued for additional 3 days after chloroquine administration.

#### **Samples collection:**

After the experiment's end time (day ten calculated from the beginning of the experiment), all study animals were anesthetized using light ether inhalation, and then sacrificed. Two separate tubes were used for blood collection (one for plasma separation with anticoagulant and the remaining tube for serum separation without anticoagulant). Tubes were then stored at -80 °C until utilized for biochemical analysis.

All rats were incised abdominally through a median abdominal incision and dissected kidneys. Ice-cold saline was used in washing kidneys, then dried and weighed. Every kidney was divided into two halves. The first half kidney was flooded in 10% tissue homogenate and settled in a phosphate buffer of 0.1 M at a 7.4 pH for renal biochemical analysis. However, the second half kidney was used for histopathological examination.

#### **Biochemical Analysis:**

The levels of urea and creatinine were estimated. The lipid peroxidation indices, including malondialdehyde; and glutathione reductase were assayed in serum samples by the spectrophotometric kits supplied by the bio diagnostic company, in Egypt.

Furthermore, malondialdehyde, superoxide dismutase, catalase, glutathione reductase, and glutathione peroxidase activities were assayed in renal tissues of rats by spectrophotometric kits supplied by the biodiagnostic company, in Egypt.

● **Estimation of creatinine and urea levels:** The urea and creatinine values were assessed by spectrophotometric analysis using the available diagnostic kits in the market (biodiagnostic company, Cairo, Egypt.)

● **Estimation of malondialdehyde:** The extent of serum lipid peroxidation was assessed by measuring malondialdehyde at 534 nm using the thiobarbituric acid reactive substances method as illustrated by (Ohkawa et al., 1979).

● **Estimation of glutathione reductase activity:**

Glutathione reductase assay (Goldberg and Spooner., 1983) was determined by measuring the oxidation rate to NADP<sup>+</sup>, which is associated with a reduction in photo absorbance at 340 nm consuming the purchased kits (Biodiagnostic, Cairo, Egypt).

- **Determination of catalase activity:**

Estimation of catalase activity was measured by spectrophotometer at 240 nm by Claiborne's method (1985).

- **Determination of superoxide dismutase:**

The activity of superoxide dismutase was estimated spectrophotometrically based on the methods of (Kakkar et al., 1984).

- **Determination of glutathione peroxidase:** The activity of glutathione peroxidase was assessed with the aid of the methods of (Rotruck et al., 1973).

- **Histopathological examination:**

Immediately upon removal of the kidneys, small sections were made and submerged in 10 % formalin solution for 24 hours intended for fixation. Alcohol with increased levels was used for drying the kidney Specimens. Xylene was used for specimen clearance purposes. Sections cut at 4µm thickness were made as two serials per slide and were then hematoxylin and eosin-stained (Bancroft and Gamble., 2008). Renal tissue sections were coded, randomized, then examined blindly, and photographed by Leica DM500 light microscope to which ICC 50 camera was attached.

#### Statistical analysis

The data were prearranged and statistically analyzed with a software statistical computer package (SPSS Version 24). Data that are normally distributed were expressed as mean ± standard deviation. One-way ANOVA test or Welch ANOVA test was utilized to compare the studied groups based on Levene's test's result of variances' homogeneity. If significant differences existed, post hoc tests were done (Tukey test after ANOVA and Games- Howell test after Welch ANOVA). Significance was assumed at  $p < 0.05$  to interpret the results (Bursac et al., 2008).

## RESULTS

- **Biochemical results**

**Effect of chloroquine and Ginkgo biloba leaves extract on the urea and creatinine, malondialdehyde, and reduced glutathione in serum samples:**

**Table (1)** compares oxidant/antioxidant

biomarkers (malondialdehyde and reduced glutathione) and urea and creatinine in serum samples.

In chloroquine group (III), a statistically significant elevated serum malondialdehyde level was noticed compared to other investigated groups ( $p < 0.05$ ). Remarkably, malondialdehyde was significantly reduced after receiving ginkgo biloba extract (group IV) than chloroquine group (III) ( $p < 0.05$ ). Reduced glutathione was significantly reduced in serum samples of chloroquine receiving group (III) ( $p < 0.05$ ). A noticeable significant elevation in reduced glutathione level in serum samples of rats was recorded after receiving GBL extract (group IV) ( $p < 0.05$ ). Conversely, no significant variance between group I (control group) & II (ginkgo biloba extract group) regarding malondialdehyde and reduced glutathione was traced ( $p > 0.05$ ).

