



**ANTI-INFLAMMATORY AND ANTIOXIDANT EFFECTS OF THE STEM BARK
AQUEOUS EXTRACT OF *RAUWOLFIA VOMITORIA* (APOCYNACEAE) IN FEMALE
WISTAR RATS**

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ABSTRACT

Rauwolfia vomitoria is widely used in African traditional medical practice in the treatment of several inflammatory-related disorders. Since, several studies have suggested that the etiology of inflammation can partly be explained by oxidative stress, this study was directed to evaluate the anti-inflammatory and antioxidant effects of the stem bark aqueous extract of *Rauwolfia vomitoria* in female Wistar rats. In sub-chronic study, cotton pellet and carrageenan induced granuloma as well as formalin induced paw oedema models were used to evaluate the anti-inflammatory potential of the plant extract. For the chronic model, we assessed the effect of the extract against Complete Freund's Adjuvant (CFA) induced inflammation. Administration of the aqueous extract (300 mg/kg) significantly reduced the formation of humid (31.25%) and dry (28.49%) granuloma in cotton pellet method compared with control. At the same dose, the plant extract also exhibited a significant sub-chronic anti-inflammatory effect by decreasing exudate volume (38.46%) and leukocytes number (53.68%) in air pouch test, and by reducing the paw oedema (49.60%) induced by formalin. Daily oral administration of the extract (300 mg/kg) or dexamethasone (1 mg/kg) for 10 and 12 days significantly inhibited by 55.98% and by 74.41% the paw oedema induced by formalin and CFA respectively when compared to control groups. CFA used alone significantly decreased reduced glutathione (GSH) level as well as superoxide dismutase (SOD) and catalase (CAT) activities in liver, kidney and spleen when compared to normal rats. Treatment with *Rauwolfia vomitoria* extract and dexamethasone significantly restored GSH, SOD and CAT in all investigated tissues. In addition, the plant extract significantly restored malondialdehyde (MDA), protein and nitrite concentrations in serum and tissues (liver, spleen and kidney) by reducing their content, as compared to CFA control group. Keeping in this view, the data suggest that *Rauwolfia vomitoria* aqueous extract may possess anti-inflammatory and antioxidant effects.

KEYWORDS: *Rauwolfia vomitoria*, inflammation, Complete Freund's Adjuvant, anti-oxidant, granuloma.

INTRODUCTION

Inflammatory disease is one of the major health problems worldwide. Oxidative damage of cellular biomolecules such as proteins and lipids is thought to play a crucial role in the incidence of several chronic inflammations.^[1] It is among causative factors in induction of many chronic and degenerative diseases including atherosclerosis, ischemic heart disease and arthritis. Arthritis disease generates many free radicals which maintain inflammations as long as possible. Although, several agents are known to treat these types

of inflammations, long-lasting use is not advisable due to the side effects.^[2, 3] Therefore, there is an urgent need to develop drugs with less adverse effects for alleviation of disorders initiated inflammation and antioxidant effects.^[4, 5] Since 1980, alternative anti-inflammatory agents and antioxidant have regained their popularity to provide new drugs. Indeed, various plant preparations have been used and claimed to prevent and treat inflammation. Most of these medicinal plants are reported to contain various biologically active principles.^[6, 7]

Rauwolfia vomitoria is a semi-deciduous tree of up to 20 m tall, with a dense rounded crown and tall, bare stem which is widespread in many countries in Africa and grown along forests of Cameroon. It is commonly known in some ethnic groups in Cameroon as *Nteu Nta Cheu* in "Bamileke"; *Etoe* in "Ewondo" and *Thauvengo* in "Babungo". The roots of this plant are used as a remedy for severe epigastric pain while leaves are used to treat headache.^[8] The stem bark has been traditionally employed in Bangou city, West region, Cameroon to treat several pathological conditions including helminthiasis, gastroenteritis, infertility, neurosis, psychosis, rheumatism and headache. We previously reported the effect of *R.vomitoria* on pain and acute inflammation^[9]. To date, there is little scientific evidence to support the traditional use of *Rauwolfia vomitoria* in the treatment of sub-chronic and chronic inflammations and the possible mechanisms involved. Therefore, this study was designed to evaluate the anti-inflammatory and antioxidant effects of the stem bark aqueous extract of *Rauwolfia vomitoria* in various models of sub-chronic and chronic inflammations.

MATERIALS AND METHODS

2.1. Plant material

2.1.1. Plant collection and identification

Plant was collected in Kepche village, Bangou city, West Region, Cameroon, in August 2010 and identified at the National Herbarium Yaounde, Cameroon by comparison with voucher specimen N^o16887/HNC.

2.1.2. Preparation of the aqueous extract of *Rauwolfia vomitoria*

The fresh stem bark of *R. vomitoria* was cut into pieces, air-dried at room temperature for two weeks after which they were pulverized using a mechanical blender. 2 kg of the powder obtained was stored in an airtight container for further use. 1 kg of powder was macerated in 9 liters of distilled water for 24 h and the filtrate obtained was evaporated in an incubator at 45 °C and 55.50 g of dark brown solid extract was obtained (yield of 2.77 %). The plant extract was dissolved in distilled water and administered to rats. Following our previous work on preliminary screening test, carrageenan-induced inflammation, 100, 200 and 300 mg/kg doses of the plant were selected.

