Evaluation of the anti-inflammatory effects of methanol extract of *Rauwofia vomitera* leaves (MERVL)using in-*vivo* based assays.

Authors Contributions

This work was carried out in collaboration among all authors. Authors UOC and ACA designed the study, performed the statistical analysis, wrote the protocol. Author UOC wrote the first draft of the manuscript. Authors FKA and AIJ managed the analyses of the study. Author UCJ and ACL managed the literature searches and the analysis of the study. Author UGW and OUE manage the analysis of the study All authors read and approved the final manuscript.

Abstract:

Background of the study: The response of the body to harmful stimuli, such as tissue damage or a pathogenic infection is known as inflammation. *Rauwofia vomitera* is a medicinal plant that is native to large areas of tropical Africa and it belongs to the family of Apocynaceae. It is a medicinal herb used in traditional Nigerian medicine to treat a variety of diseases, including inflammatory disorders.

Aim of the study: The present study investigated the anti-inflammatory effects of methanol extract of*Rauwofia vomitera* leaves (MERVL) using in-vivo based assays.

Methodology: The anti-inflammatory effect of (MERVL) was ascertained using the Egg albumin - induced inflammation using *in vivo* assay. A total of thirty (30) adult male albino rats (130-170g) were divided into five (5) groups of six (6) rats each for the in-vivo anti-inflammatory assay. Group 1 normal; Group 2 was given 10 mg/kg body weight of Indomethacin and Groups 3, 4 and 5 were given 100, 200 and 400 mg/kg body weight of MERVL) respectively. Acute toxicity was carried out using standard method.

Place and Duration of the Study: Department of Pharmacology Lab, Enugu State of University of Science and Technology Agbani Nigeria, between March 2021 and August 2021.

Results:

The percentage yield of the extract was 10.8%. The result of the quantitative and qualitative phytochemical screening of methanol extract of *R. vomitoria* leaves showed that Phenol (1590 ± 55.66) Flavonoid (1168 ± 11.43) and Tannin (1466 ± 40.73) were present in high concentration whereas, Alkaloid (549 ± 4.21) Glycoside (315 ± 61.38) and Terpenoid (162 ± 1.38) were present in moderate concentration. Saponin (1.16 ± 0.23) and Steroid (0.84 ± 0.03) were present in low concentrations. Acute toxicity tests showed no toxicity and mortality at doses up to 5000 $mgkg^{-1}$. A significant ($P < 0.05$) reduction in the mean paw oedema was observed for all the treatment groups from 1 hour to 12 hours when compared to the normal group.The result shown that groups treated with 100, 200 and 400 mg/kg b.w of extract inhibit the inflammation in a non-dose dependent manner with percentage mobilization of 5.7%, 14.1% and 6.2% respectively.**Conclusion:** The study's results demonstrate that MERVL exhibits remarkable anti-inflammatory and anti-oxidant activities and the extract yielded a better reduction in edema and increases mobilization in leukocyte in response to agar suspension. It also demonstrates that MERVL has a modulatory effect on the vascular changes that accompany inflammation. The plant's anti-inflammatory properties could potentially result from the

interaction of different photochemical substances present.The findings suggest that, if used, the plant may serve as a source of anti-inflammatory agents.

Keywords*: Rauwofia vomitera,* Egg albumin, Anti-inflammatory, Leucocyte mobilization, Methanol extract.