Regarding serum levels of urea and creatinine levels, they were significantly elevated in the chloroquine group (III) compared to other studied groups ( $p < 0.05$ ). Administration of ginkgo biloba extract before chloroquine causes a significant reduction in serum urea and creatinine levels ( $p < 0.05$ ). In contrast, no significant difference between group I (control) and group II (ginkgo biloba extract) as regards urea and creatinine values was recorded ( $p > 0.05$ ).

**Effect of chloroquine and ginkgo biloba extract on the oxidative stress and antioxidant biomarkers in renal tissue:**

**Table (2)** demonstrates a comparison of oxidant/antioxidant biomarkers (malondialdehyde, superoxide dismutase, reduced glutathione, catalase, and glutathione peroxidase) in renal tissue.

A statistically significant elevation of malondialdehyde in the chloroquine group (III) was noticed in renal tissue samples than in other investigated groups ( $p < 0.05$ ). Noticeably, malondialdehyde was significantly reduced after receiving ginkgo biloba extract (group iv) than chloroquine receiving group (III) ( $p < 0.05$ ).

**Table (1):** Comparison of renal functions and oxidant/antioxidant biomarkers among the studied groups in serum samples

Biomarker		Group I (n=10)	Group II (n=10)	Group III (n=10)	Group IV (n=10)
Malondialdehyde (nmol/ml)	mean±SD	13.86 ± 0.45 <sup>cd</sup>	13.83 ± 0.4 <sup>cd</sup>	21.9 ± 1.8 <sup>abd</sup>	17.7 ± 1.1 <sup>abc</sup>
Reduced glutathione (mg/dl)	mean±SD	3.9 ± 0.65 <sup>cd</sup>	4.18 ± 0.73 <sup>cd</sup>	1.18 ± .41 <sup>abd</sup>	3.25 ± .54 <sup>abc</sup>
Creatinine (mg/dl)	mean±SD	0.35 ± 0.008 <sup>cd</sup>	0.37 ± 0.006 <sup>cd</sup>	0.89 ± 0.04 <sup>abd</sup>	0.71± 0.02 <sup>abc</sup>
Urea (mg/dl)	mean±SD	26.37 ± 0.97 <sup>cd</sup>	27.07 ± 0.47 <sup>cd</sup>	62.67 ± 1.01 <sup>abd</sup>	45.16 ± 1.09 <sup>abc</sup>

Data are represented as mean+ SD (n= 1 rats in each group). Statistical analysis was carried out using one-way ANOVA with Tukey's post hoc test, SPSS computer program. <sup>a-d</sup> Significant difference between groups at \*p < 0.05. <sup>a</sup>: significance from group I; <sup>b</sup>: significance from group II; <sup>c</sup>: significance from group III; <sup>d</sup>: significance from group IV.

However, no statistically significant difference between the control group (I) & II (ginkgo biloba extract group) was noticed. Similarly, other antioxidant markers (superoxide dismutase, reduced glutathione, catalase, and glutathione peroxidase) were significantly reduced in renal tissue of chloroquine only receiving (group III) than other studied groups (p<0.05). A noticeable significant elevation was recorded in the levels of the fore-mentioned markers in the renal tissue of rats after receiving ginkgo biloba extract (group IV). Though, the difference between the control group (I) & ginkgo biloba extract group (II) was insignificant (p>0.05).

#### Histopathology results:

Renal tissue sections belonging to the control and ginkgo biloba extract groups (group I and II, respectively) showed minor histopathological abnormalities (fig 1 & 2).

Conversely, kidney sections of the chloroquine group (III) revealed vascular congestion, focal perivascular mononuclear inflammatory cellular infiltration, interstitial edema, and inflammation. Evidence of tubular injury was detected in the form of tubular cloudy swelling and hydropic degeneration. Tubular cystic dilatation, focal loss of the lining epithelium, focal tubular necrosis, and tubular casts were traced in some sections (fig 3&4).

Examination of renal tissue sections from rats pre-treated with ginkgo biloba extract before exposure to chloroquine (group IV) revealed improvement of pathological changes with less vascular congestion, reduction of the interstitial inflammatory infiltrate, and tubular injury (Fig 5).

**Table (2):** Comparison of oxidant/antioxidant biomarkers among the studied groups in renal tissue

Biomarker		Group I (n=10)	Group II (n=10)	Group III (n=10)	Group IV (n=10)
Malondialdehyde (nmol/ml)	mean±SD	10.86 ± 0.46cd	10.84 ± 0.5cd	18.9 ± 1.9abd	14.7 ± 1.06abc
Superoxide dismutase (u/gm tissue)	mean±SD	7.69 ± 0.61cd	7.67 ± 0.53cd	3.14 ± 0.28abd	5.1 ± 0.27abc
Reduced glutathione. (mg /gm tissue)	mean±SD	42.19 ± 0.67cd	42.58 ± 0.42cd	18.98 ± 0.35abd	31.05 ± 1.18abc
Catalase (u/gm tissue)	mean±SD	34.37 ± 1.02cd	35.48 ± 0.82cd	13.82 ± 1.69abd	28.28 ± 1.76abc

Data are represented as mean+ SD (n= 10 rats in each group). Statistical analysis was carried out using one-way ANOVA with Tukey's post hoc test, SPSS computer program. <sup>a-d</sup> Significant difference between groups at \*p < 0.05. <sup>a</sup>: significance from group I; <sup>b</sup>: significance from group II; <sup>c</sup>: significance from group III; <sup>d</sup>: significance from group IV.

