IJP (2018), Vol. 5, Issue 7 **(Research Article)**

Received on 03 March 2018; received in revised form, 24 March 2018; accepted, 30 March 2018; published 01 July 2018

ANALGESIC ACTIVITY OF HYDROMETHANOLIC EXTRACT OF *FICUS POLITA* LEAVES

A. N. Ukwuani-Kwaja *, A. A. Abdulganiyu, and I. K. Babatunde

Department of Biochemistry, Faculty of Science, Kebbi State University of Science and Technology, Aliero. P. M. B.1144. Kebbi State, Nigeria.

Keywords:

Mean latency,
Acetic acid, Acute toxicity,
Analgesic activity, *Ficus polita*

Correspondence to Author:
A. N. Ukwuani-Kwaja

Department of Biochemistry,
Faculty of Science, Kebbi
State University of Science and
Technology, Aliero. P.M.B.1144.
Kebbi State, Nigeria.

E-mail: pinknnenna@gmail.com

ABSTRACT: *Ficus polita* leaves have been used in traditional medicines to increase physical strength, work productivity and longevity, resistance to high altitude sickness, fatigue, depression, anemia, gastrointestinal ailments, infections, and nervous system disorders. The objective of this study was to evaluate the analgesic activity of hydromethanolic extract of *F. polita* leaves in rats using acetic acid-induced writhing and tail flick immersion methods. The phytochemical screening of the extract revealed the presence of flavonoids, saponins, terpenoids, glycosides, resins, steroids, balsams and pholobatannins. Acute oral toxicity study resulted in no mortality after oral administration of 3000 mg/kg of the extract. Based on the tail flick immersion test, *Ficus polita* hydromethanolic leaves extract and the standard drug, diclofenac showed significant ($P<0.05$) analgesic activity by prolonging the mean latency time when compared to the control. However, a significantly ($P<0.001$) prolonged mean reaction latency to pain in morphine-treated groups was not comparable to the extract and diclofenac treated groups. The extract and the standard drugs significantly ($P<0.001$) inhibited the acetic acid-induced abdominal writhing at all doses tested compared to control. The prolonged reaction latency and inhibition of pain in *Ficus polita* leaves extract treated groups may be correlated with its significant analgesic potential and thus validates its traditional uses.

INTRODUCTION: Pain is defined as an unpleasant sensory and emotional experience associated with actual and potential tissue damage, or described in terms of such damage, or both [1]. Non-steroidal anti-inflammatory drugs (NSAIDs) and opiates are drugs commonly used to treat or reduce pain and have been associated with adverse effects such as ulceration, gastrointestinal disturbances, addiction, renal damage, respiratory depression, *etc*.

QUICK RESPONSE CODE

DOI:
10.13040/IJPSR.0975-8232.IJP.5(7).404-08

The article can be accessed online on
www.ijpjournal.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.IJP.5(7).404-08

This has lead to increasing interest and need to search for treatment alternatives with little or no side effects. The use of herbal medicine is gaining support and recognition across the world because most of these products are believed to have bioactive compounds responsible for healing various diseases without any side effects and at a lower cost [2]. *F. polita* is an edible evergreen shrub or small tree usually growing in lowland rainforest and gallery forest of West and Central Africa.

Traditionally its leaves are used in the treatment of infectious diseases, abdominal pains and diarrhea [3]. Previous researches have reported this plant to possess antibacterial [4], anti-inflammatory [5], anti-HIV [6] and *in-vitro* antimalarial [7] activities. In Kebbi state, Nigeria, it is used to increase stamina and relief pain.

However, there is no scientifically validated data on this folkloric claim. Therefore, the study aimed to evaluate the analgesic potential of *Ficus polita* leaves extract in Albino rats.

MATERIALS AND METHODS:

Plants Materials: The leaves of *Ficus polita* were collected from Aliero Local Government area of Kebbi state, Nigeria in February 2016 and identified by Dr. Dramendrah Singh of Botany unit, Biological Sciences Department of Kebbi State University of science and technology and a voucher specimen number 126 was deposited in the herbarium.

