

Effect of *Ginkgo biloba* Leaf Extract (EGb 761) on Changes in Haematological Parameters and Erythrocyte Osmotic Fragility in Hypotonic and Chlorpyrifos Exposed Rats

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Abstract

BACKGROUND: Standardized leaf extract of *Ginkgo biloba* (EGb 761[®]) is a modern herbal medicinal product. It is one of the bestselling and popular botanical drug preparations in the world.

OBJECTIVES: The study was carried out to evaluate the phytochemical constituents of *Ginkgo biloba* leaf extract (EGb 761[®]), to determine the effect of the extract on erythrocyte osmotic fragility under hypotonic stress, and to investigate the effect of the extract on haematological parameters and erythrocyte osmotic fragility in rats experimentally exposed to chlorpyrifos-induced oxidative stress.

METHODS: A total of 35 adult albino rats, divided into 7 groups of 5 rats each, were used. Group A served as control, Groups B and C were pre-treated and post-treated with vitamin E respectively, while Groups D and E were pre-treated and post-treated with EGb 761[®] respectively before administration of chlorpyrifos. Groups F and G were only treated with the extract and chlorpyrifos respectively.

RESULTS: The findings showed that there was no significant difference in erythrocyte osmotic fragility between pre-treatment and post-treatment with EGb 761 in chlorpyrifos induced oxidative stress ($P > 0.05$). However, the erythrocytes of rats treated with EGb 761[®] were significantly more resistant to hypotonic haemolysis in comparison to vitamin E and control ($P < 0.05$). Pre-treatment with EGb 761 resulted in significantly higher packed cell volume, haemoglobin concentration, and red blood cell counts than pre-treatment with vitamin E and control ($P < 0.05$). Also, post-treatment with EGb 761[®] significantly increased total protein in comparison to control ($P < 0.05$).

CONCLUSIONS: It was concluded that EGb 761[®] administration resulted in increased erythrocyte resistance to haemolysis and increased blood cellular components.

KEYWORDS: Chlorpyrifos, *Ginkgo biloba*, haematology, rats, vitamin E

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Received: 2019-04-19

Accepted: 2019-08-19

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How to Cite This Article

Abdulrazak, S., Abdulkadir Nuhu, A., & Israila Yashim, Z. (2019). Effect of *Ginkgo biloba* Leaf Extract (EGb 761) on Changes in Haematological Parameters and Erythrocyte Osmotic Fragility in Hypotonic and Chlorpyrifos Exposed Rats. Iranian Journal of Veterinary Medicine, 13(4), 331-341.

Introduction

Ginkgo biloba has existed for millions of years. The plant belongs to the family Ginkgoaceae and it originated from Asia (Nuhu, 2014). The standardised extract of *Ginkgo biloba* (EGb 761[®]) with well defined components used in this study is one of the most accepted and widely used herbal medicinal products in the world (Xie et al., 2014). The extract is considered relatively non-toxic and safe for consumption (Abdulrazak et al., 2018). The major constituents of the extract are flavonoids (Nuhu et al., 2017) and terpenoids (Van beek, 2002). The flavonoid fraction is composed of quercetin, kaempferol, isorhamnetin and glycosides, while the terpenoid fraction contains the ginkgolides A, B, C and J, as well as bilobalide. These compounds in EGb 761[®] are actively involved in the regulation of cellular metabolism, cell protective effect against toxins, and assist in balancing physiological status under oxidative stress (Yoshikawa et al., 1999), unlike most synthesized drugs with only one target function in their mechanism of action (He et al., 2009). The mechanisms of action of the extract are related to its antioxidant effect, and anti-platelet activating factor (Anti-PAF) activity (Smith and Luo, 2004). He et al. (2009) investigated the effects of EGb 761 on the properties of human red blood cells in the presence and absence of amyloid peptide (A β 25-35), peroxide and hypotonic stress. The findings reported that the protective effect of EGb 761[®] on red blood cells was dependent on the type of external stress present. According to Chan et al. (2007), antioxidant protective ability of EGb 761[®] is related to its ability to inhibit nitric oxide-stimulated protein kinase C (PKC) activity.