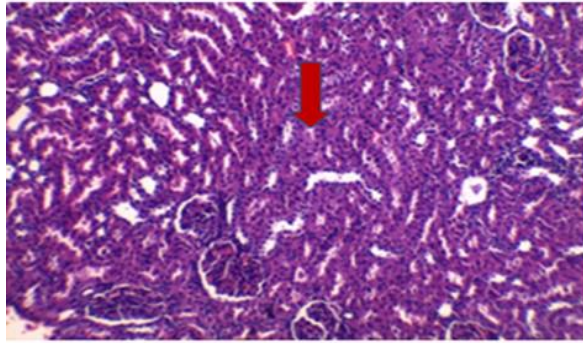


Figure (1): A section from kidney of control group showing closely packed tubules (red arrow) with normal tubular epithelial lining (H&EX200)

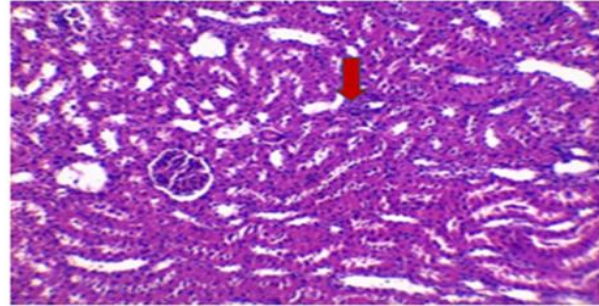


Figure (2): A section from kidney of Gingko biloba receiving rats showing closely packed renal tubules (red arrow) lined by unremarkable cuboidal to low columnar cells (H&E X200)

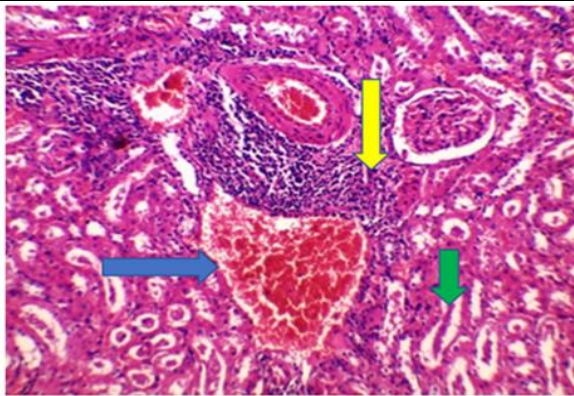


Figure (3): A section from kidney of chloroquine only receiving rat showing vascular congestion with interstitial haemorrhage (blue arrow), marked tubulointerstitial nephritis with perivascular inflammatory infiltrate (yellow arrow) and intratubular hyaline casts (green arrow) (H&EX200)

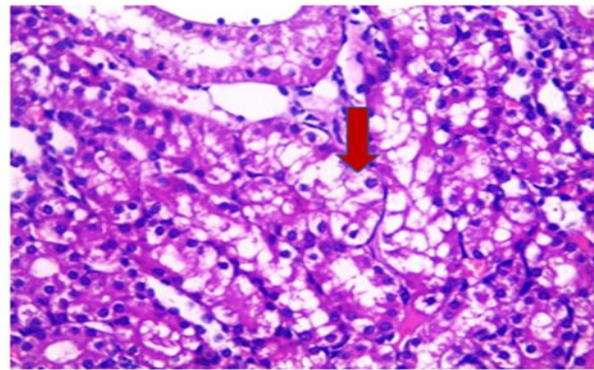


Figure (4): A section from kidney of chloroquine only receiving rat showing hydronic degeneration and cloudy swelling of the renal tubular epithelium (red arrow) (H&EX400)

