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Research Article

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Anti-inflammatory and toxicity studies of the ethanolic extract of the leaves of *Ficus platyplla* Del (Moraceae)

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ABSTRACT

The result of the phytochemical screening of the leaf powder revealed the presence of carbohydrate, saponins, tannins, flavonoids, steroids and alkaloids, while cardiac glycoside and anthraquinones were absent. The LD_{50} determined by Lorke's method revealed that at the end of stage I studies, no death was recorded within 24 hours post administration of extract. In stage II of the study, 5000mg/kg produced death within 24 hours post administration of the extract. The anti-inflammatory result obtained revealed that the Ethanolic extract of leaf powder of Ficus platyphylla Del produced anti-inflammatory activities against rat paw oedema. The extract started having significant anti-inflammatory effect from 3 hours post administration. This was observed from the percentage inhibition and the statistical analysis. The extract at doses of 300, 600 and 1200 mg/kg significantly ($P < 0.01$) and dose-dependently inhibited egg albumin-induced rat paw oedema compared with the control (Aspirin) at 5 hr post-egg albumin administration. The order of inhibition was 1200 > 600 > 300mg/kg (50.1, 47.8, and 40.3%) respectively while the reference; aspirin (10 mg/kg) gave an inhibition of 39.98%. The results showed that the extract contains some pharmacologically active constituents and that there is scientific basis for the traditional use of the decoction for inflammation, diarrhea, colds etc.

Key words: *Ficus platyphylla* extract; inhibition; LD_{50}; anti-inflammatory; dose dependent

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INTRODUCTION

One of the most important uses of plants known to man is their medicinal value which has constituted an important part of traditional medicine which is part of African heritage. Medicinal plants have been used for a long time in the development of new drugs and continue to play an important role in drug discovery process [1]. People have relied on plants for staying healthy and treating illness for a long time. The World Health Organization (WHO) has estimated that about 80% of the world's population in rural areas relies chiefly on traditional medicine [2]. In Nigeria, majority of citizens still use medicinal plants and visit traditional practitioners for their health care need [3].

Chronic inflammatory diseases still remain of the world's major health challenges [4, 5, 6]. Inflammation is the response of living tissues to injury and it involves a complex array of enzyme activation, mediator release, and extravasations of fluid, cell migration, tissue breakdown and repair [7,8]. Due to its implication in virtually all human and animal diseases, inflammation has become the focus of global scientific research, more so, since the currently used anti-inflammatory agents both steroidal and non-steroidal are prone to evoking serious adverse reactions [9, 5, 10]. Attention is however being drawn to the investigation of the safety and efficacy of plant based medicaments used in the traditional medicine due to its affordability, with minimal side effects [9, 11].

The use of plants as medicine is older than recorded history. Many products obtained from plants have been used without finding out their safety or toxicity to the consumers. A toxic substance is any chemical entity that causes

structural or functional changes in the host that are deleterious to health [12]. Thus the needs to assess the safety of the plant extract. As mute witness to this fact marshmallow root, hyacinth, and yarrow have been found carefully tucked around the bones of a Stone Age man in Iraq[13].

Ficus platyphylla Del is a large tree with very large broad leaves and small figs with stalk often considerably longer than the fig measuring upto 18m in height and 6m in diameter. *F. platyphlla* is savanna woodland, often in rocky, up to an altitude of 750m [14]. Its distribution extends from Senegal to Ethiopia and Somalia (Ethiopia, Somalia, Sudan, Nubia, Uganda, Cote D'Ivoire, Cameroon, Gambia, Ghana, Guinea – Bissau, Mali, Niger, Nigeria, Senegal, Togo) [15]. The plant *F. platyphylla* is found abundantly in savanna habitat especially in Northern Nigerian and parts of Africa is traditionally used to treat a lot of ailments such as diarrhea, inflammation, convulsion, colds, sexually transmitted diseases etc. Other economic uses include the fruit as food, leaves as fodder, dyes, gum as masticulary for hunting and fishing apparatus, latex as rubber, bark as astringent, tannins, fibre etc [16].