Chlorpyrifos is widely used today especially in northern Nigeria, as a pesticide,

and it has been known to induce oxidative stress (Nuhu et al., 2017). Exposure in both humans and animals is commonly via food products. It elicits many adverse effects, some of which are: haemotoxicity, genotoxicity, neurochemical and neurobehavioural changes (Uchendu et al., 2012). United States Environmental Protection Agency restricted some of its domestic use in 2000 based on human health risk (Uchendu et al., 2012). Some of the symptoms of chlorpyrifos poisoning in human and animals include headache, dizziness, salivation, unconsciousness, convulsion and death (Akhtar et al., 2009).

The underlying principle behind the therapeutic action of the *Ginkgo* leaf extract on chronic ailments (such as oxidative stress, poisoning, neurodegenerative and cardiovascular diseases) has focused on its antioxidant effects (Xie et al., 2014), which are elicited via two mechanisms of action; inhibition of free radical formation, and free radical scavenging activity. It scavenges reactive oxygen species (ROS) such as hydroxyl radicals (OH[·]), peroxy radical (ROO[·]), superoxide anion radical (O^{2-·}), nitric oxide radical (NO[·]), hydrogen peroxide (H₂O₂), and ferryl ion species (Mahadevan and Park, 2007). The extract can also enhance activities of antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase, catalase, and/or heme oxygenase-1, thereby indirectly contributing as an antioxidant (Mahadevan and Park, 2007). It has been suggested that *Ginkgo* leaf extract increases expression of mitochondrial enzymes, NADH dehydrogenases, which can influence ROS generation in the mitochondria.

This study aimed to evaluate the phytochemical constituents of EGb 761[®], effect of the extract on haematological parameters and

erythrocyte osmotic fragility in rats experimentally exposed to chlorpyrifos-induced oxidative stress, and also to determine the effect of the extract on erythrocyte osmotic fragility under hypotonic stress.

Materials and Methods

Phytochemical Screening of the Extract

Phytochemical screening of the standardised extract of *Ginkgo biloba* leaf extract (EGb 761®) (Nutra Green Biotechnology Co. Ltd, United Kingdom) was carried out using standard methods (Silva et al., 1998; Trease and Evans 2002).

Experimental Animals and Management

Albino rats, weighing between 180 - 200 g, were acclimatised for 2 weeks prior to the experiments; the rats were given access to pellets of feed prepared from grower's mash, maize bran and groundnut cake at the ratio of 4:2:1 and water was provided *ad libitum*. The feed was produced in the Animal section of the Department of Veterinary Physiology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria. All animal experimentation was done in accordance with Ahmadu Bello University Animal Use and Care Guidelines. Ethical clearance was obtained with approval number ABUCAUC/2016/015 from Committee on Animal Use and Care, Ahmadu Bello University, Zaria.

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 (100 mg/ml), *Ginkgo biloba* leaf extract (100 mg/ml), and chlorpyrifos (10.6 mg/ml) used for this study were 100 mg/kg, 100 mg/kg, and 10.6 mg/kg, respectively. Timing and the choice of treatment doses for the study were in-

formed by the acute toxicity study ($L.D_{50} > 5000$ mg/kg) and several trial experiments, as supported by previous studies (Sarıkçioğlu et al., 2004, Biddlestone et al., 2007 and Ambali et al. 2012).