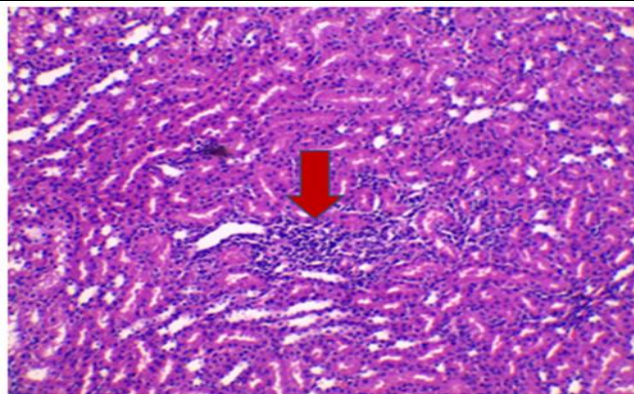


Figure (5): A section from kidney of combined Gingko biloba and chloroquine receiving rats showing less interstitial nephritis with less tubular injury (H&EX200)

## **DISCUSSION**

The kidney plays a fundamental role in normalizing human body functions (white et al., 2020). Renal disorders constitute a major source of morbidity and mortality (Sneha et al., 2019). Xenobiotics produce their direct renal tubular, and papillary toxicity in a dose-dependent way owing to their higher concentrations during the excretion process (George et al., 2017; Mahmoudi et al., 2021).

Drug-induced renal dysfunction is frequently described yet with an unknown mechanism to date. Thus, it is a critical issue to be aware of medications with nephrotoxic potential since, if caught early enough, kidney damage can be reversed (Mahmoudi et al., 2021).

Chloroquine is a popular traditional antimalarial drug. Since 2019, novel trials have been made to use chloroquine as a potential off-label therapeutic modality for COVID-19 owing to potential antiviral action (Ferner et al., 2020; Mahmoudi et al., 2021).

Serious renal harm has been described with chloroquine therapy (Thorogood et al., 2007).

Various chloroquine-related histological and functional kidney alterations occur secondary to incorrect renal tubular withholding of sodium and chloride and renal active hormone fluctuations (Akuodor et al., 2018). Lately, the consumption of herbal antioxidant remedies in alleviating drug-mediated nephrotoxicity has gained a great concern. Natural therapies are currently being used to treat drug-induced nephrotoxicity since they are less expensive, more readily available, and safer (Fattiny and Al-Amri., 2019).

Ginkgo biloba leaves extract is widely recognized for its antioxidant, free radical hunting, and anti-apoptotic characteristics (Hsu et al., 2009).

The current investigation aimed to assess the nephrotoxic effect of chloroquine and the potential nephroprotective role of ginkgo biloba extract on the kidney of male albino rats.

According to the results of this investigation, the elevated blood levels of urea and creatinine in chloroquine-treated rats indicate renal functional impairment induced by chloroquine (Kumar et al., 2000). Treatment of chloroquine-treated rats (200 mg/kg) of ginkgo biloba extract for 7 days before and 3 days following chloroquine administration was found to safeguard the kidney function as evidenced by significant improvement of serum

urea and creatinine levels.

In the current study, chloroquine treatment resulted in a state of peroxidation of membrane lipids and oxidative stress-mediated kidney tissue injury. The considerable increase in renal and serum malondialdehyde levels in the chloroquine group (group III) compared to other investigated groups confirmed this. The levels of renal antioxidant markers (superoxide dismutase, reduced glutathione, catalase, and glutathione peroxidase) and serum reduced glutathione were significantly reduced in the chloroquine group. chloroquine -treated rats that were pre-received protective ginkgo biloba extract (group IV) displayed notable amelioration against chloroquine -renal oxidant injury.

This was evidenced by a significant decrease in renal malondialdehyde levels and elevation of the levels of renal antioxidant markers (superoxide dismutase, reduced glutathione, catalase, and glutathione peroxidase).

Membrane lipids, however, are extremely vulnerable to the insult of highly reactive radicals. It is generally recognized that lipid peroxidation indices testing is a practical way to quantify oxidative damage. Chloroquine's oxidative stress causes an overabundance of free radicals, promoting lipid peroxidation and reducing antioxidant activities that are enzyme and non-enzyme mediated ending in renal insult (Giovannella et al., 2015; Gregório et al., 2021; Brkić et al., 2022).

The exaggerated activities of sod, reduced glutathione, catalase, and glutathione peroxidase in ginkgo biloba extract-administered rats might be ascribed to its free radical hunting properties. Hence, ginkgo biloba extract could capture free radicals generated by chloroquine, consequently decreasing the antioxidant enzyme utilization.

Furthermore, ginkgo biloba extract enhances blood supply, ameliorates inflammatory response, and reduces platelet aggregation (Fang et al., 2021). The presence of flavonoids, steroids, diterpene terpenoids, catechins, flavone glycosides, and other naturally occurring chemicals in ginkgo biloba extract is thought to be responsible for these favorable effects (Sndos et al., 2019). These

elements could restore the equilibrium between the antioxidants and oxidants and scavenge free radicals, therefore preventing and alleviating many illnesses caused by oxidative damage (Tousson et al., 2014). Renal tissue of the chloroquine-intoxicated group exhibited marked morphological changes including vascular congestion, focal perivascular mononuclear inflammatory cellular infiltrates, tubular injury, interstitial edema, and inflammation.

