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# *Gingko biloba* Leaf Extract Mitigates Chlorpyrifos-Induced Oxidative Stress in Albino Rats

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**ABSTRACT:** *Gingko biloba* leaf extract is one of the most popular botanical drug preparations in the world due to its reliable biological activities. The present study was carried out to evaluate the *in vivo* prophylactic and therapeutic antioxidant activities of *Gingko biloba* leaf extract in rats experimentally exposed to chlorpyrifos-induced oxidative stress through evaluating glutathione concentrations, malondialdehyde concentration, superoxide dismutase, and catalase activities. Exactly 35 adult albino rats randomly divided into 7 groups of 5 animals each were used for this study. The results indicated that pre-treatment with *Gingko biloba* leaf extract significantly ($P<0.05$) increased the activity of superoxide dismutase, and level of glutathione and decreased serum malondialdehyde concentration compared to groups pre-treated with vitamin E. Similarly, pre treatment with *Gingko biloba* leaf extract significantly ($P<0.05$) increased the activity of superoxide dismutase and level of glutathione and lowered serum malondialdehyde concentration when compared to post treatment with *Gingko biloba* leaf extract. However, catalase activity was not significantly affected by the treatments. The results showed that the extract has both prophylactic and therapeutic activities, but its prophylactic activity was more expressed than the therapeutic activity. Also, the prophylactic antioxidant activity of the extract was more than that of vitamin E, a reference antioxidant in this study which is a fat-soluble vitamin with powerful antioxidant properties. These results further justify the use of *Gingko biloba* leaf extract in both medical and ethnomedical practices for the treatment of various disorders.

**Keywords**: Antioxidants, Catalase, Chlorpyrifos, *Gingko biloba* leaf extract, Glutathione, Malondialdehyde, Superoxide dismutase, Vitamin E

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## INTRODUCTION

Reactive Oxygen Species (ROS) are a class of molecules that are derived from the metabolism of oxygen and exist inherently in all aerobic organisms. They are responsible for toxic effects in the body through various tissue damages and are formed either by the loss of a single electron from a non-radical or by the gain of a single electron by a non-radical species (Fermino et al., 2015). Oxidative stress is the steady state level of oxidative damage in a cell, tissue, or organ, caused by the reactive oxygen species. This damage can affect a specific organ or the entire organism. Oxidative stress is caused by an imbalance between the production of reactive oxygen species and endogeneous antioxidants (Yoshikawa et al., 1999).

The fate of Gingko biloba leaf extract (EGb 761) in the body after ingestion is of interest in order to investigate their antioxidant properties (Lena and Barlow, 2003). The extract is widely used as a health supplement (Ibrahim and Nuhu, 2016). Previous works by ilhan et al. (2004), He et al. (2014), and Sarikcioglu et al. (2004) have studied the antioxidant activity of EGb 761 and these reports are of great importance (Boligon et al., 2013). Unstable free radical species react with cellular components and the production of these species is balanced by the presence of defense mechanisms consisting of enzymatic and non-enzymatic components (Imai and Nakagawa, 2006) such as Superoxide Dismutases (SOD), catalase (CAT),

glutathione (GSH), and other components (Spadiene et al., 2012).

Chlorpyrifos (0, 0- diethyl 0-(3, 5, 6- tricloro-2-pyridinol) phosphorothionate) is a broad spectrum chlorinated pesticide, used extensively in agriculture and has numerous adverse and toxic effects among which is oxidative stress (Mehta et al., 2009). Chlorpyrifos has multiple effects on the target cells including generation of reactive oxygen species and induction of intracellular oxidative stress (Bebe and Panemanogalore, 2003). The increased use of chlorpyrifos in agriculture with its attendant consequence on the health and well-being of man, animals and the environment is alarming especially in developing countries; there is an urgent need to identify more agents that can mitigate the adverse health consequence posed by long and short-term exposure to chlorpyrifos (Uchendu et al., 2012).

This study aimed to compare the in-vivo therapeutic and prophylactic antioxidant activities of Gingko biloba leaf extract vis-à-vis vitamin E, an endogenous antioxidant against chlorpyrifos induced oxidative stress.