For the effects of the extract on haematological parameters and on erythrocyte osmotic fragility in chlorpyrifos-exposed rats, thirty-five albino rats were divided into seven groups of five rats each; Group A (Distilled water, 5 ml/kg) served as untreated control and administered only distilled water. Group B (VE+CPF) was pre-treated with vitamin E and then administered chlorpyrifos 30 min later, Group C (CPF+VE) was administered with chlorpyrifos and then vitamin E 30 min later, Group D (Extract+CPF) was pre-treated with the extract, and then with chlorpyrifos 30 min later, Group E (CPF+Extract) was administered with chlorpyrifos and the extract 30 min 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 orally once daily for two weeks. The administered doses in ml of vitamin E, *Ginkgo biloba* leaf extract and chlorpyrifos used for this study were obtained by the formula;

$$\text{Doses (ml)} = \frac{\text{Body weight (kg)} \times \text{Dosage (mg/kg)}}{\text{Concentration (mg/ml)}}$$

Consequently, each animal received 0.18-0.20 ml dose of the respective treatment type per day depending on their individual body weight (180-200 g).

In the erythrocyte osmotic fragility study of non-chlorpyrifos-exposed rats, fifteen rats were divided into 3 groups of five animals each. Groups A, B, and C were administered with *Ginkgo biloba* leaf extract, Vitamin E,

and isotonic saline at a dosage of 100 mg/kg, 100 mg/kg, and 5 ml/kg respectively. The regimens were administered once daily by oral gavage for a period of 2 weeks.

The animals were sacrificed by jugular venesection at the completion of the experiments. Blood samples were collected in clean plastic centrifuge tubes containing heparin as anticoagulant for haematological assay and erythrocyte osmotic fragility test.

Determination of blood cellular components

Determination of packed cell volume (PCV) was done as described by Weiss and Wardrop (2010), with increased centrifugation time as a slight modification. Erythrocytes and leucocytes counts were done using a haemocytometer. Haemoglobin meter (XF-1C-China) was used to determine haemoglobin concentration (Hb), and the mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentrations (MCHC) were then calculated.

Determination of Erythrocyte Osmotic Fragility

Erythrocyte osmotic fragility was determined according to the method as described by Buhari et al. (2014). Exactly 5 ml of varying concentrations of sodium chloride (NaCl) solutions with phosphate buffer (3.22 g/L) at a pH of 7.4 (0.0, 0.1, 0.3, 0.5, 0.7, and 0.9%) were prepared in sets of 6 centrifuge tubes each. Blood (20 μ L) was added to each concentration of the test solution in each tube. The contents were mixed and incubated at room temperature for 30 min and then centrifuged at $3000 \times g$ for 10 min. The concentration of haemoglobin in the supernatant solution of each tube was measured at 540 nm using a spectrophotometer (Spectronic-20, Philip Harris Limited, Shenstone,

England) by reading the absorbance. By assuming that the haemolysis of haemoglobin in 0.0% NaCl solution was 100%, percentage haemolyses of the various treatment groups were then calculated, as described by Deriyeli et al. (2004).

Data Analysis

Data were expressed as mean \pm standard error of mean (SEM) 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.

Results

Phytochemical Screening of the Extract

The result of phytochemical screening is presented in Table 1. Phytochemical screening of EGb 761[®] revealed the presence of carbohydrates, glycosides, saponins, triterpenes, tannins, flavonoids, and alkaloids. However, the result shows that the extract did not contain anthraquinones and steroids.

Effect of *Ginkgo biloba* Leaf Extract on Haematological Parameters

The results of haematological parameters are presented in Tables 2 and 3. Packed cell volume and haemoglobin were significantly ($P < 0.05$) higher in Group D when compared to Groups A, B, and C. Similarly, total red blood cell count was significantly ($P < 0.05$) higher in Group D when compared to Groups A, B, C, and F. Total protein was significantly ($P < 0.05$) higher in Group D and E, compared to Group A. There was no significant ($P < 0.05$) effect of EGb 761[®] administration on total and differential leucocyte counts.