Similarly, Pari and murugan, (2006) reported that chloroquine-treated rats showed numerous hemorrhagic and necrotic areas, and cloudy swelling of renal tubules that were thought to be the result of chloroquine -induced oxidative damage.

The accumulated reactive radicals induced by peroxidation of membrane phospholipids can end in cytotoxicity (Kaneko et al., 2003). Pre-administration of ginko biloba extract could potentially overcome the histopathological changes in renal tissue, and preserve structural integrity evidenced by improving vascular congestion, interstitial inflammation, and tubular injury.

The ginko biloba extract could improve the renal function in the chloroquine -intoxicated group by lowering lipid peroxidation and reversing oxidative damage through its free radical catching effect and increasing the renal oxidants.

#### **CONCLUSION**

In the light of current study results, ginko biloba leaves is strongly suggested to be potentially protective against chloroquine -induced oxidative stress dysfunction in rats' renal tissue owing to its fair antioxidant activity. Chloroquine could potentially recover renal functions and preserve the renal cellular layout. These effects were produced by ginko biloba leaves extract efficacy in reducing serum creatinine and urea values and decreasing renal malondialdehyde. Moreover, chloroquine could increase antioxidant markers (superoxide dismutase, reduced glutathione, catalase, and glutathione peroxidase) significantly.

#### **RECOMMENDATIONS:**

Finally, the authors recommend future planned prospective studies on humans for further confirmation of the role of ginko biloba leaves extract on chloroquine-induced nephrotoxicity.

#### **CONFLICT OF INTEREST**

Authors declared no conflict of interest.

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#### **REFERENCES**

- Al Kury, L.T., Dayyan, F., Shah, F.A., Malik, Z., Khan Khalil, A.A, Alattar, A., et al., (2020): Ginkgo biloba extract protects against methotrexate-induced hepatotoxicity: a computational and pharmacological approach. *Molecules*, 25(11): 2540.
- Akuodor, G.C., Eban, L.K., Ajoku, G.A., Nwobodo, N.N., Akpan, J.L. et al., (2018): Antidiabetic and antihyperlipidemic potential of ethanol extract of *Salacia lehmbachii* stem bark in alloxan-induced diabetic rats. *Journal of Basic and Clinical Physiology and Pharmacology*, 30(2):239-244.
- Bancroft, J.D. and Gamble, M., (2008): Theory and practice of histological techniques. 6<sup>th</sup> edition., Churchill Living stone: Elsevier Health Science, Philadelphia USA: 121 – 134.
- Brkić B.M., Rovčanin, B., Stojanović, M., Srebro, D., et al., (2022): Chloroquine Attenuates Oxidative Stress in Gentamicin-Induced Nephrotoxicity in Rats. *Dose-Response*, 20(3):1-9.
- Bursac, Z., Gauss, C. H., Williams, D. K. and Hosmer, D. W.(2008): Purposeful selection of variables in logistic regression. *Source code for biology and medicine*, 3: 17.
- Celik, I., Cihangiroglu, M., Ilhan, N., Akpolat, N., Akbulut, H.H., (2005): Protective effects of different antioxidants and amrinone on vancomycin induced nephrotoxicity. *Basic & clinical pharmacology & toxicology*, 97(5):325-32.
- Claiborne, A. (1985): Catalase activity. In: Handbook of methods for oxygen radical research, Greenwald RA (ed). CRC Press, Boca Raton; 283-284.
- de Barros, C.M., de Faria Almeida, C.A., Pereira, B., Mancini Costa, K.C., Pinheiro, F.A. et al., (2020): COVID-19 Pandemic - A Narrative, Review of the Potential Roles of Chloroquine and Hydroxychloroquine. *Pain Physician*, 23: 351-366.
- Dubey, A.K., Shankar, P.R., Upadhyaya, D., Deshpande, V.Y., (2004): Ginkgo biloba-