2.1.3. Quantitative phytochemical analysis

2.1.3.1 Total phenolic content

Total phenolic content present in *Rauwolfia vomitoria* extract was determined using the Folin-Ciocalteu method.^[10] The reaction mixture contained: 200 µl of diluted extract (1 mg/ml), 800 µl of freshly prepared diluted Folin-Ciocalteu reagent and 2 ml of 7.5 % sodium carbonate. The final mixture was diluted to 7 ml with distilled water. The mixtures were kept in the dark at ambient conditions for 2 h to complete the reaction. Total polyphenols content was calculated using the following equation $Y=0.004X$, $R^2=0.979$, based on the calibration curve, where Y was absorbance. The

absorbance was at 765 nm. The results were expressed as mg of quercetin/g of dried extract (mg of QT/g DE).

2.1.3.2 Total flavonoids content

Total flavonoids content was determined using aluminum chloride (AlCl₃) according to a known method using quercetin as a standard.^[10] The extract (0.1 ml of 1 mg/ml) was added to 0.3 ml of distilled water followed by 5% NaNO₂ (0.03 ml). After 5 min, the reaction mixture was treated with 0.2 ml of NaOH (1 mM). Finally, the reaction mixture was completed to 1 ml with distilled water and the absorbance was measured at 510 nm. Total flavonoids content was calculated using the following equation $Y=4.535X$, $R^2=0.995$, based on the calibration curve, where Y was absorbance. The results were expressed as mg of quercetin/g of dried extract (mg of QT/g DE).

2.1.3.3. Total flavonols content

Total flavonols in the extract were estimated using a known method with modification.^[10] To 2.0 ml of sample (standard), 2.0 ml of 2% AlCl₃ ethanol and 3.0 ml (50 g/l) sodium acetate solutions were added. The absorption at 440 nm was read after 2.5 h of incubation at 20°C. Extract samples were evaluated at a final concentration of 0.1 mg/ml. Total flavonols content was calculated as quercetin (mg/g) using the following equation $Y=0.002X$, $R^2=0.936$, based on the calibration curve, where Y was absorbance and the concentration was expressed as mg of quercetin/g of dried extract (mg of QT/g DE).

2.1.3.4. Total vitamin C content

Total vitamin C content determination was accomplished according to the method of Ghate in 2013.^[11] In brief, aliquots of extract (1 mg/ml in distilled water) were mixed with 1 ml of 2, 4-dinitro-phenylhydrazine reagent and was incubated at 95°C for 15 min. After incubation, 5 ml of 85% H₂SO₄ was added dropwise to the reaction mixture in a cold condition. After 30 min, the absorbance was measured at 520 nm. All tests were performed three times. The results were expressed as mg of vitamin C/g of dried extract (mg of VC/g DE).

2.1.3.5. Total alkaloid content

Quantification of alkaloid content of extract was done using the protocol of Hazra in 2008.^[12] To 1 ml of extract (1 mg/ml in distilled water), 0.1 ml of FeCl₃ (2.5 mM FeCl₃ in 0.5 M HCl) was added followed by addition of 0.1 ml of 1,10-phenanthroline. After incubation for 30 min at 70 °C the absorbance was measured at 500 nm. All tests were performed three times. The alkaloid content was quantified from the berberin standard graph.

2.2. Animals

Female Wistar rats were used for all experiments. They were housed in plastic cages light/dark cycle at ambient temperature. The animals were fed with standard food and water *ad libitum* and fasted for 16 hours (with free access to water) before anti-inflammatory tests. The

experimental protocol was in conformity with the guidelines of the Cameroon National Ethical Committee on the use of laboratory animals for scientific research (CEEC Council 86/609).

2.3. Anti-inflammatory activities of *Rauwolfia vomitoria*

2.3.1. Cotton pellet- induced granuloma

Effect of *Rauwolfia vomitoria* against cotton pellet-induced granuloma was tested, according to the method of D'Arcy in 1960. ^[13] Adult female Wistar rats weighting 140-170 g were randomly divided into five groups of five in each. Extract, diclofenac and distilled water were administered *per os* 30 min prior to implantation of cotton pellets. Two sterilized cotton pellet weighting 10 mg each were implanted subcutaneously into the both sides dorsal area of each after anesthetized rat (0.3 ml of valium and 0.15 ml of ketamin). Group 1 received distilled water at the dose of 10 ml/kg (Control). Groups 2, 3 and 4 received aqueous extract at the doses of 100, 200 and 300 mg/kg, respectively. Group 5 received diclofenac at the dose of 3 mg/kg (standard). All treatments were given by gastric gavage for 7 consecutive days from the day of cotton implantation. On the 8th day, rats were anesthetized and pellets together with the granuloma tissues were carefully removed surgically and weighted (humid weight). After dried in an incubator at 60 °C, cotton-pellets were weighted (dry weight). The percentage of inhibition of the formation of granuloma tissue was calculated using the following formula.

$$\%I = (1 - W_t / W_c) \times 100$$

Where, %I = Percentage of Inhibition; W_t = Average weight of pellets in treated group;

W_c = Average weight of pellets in control group.