## MATERIALS AND METHODS

### Experimental Animals and Management

Albino rats weighing an average of 180 g were obtained from Animal House, Department of Veterinary Physiology, Ahmadu Bello University, Zaria. The animals were conditioned to the laboratory environment in plastic cages for at least 2 weeks before the commencement of the experiment. All animals were maintained on standard rat diet and water was provided ad-libitum. All animal experimentation was done in accordance with Ahmadu Bello University Animal Use and Care Guidelines. Ethical clearance with approval number ABUCAUC/2016/015 was obtained from Committee on Animal Use and Care, Directorate of Academic Planning and Monitoring, Ahmadu Bello University, Zaria before the commencement of the study.

### Experimental design

The method of Ambali et al. (2012) was used with modifications to accommodate both the pre-treatment and post-treatment activity of EGb 761 vis-à-vis the treated control. The dosages for Vitamin E, Gingko biloba leaf extract, and chlorpyrifos used for this study were 100 mg/kg, 100 mg/kg, and 10.6 mg/kg respectively. Exactly 35 albino rats of 180 g average weight were divided into seven groups of five rats each; Group A (Distilled water 5 ml/kg) served as untreated control and was given only distilled water. Group B (VE+CPF) was pre-treated with vitamin E and then administered chlorpyrifos 30 minutes later, Group C (CPF+VE) was administered with chlorpyrifos and then vitamin E 30 minutes later, Group D (Extract+CPF) was pretreated with the extract, and then with chlorpyrifos 30 minutes later, Group E (CPF+Extract) was administered with chlorpyrifos and the extract 30 minutes later, Group F (extract only) was administered with the extract only and Group G (CPF only) was administered with chlorpyrifos only. The regimens were administered once daily by oral gavage for two weeks.

The animals were sacrificed by jugular vein incision at the completion of the experiment. Blood samples in clean plastic centrifuge tubes were collected without anticoagulant and allowed to stand for 15 minutes. The blood samples collected were allowed to coagulate. After coagulation, the samples were centrifuged at 2,000 × g for 10 minutes. The clear supernatants were obtained as the sera and used for the determination of malondialdehyde (MDA) Concentration, CAT activity, SOD, and GSH Concentration.

### Determination of Oxidative Stress Biomarkers

**MDA determination.** This was done as described by Ohkawa et al. (1979) with modification by Atawodi (2011). Exactly 2 $cm^3$ of 15%Trichlorocetic Acid (TCA) was measured into a test tube, 2 $cm^3$ of Thiobarbitutric Acid (TBA) and 100 µL of serum homogenate were added. The mixture was incubated at 80$^oC$ for 30 minutes in a water bath and allowed to cool for 30 minutes, followed by centrifugation at 3000 rpm for 10 minutes. A clear supernatant was collected and the absorbance was determined at 535 nm in a spectrophotometer (Spectronic-20, Philip Harris Limited,

Shenstone, England).

**CAT activity.** Catalase activity was determined using the method of Abebi (1974). Exactly 10 µl of serum was added to a test tube containing 2.80 $cm^3$ of 50 mM potassium phosphate buffer (pH 7.0). The reaction was initiated by adding 0.1 $cm^3$ of freshly prepared 30 mM $H_2O_2$ and the decomposition rate of $H_2O_2$ was measured at 240 nm for 5 minutes on a spectrophotometer. A molar extinction coefficient (E) of 0.041 $mM^{-1}cm^{-1}$ was used to calculate the catalase activity.

**SOD activity.** SOD activity was determined by the method described by Fridovich (1989). Exactly 114.3 g of $Na_2CO_3$ and 4.2 g of $NaHCO_3$ were dissolved in distilled water and made up to 100 $cm^3$ in a volumetric flask (buffered with 0.05M carbonate, pH 10.2). About 0.01g of

0.3 mM adrenaline was dissolved in 17 $cm^3$ of distilled water.