- an appraisal. Kathmandu University medical journal ,2(3):225.
- Fang, C., Lou, D., Zhou, L., Wang, J., Yang, B., He, Q., Wang, J. and Weng, Q., (2021):** Natural products: potential treatments for cisplatin-induced nephrotoxicity. *Acta Pharmacologica Sinica*, 42(12):1951-1969.
- Fattiny, S.Z.A. and AL-Amri, S.M., (2019):** Impact of Ginkgo biloba leaves Extract on Renal Toxicity Induced by Amiodarone in Male Rats. *International Journal of Pharmaceutical and Phytopharmacological Research*,9(6):1-9.
- Ferner, R.E. and Aronson, J.K., (2020):** Chloroquine and hydroxychloroquine in covid-19. *British Medical Journal*, 369: 1432. <https://doi.org/10.1136/bmj.m1432>
- Food and Drug Administration (2020):** Request for emergency use authorization for use of chloroquine phosphate or hydroxychloroquine sulfate supplied from the strategic national stockpile for treatment of 2019 coronavirus disease. <https://www.fda.gov/media/136534/download>.
- George, B., You, D., Joy, M.S. and Aleksunes L.M., (2017):** Xenioitic transporters and kidney injury. *Advanced Drug Delivery Reviews*, 116: 73–91.
- Giovanella, F., Ferreira, G.K., de Pr’a, S.D., et al., (2015):** Effects of primaquine and chloroquine on oxidative stress parameters in rats. *Anais da Academia Brasileira de Ciências*, 87(2):1487-1496.
- Gregório, P., da Cunha, R.S., Biagini, G., et al., (2021):** Chloroquine may induce endothelial injury through lysosomal dysfunction and oxidative stress. *Toxicol Appl Pharmacol*,414:115412.
- Goldberg, D.M. and Spooner, R.J., (1983):** Method for the determination of glutathione reductase. *Methods of Enzymatic Analysis*, 3(3):258–265.
- Guan, H., Qian, D., Ren, H., Zhang, W., Nie, H., Shang, E. et al., (2014):** Interactions of pharmacokinetic profile of different parts from Ginkgo biloba extract in rats. *Journal of ethnopharmacology*,155(1):758-768.
- Hsu, C.L., Wu, Y.L., Tang, G.J., Lee, T.S., Kou, Y.R., (2009):** Ginkgo biloba extract confers protection from cigarette smoke extract-induced apoptosis in human lung endothelial cells: role of heme oxygenase-1. *Pulmonary pharmacology & therapeutics*, 22(4):286-296.
- Hughes, D.A. (2020):** Acute chloroquine poisoning: A comprehensive experimental toxicology assessment of the role of diazepam. *British Journal of Pharmacology*, 177:4975–4989.
- Kakkar, P., Das, B. and Viswanathan, P.N., (1984):** A modified spectrophotometric assay of superoxide dismutase. *Indian Journal of Biochemical Biophysics*, 21:130–132.
- Kaneko, T., Tahara, S. and Takabayashi, F., (2003):** Suppression of lipid hydroperoxide-induced oxidative damage to cellular DNA by esculetin. *Biological and Pharmaceutical Bulletin*, 26 (6):840–4.
- Kim, K. S., Lee, H. A., Cha, S. W., Kwon, M. S., & Kim, E. J. (2010):** Blockade of hERG K (+) channel by antimalarial drug, primaquine. *Archives of Pharmacal Research*, 33(5): 769–773.
- Klouda, C. B. and Stone, W.L., (2020):** Oxidative Stress, Proton Fluxes, and Chloroquine/ Hydroxychloroquine Treatment for COVID-19. *Antioxidants*, 9(9): 894.
- Kumar, K.K., Naidu, M.U.R, Shifow, A.A. and Ratnakar, K.S., (2000):** Probuocol protects against gentamicin-induced nephrotoxicity in Rats. *Indian Journal of Pharmacology*, 32(2), 108–113.
- Lebin, J.A. and Le Saint, K.T., (2020):** Brief Review of Chloroquine and Hydroxychloroquine Toxicity and Management. *Western Journal of Emergency Medicine*, 21(4): 760-763.
- Li, Y., Xiong, Y., Zhang, H., Li, J., Wang, D., Chen, W. et al., (2017):** "Ginkgo biloba extract EGb761 attenuates brain death-induced renal injury by inhibiting proinflammatory cytokines and the SAPK and JAK-STAT signalings," *Scientific Reports*, 7: 45192.|
- Luzzi, G.A. and Peto, T.E., (1993):** Adverse effects of antimalarials. An update. *Drug Safety*, 8(4):295-311.
- Mahmoudi, J., Sadigh-Eteghad, S., Salehi-Pourmehr, H., Gharekhani, A. and**