Worldwide, ficus is one of the largest genera of flowering plants. Members of the genus are usually treated as a separate tribe within Moraceae because of their unique inflorescence and wasp – dependent system of pollination [17].

EXPERIMENTAL SECTION

The leaves of the plant *Ficus platyphylla* Del were collected at Babale District of Jos North Local Government Area of Plateau State (North Central Nigeria) in June, 2012. The collection was done manually by hand and the plant materials were identified, and authenticated at Federal College of Forestry, Jos, Plateau State, (North Central Nigeria), by Mr. J.J. Azila, where a plant press was made and kept in their Herbarium with a given voucher number, FHJ 0195.

70g of the leaf powder was weighed and mounted on a Soxhlet apparatus and extracted with 70% ethanol for 48 hours. The resulting solution was evaporated to dryness on a water bath and the percentage yield determined. The extract was wrapped aseptically with a foil paper and stored in a refrigerator to be used for the toxicity and anti-inflammatory examination.

Albino mice and rats of both sexes weighing between 17-25g and 15-18g were obtained from National Veterinary Research Institute, Vom, Plateau state, Nigeria and kept in the animal house of the Department of Pharmacology, University of Jos. The animals were kept in a well-constructed cage that allowed freedom of movement for one week for acclimatization to the laboratory conditions before commencement of study. Water and animal feed were provided and libitum throughout the period of acclimatization and the study.

ACUTE TOXICITY STUDIES

Stage I

The Lorke's method of lethal dose at 50 (LD_{50}) determination was used [18]. A total of nine mice were used. They were divided into three groups of three mice each. Doses of 10mg/kg, 100mg/kg, 1000mg/kg of the extract were administered intraperitoneally (IP) and then the mice were observed for behavioral manifestation of acute toxicity and death within 24 hours post administration.

Stage II

This stage depended on the outcome of the previous stage, whether or not death was observed. A total of three mice were used. They were divided into three groups of one mouse each. The doses were varied between 1,500 mg/kg, 2,900 mg/kg, and 5,000 mg/kg and the mice were observed again for death as the index of toxicity. The LD_{50} was calculated by taking the square root of the product of the highest dose that recorded no death and the lowest lethal dose respectively.

$LD_{50} = \sqrt{}$ Highest dose that record no death x Lowest dose that recorded death

ANTI-INFLMMATORY SCREENING

The anti-inflammatory activity of the aqueous leaf extract was investigated using the egg-albumin induced paw oedema test [19]. Albino rats weighing between 110-150g were divided into five groups of four each. Based on the weight of the animals, the dose to be administered to each group was calculated. The animals (rat) were pre-treated with normal saline, Aspirin 10mg/kg and the extract administered intraperitoneally. The paw size was measured with the aid of a vernier caliper at 60, 120, 180, 240 and 300 min after sub-planter injection of 0.1ml egg white. These measurements were used to estimate the degree of inflammation and percentage inhibition of oedema at intervals after the administration of egg albumin.

STATISTICAL ANALYSIS

- Data were reported as mean ± SEM with N indicating the number of animal used. Differences between the standard and test groups were tested by the student's test, with the level of significance set as P<0.01.
- Data were also reported as percentage inhibition of the anti-inflammatory effect of the test drug with respect to the control drug.

RESULTS AND DISCUSSION

LD_{50} DETERMINATION BY LORKE'S METHOD

STAGE I

TABLE 1

GROUP 1	Dose(mg/Kg)	Weight(g)	Result	Remarks
MICE 1	10	17	No death	
MICE 2	10	17	No death	3/3
MICE 3	10	17	No death	

TABLE 1I

GROUP 2	Dose(mg/Kg)	Weight(g)	Result	Remarks
MICE 1	100	17	No death	
MICE 2	100	17.5	No death	3/3
MICE 3	100	18	No death	

TABLE 1II

GROUP 3	Dose(mg/Kg)	Weight(g)	Result	Remarks
MICE 1	1000	17.5	No death	
MICE 2	1000	18	No death	3/3
MICE 3	1000	17	No death	

STAGE II

TABLE 1V

GROUP 3	Dose(mg/Kg)	Weight(g)	Result	Remarks
MICE 1	1500	18	No death	1/1
MICE 2	3000	19	No death	1/1 2/3
MICE 3	5000	18.5	Death	0/1

At the end of stage I of the LD_{50} studies, no death was recorded within 24 hours post administration of extract. In stage II of the study 5000mg/kg produced death within 24 hours post administration of the extract.