Table 1. Phytochemical Screening of *Ginkgo biloba* Leaf Extract (EGb 761)

Constituents	Inference
Carbohydrates	+
Anthraquinones	-
Glycosides	+
Saponins	+
Steroids	-
Triterpenes	+
Tannins	+
Flavonoids	+
Alkaloids	+

+ Present

- Absent

Table 2. Effect of *Ginkgo biloba* Leaf Extract on Hematological Parameters (Mean \pm Standard Error of Mean)

	RED BLOOD INDICES						
	PCV (%)	HGB (g/dL)	TP (g/dL)	TRBC ($\times 10^{12}/L$)	MCV (fL)	MCH (pg)	MCHC (g/dL)
Group A	39.60 \pm 0.92 ^a	13.16 \pm 0.31 ^a	7.65 \pm 0.89 ^a	6.62 \pm 0.15 ^a	59.82 \pm 0.27	19.90 \pm 0.09	33.24 \pm 0.02
Group B	39.50 \pm 2.59 ^a	13.13 \pm 0.86 ^a	8.60 \pm 0.21	6.65 \pm 0.37 ^a	59.33 \pm 0.92	19.70 \pm 0.31	33.23 \pm 0.02
Group C	38.50 \pm 3.17 ^a	12.80 \pm 1.05 ^b	8.50 \pm 0.50	6.55 \pm 0.60 ^a	59.00 \pm 1.23	19.63 \pm 0.38	33.23 \pm 0.04
Group D	49.25 \pm 1.70 ^b	16.23 \pm 0.63 ^a	10.05 \pm 0.12 ^b	8.35 \pm 0.29 ^b	59.03 \pm 1.12	19.43 \pm 0.38	32.95 \pm 0.35
Group E	43.25 \pm 1.25	14.38 \pm 0.41	10.20 \pm 0.36 ^b	7.15 \pm 0.14	60.45 \pm 0.56	20.11 \pm 0.17	33.28 \pm 0.02
Group F	40.50 \pm 1.04	13.45 \pm 0.35	8.80 \pm 0.42	6.67 \pm 0.13 ^a	60.65 \pm 0.49	20.15 \pm 0.18	33.25 \pm 0.02
Group G	41.20 \pm 1.93	13.70 \pm 0.63	8.04 \pm 0.53	6.90 \pm 0.33	59.72 \pm 0.19	19.84 \pm 0.06	33.20 \pm 0.04

Tukey's test: Means having different superscript (a,b) letters are significantly ($P < 0.05$) different

PCV= Packed cell volume, HGB=haemoglobin, TP=total protein, TRBC=total red blood cell count, MCV= mean corpuscular volume, MCH= mean corpuscular haemoglobin, MCHC= mean corpuscular haemoglobin concentration

NOTE:

- 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)

Table 3. Effect of *Ginkgo biloba* Leaf Extract on White Blood Cells and Differential Count Parameters (Mean \pm Standard Error of Mean)

	Differential Counts					
	TWBC ($\times 10^{12}/L$)	Lymph (%)	Neutro (%)	Mono (%)	Eosino (%)	Band (%)
Group A	6.92 \pm 0.95	80.80 \pm 2.87	12.80 \pm 2.13	4.40 \pm 0.40	1.20 \pm 0.58	0.40 \pm 0.40
Group B	5.87 \pm 0.89	84.00 \pm 0.70	13.25 \pm 1.79	2.75 \pm 2.42	0.00 \pm 0.00	0.00 \pm 0.00
Group C	9.25 \pm 1.04	79.25 \pm 2.78	16.25 \pm 2.05	3.01 \pm 1.47	0.75 \pm 0.25	0.75 \pm 0.47
Group D	7.00 \pm 0.73	83.25 \pm 1.37	13.75 \pm 0.25	2.75 \pm 1.37	0.00 \pm 0.00	0.00 \pm 0.00
Group E	10.01 \pm 2.14	85.25 \pm 1.79	12.50 \pm 1.25	1.25 \pm 0.94	0.00 \pm 0.00	1.00 \pm 1.00
Group F	9.85 \pm 2.83	87.50 \pm 2.53	10.00 \pm 2.79	1.50 \pm 0.95	1.00 \pm 0.40	0.00 \pm 0.00
Group G	10.46 \pm 2.08	87.20 \pm 1.98	9.00 \pm 1.54	2.01 \pm 1.26	0.60 \pm 0.40	1.20 \pm 0.73