Exactly 0.1 $cm^3$ of serum was diluted in 0.9 $cm^3$ distilled water to make 1:10 dilution of microsome. An aliquot of 0.2 $cm^3$ of the diluted microsome was added to 2.5 $cm^3$ of 0.05 M carbonate buffer. The reaction was started with the addition of 0.3 $cm^3$ of 0.3 mM adrenaline. The reference mixture contained 2.5 $cm^3$ of 0.05M carbonate buffer, 0.3 $cm^3$ of 0.3 mM adrenaline and 0.2 $cm^3$ of distilled water. The absorbance was measured over 30 seconds up to 150 seconds at 480 nm.

**GSH concentration.** This measurement was carried out as described by Rajagopalan et al. (2004). Exactly 150 µl of serum was added to 10% Trichloroacetic (TCA) acid and centrifuged at 1500× g for 5 minutes and 1 $cm^3$ of the supernatant was treated with 0.5 $cm^3$ of Ellman's reagent and 3 $cm^3$ of phosphate buffer (0.2 M, pH 8.0). The absorbance was read at 412 nm. The quantity of glutathione concentration was deduced.

### Data Analysis

Data were expressed as mean ± standard error of mean (S.E.M) and then analysed by one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. The analyses were done using Graphpad Prism version 5. Values of P < 0 .05 were considered significant.

### Ethics Committee Approval

This experimental research was approved by the Committee on Animal Use and Care, Directorate of Academic Planning and Monitoring, Ahmadu Bello University, Zaria. Ethical clearance with approval number ABUCAUC/2016/015 was obtained for this experiment.

## RESULTS

### Malondialdehyde Concentration

Figure 1 depicts the effect of Gingko biloba leaf extract (EGb 761) on malondialdehyde concentration. The concentration was significantly lower (P<0.05) in Group D when compared with Group B.

### Superoxide Dismutase Activity

Figure 2 presents effect of Gingko biloba leaf extract (EGb 761) on superoxide dismutase activity. The result shows significantly lower (P<0.05) superoxide dismutase level of Groups B, C, and E respectively when compared to Group D.

### Catalase Activity

Figure 3 shows the effect of Gingko biloba leaf extract (EGb 761) on catalase activity. The difference in catalase activity was not statistically significant between the groups.

### Glutathione Concentration

Figure 4 depicts the effect of Gingko biloba leaf extract (EGb 761) on glutathione concentration. Glutathione concentration was significantly higher (P<0.05) in Group D when compared to Groups B, C, E, F, and G respectively.

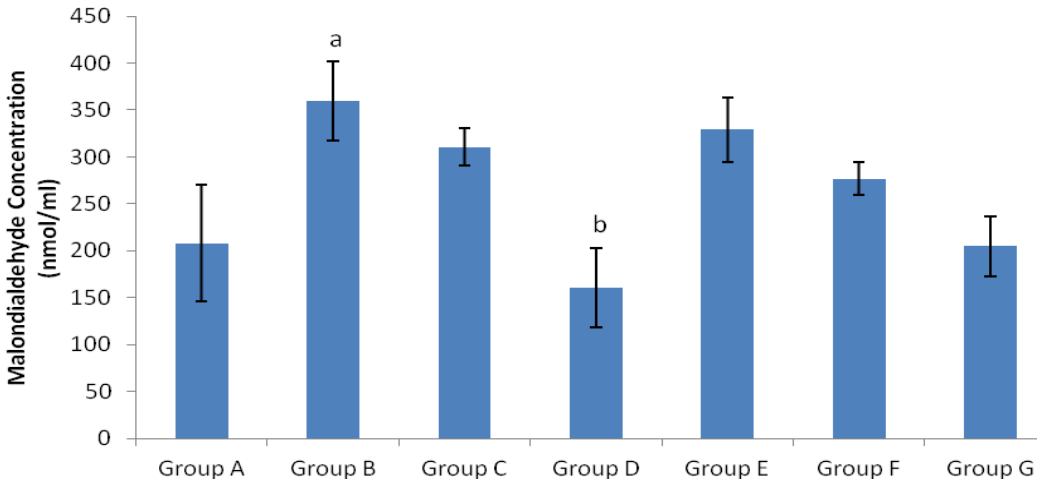


**Figure 1:** Effect of *Gingko biloba* leaf extract (EGb 761) on malondialdehyde concentration. Bars having different letters (a,b) are significantly different (P<0.05). Group A= Distilled water; Group B= Vitamin E+ Chlorpyrifos; Group C= Chlorpyrifos + Vitamin E; Group D= Extract+ Chlorpyrifos; Group E= Chlorpyrifos +Extract; Group F= Extract only; Group G= Chlorpyrifos only