2.3.2. Carrageenan- induced granuloma

The anti-inflammatory potential of the extract was tested in carrageenan induced granuloma according to the modify procedure of Ghost in 2000. ^[14] Adult female wistar rats weighting 140-170 g were randomly divided into five groups of five in each. Extract, diclofenac and distilled water were administered *per os* 30 min prior to injection of air and carrageenan. Sub-cutaneous dorsal granuloma pouch was made in ether anesthetized rats by injecting 6 ml of air, followed injection of 4 ml carrageenan (2 %). On day 1: Group 1 or control received distilled water (10 ml/kg). Groups 2, 3 and 4 received aqueous extract at the doses of 100, 200 and 300 mg/kg, respectively. Group 5 received diclofenac as drug reference (3 mg/kg). All treatments were orally administered for 7 consecutive days from the day of carrageenan injection. On the 8th day, rats were anesthetized and exudates were taken out by siringe. The leukocyte count from exudates was carried out using Neubauer chamber and results were expressed as leukocytes per mm³ of exudative fluid.

2.3.3. Formalin- induced paw oedema

To determine the effect of *R. vomitoria* against formalin-induced paw oedema, adult female Wistar rats weighting 120-130 g were used. Animals were fasted for 16 hours; these female rats were randomly divided into five groups of five in each. The plant extract, distilled water and dexamethasone were administered *per os* 1 h before the injection of formaldehyde and once a day for 10 days. ^[15] Group 1 or control received distilled water (10 ml/kg). Groups 2, 3 and 4 received aqueous extract at the doses of 100, 200 and 300 mg/kg, respectively. Group 5 received dexamethasone (1 mg/kg, drug reference). The linear circumference was drawn with permanent marker on the rat's right hind paw. Inflammation was induced by injecting formaldehyde (2%, 0.1 ml) into right hind limb of each rat under the subplantar aponeurosis on day zero and third day to induce progressive swelling of paw. Measurement of paw size was done by mean of volume displacement technique using plethysmometer 37140 Ugo Basile, *Italia* immediately before injection and 0, 2nd, 4th, 6th, 8th and 10th day after formaldehyde injection. The percentage of inhibition of oedema was obtained for each group using the following ratio.

$$\%I = \frac{(V_t - V_o)_{\text{control}} - (V_t - V_o)_{\text{treated}}}{(V_t - V_o)_{\text{control}}} \times 100$$

Where, %I = Percentage of Inhibition; V_t = Average volume for each group after treatment;

V_o = Average volume obtained for each group before treatment.

2.3.4. Complete Freund's Adjuvant-induced inflammation

Adult female Wistar rats weighting 120-130 g and fasted for 16 hours were used for this experiment. These rats were randomly divided into 6 groups of five in each. The methodology used was that of Zhaoliang in 2000 ^[16] with some modifications. Group 1 were normal rats. Group 2 received distilled water (10 ml/kg control). Groups 3, 4 and 5 received aqueous extract at the doses of 100, 200 and 300 mg/kg, respectively. Group 6 received dexamethasone (1 mg/kg, reference drug). The linear circumference was drawn with permanent marker on the rat's right hind paw. Inflammation was induced by injecting 0.1 ml of Complete Freund's Adjuvant (CFA) into right hind limb of each rat under the subplantar aponeurosis (Groups 2 to 6). Measurement of paw size was done by mean of volume displacement technique using plethysmometer (*Ugo Basile, Italia*) on day 1 before CFA injection and 1, 2, 3 and 4 h after CFA injection to see the degree of swelling. The treatment, excepted group 1, was initiated on day 9, when the joints were supposed to be well inflamed. ^[17] The volume of paws was measured every 4 days from day 1 to day 21 and the percentage of inhibition of oedema was calculated as previously mentioned.

At the end of the experiment, rats were killed by decapitation and the blood was collected for nitrite serum

determination. Liver, spleen and kidney were removed and homogenised in Tris–phosphate buffer (0.1 M, pH 7.4) to measure reduced glutathione (GSH), catalase (CAT), superoxide dismutase (SOD), malondialdehyde (MDA), proteins and nitrites.