Means within the same column are not statistically different at 95% CL ($\alpha=0.05$)

TWBC= Total white blood cell count, Lymph= Lymphocytes, Neutro= Neutrophils, Eosino=Eosinophils, Mono= Monocytes, Band=Band cells.

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NOTE:

- 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)

Effect of *Ginkgo biloba* Leaf Extract Erythrocyte Osmotic Fragility

The result of the effect of EGb 761 on erythrocyte osmotic fragility in chlorpyrifos induced oxidative stress in rats is presented in Table 4. This result indicated that the differences in percentage haemolysis were not statistically significant ($P>0.05$) between all compared groups. All groups have the same minimum erythrocyte osmotic fragility level at 0.9% NaCl concentration which is isotonic, with Group F having the lowest percentage haemolysis vis-à-vis other groups. Group G has the highest erythrocyte osmotic fragility level at 0.1% NaCl concentration while the other groups have the highest erythrocyte osmotic fragility

level at 0.0% NaCl concentration, both of which are hypotonic.

Table 5 shows the results of the effect of *Ginkgo biloba* leaf extract on erythrocyte osmotic fragility. Results of this study indicated that Groups A and B have lowest percentage haemolysis at 0.9% NaCl concentration which was isotonic and served as reference in this study, with Group A having the overall lowest value compared to other groups. Also, Group A has significantly ($P<0.05$) lower percentage of haemolysis in hypotonic medium at 0.5, 0.3, and 0.1% NaCl concentration respectively, when compared to Group C. Group B has significantly ($P<0.05$) lower percentage haemolysis at 0.5% NaCl concentration when

compared to Group C. Group A has significantly ($P<0.05$) lower percentage haemoly-

sis at 0.7, 0.3, and 0.1% NaCl concentration respectively when compared to Group B.

Table 4. Effect of *Ginkgo biloba* Leaf Extract on Erythrocyte Osmotic Fragility of Chlorpyrifos-Exposed Albino Rats, Measured as Percent Haemolysis (Mean \pm Standard Error of Mean)

	0.9% NaCl	0.7% NaCl	0.5% NaCl	0.3% NaCl	0.1% NaCl	0.0% NaCl
Group A	10.22 \pm 0.79	11.57 \pm 0.77	15.43 \pm 1.21	78.63 \pm 4.88	89.47 \pm 2.88	100.00 \pm 0.00
Group B	5.00 \pm 1.37	8.04 \pm 1.37	7.86 \pm 1.58	81.67 \pm 8.10	90.96 \pm 4.42	93.97 \pm 4.65
Group C	10.34 \pm 0.83	18.19 \pm 6.32	19.81 \pm 5.61	75.13 \pm 11.01	95.56 \pm 4.44	98.00 \pm 0.95
Group D	6.21 \pm 0.49	9.50 \pm 2.19	18.63 \pm 1.76	71.06 \pm 10.36	79.55 \pm 10.38	96.13 \pm 2.24
Group E	5.43 \pm 1.29	8.40 \pm 2.59	13.13 \pm 5.21	89.30 \pm 1.48	92.21 \pm 6.95	99.73 \pm 0.26
Group F	4.07 \pm 1.56	5.77 \pm 1.27	31.15 \pm 23.02	86.91 \pm 6.44	81.39 \pm 12.86	86.92 \pm 12.58
Group G	4.11 \pm 0.89	6.71 \pm 1.91	17.39 \pm 6.38	92.22 \pm 6.11	97.94 \pm 1.64	89.49 \pm 4.12

Means within the same column are not statistically different at 95% CL ($\alpha=0.05$)

NOTE:

- 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)

Table 5. Effect of *Ginkgo biloba* Leaf Extract on Erythrocyte Osmotic Fragility of Non- Chlorpyrifos-Exposed Albino Rats, Measured as Percentage Haemolysis (Mean \pm Standard Error of Mean).