Preparation of Plant Extract: The leaves of *Ficus polita* were chopped into small pieces with a knife, dried under mild sunlight for complete dryness and pulverized into a coarse powder using mortar and pestle. The extraction was done by cold maceration method in 50% methanol for 72 h with intermittent shaking. The extract was filtered using a muslin cloth, and the filtrate was oven dried at a temperature of 45 ºC to complete dryness. The dried sample was stored in a refrigerator for further use.

Phytochemical Analysis: Chemical tests were carried out on the hydromethanolic leaves extracts using standard procedures to identify the constituents by characteristic color changes [8, 9, 10]. All chemicals used are of analytical grade.

Animals: White albino rats (Wistar stock) were obtained from the Department of Zoology, Usmanu Danfodio University. Sokoto, Nigeria. The rats were transported to Animal House, Faculty of Science, Kebbi State University of Science and Technology Aliero.

They were allowed to acclimatize for two weeks before commencement of the study. The animals were fed on Rodent pellet (Vital feeds, Nigeria) and water *ad libitum* throughout the study period. The animals were maintained according to the animal ethics committee guidelines of the University.

Acute Oral Toxicity Study (LD_{50}): Acute oral toxicity test was performed as per the OECD-423 guidelines (acute toxicity class method). Five (5) rats of either sex selected by random sampling technique were used for this study. The animals were fasted over night providing only water, after which freshly prepared leaves extract was administered orally at a dose level of 3000 mg/kg body weight to each rat at 48 h interval respectively and subsequently observed for 14 days [11].

Analgesic Activity:

Acetic Acid Inducing Writhing Test: The peripheral analgesic activity of leaves extract of *F. polita* was measured using the acetic acid-induced writhing test in rats. The acetic acid-induced abdominal writhing test was performed according to the procedure described previously by Koster *et al.* [12] Twenty-five (25) rats were divided into 5 groups of rats each. Group 1 was given 5 ml/kg of normal saline and served as the control while Groups 2 and 3 received 200, and 400 mg of leaves extract per kg of body weight orally respectively. Groups 4 and 5 served as the positive control receiving diclofenac 50 mg/kg and morphine 10mg/kg respectively. Thirty min later, rats in all the groups were treated with acetic acid (0.7% v/v, 1 ml per 100 g body weight i.p.). Five min after acetic acid injection, rats were placed in individual cages, and the number of abdominal contractions was counted for each rat for 10 min. Percentage inhibition of writhing was calculated using the formula:

% Inhibition of pain response = Mean no of writhing in control – Mean no of writhing in test / Mean no of writhing in control × 100

Tail Immersion Test: The method described by Kumar and Shankar [13] was used for this study. The animals were divided into five groups of five animals each. Group 1 served as normal control and received distilled water (5 ml/kg), Groups 2 and 3 served as extract treatment groups and were administered with 200 mg/kg and 400 mg/kg leaves extract respectively.

Groups 4 and 5 served as a reference group and received diclofenac (50 mg/kg, p.o) and morphine (10 mg/kg i.p) respectively. The tail withdrawal from the heat (flicking response) is taken as the endpoint. A cut off period of 15 s is observed to avoid damage to the tail. The measurements of withdrawal time were conducted at 30, 60, 90 and 120 min after administration of drugs and % inhibition was calculated as:

$$\% \text{ Inhibition} = T_1 - T_0 / T_0$$

Where, T_1 is post-drug latency and T_0 is pre-drug latency.

Statistical Analysis: Values for analgesic activity were expressed as Mean ± SEM. Analysis of variance (ANOVA) followed by Dunnett-test was used to statistically analyze the data. P values less than (P<0.01) and (P<0.05) were considered significant for acetic acid-induced test and tail immersion test respectively.

RESULTS:

Extraction: The percentage yield of hydro-methanolic leaves extract of *Ficus polita* was 27.9% which indicates that the plant could be a good source of medicine as it contains sufficient amount of components that could be used for herbal formulation.