2.4. Anti-oxidant activity

2.4.1. Measurement of some biomarker on oxidative stress was as follow

The procedure to estimate the reduced glutathione (GSH) level was done follow to the method described by Ellman in 1959.^[18] The activity of catalase in the tissues was determined by the method of Sinha in 1972.^[19] SOD activity was measured in supernatant of homogenate by the method of Misra and Fridovich in 1972.^[20] The MDA levels were determined by the method of Ohkawa in 1979.^[21] The protein content in the homogenate was determined by the method of Gornal in 1949.^[22] The

presence of nitrite, a stable oxidized product of Nitric oxide (NO), was determined as according to the method described by Kim in 2001 and Slack in 1987.^[23,24]

2.5. Statistical analysis

All values were presented as mean \pm S.E.M of five rats. Differences between means were assessed by Two-way analysis of variance (ANOVA), followed by benferroni post test using Graph pad prism 5.03. Different significant was considered at $P < 0.05$.

RESULTS

3.1. Quantitative phytochemical analysis

Quantitative phytochemical analysis of aqueous extract of *Rauwolfia vomitoria* revealed the quantity per g dried extract (DE) of some bioactive phytochemicals such as, polyphenols, flavonols, flavonoids, vitamin C and alkaloids (Table 1).

Table 1: Quantitative phytochemical analysis of the stem bark aqueous extract of *R. vomitoria*

Total polyphenols (mg of QT/g DE)	Total flavonols (mg of QT/g DE)	Total flavonoids (mg of QT/g DE)	Total vitamin C (mg of VC/g DE)	Total alkaloids (mg of BER/g DE)
127.83 \pm 1.96	76.83 \pm 1.33	20.02 \pm 0.00	46.67 \pm 1.84	43.70 \pm 1.61

Results are expressed as mean \pm S.E.M, n=3 for tests performed three times. DE: Dried extract. BER: Berberin. VC: Vitamin C. QT: Quercetin.

3.2. Anti-inflammatory activity of *R. vomitoria*

3.2.1. Cotton pellet-induced granuloma

Results of the anti-inflammatory effect of the aqueous extract on cotton pellet-induced granuloma are presented in table 2. A significant inhibition of humid and dried granuloma was observed with the different doses of the extract and diclofenac when compared to control group. The highest dose of extract (300 mg/kg) and standard drug (diclofenac) reduced respectively by 31.25% and 34.69% the formation of granuloma tissue. Furthermore, the extract (300 mg/kg) and diclofenac (3 mg/kg) also significantly decreased the dried weight granuloma formation by 28.49% and 30.96% respectively.

Table 2: Effect of oral administration of the aqueous extract of *Rauwolfia vomitoria* on cotton pellet-induced granuloma in rats.

Treatment	Dosage (mg/kg)	Humid granuloma weight (g)	Dry granuloma weight (g)
Control	-	2.50 \pm 0.11	0.73 \pm 0.07
Extract	100	2.01 \pm 0.11 ^A (19.47)	0.53 \pm 0.06 ^A (27.12)
Extract	200	1.80 \pm 0.10 ^B (27.80)	0.56 \pm 0.05 ^A (22.73)
Extract	300	1.72 \pm 0.11 ^X (31.25)	0.52 \pm 0.06 ^A (28.49)
Diclofenac	3	1.63 \pm 0.12 ^X (34.69)	0.50 \pm 0.05 ^A (30.96)

Results are expressed as mean \pm S.E.M, n=5. Percentages of inhibition are in brackets. The statistical analysis was performed on absolute data. ^Ap<0,05, ^Bp<0,01 and ^Xp<0,001, significantly different compared to control.

3.2.2. Carrageenan-induced granuloma.

As shown in table 3, the extract of *R. vomitoria* produced significant reduction of the exudat volume at the doses of 100, 200 and 300 mg/kg at the rate of 33.55, 33.63 and 38.46% respectively when compared to control. Additionally, the total leukocyte count for the plant

extract (200 and 300 mg/kg) was respectively 3250 and 2200 compared to control (6250). Diclofenac (3 mg /kg) reduced by 39.56% the exudative fluid volume and the total leukocyte count was 1650.

Table 3: Effect of oral administration of aqueous extract of *Rauwolfia vomitoria* on carrageenan- induced granuloma in rats.

Treatment	Dosage (mg/kg)	Exudative fluid volume (ml)	Total leukocyte count (per mm ³ of exudative fluid)
Control	-	0.91 ± 0.07	6250 ± 121.44
Extract	100	0.63 ± 0.06 ^A (30.55)	4750 ± 128.45
Extract	200	0.60 ± 0.07 ^A (33.63)	3250 ± 121.45 ^B
Extract	300	0.56 ± 0.09 ^B (38.46)	2200 ± 53.38 ^X
Diclofenac	3	0.55 ± 0.05 ^B (39.56)	1650 ± 46.37 ^X

Results are expressed as mean ± S.E.M, n=5. Percentages of inhibition are in brackets. The statistical analysis was performed on absolute data. ^Ap<0.05, ^Bp<0.01 and ^Xp<0.001, significantly different compared to control.

3.2.3. Formalin-induced paw oedema

The anti-inflammatory activity of *R. vomitoria* against sub-chronic paw oedema induced by formalin is summarized in Fig. 1. The extract (100 and 200 mg/kg) produced significant anti-inflammatory activity on days 6 and 10 compared to control untreated group. The plant extract (300 mg/kg) and dexamethasone significantly inhibited the paw oedema from the 2nd to 10th day. The maximum inhibition was 49.60% for the extract (300 mg/kg) and 60.90% for dexamethasone.

