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## Original Research

### Anti-inflammatory activity of *Russelia equisetiformis* Schlecht & Cham: identification of its active constituent

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#### Abstract

**Aim:** The present research work was carried out to isolate and identify the chemical constituent responsible for the anti-inflammatory activity of *Russelia equisetiformis*.

**Method:** Isolation was carried out using column chromatography. The structure of the isolated compound was identified and established using the available spectroscopic techniques including high-resolution electron mass spectrum. The anti-inflammatory activity was evaluated using egg albumin-induced paw edema, formaldehyde-induced arthritis and cotton pellet granuloma in-vivo tests. Prednisolone was used as a standard drug.

**Results:** Lupeol was isolated as colorless crystals (mp 213-215°C). Lupeol at the doses of 10, 20, 40 mg/kg produced significant ( $P < 0.05$ ) and dose-dependent inhibition of egg albumin-induced edema and edematous response to arthritis. In chronic model of granuloma pouch in rat, lupeol (20 and 40 mg/kg), significantly ( $P < 0.05$ ) and also dose-dependently reduce the granuloma weight.

**Conclusion:** These findings suggest that lupeol isolated from extract of *Russelia equisetiformis* possesses anti-inflammatory activity in acute and certain aspects of chronic inflammation.

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## INTRODUCTION

*Russelia equisetiformis* (Schlecht & Cham) belongs to the family Scrophulariaceae [1]. It is a native of Tropical America, commonly known as firecracker, coral and fountain plant and may be cultivated easily, freely naturalized in sandy clearings, long streets, roadsides and grow accidentally in quite a number of places by the roadsides [2, 3]. It is an evergreen shrub with tiny dark green leaves, scale-like in structure, growing on thin rush-like stems. The branches are arched, bearing 1-2 inch long tubular flowers. The plant blooms abundantly round the year, if given optimum conditions [4]. Medicinally, the plant is used for the treatment of diabetes and leukemia in Southwestern,

Nigeria [5]. Personal communication with the tribes in this part of Nigeria revealed that, the whole plant is used for the treatment of pain and inflammation.

The methanol extract of the whole plant is reported to have both analgesic and anti-inflammatory activities [6]. Phytochemically, the plant is reported to contain triterpenes of lupane type [7]. The aim of this study is to isolate compound responsible for the anti-inflammatory activity of *Russelia equisetiformis*. Therefore, the major compound of n-hexane extract of *R. equisetiformis* was isolated and identified as lupeol and was studied for anti-inflammatory activity.

## MATERIALS AND METHODS

The experimental protocols and procedures used in this study were approved by the Ethical committee, University of Ibadan, Ibadan, Nigeria and conform to the guideline of the care and use of animals in research and teaching (NIH publications no 85-93, revised 1985).

### Plant material

The plant sample was collected in the month of October, 2010 from Bodija in the South West of Nigeria. The plant was identified in the herbarium of the Forest Research Institute; Ibadan, Nigeria, where voucher specimen was deposited with voucher's number 106998. The plant sample was air-dried at room temperature, and reduced to powdery form using electric blending machine.

### Extraction and isolation of lupeol

A 400 g of powdered whole plant material of *R. equisetiformis* was extracted with 100% methanol in the cold for 72 h; it was filtered and concentrated to dryness using rotary evaporator. The crude methanol extract was re-dissolved in water and partitioned successively with four organic solvents to obtain n-hexane, dichloromethane, ethylacetate and n-butanol fractions. These fractions were screened for anti-inflammatory activity using the method described in the section on anti-inflammatory tests. The n-hexane was found to be the most active fraction. 10g of hexane fraction packed in a column (92x6cm) with silica gel 60 (0.2-0.5mm) and was bulked using 100% hexane and subsequently hexane: ethylacetate at ratio 90:10, 80:20 and 70:30. Fractions obtained were into 3 different subfractions. The fraction (2.23g) showing TLC profile of lupeol was purified in a column (36x1.5cm) using silica gel 60(0.040-0.06mm) eluted with 50% hexane: ethylacetate in step 85:15, 75:25 and 65:35. TLC was sprayed with 1 % vanillinsulphuric acid reagent and heated to 110°C for 5 min to confirm the presence of lupeol. The structure of compound was established using the available spectroscopic techniques including high-resolution electron mass spectrum 1H NMR ( $\delta$  [CDCl<sub>3</sub> 300MHz, 298K], 13C NMR. ( $\delta$ [CDCl<sub>3</sub> Hz, 298K]. Rf 0.32(hexane: Ethyl acetate 9:1 v/v). The isolated compound obtained was freshly prepared as a fine homogenized suspension in Tween-80 (2% v/v).

### Animals

Wistar rats of either sex weighing about 120-160 g, divided into groups of six animals each, were used for this study. The animals were housed in a well ventilated pre-clinical animal house, College of Medicine, University of Ibadan, and were acclimatized in the laboratory for two weeks before experimentation,

and fed with standard diet (Ladokun Feeds Nigeria Ltd) and water *ad libitum*.

