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Prophylactic Administration of *Ginkgo biloba* Leaf Extract (EGb 761) Inhibits Inflammation in Carrageenan Rat Paw Edema Model

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ABSTRACT

Acute toxicity and anti-inflammatory effect of *Ginkgo biloba* leaf extract (EGb 761) were carried out in this study. The anti-inflammatory activity was studied using the carrageenan model whereby twenty rats were randomly divided into four groups of five animals each. Groups one and two were administered the EGb 761 extract at 500 mg/kg and 250 mg/kg, respectively. Rats in groups three (positive control group) and four (non-treated control group) were given piroxicam (10 mg/kg) and normal saline (5 ml/kg), respectively. Oedema was induced by injecting 100 µl of fresh carrageenan into the right plantar surface of the hind paw of each rat 30 minutes after administration. The acute toxicity tests result showed that the extract is safe at 5000mg/kg dose. *Ginkgo biloba* leaf extract caused a significant ($P<0.05$) decrease in the size of the paw oedema when compared to control. Of interest, EGb 761 at 250 mg/kg was as effective as, or better than piroxicam (10 mg/kg). These findings further justify the use of *Ginkgo biloba* leaf extract in both medical and ethnomedical practice and may be used in treatment of inflammation.

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ABBREVIATION

EGb 761	-	***Ginkgo biloba* leaf extract**
g	-	**Gram**
GABA	-	**Ɣ- aminobutyric acid**
IL	-	**Interleukin**
IL-4	-	**Interleukin-4**
IL-6	-	**Interleukin-6**
LD_{50}	-	**Lethal dose 50**
mg/kg	-	**Milligram per kilogram**
ml/kg	-	**Milliliter per kilogram**
NO	-	**Nitric oxide**
PG	-	**Prostaglandin**
SEM	-	**Standard error of mean**
µL	-	**Microlitre**

INTRODUCTION

Inflammation is the body's physiologic defense mechanism against infection, burn, toxic chemicals, allergens or other noxious stimuli [1, 2]. Diseases and disorders are manifested through inflammatory responses as the body recognizes the injury and prepares to repair the damage [3]. Endogenous mediators like prostaglandins, histamine, serotonin, bradykinin, etc. are liberated when inflammation occurs. Prostaglandins (PG) indicate and modulate the body's response to inflammation. These substances can elicit pain response which in turn causes dropped muscular activities [4].

Medicinal plants have provided biologically relevant products for centuries, and are still a source for new medicines [5]. *Ginkgo biloba* is a widely used plant in treatment of asthma, bronchitis, hearing loss, tuberculosis, cognitive dysfunction, stomach pain, skin problems, and anxiety [5, 6, 7]. *Ginkgo biloba* leaf extract (EGb 761) contains flavonoids and triterpenes as the main active ingredients, and these substances possesses anti-inflammatory activity [8]. The extracts of *Ginkgo biloba* is said to have promising anti-inflammatory effect. Although it involves other mechanisms, interleukin (IL) is one of the most important in the anti-inflammatory functions of *Ginkgo biloba* [9]. Haines et al. [10] showed that the synergistic interaction of *Ginkgo biloba* leaf extract (EGb 761), astaxanthin and vitamin C suppress respiratory inflammation in asthmatic guinea pigs.

Bao et al. [11] reported that EGb 761 alleviate inflammatory reactions. This is done as a result of heightened activity of Interleukin-4, an anti-inflammatory cytokine, and inhibition of Interleukin-6 (IL-6), an inflammatory cytokine by dual activity. Using carrageenan model, Thorpe et al. [12] reported that EGb 761 has anti-inflammatory activity. Similarly, Ou et al. [13] also reported that inflammatory processes resulting from oxidized low density lipoproteins-induced oxidative stress in vascular endothelial cells were ameliorated by the administration of *Ginkgo biloba* extract.