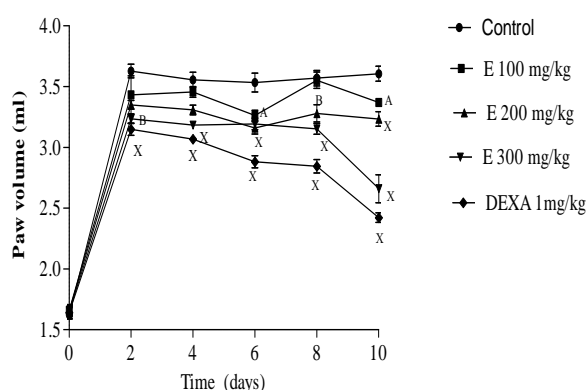


Figure 1: Effect of the oral administration of aqueous extract of *Rauwolfia vomitoria* on formalin-induced paw oedema in rats.

Each point represents the mean ± S.E.M, n=5. The statistical analysis was performed on absolute data. ^Ap<0.05, ^Bp<0.01 and ^Xp<0.001, significantly different compared to control. E: Extract. DEXA: Dexamethasone.

3.2.4. Effect of the oral administration of aqueous extract of *Rauwolfia vomitoria* on Complete Freund's Adjuvant-induced paw oedema

Complete Freund's Adjuvant (CFA) injected into the hind paw of rats induced marked oedema during the 21 days of experiment period. In control group, the level of oedema volume (1.66 ml, initially) reaches the maximum of 4.36 ml after 17 days of adjuvant inoculation (Fig. 2). Dexamethasone (reference drug, 1 mg/kg) significantly

inhibited CFA-induced paw oedema at intervals of this study when compared to control and normal groups. Treatment with extract (200 and 300 mg/kg) showed significant inhibition on paw oedema from the 13th day to the 21st day compared to control and normal groups.

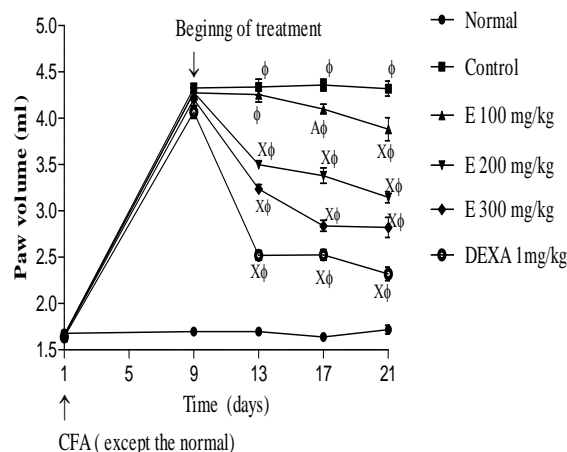


Figure 2: Effect of the oral administration of aqueous extract of *Rauwolfia vomitoria* on Complete Freund's Adjuvant-induced paw oedema.

Each point represents the mean ± S.E.M, n=5. The statistical analysis was performed on absolute data. ^Ap<0.05, ^Bp<0.01 and ^Xp<0.001 significantly different compared to control. ^ap<0.05, ^Ωp<0.01 and ^Φp<0.001, significantly different compared to normal. E: Extract. DEXA: Dexamethasone.

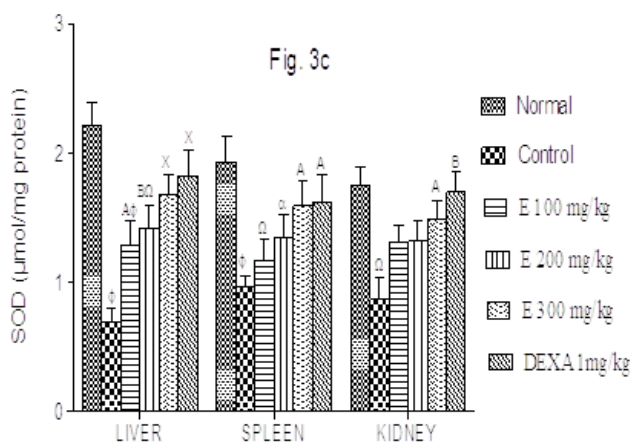
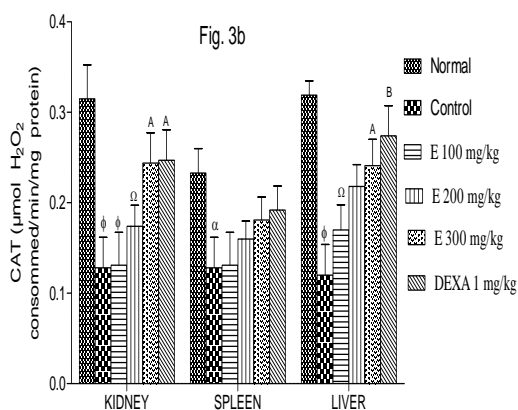
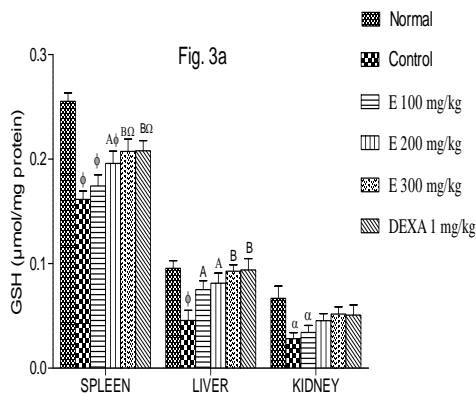
3.3. Anti-oxidant activity of *R. vomitoria*