Phytochemical Analysis of the Hydromethanolic Leaves Extract of *Ficus polita*: In this study, phytochemical tests revealed the presence of some secondary metabolite **Table 1**.

Results of Acute Oral Toxicity Study (LD_{50}) of *Ficus polita*: The hydromethanolic leave extract of *Ficus polita* revealed no mortality, nor toxic signs or symptoms at 3000 mg/kg body weight. However, treated rats showed confusion, scratching of their nostrils and head, and restless movement in the first 30 min only after which no other observation was recorded during the study period. The LD_{50} was estimated to be greater than 3000 mg/kg body weight of rats.

Acetic Induced Abdominal Writhing Test: Dose-dependent analgesic effect was exhibited at all the extract tested dose **Table 2** which was significantly (P<0.01) different from the control but not significantly different (P>0.05) from the diclofenac-treated rats. However maximum mean percentage inhibition of writhing (94.74%) was recorded by morphine-treated rats.

Tail Flick Immersion Test: In the tail immersion test, hydromethanolic leaves extract of *F. polita* and standard drugs significantly (p<0.05) increased the pain reaction time in a time-dependent manner to thermal stimulus compared to control.

TABLE 1: PHYTOCHEMICAL ANALYSIS OF *FICUS POLITA* LEAVES EXTRACT

Secondary metabolite	Inference
Carbohydrate	+
Phenol	ND
Flavonoids	+
Tannins	ND
Saponins	+
Alkaloids	ND
Terpernoids	+
Anthraquinone	ND
Resins	+
Steroids	++
Balsams	+
Phloboannins	+
Glycosides	+

Key = + means Present; ND means Not Detected or Not Present

TABLE 2: ANALGESIC ACTIVITIES OF *F. POLITA* LEAVES USING THE METHOD OF ACETIC ACID - INDUCED ABDOMINAL WRITHING TEST

Group	Dose	No of writhing	Percentage inhibition (%)
Normal saline	5 ml/kg	19 ± 1.47	-
F. polita leaves	200 mg/kg	9 ± 0.62**	52.63
F. polita leaves	400 mg/kg	6.25 ± 0.85**	67.37
Diclofenac	50 mg/kg	10.75 ± 0.75**	43.16
Morphine	10 ml/kg	1.00 ± 0.00**	94.74

Results were expressed as the mean value ± standard error of the mean (SEM). **Significant differences (P<0.01) compared to control.

TABLE 3: ANALGESIC ACTIVITY OF *FICUS POLITA* ON TAIL FLICK IMMERSION IN ALBINO RATS

Groups	Dose	30 min	45 min	60 min	75 min
		Mean latency time (sec)			
Control	5ml	4.5 ± 0.29	3.75 ± 0.25	2.25 ± 0.25	1.50 ± 0.29
F. polita leaves	200mg/kg	9.5 ± 0.65**	7.75 ± 0.48**	5.75 ± 0.48	3.5 ± 0.65
F. polita leaves	400mg/kg	10.0 ± 0.82**	7.75 ± 0.48**	4.5 ± 0.64	2.0 ± 0.41
Diclofenac	50mg/kg	9.75 ± 0.85**	6.0 ± 0.58**	4.25 ± 0.48	2.25 ± 0.25
Morphine	10mg/kg	42.5 ± 3.2***	33.75 ± 2.39***	25.0 ± 3.54***	16.75 ± 1.19***

Results were expressed as the mean value ± standard error of the mean (SEM). Differences between control and experimental groups were determined by one-way analysis of variance (ANOVA), followed by Dunnett Multiple Comparisons Test. **: P<0.01 and ***: P<0.001 show the level of significant difference compared to control.

The peak latency effect was recorded at the first 30 min. Overall maximum analgesic effect was recorded at the morphine-treated rats maintaining a higher pain reaction time (42.5 - 16.75 sec) from 30 to 75 min post administration.