### Evaluation of Anti-inflammatory activity

#### *Egg albumin-induced paw edema in rats*

The rat paw edema method as described by [8] was used. Acute inflammation was measured in terms of change in volume of the rat hind paw [9] induced by sub-plantar injection of egg albumin [10 11] Animals of six per group received 10, 20 or 40 mg/kg of lupeol administered intraperitoneally. Thirty minutes later, edema was induced with 0.1 ml of fresh undiluted egg albumin injected into the sub plantar region of the right hind paw of the rats. The volume of distilled water displaced by the treated paw was measured before and after 3 and 24 h induction of edema. Control groups received either equivalent volume of vehicle ((2% v/v) or prednisolone (5 mg/kg). Inflammation was assessed as the difference between the zero time volume of the treated paw ( $V_0$ ) and the volume at various times ( $V_t$ ) after the administration of the phlogistic agent. Percentage inhibition of edema was calculated using [12 13] relation.

$$\text{Inhibition of edema (\%)} = 100 \times \left(1 - \frac{ax}{by}\right)$$

Where a = mean paw volume of treated rats at various time after egg albumin injection; x = mean paw volume of treated rats before egg albumin injection; b = mean paw volume of control rats at various time after egg albumin injection; y = mean paw volume of control before albumin injection

#### *Arthritis induced by formaldehyde in rats*

The formaldehyde-induced arthritis method of [14] was used. On day 1, adult rats of six per group received 10, 20 or 40 mg/kg of lupeol administered i.p. Thirty minutes later; arthritis was induced by sub plantar injection of 0.1 ml of 2.5% formaldehyde solution and repeated on day 3. Arthritis was assessed by measuring the volume of distilled displaced by the paw before induction of arthritis and after induction once every day for 10 days. Control animals received either i.p. Prednisolone (5 mg/kg) or equivalent volume of vehicle (2%, v/v Tween 80). The edematous response was quantified as the area under the curve (AUC) of the time-course of the arthritic event. The AUC was calculated using the trapezoidal rule. The level of inhibition of arthritis was calculated using the relation

$$\left[1 - \frac{(\text{AUC}_t)}{\text{AUC}_c}\right] \times 100$$

Where AUC<sub>t</sub> = AUC of the control group; AUC<sub>c</sub> = AUC of the treated group.

#### *Cotton-pellet granuloma test in rats*

The effect of lupeol isolated from the plant's extract on chronic inflammation was evaluated using cotton-pellet

granuloma test in rats [15]. On day 1, rats received 10, 20 or 40 mg/kg i.p. of lupeol. Control animals received either Prednisolone (5 mg/kg) or equivalent of vehicle (2% v/v Tween 80). Thirty minutes later autoclaved cotton pellets 50±1 were implanted on the back of the rats under diethyl ether anesthesia. Drugs were administered daily for 7 days. On day 8, animals were killed by overdose of ether. The pellets were dissected out, dried in an oven at 60° C and weighed to determine the level inhibition of granuloma. The level of inhibition of granuloma tissue development was calculated using the relation:

$$\frac{T_c - T_x}{T_c} \times 100$$

### Data analysis

Data were expressed as mean ± SEM. Statistical analyses was performed by one-way, ANOVA followed by Dunnett' s test. P values < 0.05 were considered significant

### RESULTS

Characteristically, lupeol showed a violet spot on TLC when sprayed with 1 % vanillin-sulphuric acid reagent and heated at 110° C for 5 min. Lupeol was isolated as colorless crystals (mp 213-215°C). The spectral data was found in accordance with the literature data of lupeol [16 17].The <sup>13</sup>C NMR spectra showed 28-carbon atoms giving a characteristic of pentacyclic saturated

system and only one unsaturated carbon absorption at δ 150.9 ppm for alkenes. The hydroxyl carbon was found at δ 78.9. The compound was identified by comparison of its NMR spectrum with literature for Lupeol, a pentacyclic compound already isolated from other plants (Fig. 1)

<sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 0.70 (3H, s); 0.76 (6H, s); 0.80 (3H, s); 0.92 (3H, s); 1.00 (6H, s);

<sup>13</sup>C-NMR (CDCl<sub>3</sub>): δ 40.0 – 48.2(C); 55.3 (CH, t); 50.4 (CH, t); 25.1 (CH<sub>2</sub>, d); 150.9 (CH<sub>2</sub>, s); 15.4 – 20.9 (CH<sub>3</sub>, s); and 78.9 (ROH, t).

Lupeol produced dose-dependent inhibition of egg albumin-induced paw edema at 10, 20 and 40 mg/kg after 24 h as compared to prednisolone (5 mg/kg) (Table 1). Investigation of the effect of lupeol on the proliferative phase of inflammation revealed that lupeol isolated from the hexane fraction demonstrated significant (*P*>0.05) inhibition of global edematous response to formaldehyde-induced arthritis in a dose-dependent manner, with maximum inhibitory effect of 56 %, greater than a standard anti-inflammatory steroid drug, prednisolone ( 50.8 %) (Table2). However, in the cotton pellet granuloma test, lupeol was not much effective as standard anti-inflammatory steroid drug (prednisolone) in inhibiting the growth of granuloma tissue (Table 3).

