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Full Length Research Paper

Phytochemical screening and anti-inflammatory effect of ethanolic and aqueous extract of *Nephrolepis biserrata* leaf on albino wistar mice

[*1]Ekong N. J., [1]Zakari B. G., [2]Ibok N. I. and [2]Okon J. E.

[1]Department of Biological Sciences, Faculty of Sciences, Federal University Wukari, Taraba State, Nigeria.
[2]Department of Botany and Ecological Studies University of Uyo, Uyo, Akwa Ibom State, Nigeria.

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Phytochemical screening of *Nephrolepis biserrata* was carried out in the laboratory in order to prove the efficacy of its use by traditional medicine practitioners to treat inflammation. Inflammation was induced using xylene and egg white albumen methods and distilled water as control. Extract preparation of *Nephrolepis biserrata* was given to mice at 100 mg/kg at one hour interval for six hours. The result of LD_{50} value of *Nephrolepis biserrata* was calculated to be 3741.66 mg/kg. Results of the phytochemical screening revealed the presence of abundant tannins, saponnins, cardiac glycosides, moderate flavonoids, terpenes, phlobatannins and anthraquinones. Results of inflammation showed that there was reduction in inflammation level during treatment in ethanolic extract. These results support the traditional use of this leaf in some painful and inflammation conditions.

Keywords: *Nephrolepis biserrata*, phytochemical screening, inflammatory, extract.

INTRODUCTION

Inflammation is a part of the complex biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells or irritants (Ferro-Mililani *et al.,* 2007). There are two types of inflammation: acute and chronic inflammation. Acute inflammation is the initial response of the body to harmful stimuli and is achieved by the increased movement of plasma and leukocytes from the blood into the injured tissues, while prolonged inflammation leads to a progressive shift in the type of cells present at the site of inflammation and is characterised by simultaneous destruction and healing of the tissues from inflammation process (Ferrero-Miliani *et al.,* 2007).

Nephrolepis biserrata is stoloniferous fern with short-erect rhizome and leaves are up to 90cm long, leathery, arching, green to yellow green while the leaflets are forked at the end (Jorgensen and Leon –Yanez, 1999). It is widely cultivated in Africa, Northern and Southern America. The leaves of this plant are boiled and eaten as vegetable. In New Guinea, roots are pounded to flour. In Tahiti, it is used for blisters, boils, abscesses and sores. In Cameroon, decoction of fronds is used to lower abdominal pains while in Nwguyana, the leaves are used for treatment of wounds and cuts (Jorgensen and Leon-Yanez, 1999).

Despite the progress made in medical research during the past decades, the treatments of many serious diseases remain one of the world's major health problems (Li *et al.,* 2003). Inflammation has become the focus of global scientific research because of its implication in virtually all human and animal dise-ases. The conventional drugs used to ameliorate these

*Corresponding Author's E-mail: joesplendid@yahoo.com

diseases are either too expensive or toxic and not commonly available to the rural folks that constitute the major populace of the world (Li *et al.,* 2003). This study therefore seeks to examine *Nephrolepis biserrata* for anti-inflammatory activity and analgesic effects since pain is the major sign of inflammation.

MATERIALS AND METHODS

Study area

Nephrolepis biserrata was collected from a village called Afaha Etok in Ibesikpo Asutan Local Government Area of Akwa Ibom State found between the longtitude 70^0 25-80^0 North and latitude 40^0 32–50^0 East. This plant was authenticated by Dr. (Mrs.) M. E. Bassey, Department of Botany and Ecological Studies-Uniuyo, Uyo, with herbarium number (*Nephrolepis biserrata*: Ibok, UUH 2694 Uyo).

Preparation of plant extract

The fresh leaves (1kg) of *N. biserrata* were collected, air dried for one week and reduced to powder form which yielded 300g. Two hundred and fifty grammes (250g) of the powder were macerated in 300ml of 50% ethanol and shaken intermittently for 72 hours after which the mixture was filtered. The filtrate was then concentrated using a water bath at 40^0C to get the extract which was 30.58mg/kg. The extract was then preserved in a refrigerator at 4^0C for further use.

Phytochemical screening

Methods of Sofowora (1993), Trease and Evans (1989) were used. The dry ethanolic extract was subjected to phytochemical screening to reveal the presence of its secondary metabolites.