3.3.1. Effect of the stem bark aqueous of *Rauwolfia vomitoria* on GSH, CAT and SOD level in CFA-induced inflammation.

The activity of *R.vomitoria* against oxidative stress is summarized in Fig.3 a, b c. GSH, CAT and SOD content was significantly reduced in CFA control tissue as compared to normal control group. Oral administration of the extract (100, 200 and 300 mg/kg) or dexamethasone (1 mg/kg) significantly increased the

level of GSH and SOD in liver. The extract (200 and 300 mg/kg) significantly increased the level of GSH in spleen. The extract at the dose of 300 mg/kg increased significantly the level of CAT in liver and kidney. It also increased significantly the level of SOD level in spleen and kidney.

Each bar represents the mean \pm S.E.M, n=5. The statistical analysis was performed on absolute data. ^Ap<0.05, ^Bp<0.01 and ^Xp<0.001 significantly different compared to control. ^αp<0.05, ^Ωp<0.01 and ^Φp<0.001, significantly different compared to normal. E: Extract. DEXA: Dexamethasone. CAT: Catalase. SOD: Superoxide dismutase, GSH: Reduced glutathione.



3.3.2. Effect of the oral administration of aqueous extract of *Rauwolfia vomitoria* on MDA, protein and nitrite levels in chronic inflammation induced by CFA

As showing in Figure 4, MDA, nitrite and protein content significantly increased in CFA control tissue and serum as compared to normal control group. Oral administration of the extract (100, 200 and 300 mg/kg) or dexamethasone (1 mg/kg) significantly decreased the level of nitrite in liver. Extract (200 and 300 mg/kg) significantly decreased the level of MDA in liver and serum as well as tissue nitrite in spleen and protein in liver and kidney. The extract at the dose of 300 mg/kg decreased significantly the level of tissue nitrite in kidney and protein in spleen.

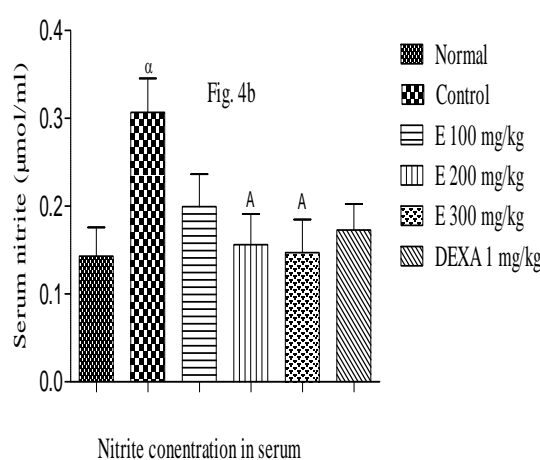
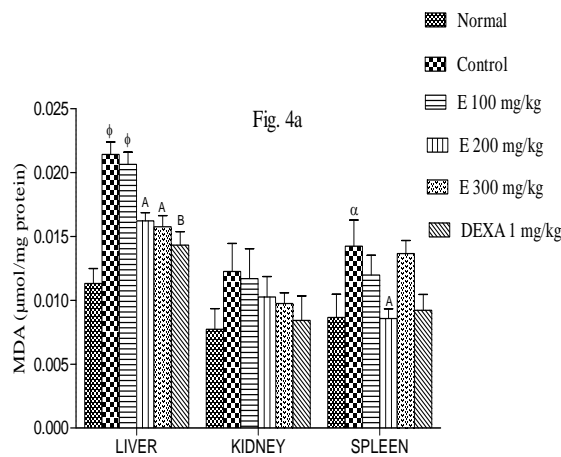


Figure 3. Effect of stem bark aqueous of *Rauwolfia vomitoria* on GSH (a), CAT (b) and SOD (c) level in CFA-induced inflammation.

