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ANALGESIC AND ANTI-INFLAMMATORY EFFECT OF THE ETHANOLIC EXTRACT OF THE LEAF OF *FICUS PLATYPHYLLA* ON MALE ALBINO MICE.

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ABSTRACT This study evaluated the analgesic and anti-inflammatory activities of ethanolic extract of *Ficus platyphylla* leaf. The median lethal dose of the ethanolic extract of *Ficus platyphylla* leaf in mice was determined using Lorke method (1983). Phytochemical screening was carried out on the extract using standard methods as described by Trease and Evans (2002). In thirty-eight albino mice used in this study, the hot plate method was used in the determination of analgesic activities while in twenty albino rats used, the egg-albumin induced paw oedema method was used in the determining the anti-inflammatory activity of the leaf extract. The result revealed the presence of phytochemicals like flavonoids, tannins, cardiac glycosides and carbohydrates in the leaf extract and the analgesic and anti- inflammatory activities potential of the leaf of the plant. The results obtained indicate that the extract administered intraperitoneally at a doses of 300, 600 and 1200 mg/kg produced a significant ($P< 0.01$) and dose–dependent inhibited oedema. The extract produced analgesic activity at the same doses mentioned above. There was significant ($P< 0.01$) analgesic activity though it was less than the standard drug. It had a clear cut dose response relationship in its effect, producing an increase in pain reaction time (PRT) of the mice used for the study. In conclusion, the extract was found to be safe as it had a median lethal dose of about 3800 mg/kg i.p in mice. The ethanolic extract of *Ficus platyphylla* leaf possesses both analgesic and anti-inflammatory activity.

Keywords: Ficus platyphylla, anti-inflammatory, analgesic, oedema, ethanolic.

INTRODUCTION

Pain is a major cause of early consultations and the most common medical symptom (Bowsher, 1987). Unfortunately, the management of pain remains a major medical concern. Inflammation is a pathophysiological response of living tissues to injury that leads to local accumulation of fluids and blood cells. It is a defense mechanism that helps the body to protect itself against infections, toxic chemicals, burns, allergens or other stimuli (Sosa *et al.*, 2002). It involves a complex array of enzyme activation, mediator release, cell migration, tissue break-down and repair (Otimenyin *et al.*, 2007). Inflammatory responses may be acute or chronic with cardinal signs of redness, swelling, heat, pain and often loss of function. The cause, area affected and the condition of the host during an inflammation depends on the timing, severity, and local character of the inflammatory response. Inflammatory responses can be provoked by physical, chemical and biological agents including medical trauma, radioactive materials, corrosive chemical, extremes of heat and cold or by infectious agents such as bacteria, viruses and other pathogenic micro-organisms (Sosa *et al.*, 2002). The drawbacks of the use of conventional anti-inflammatory agents have led researchers into developing interest in natural plants that possess anti-inflammatory property without significant adverse effect. Since opioids and Non-steroidal anti-inflammatory drugs (NSAIDS) have many side effects, many medicinal plants have found their application in managing pain and various disease conditions. One of such plants is *Ficus platyphylla* used locally in the management of pain and inflammation for years, but efficacy studies on the leaf of this widely used medicinal plant has not been scientifically substantiated, hence this study. *Ficus platyphylla* Del, family moraceae known as 'Epo-obo' among Yoruba's in South West Nigeria and as 'Gamji' in Hausa, is widely distributed throughout the Savannah region. The common name is Gutta percha tree (Olugbenga *et al.*, 2011). Preliminary phytochemical screening of the stem bark of this plant shows the presence of saponins, flavonoids and tannins (Amos *et al.*, 2001). It is highly reputed for its numerous medicinal uses which includes the following: It is used in traditional medicine for treatment of convulsive disorder. The extract of this plant is used in

Hausa ethno medicine to treat mental illness, dysentery, cough, diarrhea, chest conditions and pain relief (Sandabe and Kwari, 2000; Wakeel *et al.,* 2004). The extracts of *F. platyphylla* have also been reported to inhibit gastro-intestinal motility (Amos *et al.,* 2001) *Ficus platyphylla* possess medicinal properties that are effective in the management of tuberculosis and cough (Kubmarawa *et al.,* 2007). The plant has also been reported to possess in-vitro anti-trypanosomal activity (Wurochekke and Nok, 2004; Atawodi, 2005). The central nervous system (CNS) activity of *F. platyphylla* has also been evaluated for the scientific basis for the use of this plant in traditional medicine for the treatment of CNS disorders (Chindo *et al.,* 2003). This study is aimed at evaluating the analgesic and anti-inflammatory potentials of the ethanolic extract of the leaf of *Ficus platyphylla*.