Experimental animals

Mice used for this study were obtained from animal house Department of Pharmacology, Faculty of Pharmacy, University of Calabar, Calabar. The twenty five mice used were assigned to 5 groups of five animals each. Food was withdrawn for 24 hours before the commencement of the tests (Amresh *et al*., 2008).

Acute toxicity test

Twenty five mice weighing 20-32g were in 5 cages (5 per cage) and handled according to standard guidelines for the use and care of laboratory animals. However, the food was withdrawn for 18 hours before the onset of the experiment according to the methods of Amresh *et al.* (2008). The five groups of mice were administered with 5000 mg/kg, 4000mg/kg, 3000mg/kg, 2000mg/kg and 1000mg/kg of the extract. The groups were observed for mortality within 24 hours and the LD_{50} value was calculated using Arimethic method of Turner (1965), Aliu and Nwude (1982).

Anti-inflammatory test

The methods of Adedapoi *et al*. (2009) were used with slight modification. Two methods of inducing inflammation were used in this study. These are Egg white albumen method and xylene method. In egg white, induced paw oedema was used as a model of acute inflammation. Albino Swiss mice of either sex, 20-32g were used after 24 hours. Inflammation of the hind paw was induced by O.1mL of fresh egg white into the inner surface of the right hind paw of the mice. The paw diameter of the injected paw was measured immediately before and immediately after injection. Measurement was done subsequently every 30 minutes over a period of 4 hours. Increase in paw diameter 3 hours after administration of egg white was adopted as the parameter for measuring inflammation. Oedema was assessed as the difference between zero time diameter of the injested paw and diameter 4 hours after administration of egg white. Measurement was done using vernier calipers. Also, the xylene method was used and inflammation was induced in mice by tropical administration of 2 drops of xylene at the inner surface of the right ear. The xylene was left to act on the animals for 15 minutes and there was 50% increase in mice ear weight.

Administration of the extract

Animals in the five groups were administered with both ethanolic and aqueous extract of *N. biserrata* for 2 hours interval for 2 days at the concentration of 100mg/kg.

Statistical analysis

Data obtained were subjected to student t-test to ascertain if there is any significant effect on the extract on inflammation. Also Statistical Package for Social Sciences (SPSS) version 17.0. One way analysis of variance was adopted for comparison, and the results were subject to post hoe test using least square deviation (LSD). The data expressed as mean ± standard error ($P<0.05$) were considered significant (Okon *et al*., 2013).

Table 1. Phytochemical screening of the ethanolic extract of *Nephrolepis biserrata* leaves

Test	Observation	Inference
Alkaloids		
Mayer's test	Milky white precipitate observed	+
Dragendorff's reagent	Reddish-brown precipitate observed	+++
Picric acid test	Reddish-brown precipitate observed	+++
Tannins		
Ferric chloride test	Blue-green colouration with precipitate observed	+++
Bromine water test	Milky white precipitate observed	+
Lead acetate test	Reddish colouration observed	++
Flavonoids		
Shinoda reduction test Colouration observed	Orange-crimson or magenta	++
Amonium test	Yellow colouration observed	++
Saponins		
Frothing test for more than 10 minutes	Frothing was observed to persist	+++
Ferling test	Brown precipitate observed	++
Phlobatannins	Red precipitate observed	+
Cardiac glycosides		
Liberman's test at the interphase	Pink colouration observed to occur	++
Kellerkiliani's test at the interphace	Brown ring colouration observed	+++
Salkowski's test	Reddish-brown coloration occurred at the interphase	++
Terpenes	A pink colouration observed at the interphase	++
Anthraquinones		
Borntrager's test	A pink-red or violet colouration observed	+
Test for combined anthra-quinones	No visible reaction observed	-

Legend: - = Absent, + = Trace, ++ = Moderate, +++ = Abundance

Table 2. Anti-inflammatory effect of the ethanolic and aqueous extract of *Nephrolepis biserrata* on xylene ear oedema in mice.