The anti-inflammatory agents of plant origin have been the major focus of most research globally. Thus, evaluation of anti-inflammatory effects of *Ginkgo biloba* leaf extract is of great importance in the effective treatment and prophylaxis of several disease conditions in both humans and animals.

MATERIAL AND METHODS

Experimental animals and Ethical approval

Albino rats weighing an average of 180 g were acclimatized for 2 weeks prior to the experiment, fed standard 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

Acute toxicity study

The method of Lorke [14] with modification was used to determine the median lethal dose (LD_{50}) of the extracts in rats. This modification involves the introduction of uniform number of rats per group and the use of 18 albino rats instead of 12 for the study. In this study, 18 albino rats were randomly allocated into 6 groups of 3 rats each. The animals were starved of food ad libitum and water for 12 hours to avoid formation of complexes with food substances. Groups 1, 2, 3, 4, 5 and 6 were treated with the extract orally at 10, 100, 1000, 1600, 2900 and 5000 mg/kg body weight respectively. Rats were observed for 48 hours for any sign of toxicity or mortality.

Anti-inflammatory study

The method as described by Suleiman et al. [15] with modification was employed. Twenty rats were randomly divided into four groups of five animals each. Groups one and two received the extract at 500 mg/kg and 250 mg/kg, respectively. Rats in groups three (positive control group) and four (non-treated control group) were given piroxicam (10 mg/kg) and normal saline (5 ml/kg), respectively. All treatments were administered by oral route. Oedema was induced by injecting 100 µL of fresh carrageenan into the right plantar surface of the hind paw of each rat 30 minutes after administration. The paw diameter was measured at 0, 30 minutes, 1, 2,3,4,5, and 6 hours after administration.

Statistical 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.

RESULTS

Acute Toxicity Study

Table 1 shows the results of acute toxicity study of *Ginkgo biloba* leaf extract (EGb 761). The extract administered at doses of 10, 100, and 1000, 1600, 2900, and 5000 mg/kg respectively did not produce any sign of toxicity or mortality. Also, *Ginkgo biloba* leaf extract (EGb 761) did not alter the behavior of the animals during the period of the study. Therefore, *Ginkgo biloba* leaf extract is considered relatively safe.

Anti-inflammatory study

Sub-plantar injections of carrageenan induced inflammation as evident in the increased paw diameter of the untreated control rats. Oedema was visible within the first 5-10 minutes of administration of carrageenan, the peak of swelling occurred approximately 2-3 hours following injection of carrageenan. *Ginkgo biloba* leaf extract produced a significant ($P<0.05$) decrease in the size of the paw oedema as shown in Table 2. The activity of *Ginkgo biloba* leaf extract was highest at 250 mg/kg after 3 hours and was comparable to Piroxicam (standard anti-inflammatory agent; 10 mg/kg).

Table 1. Acute toxicity study of *Ginkgo biloba* leaf extract (EGb 761)

Groups	Dose/Day	Mortality (x/N)
Group 1	10 mg/kg	0/3
Group 2	100 mg/kg	0/3
Group 3	1000 mg/kg	0/3
Group 4	1600 mg/kg	0/3
Group 5	2900 mg/kg	0/3
Group 6	5000 mg/kg	0/3

*Group 1 (10 mg/kg Extract); Group 2 (100 mg/kg Extract); Group 3 (1000 mg/kg Extract); Group 4 (1600 mg/kg Extract); Group 5 (2900 mg/kg Extract); Group 6 (5000 mg/kg Extract).