2.0 METHODS

2.1 Collection of Plant Materials

The fresh leaves of *F. platyphylla* plant were collected from Babale, a village in Jos North Local Government Area of Plateau State, Nigeria. It was identified and authenticated by a competent curator at the Federal College of Forestry, Jos, assigned a voucher specimen number FJ 0195, and deposited at their herbarium.

2.2 Extraction of the Plant

The leaves were dried under shade and reduced into a coarse powder using a mortar and pestle. Hundred grams (100g) of the powder was subjected to soxhlet extraction using ethanol (70% $^{v}/_{v}$) and the filtrate was evaporated in a water-bath and the yield of the extract obtained. The extract was wrapped aseptically in a foil paper and stored properly.

2.3 Experimental Animals

Thirty-eight (38) albino mice and twenty (20) rats of both sexes weighing between 17-25g and 100-150g respectively were obtained from National Veterinary Research Institute, Vom, Jos and kept in the animal house of the Department of Pharmacology University of Jos. The animals were kept in well-constructed cage that allowed freedom of movement for one week for acclimatization before commencement of study. The animals were fed with animal feed and allowed free access to clean fresh water in bottles *ad libitum* throughout the period of acclimatization and the study. The study was undertaken after obtaining approval and ethical clearance from the institutional Animal Ethics Committee of University of Jos, Nigeria.

2.4 Phytochemical Screening

Phytochemical screening for major phytoconstituents was undertaken using standard qualitative methods as described by Trease and Evans, (2002). Tests were conducted for the presence of saponins, tannins, flavonoids, cardiac glycoside, alkaloids, resin, steroids, anthraquinone, carbohydrate in the leaf extract.

2.5 Acute toxicity studies

2.5.1 Median Lethal Dose (LD_{50}) Determination

Phase I

The Lorke's method of median lethal dose (LD_{50}) determination was used (Lorke, 1983). A total of nine mice were used. They were divided into three groups of three mice each. Doses of 10 mg/kg, 100 mg/kg, 1000 mg/kg of the extract were administered intraperitoneally (i.p) and then the mice were observed for behavioral manifestation of acute toxicity or death within 24 hours post administration.

Phase II

This stage depended on the outcome of Phase I, whether or not death was observed. A total of nine mice were used. They were divided into three groups of three mice each. The doses of 1600 mg/kg, 2900 mg/kg and 5000 mg/kg were administered 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.

LD50

$$= \sqrt{highest\ dose\ that\ record\ no\ death\ X\ Lowest\ dose\ t}$$

2.6 Anti-Inflammatory Screening

The anti-inflammatory activity of the ethanolic leaf extract was investigated using the egg-albumin induced paw oedema test (Yamini et al., 2010). Twenty albino rats weighing between 100-150g were divided into five groups of four. Based on the weight of the animals, the dose to be administered to each group was calculated. The control group received the vehicle only (0.1ml normal saline i.p). The standard group was pretreated with aspirin 10 mg/kg i.p whereas, the remaining three groups that served as test received the ethanolic extract of *Ficus platyphylla* leaves at doses of 300 mg/kg, 600 mg/kg and 1200mg/kg i.p respectively. 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.

2.7 Analgesic Screening

2.7.1 Hot Plate Method

The method described by Turner (1971) was used in this study. Twenty mature albino mice of both sexes were divided into five groups of four mice per group. The control group received the vehicle only (0.1ml Normal saline i.p). The standard group received pentazocine 1.5 mg/kg i.p whereas, the remaining three groups that served as test received the ethanolic extract of *F.platyphylla* leaves at doses of 300 mg/kg, 600 mg/kg and 1200 mg/kg i.p respectively. The mice were placed in a glass beaker on an aluminum hot plate at 55 ± 1^0C for a maximum time of 30s. The pain reaction time (PRT) was recorded which represented the time taken for the mice to react to the pain stimulus. The response to pain stimulus considered, included jumping and licking of hind foot. Thirty minutes after drug and extract administration, PRT for each mouse was again determined and recorded.