- Ziaee, M., (2021):** Nephrotoxicity of Chloroquine and Hydroxychloroquine in COVID-19 Patients. *Adv Pharm Bull*, 11(1):6-7.
- Murugavel, P. and Pari, L., (2004):** Attenuation of chloroquine-induced renal damage by  $\alpha$ -lipoic acid: possible antioxidant mechanism. *Renal Failure*, 26(5): 517–524.
- Ohkawa, H., Ohishi, N. and Yagi, K., (1979):** Assay for lipid peroxidation in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry*, 95(2):351–358.
- Öztürk, G., Anlar, Ö., Erdoğan, E., Kösem, M., Özbek, H., Türker, A., (2004):** The effect of Ginkgo extract EGb761 in cisplatin-induced peripheral neuropathy in mice. *Toxicology and applied pharmacology*, 196(1):169-175.
- Pari, L. and Murugan, P., (2006):** Tetrahydrocurcumin: Effect on Chloroquine-Mediated Oxidative Damage in Rat Kidney. *Basic & Clinical Pharmacology & Toxicology*, 99(5): 329–334.
- Plantone, D. and Koudriavtseva, T., (2018):** Current and future use of chloroquine and hydroxychloroquine in infectious, immune, neoplastic, and neurological diseases: a mini-review *Clinical Drug Investigations*, 38: 653–671.
- Reeves, P.G., Nielsen, F.H. and Fahey, G.C., (1993):** AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. *Journal of Nutrition*, 123(11):1939-1951.
- Rotruck, J.T., Pope, A.L., Ganther, H.E., Swanson, A.B., Hafeman, D.G., and Hoekstra, W.G., (1973):** Selenium: biochemical role as component of glutathione peroxidase. *Science*, 179:588-590.
- Sneha, D., Neeru, V., and Sunil, S., (2019):** Kidney disorders and management through herbs: A Review *Journal of Phytopharmacol*, 8(1): 21-27.
- Song, J., Liu, D., Feng, L., Zhang, Z., Jia, X., and Xiao, W., (2013):** Protective Effect of Standardized Extract of Ginkgo biloba against Cisplatin-Induced Nephrotoxicity. *Evidence-Based Complementary and Alternative Medicine*; 846126. doi: 10.1155/2013/846126.
- Thorogood, N., Atwal, S., Mills, W., Jenner, M., Lewis, D.A., Cavenagh, J.D., et al., (2007):** The risk of antimalarials in patients with renal failure. *Postgraduate Medical Journal*, 83:e8.
- Tousson, E., Atteya, Z., El-Atrash, E., Jeweely, O.I., (2014):** Abrogation by Ginkgo Byloba leaf extract on hepatic and renal toxicity induced by methotrexate in rats. *Journal of Cancer Research and Treatment*, 2(3):44-51.
- Wang, J., Zhang, Y., Zhou, M., Zheng, M., Cui, J., Liu, Z., Liu, C. and Liu, S., (2020):** Rapid screening and evaluation of XOD inhibitors and O<sub>2</sub><sup>•-</sup> scavenger from total flavonoids of Ginkgo biloba leaves by L.C.–M.S. and multimode microplate reader. *Biomedical Chromatography*, 34: 4852.
- White, N.J., Watson, J.A., Høglund, R.M., Chan, X.H., Cheah, P.Y. et al., (2020):** COVID-19 prevention and treatment: A critical analysis of chloroquine and hydroxychloroquine clinical pharmacology. *PLoS medicine*, 17(10): e1003252.

## التأثير الكلي السام للكوروكين (العلاج التجريبي لكوفيد19) والدور الوقائي المحتمل لمستخلص الجنكوبيلوبا في ذكور الجرذان البيضاء

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### مقدمة:

تم استخدام الكوروكين (4-أمينوكينولين) تاريخياً لعلاج حالات الملاريا والوقاية منها. في عام 2019، أعيد استخدام الكوروكين والهيدروكسي كوروكين كطريقة علاجية محتملة لفيروس كوفيد 19 بسبب نشاطه المضاد للإلتهابات والمضاد للفيروسات الذي يمنع تكاثر الفيروس. وتشكل أعراض التسمم الحاد بالكوروكين خطراً على الحياة تسبب الجرعات السامة من الكوروكين ضرراً خلوياً مؤكسداً مباشراً أو غير مباشراً محدثاً تلفاً للعديد من الأعضاء بما في ذلك. يعتبر مستخلص أوراق الجنكه بيلوبا دواء عشبي شائع يحتوي على مستويات عالية من مضادات الالتهابات ومضادات الأكسدة وله قدرة على الارتباط بالجذور الحرة.

### الهدف من البحث:

تهدف الدراسة الحالية إلى تقييم التأثير الكلي السام للكوروكين وتقييم الدور المحتمل للوقاية الكلية لمستخلص الجنكوبيلوبا على كلية ذكور الجرذان البيضاء.

