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In vivo Anti-Inflammatory Effect of Zapoteca portoricensis (Jacq) HM Hernández

Abstract

Purpose: To evaluate the anti-inflammatory activity of the root extracts and fractions of *Zapoteca portoricensis*.
Methods: The root of *Z. portoricensis* was extracted with methanol and the extract (ME) subjected to activity-guided fractionation to obtain chloroform (CF), ethyl acetate (EF) and methanol (MF) fractions. CF was further separated into four column fractions (CF1-CF4). The crude methanol extract and all the fractions were screened for anti-inflammatory activity using egg-albumin induced rat paw edema as a model of inflammation.
Results: The crude methanol extract (200 mg/kg) exhibited a significant ($P<0.01$) anti-inflammatory effect with edema inhibition of 71.9 % at 3 h. At 200 mg/kg, CF exhibited high and significant ($P < 0.01$) inhibition of edema (59.9 % at 3 h). EF (200 mg/kg) exhibited moderate inhibition of edema (29.8 % at 3 h) while MF (200 mg/kg) did not show any edema inhibition at 3 h. The column fractions CF1, CF3 and CF4 showed high and significant ($P < 0.01$) inhibition of edema (62.3, 60.9 and 66.7 %) respectively. The activities of these fractions are significantly higher than that of acetyl 100 mg/kg salycilic acid (45.6 % at 3 h). These column fractions on phytochemical analysis were shown to contain mainly terpenoids and steroids.
Conclusion: *Zapoteca portoricensis* possesses significant anti-inflammatory activity in acute inflammation in rats. The terpenoids and steroids present in the column fractions may be responsible for the activity.

Keywords: *Zapoteca portoricensis*; Anti-inflammatory, Egg albumen-induced edema, Terpenoids; Steroids.

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Introduction

Inflammation is a complex reaction through which the body responds to damage to its cells and vascularized tissues. It is the body's reaction to the invasion of an infectious agent, antigen challenge or traumatic injury [1]. Early investigators considered inflammation as a primary host defense system [2]. From this point of view, inflammation is the key reaction of the innate immune response but in some cases it can lead to death, as in anaphylactic shock, or debilitating diseases, as in arthritis and gout. Non-steroidal anti-inflammatory drugs (NSAIDs) have been the mainstay of the treatment of rheumatoid arthritis and other forms of

inflammatory disorders. At present, the use of NSAIDs is limited by their gastrointestinal side effects which include stomach upset, abdominal pain, ulcers, and even gastrointestinal bleeding [3]. Research efforts are therefore currently directed towards studying many promising areas of new treatment approaches for inflammatory disorders. This search for alternative means of managing inflammatory disorders has led to the extensive investigation into the world's rich vegetation for newer and better anti-inflammatory agents. Recent studies have shown that a lot of medicinal plants hold enormous potential as sources of novel anti-inflammatory compounds.

Zapoteca portoricensis (Jacq) HM Hernández (Fabaceae) is a glabrous seasonal shrub with slender unarmed branches. The plant is a native of West Africa, West Indices and Atlantic Coast of America [4]. Aqueous and alcohol extracts of the plant are used traditionally in Southern Nigeria as antidiarrhoea, anticonvulsant, antispasmodic and in the treatment of tonsillitis [5]. The antimicrobial activity of the crude methanol extract has been reported in a previous study [5]. But the traditional use of this extract in the management of tonsillitis, a disease condition associated with microbial inversion and inflammation of the tonsils stimulated our interest in investing the anti-inflammatory constituents of the root extracts and fractions of the plant. In the present study, we report the anti-inflammatory activity of its solvent and column fractions.

Experimental

Plant material

The roots of *Zapoteca portoricensis* were collected between March and April 2008 from Orba, Nsukka, Enugu State, Nigeria. The plant material was authenticated by Mr. Alfred Ozioko of the Centre for Ethenomedicine and Drugs Development, a subsidiary of Bioresources Development and Conservation Program (BCDP), Nsukka, Enugu State. The material was cleaned, air-dried and pulverized.

Chemicals

All the regents were of analytical grade and were used as such without further purification. These included chloroform, methanol, ethyl acetate (Aldrich), silica gel (70-230 mesh), acetyl salicylic acid and Tween 80. Freshly distilled water was used throughout.