Table 2. Effect of *Ginkgo biloba* leaf extract on carrageenan induced acute inflammation measured as paw size in mm (mean ± SEM)

Items	0 hr *	0.5 hr	1 hr	2 hrs	3 hrs *	4 hrs *	5 hrs *	6 hrs *
Group A	3.32 ± 0.18^{a}	4.89±0.23	5.71±0.25	5.99±0.20	5.94±0.30	5.23±0.39	4.48±0.17	3.72±0.19
Group B	3.00±0.14	4.64±0.40	5.14±0.32	5.97±0.38	5.60±0.56	4.60 ± 0.35^{a}	3.92 ± 0.25^{a}	3.55 ± 0.17^{a}
Group C	2.46 ± 0.18^{b}	4.36±0.22	4.72±0.18	5.52±0.09	5.13 ± 0.16^{a}	4.91±0.16	4.02 ± 0.20^{a}	3.47 ± 0.13^{a}
Group D	2.55 ± 0.13^{b}	5.02±0.11	5.65±0.25	6.67±0.48	6.83 ± 0.49^{b}	6.16 ± 0.30^{b}	5.30 ± 0.30^{b}	4.34 ± 0.21^{b}

*ANOVA: Indicates that Comparism for all groups is statistically significant ($P<0.05$) within the same column. Tukey's test: Means having different superscript (^{a,b}) letters are significantly different ($P<0.05$). Group A (500 mg/kg Extract); Group B (250 mg/kg Extract); Group C (Piroxicam (10 mg/kg); Group D (Normal saline (5 ml/kg).

DISCUSSION

Acute Toxicity Study

Toxicological study is first assayed to determine the safety of drugs and plant products for human and animal use [15]. The calculated LD_{50} of *Ginkgo biloba* leaf extract (EGb 761) was greater than 5000 mg/kg. This value falls within the practically non-toxic range [14]. Doses up to 5000 mg/kg, orally administered, did not alter the behavior of the animals during the period of the study, thus, the extract was considered relatively safe.

This finding was consistent with the outcome of a similar study carried out by Salvador [16], who reported that the LD_{50} of standardized *Ginkgo biloba* extract administered orally to mice was 7,730 mg/kg. He

also reported no organ damage or impairment of hepatic or renal function when *Ginkgo biloba* extract was administered orally over 27 weeks to rats and mice at doses ranging from 100 to 1,600 mg/kg.

Anti-inflammatory study

Results from this study suggest *Ginkgo biloba* leaf extract possessed anti-inflammatory effect. This may be as a result of inhibition of inflammatory mediators, such as nitric oxide (NO), prostaglandins, and proinflammatory cytokines into the paw tissue, because evidence shows that *Ginkgo biloba* and its constituents suppress induction of these mediators [17].

Of interest, EGb 761 at 250 mg/kg was as effective as, or better than piroxicam (10 mg/kg). However, administration of higher dose (500 mg/kg) of the extract did not produce such or higher anti-inflammatory effect. This may not be unconnected to the reports of Ivic et al. [18] and Kiewert et al. [19] that EGb 761 contains triterpenes; ginkgolides and bilobalide, and these active components at higher doses are known antagonists at both glycine and Ɣ- aminobutyric acid (GABA) in the body, which are neurotransmitters that are known to inhibit the activities of neurons that activate the release of inflammatory agents and regulate inflammation in the body.

Our finding is consistent with the work of Abdel Salam et al. [20] and Han [21], who reported that oral administration of *Ginkgo biloba* extract significantly reduced carrageenan induced paw oedema. Other studies have shown that treatment with *Ginkgo biloba* extract (30–120mg/kg; orally) reduced inflammation and acute colonic damage induced by acetic acid [22]. Similar studies on the anti-inflammatory properties of flavonoids, quercetin and kaempferol have also demonstrated reduced carrageenan-induced hind paw oedema in mice [23]. However, our result disagrees with the findings of Biddlestone et al. [24], who reported that *Ginkgo biloba* had no effect on paw oedema regardless of dose or duration of administration.

CONCLUSION

This study shows that *Ginkgo biloba* leaf extract (EGb 761) is practically non-toxic and is considered relatively safe. Also, the extract possessed prophylactic anti-inflammatory effect and was as effective as, or better than Piroxicam, a standard anti-inflammatory drug.

DECLARATIONS

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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.

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.

Consent to Publish

Not applicable

Competing Interests

The authors declare that there is no conflict of interest.

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