1.0 INTRODUCTION:

The response of the body to harmful stimuli, such as tissue damage or a pathogenic infection, was regarded as inflammation. [1] There are two stages to this response: acute inflammation and chronic inflammation, each having its own traits.[2] The characteristics of the inflammatory phase are discussed in a variety of models designed for the study of medications or natural products with anti-inflammatory action. When an inflammatory drug is injected into a rat's hind paw, the compound's capacity to prevent edoema is commonly utilised to assess its anti-inflammatory effectiveness.[3]. In order to initiate rat paw inflammation, the researchers used fresh egg white with references to explain the mechanism of edoema production [4]. The egg white contains protein such as ovalbumin, Ovo transferrin, ovomucoid, ovomucin, and lysozyme [5]. These proteins have been identified as the main allergen in egg white,[6] which is suspected to cause an inflammatory response if injected into the rat's paw. Furthermore, carrageenan has a well-established method for inducing an inflammatory response, which makes it a popular stimulator of inflammation.[7] Rat paw edoema was caused by carrageenan in two stages. First to two hours after induction, histamine, serotonin, and enhanced local prostaglandin production mediated the early phase. Bradykinin, leukotrienes, leukocyte infiltrations, and inducible cyclooxygenase mediated the later phase, which took place three hours later. [8]. This research was aimed to investigated the anti-inflammatory effects of methanol extract *Rauwofia vomitera* leaves (MERVL) using in-vivo based assays. The outcome may offer initial insights into the inflammatory pathways behind animal models of inflammation generated by egg white. Prior research has demonstrated that anti-inflammatory medicinal plants can reduce oxidative stress and strengthen the immune system.[9]. The leaf of Rauwofia vomitera (Apocyanaceae) is one of these plants that has anti-inflammatory characteristics. This particular species of vomitoria belongs to the Apocyanaceae family. Other names for it include swizzle, snake root, and serpent wood [10]. It is referred to as asofeyeje in the regional languages of Western Nigeria [11]. This plant's main phytochemical components are reducing sugars, polyphenols, glycosides, and alkaloids [12]. Rauwolfine, reserpine, rescinnamine, serpentine, ajmaline serpentinine, steroid-serposterol, and saponin are among R. vomitoria's active alkaloids [13]. R. vomitoria is a common herb that has been traditionally used for psychiatric care in Nigeria. It has been utilised over the years for the treatment of mental diseases and hypertension [10]. Rauwolfia vomitoria is used medicinally for the treatment of inflammation because of its phytochemical composition. For instance, a number of investigations have demonstrated that alkaloids inhibited the proliferation of lymphocytes stimulated by antigen and mitogen, the cytotoxicity of natural killer.

2. Materials and Methods

2.1. Collection of Plant Material and Extraction Procedure

On March 13, 2021, when this study was being conducted, fresh leaves of Rawuofia vomitera were found on the Enugu State University of Science and Technology campus. Mr. A. O. Ozioko of the International Centre for Ethnomedicine and Drug production (InterCEED), Nsukka, Nigeria, verified the authenticity of the newly cut leaf. The plant with voucher specimen number INTERCEED/002 was placed at the InterCEED Herbarium.The plant was gathered, cleaned, and dried in the shade. Using a mechanical grinder, the dried leaves were ground into a powder. Using a maceration flask, a weighed quantity (1000 g) was macerated in 2.5 L of absolute methanol. After being stirred frequently for 72 hours, it was filtered using a muslin cloth into a flask with a flat bottom. Whatman No. 1 filter paper was used to perform the filtration. To create the crude ethanol extract, the extract was concentrated using a rotary evaporator at a temperature of 45°C. The concentrated extract was kept in the refrigerator in a labelled sterile reagent bottle at a temperature of 2 to 40 C.

2.1.1Chemicals and reagents:

Chemical used for this study were of the analytical grade and products of May and Baker England, British Drug House (BDH) England, Fluka Germany, Burgoyne, India, Harkin and Williams, England and Sigma Aldrich.

2.1.2. Experimental Animals

Adult Male Wistar rats (130–170 g), obtained from the animal house of the Department of Veterinary Medicine, University of Nigeria Nsukka, were utilised for in-vivo anti-inflammatory activities. Swiss albino mice (26–32 g) were used for toxicity testing. They spent seven days getting used to the laboratory environment and being confined in metal steel cages prior to the investigations. The rats were given water ad labitum and standard grower's mash rat pellets (Grand Cereals LTD, Enugu, Nigeria).

2.2. Phytochemical Analysis

Various qualitative and quantitative screening tests were carried out to evaluate the phytochemical composition of the crude extract using procedures outlined by Trease and Evans [15]. ,Harborne [16].

2.2.1. Acute Toxicity Study

In order to establish the fatal dose range of the extract, the acute toxicity of the fraction was investigated and the median lethal dose (LD50) was estimated using the Lorke [17] technique with certain modifications. Eighteen (18) Swiss albino mice were used in the investigation; they were given access to water but famished for eighteen hours. Six (6) groups of three mice each were created, and MERVL was given to each group at different dose levels (10, 100, and 1000 mg/kg for phase one and 1600, 2900, and 5000 mg/kg for phase two). The animals were observed for the following 24 hours for signs of toxicity, such as anxiety, dullness, lack of coordination, and behavioural changes.

2.2.2. Experimental Design

For the study, a total of thirty (30) male Wistar albino rats were used. They were divided into five (5) groups, each with six (6) rats, and were given the following treatment:

Group 1: Normal control

Group 2: Received 10 mg/kg body weight of indomethacin (standard drug)

Group 3: Received 100 mg/kg body weight of (MERVL).

Group 4: Received 200 mg/kg body weight of (MERVL).

Group 5: Received 400 mg/kg body weight of (MERVL).