Laboratory animals

Albino rats (130-200 g) of either sex purchased from the animal house of the Department of Veterinary Parasitology and Enthomology, University of Nigeria were used for the experiment. The animals were housed in metallic cages and were fed with standard pelletised feed (Grand Creals and Oil Mill Nig Ltd) and water *ad libitum.* The use and care of laboratory animals were conducted in accordance with internationally accepted best practices as contained in the European Community guidelines (EEC Directive of 1986; 86/609/EEC) and approved by the local Ethics Committee of our institution.

Extraction and fractionation

About 800 g of the pulverized dried roots of *Z. portoricensis* was extracted with 3 L of methanol at room for 7 days. The extract (ME) was filtered with Whatman No 1 filter paper and concentrated *in vacuo* using a rotary evaporator. The dried methanol extract (20 g) was fractionated in a glass column (150 cm x 1.5 cm) packed with 200 g of a slurry of silica gel (70-230 mesh). The column was eluted in succession with 500 mL chloroform, 600 mL ethyl acetate and 500 mL methanol to obtain chloroform (CF), ethyl acetate (EF) and methanol (MF) fractions, respectively. These fractions were screened for anti-inflammatory activity in rats.

Column chromatographic separation

About 5 g of the chloroform fraction was chromatographed on silica gel (70-230 mesh, 200 g) packed into a glass column (1.5 x 150 cm) with the bed of 100 cm height. The elution was performed with gradient mixtures of hexane: chloroform 1:3 (400 mL); 1:6 (400 mL); 1:9 (400

mL); chloroform: ethyl acetate 1:1 (400 mL), 1:2 (400 mL), 1:5 (400 mL) and finally ethyl acetate: methanol 1:1 (400 mL), 1:3 (400 mL), 1:5 (400 mL), 1:7 (400 mL). Aliquots of 20 mL were collected and monitored with TLC. Similar fractions were combined to get four major column fractions CF1 – CF4. These fractions were also screened for anti-inflammatory activity in rats.

Phytochemical tests

Phytochemical analysis was carried out on the pulverized root, the methonol extract, and the solvent and column fractions using the procedure outlined by Harbourne [6].

Acute toxicity (LD_{50}) test

The oral acute toxicity of the methanol extract was determined according to the method described by Lorke [7]. Briefly, albino mice (20-30 g) of either sex were divided into 3 groups of 3 animals per group. The extract dispersed in 10% v/v tween 80 was administered to the mice at doses of 10, 100 and 1000 mg/kg and the animals were monitored for 24 h for gross behaviour and mortality.

From the result of the first phase, doses of 140, 225, 370 and 600 mg/kg were administered orally to 4 groups of 3 mouse per group. The animals were monitored for 24 hours for mortality. The LD_{50} was calculated as the geometric mean of the maximum dose that caused 0% death and the minimum dose that caused 100% death.

Anti-inflammatory tests

The acute anti-inflammatory activity of the extract and fractions was evaluated using egg-albumin induced rat paw edema model as previously described [8, 9]. The animals used for the experiment were fasted for 12 hr and deprived of water throughout the period of the experiment in order to minimize variability in edematous response [10]. The extract, solvent and column fraction were solubilized in 10% v/v tween 80 in distilled water and administered orally at a dose of 200 mg/kg to the rats (n = 5). The control groups received 0.4 ml of 10% v/v tween 80 or 100 mg/kg acetyl salicylic acid. One hour post administration of the extract and fractions, 0.10 mL fresh undiluted egg-albumin was administered in the sub-plantar region of the rat hind paw. Paw volume were measured by water displacement method at 0, 1, 2, 3 and 4 hr after egg-albumin injection. The percent inhibition of edema was calculated using the formula:

$$\%\ Inhibition = \frac{(V_0 - V_t)}{V_0} \times 100$$

where V_t is the volume of edema at corresponding time and V_0 is volume of edema of control at the same time [9].

Statistical analysis

The data obtained were analyzed using student's *t*-tests and expressed as mean ± SEM. Differences between means were considered significant at $P<0.05$ [11].

Results

The result of phytochemical analysis of the crude extracts and fractions of *Zapoteca portoricensis* root is shown in Table 1. Crude methanol extract shows the presence of alkaloids, terpenes, steroids, saponins and glycosides. The column fractions showed the presence of only steroids and terpenes.