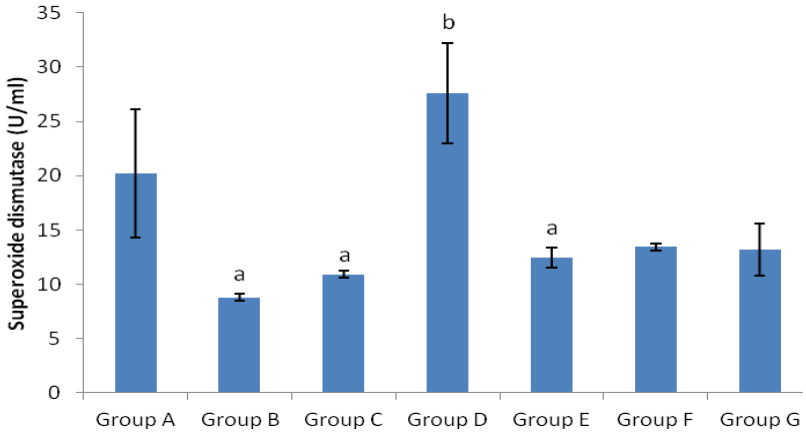


**Figure 2:** Effect of *Gingko biloba* leaf extract (EGb 761) on superoxide dismutase activity. Bars having different letters (a,b) are significantly different (P<0.05). Group A= Distilled water; Group B= Vitamin E+ Chlorpyrifos; Group C= Chlorpyrifos + Vitamin E; Group D= Extract+ Chlorpyrifos; Group E= Chlorpyrifos +Extract; Group F= Extract only; Group G= Chlorpyrifos only

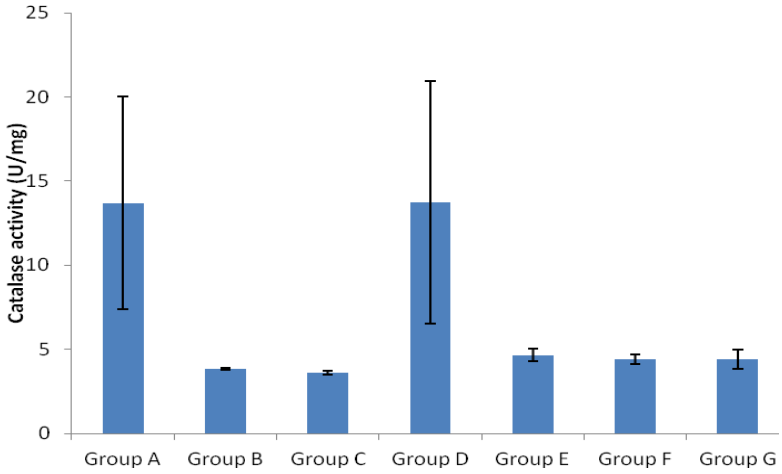


**Figure 3:** Effect of *Gingko biloba* leaf extract (EGb 761) on catalase activity. Bars are not statistically different at 95% CL (α=0.05). Group A= Distilled water; Group B= Vitamin E+ Chlorpyrifos; Group C= Chlorpyrifos + Vitamin E; Group D= Extract+ Chlorpyrifos; Group E= Chlorpyrifos +Extract; Group F= Extract only; Group G= Chlorpyrifos only