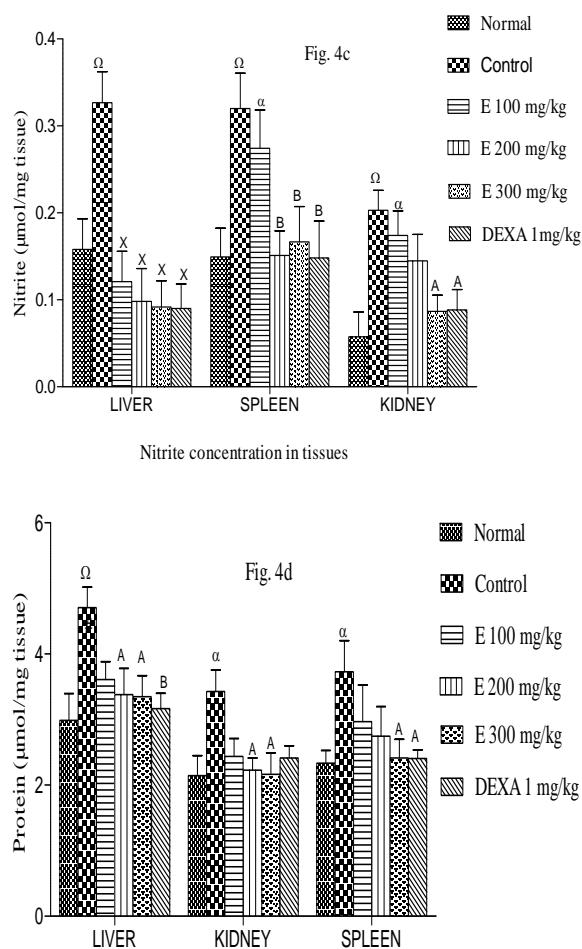


Fig.4. Effect of stem bark aqueous of *R. vomitoria* on MDA (a); nitrite (b, c) and protein (d) level in CFA-induced inflammation.

Each bar represents the mean \pm S.E.M, $n=5$. ^A $p<0.05$, ^B $p<0.01$ and ^X $p<0.001$ significantly different compared to control. ^α $p<0.05$, ^Ω $p<0.01$ and ^Φ $p<0.001$, significantly different compared to normal. E: Extract. MDA: Malondialdehyde. DEXA: Dexamethasone.

DISCUSSION

Inflammatory disease is one of the major health problems worldwide. Inflammation is the body defense mechanism. Sub-chronic inflammation is a reaction arising when the acute response is insufficient to eliminate pro-inflammation agents. Sub-chronic inflammation includes a proliferation of fibroblast and infiltration of exudation.^[25, 26] The effects of aqueous extract of *R. vomitoria* were evaluated in sub-chronic inflammation induced by cotton pellet, carrageenan, formalin and Complete Freund's Adjuvant.^[27] The aqueous extract of *R. vomitoria* shows significant anti inflammation activity in cotton pellet induced granuloma at all doses. Thus, it is found to be an effective drug in sub-chronic inflammation. These results are similar to those of Nwaehujor *et al* in 2014^[28] which have shown that the methanol extract of the leaves of *Bridelia micrantha* has anti-oedema activity against sub-chronic

inflammation induced by cotton pellet. Our plant extract contained 20.02 mg/g of dried extract of flavonoids. Flavonoids are well known to possess anti-inflammatory effects. In fact, among the phenolic compounds, flavonoids are known to display anti-inflammatory and anti-oxidant effects by inhibiting lipid peroxidation.^[29] To assess its efficacy against inflammatory proliferative phase, instead of croton oil or turpentine oil, carrageenan was used as the irritant.^[30] Treatment of carrageenan induced-granuloma with aqueous extract at the doses of 100, 200 and 300 mg/kg as well as diclofenac significantly reduced the volume of exudative fluid as compared to group 1 (control) and decreased the number of polymorphonuclear leukocytes indicating its effectiveness against development of proliferate cells and infiltration of leukocytes. The presence of flavonoids in our plant extract might justify these results. This could be probably explained by the reduction of prostaglandin levels thus reducing the inflammation events.^[31] It is reported that, flavonoids and their related compounds exhibit inhibition of arachidonic acid peroxidation, which chemical mediators are implicated in this type of inflammation.^[32] It suggests that flavonoids were responsible for the effects observed in this model of inflammation. Though, the chemical mediators of this type of response are unknown, protein synthesis is necessary for the formation of granuloma.^[33, 34] Kinin is said to be the main mediator of exudates formation, as it both vasodilators and increases vascular permeability in the early stages of inflammation.^[35] It may therefore be said that aqueous extract might possess anti-kinin like activity. It is well known that inhibition of oedema induced by formaldehyde in rats is one of the best suitable test procedures for chronic anti-inflammatory agents as it closely resembles human inflammation.^[34] Aqueous extract as dexamethasone, reference drug, in dose dependent manner significantly inhibited the inflammation on the 10th day. These results suggested that the plant extract might possess anti-proliferative and/or anti-inflammatory activities. Dimo *et al* in 2005^[15] obtained the similar result with the leaf extracts of *Kalanchoe crenata* Andr. They suggested that plant might act on proliferative phase of inflammation. Further experiments may be needed to clarify this point. In model of CFA-induced chronic inflammation, a number of inflammatory mediators are released, namely prostaglandin, free cytokines and substance P.^[34,35] The aqueous extract at the doses of 200 and 300 mg/kg and dexamethasone significantly reduced inflammation from the 13th day to the 21st day. It is possible that aqueous extract might be inhibiting some of these mediators. Moreover, the extract exhibited inhibition during the phases of inflammation,^[9] which suggests that it might possess potential antihistaminic, antiserotonin and antiprostaglandin activities. Similar result has been previously obtained by Agathe *et al* in 2004^[36] while working with aqueous extract of *Sclerocarya birrea* in inflammation induced by Complete Freund's Adjuvant. Indeed, the phytochemical analysis of the aqueous extract of *R. vomitoria* contain alkaloids, flavonols,