The results of the acute anti-inflammatory activity study on the extract and fractions of *Zapoteca portoricensis* root are shown in Table 2. At 200 mg/kg (p.o) the crude methanol extract exhibited a significant ($P<0.01$) anti-inflammatory effect with edema inhibition of 71.9% at 3 h. The oral LD_{50} of this extract was calculated to be 470 mg/kg. At 200 mg/kg (po), CF exhibited high and significant ($P < 0.01$) inhibition of edema (59.9 % at 3 h). EF (200 mg/kg, po) exhibited moderate inhibition of edema (29.8% at 3 h) while MF (200 mg/kg, p.o) did not show any edema inhibition at 3 h. The column fractions CF1, CF3 and CF4 at 200 mg/kg (po) showed high and significant ($P < 0.01$) inhibition of edema (62.3, 60.9 and 66.7%)

Table 1: Extracts fractions and phytochemical constituents of *Z portoricensis*

Test	MeOH Extract	$CHCl_3$ Fraction	Ethylacetate Fraction	MeOH Fraction	CF_1	CF_3	CF4
Alkaloids	++	-	+	++	-	-	-
Terpenoids	+	+	+	+	++	++	+
Steroids	+	++	++	+	++	+	+
Glycosides	+	-	++	+	-	-	-
Flavonoids	-	-	-	-	-	-	-
Tannins	-	-	-	-	-	-	-
Saponins	+	+	+	+	-	-	+

- = not detected, + = detected in moderate quantity, ++ = detected in abundant quantity

Table 2: Anti-inflammatory effects of methanol extract and fractions of *Z. portoricensis* on egg-albumin induced rat paw edema

Treatment	Dose (mg/kg)	Edema Volume (mL) (Mean ±SEM		
		1 h	2 h	3 h
Tween 80 (10%)	0.40 ml	1.09 ± 0.08	0.99 ± 0.09	0.57 ± 0.04
MeOH extract	200	0.82±0.08 (24.8)*	0.41 ± 0.04 (58.6) **	0.16 ± 0.06 (71.9) **
$CHCl_3$ Fraction	200	0.98 ± 0.08 (10.1)	0.79 ± 0.09 (21.2)	0.24 ± 0.09 (57.9) **
Ethyl acetate fraction	200	1.03 ± 0.08 (5.5)	0.74 ± 0.11 (25.3)	0.40 ± 0.05 (29.8) *
MeOH fraction	200	0.92 ± 0.09 (15.6)	0.91 ± 0.05 (8.1)	0.67 ± 0.04 (-)
CF1	200	0.54 ± 0.13 (50.5)*	0.42 ± 0.13 (57.6)*	0.21 ± 0.08 (63.2) **
CF3	200	0.57± 0.08 (47.7)	0.21 ± 0.09 (78.8)*	0.21±0.1(60.87)**
CF4	200	0.55± 0.11 (49.5)	0.35 ± 0.13 (64.6)*	0.19 ± 0.1 (66.7) **
Acetylsalicylic acid	100	0.88 ± 0.09 (19.3)	0.35 ± 0.12 (64.7)*	0.31 ± 0.08 (45.6) *

**P<0.05, **P<0.01, (n=5): significant compared to the vehicle treated group. Values in parenthesis represent percent inhibition of edema.*

respectively. The anti-inflammatory effect of the crude methanol extract, chloroform fractions and its column fractions are significantly ($P < 0.05$) higher than that of aspirin.

Discussion

Zapoteca portoricensis (Fabaceae) root extract is used in folkmedicine as anti-diarrhoea, anticon-vulsant, antispasmodic and in management of tonsillitis. In the present study, we have investigated the anti-inflammatory effect of the extract and fractions of this plant material. Our results have shown that this plant material possesses very high and significant anti-inflammatory activity. In an earlier report by Nwodo and Uzochukwu [5], the crude methanol root extract of this plant was shown to exhibit antimicrobial effect against some strains of both gram positive and gram negative bacteria. This observation together with our present report seem to validate the folkloric use of this plant material in the management of tonsillitis, a disease condition known to be associated with inflammation and microbial inversion. The oral LD_{50} of this extract was calculated to be 470 mg/kg and this value indicates a fairly safe extract [12].

The phytochemical investigation of the crude methanol extract revealed the presence of alkaloids, steroids, terpenoids, saponins and glycoside (Table 1). Some of these phytoconstituents have been shown in several studies to possessing anti-inflammatory activity [13-15]. Steroids and triterpenoids are known to exert their anti-inflammatory effect by inhibiting phospholipase A_2, a key enzyme of arachidonic acid metabolism, thereby stopping prostaglandin synthesis [16]. Some plant steroids have also been shown to stabilize lysozomal membranes thereby inhibiting the release of pro-inflammatory mediators [17]. Alkaloids and flavonoids have also been shown to possess anti-inflammatory activity by inhibiting the action of arachidonic acid metabolism via the cyclooxygenase and 5-lipoxygenase pathways [18, 19]. Flavonoids also inhibit inflammation by lysozomal membrane stabilization and also by inhibiting migration of leucocytes to the site of inflammatory stimulus [20, 21. Interestingly, we observed in this study that the chloroform fraction (the most active fraction) contained mainly steroids and triterpenoids. It is possible that the observed high anti-inflammatory activity of this fraction is related to the presence of these phytoconstituents. In the present study, we decided to fractionate this fraction further with the view of isolating the anti-inflammatory principles.