2.8 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 at P <0.01.

SEM = $^S/\sqrt{N}$ S = Standard deviation, N = Number of animals

3.0 RESULTS

3.1 Phytochemical screening

After extraction of the leaf with ethanol (70% v/v), the yield of the extract was 29.56g while the percentage yield was 16.42g. The phytochemical analysis revealed the presence of flavonoids, tannins, cardiac glycosides and carbohydrates in the leaf extract of *F. platyphylla*.

Table 1: Phytochemical screening of ethanolic leaf extract of *Ficus platyphylla*

Phytochemical Constituents	Results
Alkaloids	–
Saponins	–
Flavonoids	+ + +
Tannins	+ + +
Carbohydrates	+ +
Cardiac Glycosides	+
Steroids	–
Anthraquinones	–

+++ Highly Present, ++ Moderately Present, + Present, - Absent

Table 2 shows the determination of LD_{50} value by Lorke's Method**.** At the end of phase I of the LD_{50} studies, no death was recorded within 24 hours post administration of extract. In phase II of the study, 5000mg/kg produced death within 24 hours post administration of the extract.

CALCULATION OF LD_{50}

Highest dose that recorded no death = 2900mg/kg, lowest dose that recorded death = 5000mg/kg

$LD_{50} = \sqrt{highest\ dose\ that\ recorded\ no\ death\ X\ Lowest\ dose\ that\ recorded\ death}$

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

$= \sqrt{14500000}$

$= 3807.89$mg/kg

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

Table 2: Determination of LD_{50} value by Lorke's method

Phase	Group	Number of mice used	Dose (mg/kg)	Remark
Phase 1	1	3	10	No death
	2	3	100	No death
	3	3	1000	No death
Phase 2	4	3	1600	No death
	5	3	2900	No death
	6	3	5000	Death

Table 3: Effect Of ethanolic leaf extract of *Ficus platyphylla* on Mice Pain Reaction Time (PRT).

Treatment	PRT 30 mins Post treatment administration
Control	2.93 ± 0.43
Pentazocine (1.5 mg/kg)	33.01 ± 4.81**
Extract (300 mg/kg)	16.38 ± 3.11**
Extract (600 mg/kg)	29.42 ± 4.88**
Extract (1200 mg/kg)	32.00 ± 2.23**

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

Table 4: Effect of ethanolic leaf extract of *Ficus platyphylla* on egg albumin induced paw oedema test.

Increase in Rat paw size

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 (2.7)	0.663 ± 0.036** (23.9)	0.542 ± 0.031** (34.1)	0.513 ± 0.024** (34.9)
Extract (300mg/kg)	0.361 ± 0.005	0.825 ± 9.054	0.700 ± 0.040 (9.2)	0.636 ± 0.035** (26.9)	0.492 ± 0.017** (40.1)	0.471 ± 0.021** (40.3)
Extract (600mg/kg)	0.341 ± 0.004	0.795 ± 0.028	0.711 ± 0.037 (7.8)	0.585 ± 0.020** (32.8)	0.434 ± 0.017** (47.2)	0.412 ± 0.013** (47.8)
Extract (1200mg/kg)	0.368 ± 0.005	0.705 ± 0.037	0.587 ± 0.049* (23.9)	0.532 ± 0.021** (38.9)	0.423 ± 0.018** (48.5)	0.394 ± 0.003** (50.1)

Paw Size (mm), N=4; Values are represented as mean ± standard error of mean (SEM); *P <0.05; **P <0.01 compared with control; Values in parenthesis indicate percentage inhibition (%).