2.2.3. Determination of the Effect of MERVL on Egg Abumin-induced Rat Paws Oedema

Using a modified version of Winter et al.'s protocol, the impact of MERVL on rat paw oedema produced by egg abumin was examined [18]. In order to guarantee consistent hydration and reduce variability in the oedematous response, wistar albino rats were fasted for eighteen hours before to the experiment. A Vernier calliper was then used to measure the rats' right hind paw size at time zero, which was prior to the induction of oedema. One hour before inducing acute inflammation, 0.1 ml of freshly produced egg albumin was used to deliver the MERVL orally. Rats' right hind paw sub-plantars were injected with egg albumin. Rats' increased right hind paw size was then measured at 0, 1, 2, 3, 4, and 5 hours following egg injection of egg albumin. The development of oedema was evaluated by comparing the paw sizes of the injected paws at time zero and at various intervals following egg albumin injection. Using the relation below, these numbers were used to calculate the percentage inhibition of edoema for each dose of the extract and for indomethacin at the various time intervals:

Paw oedema= (Vt - Vo)

Vo=Size of paw oedema at time zero

Vt=Size of paw oedema at time t [0, 1, 2, 3, 4, 12hr]

Percentage inhibition of oedema=(vt-vo) Toxic group-(vt-vo) Treated groups

2.2.4 Leucocytes Mobilization Test in Rats

It was established whether the MERVL leaves had an impact on in vivo leukocyte mobilisation triggered by an inflammatory stimulus using the method outlined by Winter et al. [18]. In this experiment, thirty (30) mature male Wistar rats weighing between 120 and 190 g were used, and it was divided into five groups of six rats each. Groups I and II received 3% tween 80 and indomethacin (10 mg/kg), respectively, while groups III, IV, and V received the extract in various quantities (100, 200, and 400 mg/kg). Three hours after the animals in the appropriate groups (n=6) received oral dosages of the extracts, tween 80, and reference medicines, each animal had an intraperitoneal injection of 0.5 ml of a 3% w/v agar solution in normal saline.Four hours after the animals were killed, 5 ml of a 5% EDTA in phosphate buffered saline solution (PBS) was used to clear the peritoneal cavities. % Leukocyte mobilization (% L.M) = $\left(1-\left(\frac{T}{C}\right)\right)x100$

Where T and C represent the leukocyte count of the treated and control groups respectively.

2.2.5 Statistical Analysis

The data obtained were analyzed using both one analysis of variance (ANOVA) in Statistical product and Service Solution (SPSS) version 22.0 and presented as Mean ±SD. Mean values with $p < 0.05$ were considered significant. The mean values were separated using Post Hoc Tests and Homogeneous subsets (Duncan).

Results

3.1 Percentage Yield of Methanol Extract of *Rauwolfia vomitoria* leaves

The percentage yield of the extract was 10.8%

3.2.1 Quantitative and qualitative phytochemical composition of methanol extract of *R. vomitoria* leaves.

The result of the quantitative and qualitative phytochemical screening of methanol extract of *R. vomitoria* leaves showed that it contained tannins, phenol, alkaloids, flavonoids, glycosides,

saponins, terpenoids, steroids as shown in Table 1. Phenol(1590 ± 55.66) Flavonoid (1168 ± 11.43) and Tannin (1466 ± 40.73) were present in high concentration whereas, Alkaloid(549 ± 4.21) Glycoside(315 ± 61.38) and Terpenoid(162 ± 1.38) were present in moderate concentration. Saponin(1.16 ± 0.23) and Steroid (0.84 ± 0.03) were present in low concentrations.

Table 1: Quantitative phytochemical constituents of methanol extract of *R. vomitoria* leaves

Phytochemical Constituents	**Quantitative remarks(mg/100g)**	**Qualitative remarks**
Tannin	1466 ± 40.73	+++
Phenol	1590 ± 55.66	+++
Alkaloid	549 ± 4.21	++
Flavonoid	1168 ± 11.43	+++
Glycoside	315 ± 61.38	++
Saponin	1.16 ± 0.23	+
Terpenoid	162 ± 1.38	++
Steroid	0.84 ± 0.03	+

n=3

3.2.2 Result on the Acute toxicity study of methanol extract of *R. vomitoria* leaves

The result as shown on Table 2 showed that the methanol extract of *R. vomitoria* leaves was not toxic and there was no sign of behavioural changes and physiological alterations even up to the dose of 5000mg/kg body weight.