DISCUSSION: Phytochemicals are natural chemical compounds produced by plants through primary and secondary metabolism which posse's biological activities beneficial to human health. Plants have the capacity to synthesise a diverse array of secondary metabolites. Many researchers have linked presence of secondary active metabolites such as flavonoids, saponins, tannins and alkaloids to analgesic activities among other properties [2, 14, 15]. It is therefore assumed that the chemical composition of *Ficus polita* leaves such as flavonoid and saponins could be responsible for the analgesic effects observed. Lethal Dose (LD_{50}) is usually an initial step in the assessment and evaluation of the toxic characteristics of a substance. Hydro-methanolic leaves extract of *Ficus polita* at a dose of 3000 mg/kg showed no mortality. Bruce [16] reported that any substance with an estimated LD_{50} between 2000 - 5000 mg/kg body weight given orally could be considered of low toxicity and is safe.

In the present study, the LD_{50} of *Ficus polita* was greater than 3000 mg/kg suggesting that the extract has very low toxicity when administered orally and as such safe for use as herbal formulation. Acetic acid-induced writhing is a well-recommended protocol in evaluating medicinal plants for their analgesic property. Acetic acid produces writhing reflex in animals by activating the chemosensitive nociceptors [17]. Pain sensation in acetic acid-induced writhing is elicited by producing localized inflammatory response due to the release of free arachidonic acid from tissue phospholipids *via* cyclo-oxygenase (COX), and producing prostaglandin specifically PGE2 and PGF2α and also the level of lipoxygenase products may also increase in peritoneal fluids [18, 19]. The increase in prostaglandin levels within the peritoneal cavity then enhances inflammatory pain by increasing capillary permeability [20]. The analgesic effect (decreased number of writhing) of hydromethanolic leaves extract of *Ficus polita* may be due to inhibition of arachidonic acid metabolites *via* cyclooxygenase.

The tail immersion test is considered to be selective for the drugs acting centrally like morphine. It has been reportedly established that any agent that causes a prolongation of pain latency using this test must be acting centrally [21]. In the present study, hydromethanolic leaves extract of *Ficus polita* exhibited significantly prolonged pain reaction time thereby suggesting a mechanism involving central pain pathways. Narcotic analgesics are known to inhibit both peripheral and central mechanism of pain, while NSAIDs inhibit only peripheral pain. The leaves extracts of *Ficus polita* exhibited both types of pain inhibition. The analgesic effect of the plants in both models suggests that they may be acting through the central and peripheral mechanism.

CONCLUSION: *Ficus polita* is a plant remedy employed in northwest Nigeria for the treatment of various diseases including pain. Hydromethanolic leave extract of *F. polita* showed the significant analgesic effect on acetic acid-induced writhing and tail immersion test in the albino rat at all doses tested. However, further study is required to the determination of the exact mechanism of action, isolation and characterization of its bioactive compound(s).

ACKNOWLEDGEMENT: The authors are grateful to Biochemistry Laboratory staff, Kebbi state university of science and technology especially Miss Rebecca M. Samuel for their time and technical support.

CONFLICT OF INTEREST: We declare that we have no conflict of interest.

REFERENCES:

1. IASP: Pain Definitions". Pain. 1979; 6 (3): 247-8. Doi: 10. 1016/0304-3959(79)90046-0.
2. Koech SC, Ouko RO, Michael NM, Ireri MM and Ngugi MP: Analgesic activity of dichloromethanolic root extract of *Clutia abyssinica* in swiss albino mice. Nat Prod Chem Res 2017; 5: 255. Doi: 10.4172/2329-6836.
3. Etkin NL and Ross PJ: Food as medicine and medicine as food: An adaptive framework for the interpretation of plant utilization among the Hausa of northern Nigeria. Soc Sci Med 1982; 16: 1559-1573.
4. Kuete V, Kamga J, Sandjo LP, Ngameni B, Poumale HM, Ambassa P and Ngadjui BT: Antimicrobial activities of the methanol extract, fractions and compounds from *Ficus polita* Vahl (Moraceae). BMC Complement Altern Med 2011; 11: 6.
5. Recio MC, Giner RM, Maiiez S, Rios JL, Marston A and Hostettmann K: Screening of tropical, medicinal plants for