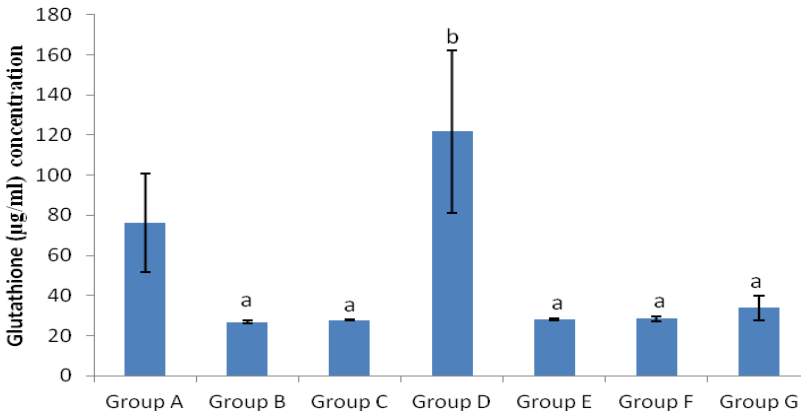


**Figure 4:** Effect of *Gingko biloba* leaf extract (EGb 761) on glutathione peroxidase concentration. Bars having different letters (a,b) are significantly different (P<0.05). Group A= Distilled water; Group B= Vitamin E+ Chlorpyrifos; Group C= Chlorpyrifos + Vitamin E; Group D= Extract+ Chlorpyrifos; Group E= Chlorpyrifos +Extract; Group F= Extract only; Group G= Chlorpyrifos only

## DISCUSSION

The underlying principle behind antioxidant properties of *Ginkgo biloba* leaf extract has focused on its mechanisms of action; directly scavenging free radicals, and indirectly inhibiting formation of free radicals (Omoregie and Osagie, 2012). Administration of chlorpyrifos elicits oxidative stress in this study which is evident by increased MDA, and decreased SOD and CAT activities, and GSH concentration. This study shows that pre treatment with EGb 761 significantly increased SOD and GSH, and also significantly decreased MDA concentration when compared with groups pre treated with Vitamin E. This implies that EGb 761 has more prophylactic antioxidant effect than Vitamin E. This is consistent with the findings of Fatani et al. (2006), Ilhan et al. (2004), Van beek (2000), and Yoshikawa et al. (1999). This study also shows that the free radical scavenging activity of EGb 761 is comparable to that of Vitamin E. DeFeudis et al. (2003) reported that EGb 761 directly scavenge reactive oxygen species such as hydroxyl radicals ($OH^{\cdot}$), peroxyl radical ($ROO^{\cdot}$), superoxide anion radical ($O^{2-\cdot}$), nitric oxide radical ($NO^{\cdot}$), hydrogen peroxide ($H_2O_2$), and ferryl ion species. This study is also consistent with the findings of Spadiene et al. (2012), Droy-Lefaix, (1997), and Kose et al. (1997) that studied the in-vitro antioxidant effect of EGb 761.

Of interest, pre treatment with EGb 761 significantly increased SOD and GSH levels when compared to post treatment with EGb 761. This may not be unconnected to the pharmacokinetics and pharmacodynamics of the extract. Previous studies on the antioxidant activity of EGb 761 have not compared the in-vivo prophylactic and therapeutic antioxidant effect in a single study. Therefore, this study being the first to do that suggests that EGb 761 has both prophylactic and therapeutic antioxidant effects. However, the study shows that antioxidant activity of EGb 761 is probably due to its ability to inhibit the formation of reactive oxygen species and prevention of cellular damage than its free radical scavenging antioxidant effect. This implies that EGb 761 has more prophylactic than therapeutic antioxidant effect.

## CONCLUSION

The findings in this study suggest that *Gingko biloba* leaf extract possesses both prophylactic and therapeutic antioxidant activities but the former was more expressed than the latter. It also shows that the extract has more prophylactic antioxidant activity than vitamin E. These results further justify the use of *Gingko biloba* leaf extract in both medical and ethnomedical practices for the treatment of various ailments.

## DECLARATIONS

### Acknowledgement

We appreciate the entire technical staff of the Departments of Veterinary Physiology, Veterinary Pharmacology and Toxicology, Ahmadu Bello University, Zaria for their technical support.

### Competing Interests

The authors declare that there is no conflict of interest.

### Authors' Contributions

AAN designed the study. SA, AAN, and ZIY carried out the experimental research, collected the data, analysed and interpreted the results. The first draft of manuscript was prepared by SA and reviewed by the rest of the authors and the final version of the manuscript was read and accepted by all the authors.

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