flavonoids and polyphenols which are responsible for anti-inflammatory properties by the inhibition of lipopolysaccharide-induced nitric oxide (NO).

Inflammation and oxidative stress are two closely related events, free radicals and reactive oxygen species (ROS) are continuously generated inside body as a result of exposure to many exogenous drugs and xenobiotics.^[37] Under normal condition, there is equilibrium between the ROS generated and the antioxidants present as the ROS generated are neutralized by endogenous antioxidants.^[38] Deleterious effects caused by ROS occur as a consequence of an imbalance between the formation and inactivation of these species leading to irregularities in cellular physiology and different pathological condition.^[38] The direct effect of the aqueous extract of *R. vomitoria* as an antioxidant was evaluated via the GSH, CAT, SOD, MDA, Protein and Nitrite in Complete Freund's Adjuvant test. GSH, CAT and SOD content were significantly reduced in CFA control tissue as compared to normal group. In the present study, significant decrease of GSH, CAT and SOD levels in tissues observed may be one of the causes for the development of CFA-induced inflammation. Lower concentrations of GSH, CAT and SOD have been implicated in inflammatory diseases by excessive accumulation of free radicals.^[38] Oral administration of the extract or dexamethasone significantly increased the level of GSH and SOD in some tissues. An observed significant increase levels of GSH, CAT and SOD in tissues of pretreated groups show that aqueous extract tends to prevent the tissues depletion of GSH and restore CAT and SOD enzymes. The similar observation was demonstrated by Arief *et al* in 2010^[39] while study the antioxidant effects of hexagamavunon-1 against carbon tetrachloride-induced hepatic injury in rats. Aqueous extract of *R. vomitoria* revealed in quantitative phytochemical analysis 127.83 mg/g of dried extract of polyphenols. This might be responsible for the observed antioxidant and anti-inflammatory properties. In addition, it was reported that polyphenolic compounds were associated with antioxidant activity and play an important role in stabilizing lipid peroxidation.^[40] Furthermore, the protective effect of *R. vomitoria* may be attributed to the potent free radical scavenging activity by the presence of 46.67 mg/g of dried extract of vitamin C. It is well established that GSH in tissue keeps up the cellular levels of the active forms of vitamin C by neutralizing the free radicals; when there is reduction in GSH the cellular levels of vitamin C is closely interlinked to each other.^[41] To understand the antioxidant effects of

plant, MDA, nitrite (tissue and serum) and proteins were investigated. The significant increase of MDA and proteins levels as well as nitrite level in control group as compared to normal rats in our study may be attributed to the development of complete Freund's adjuvant-induced inflammation related to arthritis. Higher concentrations of MDA, nitrite and proteins have been implicated in inflammatory diseases. Aqueous extract and dexamethasone significantly reduced the level of MDA, nitrite and protein in treated groups compared to control group in a dose dependent manner. The present findings are supported by the fact that our plant extract inhibits the production of nitric oxide (NO) and pro-inflammatory mediators by activated cells from various tissues.^[38] These results corroborate those previously obtained by Arief *et al* in 2010^[39] while working on evaluation of antioxidant activity of hexagamavunon-1 against carbon tetrachloride-induced hepatic injury in rats. *R. vomitoria* extract exhibited a potent free radical scavenging in CFA- induced chronic inflammation. Prophylactic treatment of inflammation induced by CFA with our aqueous extract significantly reduced nitrite levels in serum and tissue. Moreover, quantitative phytochemical investigation of the aqueous stem bark extract of *R. vomitoria* indicates the presence of metabolites such as alkaloids, polyphenols, known to possess anti-inflammatory and antioxidant activities. In fact, the antioxidant activity of polyphenolic compounds is well known and is mainly due to their redox properties, which play an important role in neutralizing free radicals, quenching singlet and triplet oxygen species or decomposing peroxides.^[40, 41]

CONCLUSION

The results of the present investigation demonstrate that aqueous extract of *Rauwolfia vomitoria* has anti-inflammatory activity in sub-chronic and chronic inflammation models. It also has antioxidant activity, which may contribute to its anti-inflammatory activity. Further work relating to its toxicity, hepatoprotective, isolation and characterization of the active constituents present in the plant and studies on various pharmacological evaluations, is under way by our research team in our laboratory.

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