Inducement of oedema

Treatment dose (mg/kg)	Weight of right ear (g)	Weight of left ear (g)	Increase in ear weight
Control (distil H_2O)	0.04 ± 0.00	0.04± 0.00	0%
Xylene	0.06 ± 0.00	0.03± 0.00	50%

Results are expressed as mean ± standard deviation of three replicates (n=5)

Table 3. Treatment of oedema

After treatment	Decrease in weight with aqueous extract		Decrease in weight with ethanolic extract	
	2 hrs (100mg/kg)	4 hrs (100mg/kg)	2 hrs (100mg/kg)	4 hrs (100mg/kg)
Control (Distil H_2O)	0.04± 0.00	0.04 ± 0.00	0.04 ± 0.00	0.43 ± 0.0047
Xylene	00.3 ± 0.00	0.030 ± 0.0170	0.017 ± 0.0047	0.013± 0.0047

Results are expressed as mean ± standard deviation of three replicates (n=5)

Table 4. Anti-inflammatory effects of the ethanolic and aqueous extract of *Nephrolepis biserrata* with egg-induced oedema in mice using vernier callipers

Treatment dose	0	0.5hr	1hr	2hr	3hr	4hr	5hr	6hr
Control (distill H_20)	0.24 ±0.00	0.22 ±0.00	0.22 ±0.00	0.22 ±0.0094	0.24 ±0.01	0.24 ±0.00	0.24 ±0.00	0.24 ±0.00
Ethanolic extract (100mg/kg)	0.25 ±0.00	0.12 ±0.00	0.11 ±0.01	0.11 ±0.01	0.08 ±0.0012	0.07 ±0.00	0.06 ±0.00	0.04 ±0.00
Aqueous exract (100mg/kg)	0.24 ±0.00	0.12 ±0.00	0.12 ±0.01	0.22 ±0.01	0.22 ±0.01	0.22 ±0.01	0.24 ±0.00	0.8 ±0.00

Results are expressed as mean ± standard deviation of three replicates (n=5)

RESULTS

The phytochemical analysis of the ethanolic and aqueous extract of *N. biseratta* leaves revealed the presence of alkaloids, tannins, flavonoids, saponins, phlobatannins, cardiac glycosides, terpenes, anthraquinones and absent in combined anthraquinones (Tab. 1)

Median lethal dose (LD_{50})

Median lethal dose revealed that *N. biserrata* had 3741.66mg/kg taken orally.

Inducement of oedema in mice

Mice showed a significant increase in ear weight by 50% with Xylene and there was no increase in the ear weight at the control group using H_2O (Tab. 2)

Assessment of anti-inflammatory effect

Treatment with *Nephrolepis biserrata* leaf extract shows decrease in ear weight in mice. Treated mice showed significant ($p<0.05$) dose dependent decrease in both aqueous and ethanolic extracts of *Nephrolepis biserrata* (Tab. 3 and 4)

DISCUSSION

In this study, the extract preparation of *Nephrolepis biserrata* given to mice at 100mg/kg at one hour interval for 6 hours produced a reduction in the level of inflammation. The leaves of *Nephrolepis biserrata* are used for treatment of blister, boils, abscesses, sores, abdominal pains, wounds and cuts (Burkill, 1985). It would follow that ethanolic extracts of *Nephrolepis biserrata* are rich in secondary metabolites, hence can function as anti-inflammatory agent, e.g. *Nephrolepis biserrata* contains tannins. Tannins are important compounds known to be potent cyclooxygenase-1-inhibitors and with anti-phlogistic activity (Xu, 1996). The mechanism of anti-inflammatory activity may be related to anti-phlogistic action of the tannins. Phenolics present in these leaves have been used since early twenties for the destruction of cancer cells and for neutropective effects of oestrogen (Esenowo *et al.,* 2010) while tubocurarine and procenbazine have been used in tincture and are used in wounds apart from their use as neuroblockers (Cause e*t al.,* 1971). Okon *et al.* (2013) who worked on *Eryngium foetidum* ethanolic leaf extract reported the presence of saponins, tannins, flavonoids, terpenes and cardiac glycosides and stated that the volatile oils, resins, flavonoids and terpenoids present in the extracts especially at high dose are known to possesses analgesic and anti-inflammatory effects which are comparable to that of standard drug. In this study ethanolic extract has been noted for the same purpose.

The LD_{50} of these leaves was 3741.66mg/kg. This figure can serve as a guide in selection of doses for pharmacological studies. The acute toxicity test is mild and it suggests that the leaves are safe for use. The ethanolic leaves extract of *N. biserrata* possess significant anti-inflammatory effects in laboratory animals

at the dose investigated. The results support the traditional use of these leaves in some painful and inflammatory conditions and suggest the presence of biological active components which may be worth further investigation and elucidation.

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