	0.9% NaCl	0.8% NaCl	0.7% NaCl	0.5% NaCl	0.3% NaCl	0.1% NaCl	0.0% NaCl
Group A	0.97 \pm 0.46	2.97 \pm 0.75	3.30 \pm 0.79 ^b	47.58 \pm 4.85 ^b	62.68 \pm 2.69 ^b	58.84 \pm 10.22 ^b	100.00 \pm 0.00 ^a
Group B	3.66 \pm 1.23	3.96 \pm 0.89	11.18 \pm 0.94 ^a	57.19 \pm 2.42 ^b	87.54 \pm 5.33 ^a	97.57 \pm 2.34 ^a	97.73 \pm 1.42 ^a
Group C	5.04 \pm 1.89	4.18 \pm 1.05	4.91 \pm 0.50 ^b	78.07 \pm 5.55 ^a	92.80 \pm 7.20 ^a	95.03 \pm 4.63 ^a	86.97 \pm 5.02 ^b

Tukey's test: Means having different superscript (a,b) letters are significantly different ($P<0.05$)

NOTE:

- Group A= (*Ginkgo biloba* leaf extract)
- Group B= (Vitamin E)
- Group C= (Isotonic saline)

Discussion

Phytochemical Screening of the Extract

Phytochemical screening of *Ginkgo biloba* leaf extract in this study showed the presence of carbohydrates, glycosides, saponins, triterpenes, tannins, flavonoids, and alkaloids. This is also consistent with the findings of other investigators (Ibrahim and Nuhu, 2016; Goh and Barlow 2002; DeFeudis and Drieu 2000). The medicinal values of *Ginkgo biloba* lie in the presence of these active compounds which have been reported to have protective effect against ailments such as cardiovascular diseases, diabetes, and oxidative stress (Abdulrazak et al., 2018; Chan et al., 2007; Saw et al., 2006).

Effect of *Ginkgo biloba* Leaf Extract on Haematological Parameters

In this study, *Ginkgo biloba* leaf extract significantly ($P < 0.05$) increased total red blood cell counts ($8.35 \pm 0.29 \times 10^{12}/L$), haemoglobin concentration (16.23 ± 0.63 g/dL) and packed cell volume (49.25 ± 1.70 %) when compared with group pre-treated with Vitamin E (39.50 ± 2.59 %, 13.13 ± 0.86 g/dL, $6.65 \pm 0.37 \times 10^{12}/L$ respectively) and control (39.60 ± 0.92 %, 13.16 ± 0.31 g/dL, $6.62 \pm 0.15 \times 10^{12}/L$ respectively). This, therefore, suggests that EGb 761® has more prophylactic protective effect on erythrocyte parameters in rats exposed to oxidative stress than vitamin E. Also, post treatment with EGb 761® significantly increased total protein (10.20 ± 0.36 g/dL) when compared to control (7.65 ± 0.89 g/dL). These findings suggest that EGb 761® has more prophylactic than therapeutic protective effect on erythrocyte parameters in rats exposed to oxidative stress. This effect may be due to accelerated erythropoiesis. However, further investigation is required to confirm this. These findings are in accordance with those reported

by Khafaga and Bayad, (2016), He et al., (2009), Abdel Baieth (2009) and He et al., (2008) that EGb 761® caused an increase in blood cellular components.

