INNSPUB

Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 24, No. 1, p. 40-50, 2024
http://www.innspub.net

RESEARCH PAPER OPEN ACCESS

Anti-inflammatory properties of *Uvaria chamae* used in traditional Beninese medicine (West Africa)

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Article published on January 07, 2024

Key words: TNFα, Anti-inflammatory activity, *Uvaria chamae*

Abstract

Inflammatory diseases are still a major public health concern. Their management is problematic due to the harmful side-effects of anti-inflammatory drugs traditionally used in conventional medicine. Medicinal plants are a credible source for research into new anti-inflammatory drug. This study aimed to explore the anti-inflammatory properties of the roots of *Uvaria chamae*. Anti-inflammatory activity was explored using the red blood cell membrane stability test and the formalin-induced œdema model in rats. TNFα assay was used to assess immunomodulatory activity. This methodology was complemented by research into antioxidant power using the DPPH test. Results indicated that the extract significantly inhibited membrane destabilization of red blood cells (61.43%), and the formation of formalin-induced paw œdema. This effect is consistent with the reduced production of TNFα (22.06%). The extract tested proved to be a good antioxidant by inhibiting the DPPH radical (IC_{50}= 0.669 mg/mL); its larvae toxicity was moderate (LC_{50} = 0.033 mg/mL). These results suggest that the ethanolic extract of *U. chamae* roots has anti-inflammatory, immunomodulating and antioxidant properties. This justifies the use of the plant in traditional Beninese medicine for the treatment of inflammatory diseases.

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Introduction

Inflammation is an immune response that scientists have been exploring for several decades. It develops following tissue damage caused by physicochemical factors or microbial infections (bacterial, viral or parasitic). It is usually a beneficial process, with the aim of eliminating the pathogen and repairing tissue damage. However, inflammation can often be harmful due to the aggressiveness of the pathogen or the excessive production of reactive oxygen species. This gives rise to oxidative stress, which is implicated in the onset of several diseases. Uncontrolled inflammation is therefore considered to be one of the pathophysiological causes of most chronic diseases, including inflammatory diseases, diabetes, cancer and cardiovascular disease (Sahlmann and Ströbel, 2016).

Modern medical treatments for inflammatory syndromes are often based on the use of non-steroidal anti-inflammatory drugs (NSAIDs) and glucocorticoids. But these drugs are often responsible for serious side effects (Heymonet, 2013).

NSAIDs, whatever their route of administration, expose patients to the risk of gastrointestinal toxicity. The higher the dosage and the longer the course of treatment, the greater the risk (Al-Shidhani *et al.*, 2015). In this context, public and scientific interest is increasingly focused on the treatment of inflammation through phytotherapy.

In Africa, *Uvaria chamae* is a plant widely used in traditional medicine to treat a range of ailments, including dysentery, infections and dysmenorrhoea. Studies have shown that *U.chamae* has antimicrobial effects (Koudokpon *et al.*, 2018; Oluremi *et al.*, 2010), antidiabetics (Emordi *et al.*, 2018), anti-fungals (Okwuosa *et al.*, 2012) and anti-malaria (Okokon *et al.*, 2006).

In Benin, *U. chamae* is used in over 30 traditional treatment recipes of several diseases (Kouchadé *et al.*, 2016). However, little scientific data exists on the anti-inflammatory activity of *Uvaria chamae*. The aim of this study was to evaluate the anti-inflammatory and immunomodulatory effects of *Uvaria chamae* root extracts *in vitro* and *in vivo*.

Material and methods

Ethical consideration

The experimental design was approved by Ethical Committee of Research unit in Applied Microbiology and Pharmacology of natural substances of the University of Abomey-Calavi of Benin (N° 0025/2018/CE/URMAPha/UAC). All experiments were performed according to the National Institute of Health's (NIH) guidelines for the care and use of laboratory animals.

Animal material

Wistar albino rats, 12-week-old adults weighing between 200 g and 250 g, acclimatized to rearing conditions in the animal house of the Research Unit in Applied Microbiology and Pharmacology of natural substances (URMAPha). They had free access to water and food and were subjected to a cycle of 12 hours of light and 12 hours of darkness. The average temperature in the animal house was between 28°C and 30°C.