B. CALCULATION OF LD_{50}

Highest dose that recorded no death = 2900mg/kg

Lowest dose that recorded death = 5000mg/kg

$$LD_{50} = \sqrt{\textit{Highest dose that recorded no death x Lowest dose that recorded death}}$$

$$= \sqrt{5000 \times 2900}$$

$$= \sqrt{14500000}$$

$$= 3807.89 \text{ mg/kg}$$

Therefore the median lethal dose (LD_{50}) of the ethanolic extract of *Ficus platyphyll*a leaves is approximately 3800 mg/kg through intraperitoneal route in mice.

EFFECT OF ETHANOLIC LEAF EXTRACT OF*FICUS PLATYPHYLLA* ON EGG ALBUMIN INDUCED INCREASE IN RAT PAW SIZE.

TABLE V

Paw Size (mm)

Treatment	0 hr	1 hr	2 hr	3 hr	4 hr	*5 hr*
Normal Saline	0.360 ± 0.021	0.698 ± 0.025	0.771 ± 0.038	0.871 ± 0.019	0.822 ± 0.012	0.789 ± 0.017
Aspirin(10mg/kg)	0.337 ± 0.013	0.886 ± 0.069	0.750 ± 0.047	0.663± 0.036**	0.542± 0.031**	0.513± 0.024**
Extract (300mg/kg)	0.361 ± 0.005	0.825 ± 9.054	0.700 ± 0.040	0.636± 0.035**	0.492± 0.017**	0.471± 0.021**
(600mg/kg)	0.341 ± 0.004	0.795 ± 0.028	0.711 ± 0.037	0.585± 0.020**	0.434± 0.017**	0.412± 0.013**
(1200mg/kg)	0.368 ± 0.005	0.705 ± 0.037	0.587± 0.049*	0.532± 0.021**	0.423± 0.018**	0.394± 0.003**

*N=4; Values are represented as mean ±standard erro r of mean (SEM); **P <0.01 compared with control*

PERCENTAGE (%) INHIBITION

TABLE VI

	2 HOURS	**3 HOURS**	**4 HOURS**	**5 HOURS**
ASPIRIN(10mg/kg)	2.7%	23.9%	34.1%	34.9%
Extract (300mg/kg)	9.2%	26.9%	40.1%	40.3%
,, 600	7.8%	32.8%	47.2%	47.8%
,, 1200	23.9%	38.9%	48.5%	50.1%

The anti-inflammatory result obtained revealed that the ethanolic extract of leaf powder of *Ficus platyphylla* Del produced anti-inflammatory activities against the paw oedema in albino rats when compared with the control (**P<0.001). This might be due to the presence of flavonoids and tannins in ethanolic leaf extract of the leaf powder. The potency was found to be inversely proportional to time (Table V) taken for reduction in the paw volume. The extract is thought to be suppressing oedema by reducing vascular permeability as the case with non-steroidal anti-inflammatory drugs [20]. The extract started having significant anti-inflammatory effect from 3 hours post administration. This was observed from the percentage inhibition and the statistical analysis. The extract at doses of 300, 600 and 1200 mg/kg significantly ($P < 0.01$) and dose-dependently inhibited egg albumin-induced rat paw oedema compared with the control At 5 hr post-egg albumin administration, the highest dose of the extract (1200 mg/kg) inhibited oedema development by 50.1%, 600 and 300 mg/kg dose inhibited oedema by 47.8% and 40.3% respectively while the reference, aspirin (10 mg/kg) gave an inhibition of 39.98%. Thus the study revealed that *Ficus platyphylla* Del has potential anti-inflammatory activity which can be further research to isolate and characterize its active constituents.

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