The result of the anti-inflammatory effect of the column fractions as shown in Table 2 indicate that the column fractions exhibited very high and significant inhibition of acute edema induced by egg albumen. The egg-albumin induced inflammatory reaction has been shown in previous studies to occur in two phases. The early phase, which begins at 1 h after administration of the phlogistic agent results from the release of histamine, serotonin and bradykinin; while the later phase (3-5 h after irritant administration) is associated with the release of mediators such as prostaglandins, protease and lysozymes [8, 22, 23]. As shown in Figure 1, in all the fractions tested, edema peaked at 1 h and gradually declined to 3 h. It is possible that these fractions may have suppressed the release or antagonize the action of the early phase mediators like histamine, serotonin and/or bradykinin.

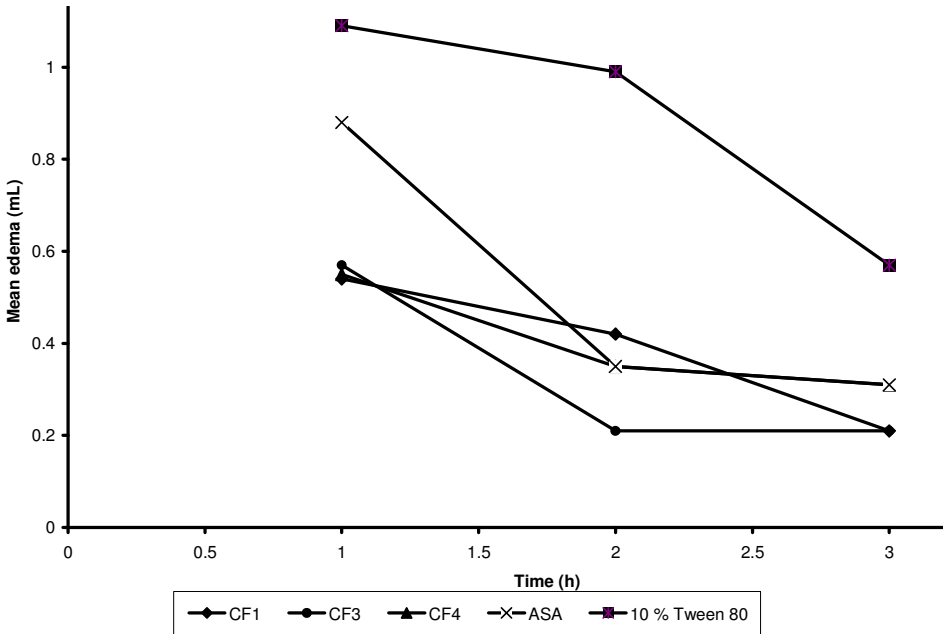

Figure 1: Effect of the column fractions (200 mg/kg) of *Z. portoricensis* and ASA (100 mg/kg) on the development of acute edema after sub-plantar injection of egg albumin in rats

Conclusion

The effect of the fractions on late phase mediators of inflammation could not be ascertained as the edema induced by egg albumen in this experiment was not sustained beyond 4 h. This observation is consistent with recent reports with egg albumen-induced edema models of inflammation [9, 17, 20]. The exact mechanisms of action, however, are subjects for further investigation.

The results of the present study have shown that the root of *Z portoricensis* possesses significant anti-inflammatory activity in acute inflammation in rats thus, justifying its use by traditional medicine practitioners in the management of tonsillitis, a disease condition associated with inflammation. Steroids and triterpenoids may be responsible for the observed anti-inflammatory activity. Purification and characterization of these potent anti-inflammatory compounds as well as elucidating the actual mechanism of anti-inflammatory activity are in process.

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Contribution of Authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. MOA was directly involved in the day to day laboratory work and data collection. NJN conceived and designed the work and supervised the laboratory work. FBC O assisted in the design of the work and in the chromatographic separation as well as assisted in the analysis of the data and putting together the final manuscript. All authors read the manuscript and approved it.

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