### طريقة البحث:

الفران التجريبية والنظام الغذائي: تمت الدراسة على أربعين فأراً بالغاً من ذكور الجرذان البيضاء. تم التوزيع العشوائي للجرذان لتصنيفها إلى أربع مجموعات (10، في أي من المجموعات). المجموعة الأولى (الضابطة). أعطيت المجموعة الثانية (أقرص من مستخلص الجنكوبيلوبا 200مجم / كجم من وزن الجسم) لمدة 10 أيام متواصلة. تلقت المجموعة الثالثة (الكوروكين) وفي نهاية اليوم التاسع. تم إعطاء المجموعة الرابعة (الكوروكين والجنكوبيلوبا) لمدة 10 أيام متتالية وجرعة فموية واحدة ومن الكوروكين في اليوم السابع. علاوة على ذلك، استمر إعطاء الجنكوبيلوبا لمدة 3 أيام إضافية عقب إعطاء الكوروكين.

**طريقه أخذ العينات:** بعد إنقضاء مدة البحث (عقب اليوم العاشر من بدء التجربة)، تم تخدير الحيوانات في المجموعات قيد الدراسة عن طريق إستنشاق الإثير الخفيف وذبحها. تم أخذ عينات الدم حتى يتم استخدامها للقياسات البيوكيميائية مثل البوريا والكرياتينين ومالونيل ألدهيد والجلوتاثيون المختزل كما تم أخذ عينات من كلي الفران في جميع المجموعات التجريبية لإستخدامها لغرض التحديد البيوكيميائي لمستويات الكلي لكل من مالونيل ألدهيد والجلوتاثيون المختزل والكاتالاز وفوق أكسيد الديسميوتاز. تم فحص الأنسجة الكليه بعد تثبيتها في شمع البارافين وصبغها بصبغات الهيماتوكسيلين والإيوسين و تم فحص المقاطع وتصويرها باستخدام عدسات المجهر الضوئي.

### النتائج:

لوحظ ارتفاع ذو دلالة إحصائية في مستويات المالونيل ألدهيد بعينات السيروم و عينات النسيج الكلي في مجموعته الكوروكين فقط (المجموعة الثالثة) مقارنة بالمجموعات الأخرى و الذي إنخفض بشكل ملحوظ بعد تلقي مستخلص الجنكوبيلوبا (المجموعة الرابعة) مقارنة بمجموعه الكوروكين فقط. بينما لوحظ إنخفاض مستويات الجلوتاثيون المختزل بشكل كبير بعينات السيروم و عينات النسيج الكلي في مجموعته الكوروكين فقط (المجموعة الثالثة) والذي إرتفع بدلاله إحصائيه بعد تلقي مستخلص الجنكوبيلوبا (المجموعة الرابعة). فيما يتعلق بمستويات البوريا والكرياتينين في عينات السيروم كانت مرتفعة بشكل ذا دلالة إحصائيه في مجموعته الكوروكين (المجموعة الثالثة) مقارنة بالمجموعات الأخرى. و الذي سجل إنخفاضاً ذا دلالة إحصائيه بعد تلقي مستخلص الجنكوبيلوبا.

لوحظ انخفاضاً ذا دلالة إحصائية بمستويات الدلائل المضادة للأكسدة قيد الدراسة وهي فوق أكسيد ديسميوتاز و الكاتالاز و الجلوتاثيون بيروكسيديز في النسيج الكلي للفران في مجموعته الكوروكين فقط (المجموعة الثالثة) والذي إرتفع بدلاله إحصائيه بعد تلقي مستخلص الجنكوبيلوبا (المجموعة الرابعة).

نتائج فحص العينات الهستوباثولوجي: كشف فحص عينات النسيج الكلي للفران التي تلقت الكوروكين فقط (المجموعة الثالثة) عن وجود إحتقان في الأوعية الدموية، وإرتشاح خلوي إنتهائي أحادي النواة حول الأوعية، وأوذما خلالية وإلتهاب. إلى جانب ذلك، تم رصد وجود إصابات في الأنابيب الكليه والتي تحسنت بعد إعطاء الجنكوبيلوبا (المجموعة الرابعة).

### الإستنتاج:

في ضوء نتائج الدراسة الحالية، يُقترح أن يكون لمستخلص الجنكوبيلوبا تأثير وقائي ضد الجذور الحرة والخلل الكلي المؤكسد الذي يسببه عقار الكوروكين في الفران و ذلك بسبب نشاطه المضاد للأكسدة. كما أن بإمكان مستخلص الجنكوبيلوبا أن يستعيد وظائف الكلي عن طريق الحفاظ على السلامة الهيكلية لخلايا الكلي الناجمة عن الكوروكين، والذي يتضح من إنخفاض مستويات الكرياتينين والبوريا في الدم بشكل كبير، وإنخفاض مستوي المالونيل ألدهيد في النسيج الكلي وزيادة العوامل المضادة للأكسدة (الجلوتاثيون المنخفض، الكاتالاز و الجلوتاثيون).