Effect of *Ginkgo biloba* Leaf Extract Erythrocyte Osmotic Fragility

The prophylactic and therapeutic antioxidant activities of EGb 761 on erythrocyte osmotic fragility in chlorpyrifos-induced oxidative stress were not significant when compared to vitamin E and control. Also, the prophylactic antioxidant activity of EGb 761 was not statistically significant vis-a-vis the therapeutic antioxidant activity of the extract. Therefore, this study suggests that the percentage of haemolysis was not statistically significant between all the groups.

However, the osmotic fragility of non-chlorpyrifos-exposed rats showed that rats treated with *Ginkgo biloba* leaf extract markedly afforded red blood cells an increased erythrocyte osmotic resistance against hypotonic stress (i.e. 0.7 % NaCl concentration has 3.30 ± 0.79 % haemolysis, 0.5% NaCl concentration has 47.58 ± 4.85 % haemolysis, 0.3% NaCl concentration has 62.68 ± 2.69 % haemolysis and 0.1% NaCl concentration has 58.84 ± 10.22 % haemolysis) when compared to the control group (4.91 ± 0.50 %, 78.07 ± 5.55 %, 92.80 ± 7.20 %, 95.03 ± 4.63 % haemolysis at 0.7, 0.5, 0.3, and 0.1 % NaCl respectively) (Table 5). In the same vein, the extract also markedly increased red blood cell resistance to hypotonic stress (i.e. at 0.7 % NaCl concentration was 3.30 ± 0.79 % haemolysis, 0.3 % NaCl concentration was 62.68 ± 2.69 % haemolysis and at 0.1 % NaCl concentration was 58.84 ± 10.22 % haemolysis respectively) when compared to vitamin E (11.18 ± 0.94 , 87.54 ± 5.33 and 97.57 ± 2.34 % haemolysis at 0.7, 0.3, and 0.1 % NaCl concentration respectively). Furthermore, vitamin E increased

red cell resistance in hypotonic medium (at 0.5 % NaCl concentration was 57.19 ± 2.42 % haemolysis) when compared to the control group (78.07 ± 5.55 % haemolysis).

These results revealed beneficial effect of EGb 761 on erythrocyte parameters; the results demonstrate that *Ginkgo biloba* leaf extract has a more protective action on red blood cells against hypotonic stress than vitamin E. Quercetin, a flavonoid and a major constituent of *Ginkgo biloba* leaf extract, and triterpenes have been reported to be responsible for this protective effect (Chan et al., 2007; He et al., 2008). However, this study disagrees with Abdel Baieth (2009), who reported that *Ginkgo biloba* leaf extract caused an increase in red cell osmotic fragility in hypotonic medium; the electromagnetic field exposure in that study as well as high dosage of EGb 761 used is likely responsible for the contrasting results. These findings are in agreement with Khafaga and Bayad (2016), Zhou et al. (2015), Furman et al. (2012) and He et al. (2008), who reported that *Ginkgo biloba* leaf extract has both a protective and disruptive action on red blood cells depending on whether an exogenous stress is present or not.

This study shows that EGb 761® has more prophylactic than therapeutic ameliorative effects on blood cellular components exposed to chlorpyrifos-induced oxidative stress, but the extract did not show any prophylactic or therapeutic effect on erythrocyte osmotic fragility in erythrocytes exposed to chlorpyrifos-induced oxidative stress when compared to vitamin E and control. However, EGb 761 has afforded red blood cells an increased osmotic resistance against hypotonic solution only. This result was better than that obtained for vitamin E, a reference antioxidant, at the same dosage. These find-

ings further justify the use of *Ginkgo biloba* leaf extract in both medical and ethnomedical practices as herbal remedy and dietary supplement.

Acknowledgments

We appreciate Abdulwahab Hashimu Yau and Yusuf Abdulraheem Oniwapele of the Department of Veterinary Pharmacology and Toxicology, Ahmadu Bello University, Zaria for their technical support.

Conflict of Interest

The authors declare that there is no conflict of interest.

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