Table 2: Acute toxicity study (LD_{50}) of methanol extract of *R. vomitoria* leaves

Phase 1	Dose (mg/kg b.w)	Mortality rate
Group 1	10	0/3
Group 2	100	0/3
Group 3	1000	0/3
Phase II		
Group 1	1600	0/3
Group 2	2900	0/3
Group 3	5000	0/3

(n=3)

3.2.3 Effect of Methanol Extract of *R. vomitoria* on Egg Albumin induced paw edema

Group treated with 100mg/kg b.w significantly (P <0.05) lowered the inflammation when compared to normal control and the extract reduced the inflammation with percentage inhibition of 43% at 12hr. Group treated with 200 and 400mg/kg b.w of extract showed no significant difference when compared with the normal control, although there is reduction in inflammation at 12hrs with percentage inhibition of 46% and 42% respectively. Moreover, group treated with 400mg/kg of extract were observed to be significantly (P < 0.05) lowered when compared with the group treated with 200mg/kg b.w of extract.

Table 3: Effect of methanol extract of *R. vomitoria* on egg albumin induced paw edema

Group	0 hour	1 hour	2 hours	3 hours	4 hours	12 hours
1-Normal control	3.09±0.30^{ab}	6.54±0.76^{ef}	6.72±0.76	5.75±0.78	5.49±0.49^{ab}	5.46±0.71
2-Standard control (indomethacin 10mg/kg)	3.42±1.06^{ab}	5.66±1.26^{def} (13%)	3.90±0.84^{bc} (42%)	3.69±0.68^{abc} (36%)	2.99±0.48^{ab} (46%)	2.81±0.74^{ab} (49%)
3-100mg/kg b.w extract	2.83±0.38^{bc}	6.51±1.63^{ef} (1%)	3.76±0.37^{abc} (44%)	3.27±0.53^{bc} (43%)	3.15±0.41^{bc} (43%)	3.10±0.36^{bc} (43%)
4-200mg/kg b.w extract	3.21±0.48^{ab}	5.30±0.10^{ab} (19%)	4.68±0.93^{cd} (30%)	3.85±0.11^{abc} (33%)	2.84±0.10^{ab} (48%)	2.95±0.21^{ab} (46%)
5-400mg/kg b.w extract	2.60±0.86^{ab}	5.28±0.01^{ab} (19%)	4.80±0.21^{cd} (29%)	3.95±0.44^{bc} (31%)	3.26±0.51^{ab} (41%)	3.16±0.61^{ab} (42%)

Results are expressed in Mean ± SD (n = 5). Mean values with different letters as superscripts(a-f) down the column are considered significant ($p < 0.05$). Percentage of inhibition are in bracket.

3.2.4 Effect of methanol extract on lipid peroxidation and antioxidant status of rats in Egg albumin- induced inflammation.

The results showed that there is significant ($p < 0.05$) increase in MDA, SOD, GSH, and CAT levels when compared with the untreated group.

Table 4: **Effect of methanol extract on lipid peroxidation and antioxidant status of rats in Egg albumin- induced inflammation.**

GROUPS	MDA (mg/dl)	SOD(IU/L)	GSH (mg/dl)	CAT(IU/L)
1	2.35±0.04^{a}	3.12±0.08^{e}	0.25±0.41^{e}	0.31±0.08^{a}
2	8.88±0.09^{e}	7.10±0.04^{a}	0.78±0.01^{d}	0.63±0.03^{c}
3	4.82±0.02^{d}	4.07±0.05^{b}	0.38±0.09^{b}	0.37±0.01^{b}
4	3.62±0.01^{c}	5.31±0.02^{c}	0.53±0.03^{c}	0.73±0.03^{d}
5	3.07±0.05^{b}	9.22±0.08^{e}	0.97±0.02^{e}	0.81±0.26^{e}

Results are expressed as means ± SD (n = 5)

Mean values with different letters as superscripts down the column are considered significant at ($p < 0.05$).

3.2.5 Effect of methanol extract of *R. Vomitoria* leaves on in-vivo agar induced leucocyte migration

The effect of methanol extract of *R. Vomitoria* leaves on in-vivo agar induced leucocyte migration as depicted in Table 5 shows that dose treated with 100, 200 and 400mg/kg b.w of extract inhibit the inflammation in a non-dose dependent manner with percentage mobilization of 5.7%, 14.1% and 6.2% respectively. The results also showed a significant reduction in TLC level when compared with the untreated control

Table 5: Effect of methanol extract of *R. Vomitoria* leaves on in-vivo agar induced leucocyte migration.