**Table 1.** Effect of lupeol isolated from *R. equisetiformis* on egg albumin-induced edema

Treatment	Dose mg/kg Or mg/kg	Edema volume (ml)		% inhibition	
		3 h	24 h	3 h	24 h
Control (2%Tween80)	10	0.36± 0.01	0.31±0.01	-	-
Lupeol	10	0.34±0.01	0.24±0.01*	5	23
	20	0.32±0.01	0.21±0.01*	11	33
	40	0.28±0.01*	0.18±0.01*	23	44
Prednisolone	5	0.25±0.01*	0.15±.0.01*	31	55

\*P< 0.05 vs. Control. Values of edema are mean ± S.E.M (n =6)

**Table 2.** Effect of lupeol isolated from *R. equisetiformis* on formaldehyde-induced arthritis in rats.

Treatment	Dose mg/kg Or mg/kg	Edema volume (ml)	% inhibition
Control (2%Tween80)	10	4.59±0.06	-
Lupeol	10	2.52± 0.03*	45.0*
	20	2.18±0.03*	52.5*
	40	2.01±0.03*	56.0*
Prednisolone	5	2.33±0.03*	50.8*

\*P< 0.05 vs. Control. Values of edema are mean ± S.E.M (n =6)

**Table 3.** Effect of lupeol isolated from *R. equisetiformis* on cotton pellet granuloma

Treatment	Dose mg/kg or mg/kg	Cotton pellet granuloma	
		Weight of granuloma (mg)	% inhibition
Control (2%Tween80)	10	186.67±1.21	-
Lupeol	10	171.34±1.24	8.0
	20	145.52±2.02*	22*
	40	128.15±2.24*	31*
Prednisolone	5	92.3±1.65*	50.6*

\*P< 0.05 vs. Control. Values of granuloma weight shown are mean ± S.E.M (n =6)

## DISCUSSION

Anti-inflammatory drugs, presently available for the treatment of various inflammatory disorders have one or more adverse or side-effects [18]. The presence of anti-inflammatory activity in triterpenes seems interesting, since they possess hydro-aromatic ring system, which is more or less similar to that of steroidal anti-inflammatory drugs, devoid of side-effects [19]. The inhibition of lupeol of the acute phase of edema caused by egg albumin, suggests that lupeol may suppress both the early and later phases of the acute inflammatory response. The lupeol may have inhibited the release or actions of the various chemical mediators such as easily histamine, 5HT, kinins and prostanoids known to mediate acute inflammation induced by phlogistic agents such as egg albumin.[20 21, 22, 23, 24, 25]. The disparity in the activity in the two chronic inflammatory models (formaldehyde-induced arthritis and cotton pellet granuloma), suggests that lupeol may predominantly affect certain aspects of inflammatory response associated with formaldehyde arthritis, while exerting little or no effect on the process involved in granulomatous inflammation. Granuloma of chronic inflammation comprises an accumulation of modified macrophages arranged in small clusters or nodular collections or surrounded by a cuff of lymphocytes [26] and is a consequence of cell-mediated immunity [27]. The inability of lupeol to inhibit granulomatous inflammation may be due to its inability to inhibit the accumulation of macrophages and lymphocytes in chronic inflammation and therefore has no effect on cellular mediated immune responses. However, these macrophages and lymphocytes at the inflammation or injury site are known to secrete peptide growth factors (PGF), which partly mediate the process of healing and repair [28]. It implies therefore, that lupeol may promote tissue repair by mediating the pro-healing actions of PGF, inhibit the expression of tissue necrotic factor (TNF), which is known to exacerbate the later stage of formaldehyde-induced arthritis [14]. Necrotic tissue on its own can perpetuate inflammation through

several mechanisms such as the release of mediators from dead or dying passenger leukocytes.[29] Thus, it is possible that lupeol isolated from *R. equisetiformis* may modulate the arthritis event through mediators' release inhibition by preventing formaldehyde-induced tissue necrosis as well as tissue destruction seen in arthritis. However, it may have little inhibitory effect on cellular response associated with chronic inflammation. Also in arthritis, there is increase in the level of lipid peroxide, superoxide dimutase (SOD), glutathione peroxidase (GPx) and catalase (CAT).[30]. Lupeol and its esters have been reported to reduce the level of the above mentioned enzyme involved in lipid peroxidation in arthritic-induced animals [31]. Therefore, it is not unlikely, that lupeol isolated from *R. equisetiformis* extract demonstrated its anti-arthritic action, by reducing the alterations induced in arthritic animals in the levels of lipid peroxide, SOD, GPx and CAT. In summary, the major compound isolated from the hexane extract of *R. equisetiformis* possesses anti-inflammatory activity and may be the reason for its traditional use in inflammatory disease conditions. This study confirms that, the compound responsible for the anti-inflammatory activity is lupeol.

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