anti-inflammatory activity. Phytother Res 1995; 9: 571-574. 10.1002/ptr.2650090807.
6. Ayisi NK and Nyadedzor C: Comparative *in-vitro* effects of AZT and extracts of *Ocimum gratissimum, Ficus polita, Clausena anisata, Alchornea cordifolia*, and *Elaeophorbia drupifera* against HIV-1 and HIV-2 infections. Antivir Res 2003; 58: 25-33. 10.1016/S0166-3542(02)00166-3.
7. Gbeassor M, Kedjagni AY, Koumaglo K, de Soma C, Agbo K, Aklikokou K and Amegbo KA: *In-vitro* anti-malarial activity of six medicinal plants. Phytother Res 1990; 4: 115-117. 10.1002/ptr.2650040309.
8. Trease GE and Evans WC: Pharmacognosy. A physician guide to Herbal medicine, Ballere Tindal, London U.K. Edition 11th, 1989; 530.
9. Harborne JB: Phytochemical methods: A guide to modern techniques of analysis. Chapman and Hall, London. Edition 3rd, 1973; 7-13, 60-89, 131-135, 186-188, 203, 279.
10. Odebeyi O and Sofowora O: Phytochemical screening of Nigerian medicinal plants. Lloydia 1978; 41: 41-234.
11. Dixon WJ: Staircase bioassay; the up and down method. Nuero Sci Biobehav Rev 1991; 15: 47-50.
12. Koster R, Anderson M and Debeer EJ: Acetic acid analgesic screening. Federation Proceedings 1959; 18: 412 -417.
13. Kumar JP and Shankar NB: Analgesic activity of *Mollugo pentaphylla* Linn. by tail immersion method. Asian J Pharm Clin Res 2009; 2(1): 61-63.
14. Kumar A, Agarwal K, Maurya KA, Shanker K and Bushra U: Pharmacological and phytochemical evaluation of *Ocimum sanctum* root extracts for its anti-inflammatory, analgesic and antipyretic activities. Pharmacognosy Magazine 2015; 11: 17-24.
15. Afsar T, Khan M, Razak S, Ullah S and Mirza B: Antipyretic, anti-inflammatory and analgesic activity of *Acacia hydaspica* R. Parker and its phytochemical analysis. BMC Complementary and Alternative Medicine 2015; 15: 136-145.
16. Bruce RD: An up and down procedure for acute toxicity testing. Fundamentals of Applied Toxicology 1985; 5: 151 - 157.
17. Onasanwo SA and Elegbe RA: Antinociceptive and Anti-inflammatory Properties of the leaf extract of *Hedranthera barteri* in rats and mice. African J Biomedical Research 2006; 2: 109-118.
18. Khan H, Saeed M, Gilani AUH, Khan MA, Dar A and Khan I: The antinociceptive activity of *Polygonatum verticillatum* rhizomes in pain models. Journal of Ethnopharmacology 2010; 127 (2): 521-527. http:// dx.doi.org/10.1016/j.jep.2009.10.003
19. Duarte I, Nakamura M and Ferreira S: Participation of the sympathetic system in acetic acid-induced writhing in mice. Brazilian Journal of Medical and Biological Research 1988; 21(2): 341.
20. Zakaria ZA and Gani AZDF: Antinociceptive, anti-inflammatory and antipyretic property of an aqueous extract of *Dicranopteris linearis* in experimental animal models. J Nat Med 2008; 62: 179-187.
21. Ibironke GF and Ajiboye KI: Studies on the anti-inflammatory and analgesic properties of *C. ambrosiodes* leaf extract in Abino rats. Inter J Pharmacology 2007; 3: 111-115.

How to cite this article:
Ukwuani-Kwaja AN, Abdulganiyu AA and Babatunde IK: Analgesic activity of hydromethanolic extract of *Ficus polita* leaves. Int J Pharmacognosy 2018; 5(7): 404-08. doi link: http://dx.doi.org/10.13040/IJPSR.0975-8232.IJP.5(7).404-08.