	DIFFERENTIALS

Group	TLC (mm3)	% Mobilization	NEU	LYM	MONO	EOSI	BASO
1-Normal control	6875±275.38		56.00±1.63	38.75±0.96	3.75±1.71	1.50±0.58	0
2-Standard control(indomethacin)	6350±525.99*	(8.3%)	56.00±1.83	39.75±1.50	2.75±0.96	1.75±0.50	0
3- 100mg/kg b. w Extract	6500±258.20*	(5.7%)	59.25±2.22	37.25±2.22	3.25±0.96	1.75±0.96	0
4-200mg/kg b. w Extract	6025±478.71**	(14.1%)	59.25±2.99	36.75±2.87	2.50±1.00	1.50±0.58	0
5- 400mg/kg b. w Extract	6475±556.03***	(6.2%)	58.50±3.11	38±2.94	2±0.82	2.±0.82	0

Results are expressed in Mean ± SD (n = 5). Percentage mobilization is expressed in brackets

NEU-Neutrophils, **LYM**-Lymphocytes, **MONO**-Monocytes, **EOSI**-Eosinophils, **BASO**-Basophils, **TLC- Total Leucocyte Test**

4.0 DISCUSION

The anti-inflammatory and anti-oxidant properties of the MERVL were determined in the current investigation using in-vivo, anti-inflammatory, and anti-oxidant based assays. Table 1 displays the plant's quantitative phytochemical analysis, which indicates that it includes different levels of tannins, phenols, flavonoids, alkaloids, terpenoids, glycosides, steroids, and saponins. It has been observed that flavonoids, which were found to be present in high concentrations in the plant,

have the ability to scavenge free radicals and alter the production of cyclooxygenase (COX-1 and COX-2), which is involved in the synthesis of prostaglandins [20].These bioactive components have been proposed to be responsible for the anti-inflammatory qualities of several therapeutic plants [21]. Through a variety of pathways, including the suppression of transcription factors and regulatory enzymes, flavanoids reduce inflammation. The activity of mediators involved in inflammation is greatly influenced by these factors [22]. Investigations of acute toxicity have demonstrated the high safety profile of oral MERVL doses. Table 2 indicates that animals were able to withstand up to 5000 mg/kg of plant extract without experiencing any fatalities. Table 3 illustrates how MERVL affects rat paw oedema brought on by egg albumin. The extract may prevent serotonin and histamine from being released since it can decrease the early stages of oedema.The oedema suppression that occurs during the second and third phases of inflammation indicates that the anti-inflammatory effect of MERVL is related to the suppression of prostaglandin and kinin production that occurs during this time, which is generated by egg albumin. This result is in accordant with Celestine et al.,[10]. The MERVL reduces vascular permeability and fluid exudation, most likely by limiting endothelial cell contraction, and suppresses oedema because these mediators increase vascular permeability and vasodilatation at the site of injury. Numerous nonsteroidal anti-inflammatory medications (NSAIDs), including aspirin and indomethacin has also involved in inflammatory regulation. Reactive nitrogen species (RNS) and other free radicals are produced when tissues are injured during inflammation, and these molecules harm cellular processes [24]. These extremely reactive radicals destroy proteins and nucleic acids in cellular membranes by oxidative mechanisms and also induce lipid peroxidation. Oxidative stress is caused by an excess of free radicals produced without a greater capacity to scavenge radicals [25]. Due to their ability to scavenge reactive oxygen species (ROS), reactive nitrogen species (RNS), and other reactive species, Rauowfia vomitera have anti-inflammatory properties [26]. A major factor in the significant mobilisation of leukocytes at the site of an injury is the increased proliferation of lymphocytes and other phagocytic cells following injury [27]. They disrupt the extracellular matrix, induce inflammation, and degrade phagocytosed material [28]. The results of which demonstrated that indomethacin at high doses inhibited leukocyte accumulation, are compatible with the use of indomethacin in our investigation, which decreased leukocyte mobilisation [29]. However, the drugs most likely inhibited the ability of the inflammatory mediators to multiply, leading to an increase in leucocyte synthesis that might relocate to the site of leucocyte mobilisation, where lymphocytes are most common. The drugs were able to respond to a harm caused by the agar suspension.

4.0Conclusion

The study's results demonstrate that MERVL exhibits remarkable anti-inflammatory and anti-oxidant activities and the extract yielded a better reduction in edema and increases mobilization in leukocyte in response to agar suspension. It also demonstrates that MERVL has a modulatory effect on the vascular changes that accompany inflammation.The plant's anti-inflammatory properties could potentially result from the interaction of different photochemical substances

present.The findings suggest that, if used, the plant may serve as a source of anti-inflammatory agents.

Data Availability The numerical data used to support the findings of this study are available from the corresponding author upon request.

Declaration of Competing Interest The authors declare that they have no competing interest that could have appeared to influence the work reported in this paper.

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