4.0 DISCUSSION AND CONCLUSION

4.1 DISCUSSION

A variety of in vitro and in vivo experiments have shown that flavonoids, tannins, and other secondary plant metabolites possess analgesic and anti-inflammatory properties in various experimental animal models (Yuan *et al.*, 2006). Thus, these compounds may contribute to the observed anti-inflammatory and anti-nociceptive effects of *F. platyphylla*. From the value obtained as the median lethal dose for the ethanolic leaf extract of *F. platyphylla* was about 3800 mg/kg i.p in mice. This suggests that the extract is safe. The method used for evaluating the analgesic activity of the extract is the hot plate method described by Turner (1971). In the hot plate model, the tolerance to pain stimulus (in this case thermal stimulus), is manifested by increase in the pain reaction time (PRT) which indicates the level of analgesia that is induced by extract or reference drug pentazocine 1.5 mg/kg i.p (Ramadran and Basinath, 1986).

From the result of this study, the ethanolic leaf extract of *F. platyphylla* significantly ($P<0.01$) increased the PRT in hot plate model in the treated groups when compared to the negative control treated group (0.1ml normal saline). Anti-nociceptive effect was observed at 30 mins in all the extract treated groups but this effect was lower than the reference drug treated group. A clear cut dose response relationship was observed as the dose of extract administered increased there was an increase in the pain reaction time compared to the pain reaction of the control. The ethanolic leaf extract of *F. platyphylla* demonstrated a good level of antinociceptive activities in the hot model used for this study. Hot plates have been used for the study of centrally acting analgesia (Woolfe and MacDonald, 1994). The effect of the extract indicates that it might be centrally acting, as centrally acting analgesic drugs elevate pain threshold of animals towards heat and pressure (Adeyemi *et al.*, 2004). The ethanolic extract of the leaves of *F. platyphylla* was evaluated in rat paw oedema models induced by egg albumin. Determination of anti-inflammatory activity is based on vernier calliper measurement of oedema produced by sub planar injection of egg albumin in the right hind paw of rat. The oedema produced in animals treated with standard drug (aspirin 10 mg/kg) and test extract were compared with the oedema of untreated control animals at constant intervals of 0, 1, 2, 3, 4 and 5 hours. Thus percentage inhibition of oedema at known intervals in treated animals was compared with percent inhibition of oedema of control. The extract (300, 600 and 1200 mg/kg) also significantly ($P < 0.01$) and dose-dependently inhibited egg albumin-induced rat paw oedema compared with the control group (Table 6). At 5 hours post-egg albumin administration, the highest dose of the extract (1200 mg/kg) inhibited oedema development by 50.06%, 600 and 300 mg/kg dose inhibited oedema by 47.8% and 40.3% respectively while aspirin (10 mg/kg, i.p), a cyclo-oxygenase inhibitor used in this assay as a reference, gave an inhibition of 39.98% (Table 7). The inflammatory response is a physiological characteristic of vascular tissue. Increased permeability seen in the inflammatory reaction results in exudation of fluid rich in plasma proteins, coagulation factors and injured tissues with subsequent oedema at the site. Exudation which is a consequence of vascular permeability is considered as major features of acute inflammation **(**Thirupathy *et al.*, 2001**)**. Histamine and other mediators of inflammation increase vascular permeability at various times after injury. Chemically induced vascular permeability can causes an immediate inflammatory reaction. This indicates that the extract possibly exhibits its anti-inflammatory action by inhibiting the synthesis, release or action of inflammatory mediators including histamine, serotonin and prostaglandin known to mediate acute inflammation induced by phlogistic agents, that are likely also involved in egg albumin induced acute oedema (Haiping *et al.*, 2008).

4.3 CONCLUSION

Based on the results obtained in this study, it could be concluded that the plant is safe for consumption, also, ethanolic leaf extract of *F. platyphylla* demonstrated significant anti-inflammatory and anti-nociceptive activities, providing a scientific basis to explain, in part, the popular use of the plant in Nigerian folk medicine. It also suggests that the extract contains bioactive constituents that could be responsible for the observed activities.

4.4 RECOMMENDATION

This study has shown that the extract possess both analgesic and anti-inflammatory effects. However, more work is required to establish the exact mechanism of action of the active principles present in the extract.

CONFLICT OF INTEREST

Authors have declared that no competing interests exist

ETHICAL APPROVAL

Ethical approval to conduct this study was obtained from the Institutional Animal Ethics Committee of the University of Jos, Plateau state, Nigeria.

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