Plant material

The plant material consists of the roots of *Uvaria chamae*. These organs were collected for in commune of Abomey-Calavi, Benin and identified in the National Herbarium of the University of Abomey-Calavi under the number AA6687/HNB by Professor YEDOMONHAN Hounnankpon, Conservator of the National Herbarium of Benin.

Preparation of extract

After collection, the organs were washed with water, then dried in a ventilated room, at room temperature and protected from light, before being cut up and ground to a powder. The powder obtained was used to prepare the extracts. To this end, 50 g of powder were macerated in 250 mL of ethanol and 250 mL of distilled water for 48 h under magnetic stirring. The macerated mixture was then filtered through cotton wool and filter paper. The filtrate obtained was then dried in an oven at 50°C for 48 h to obtain a dry hydroalcoholic extract (Dougnon *et al.*, 2021; Fanou *et al.*, 2022). The extract was kept refrigerated at 4°C in an airtight bottle until use.

In vitro anti-inflammatory activity

Red blood cell membrane stability test

The *in vitro* anti-inflammatory activity of the extract was assessed using the method described by Falade *et al.* (2005). The assessment was carried out in two stages:

Preparation of erythrocytes and drugs

Fresh bovine blood was collected on anticoagulant containing sodium citrate (3.8% w/v). Blood samples were then centrifuged at 3000 rpm for 5 min at room temperature. The supernatant was carefully removed (plasma and leukocytes). Isotonic saline (0.9% NaCl) was added to the pellet. The mixture is centrifuged again at 3,000 rpm for 5 minutes at room temperature; the same process is repeated until a clear supernatant is obtained. The final pellet is completely freed from the clear supernatant. A 10% (v/v) globular suspension is prepared in 0.9% NaCl. Diclofenac and ibuprofen were used as reference molecules. Molecules and the hydroalcoholic extract of *Uvaria chamae* were concentrated to 2.5 mg/mL from isotonic solution (0.9%).

Determination of the percentage of membrane stabilization of extracts

The membrane stabilization activity of the extract was assessed using the method described by Falade *et al.* (2005). This consisted of using 3 series of hemolysis tubes: two of 11 tubes, each intended to receive one of the reference drugs, and the last reserved for the extract under study, comprising 10 tubes (sample tubes, control tubes and haemato control tube); 1 mL of PBS (pH 6.8) and 2 mL of 0.25% NaCl were then added to each tube. Increasing volumes of the reference drugs (0.2 mL, 0.4 mL, 0.6 mL, 0.8 mL and 1 mL) were then added to the contents of the tubes, avoiding addition to the haematie-control tube. The mixture was then incubated at 37°C for 10 minutes. Next, 0.5 mL of the 10% globular suspension was added to the sample and haemato control tubes; the volume of each tube was then adjusted to 4.5 mL with the distilled water solution. The mixtures thus prepared were incubated at 56°C for 1 h. After being cooled under running water, they were centrifuged at 5000 rpm for 5 min. The supernatant was collected and the optical density (OD) measured at 546 nm using a spectrophotometer. Membrane stability was expressed as a percentage using the following formula:

100-((OD of test drug-OD of control drug)*100)/(OD control haematie).

With DO control haematie corresponding to 100% lysis or 0% stability.

In vivo anti-inflammatory activity

Paw œdema was induced in rats using 2% formalin (Foughalia Amina, 2017; Rahmani *et al.*, 2016). Injection under the plantar pad of the rat's right hind leg provoked an inflammatory reaction. Sixteen (16) rats Wistar were used. The rats were fasted for 16 hours before experimentation and divided into 4 lots of 4: lot 1: positive control (formalin), lot 2: reference lot (reference substance: diclofenac 400 mg/Kg), lot 3: test lot (hydroalcoholic extract of *U.chamae* 400 mg/kg), lot 4: test lot (hydroalcoholic extract of *U.chamae* 800 mg/kg). For each rat, the circumference (Co) of the right hind paw was measured. The various treatments were then administered by gavage at a rate of 1 mL per 100 g of body weight. This volume was topped up with distilled water to a total volume of 3 mL, ensuring uniform hydration in all rats and minimizing individual variations in response. One hour after treatment by gavage, each rat in lots 1 to 4 was injected with 0.1 mL of 2 % formalin solution under the plantar pad of the right hind paw. The animals were then returned to the cage. The evolution of inflammation was determined by morphological and immunological parameters.

Morphological parameters

After injection of 2 % formaldehyde, the evolution of œdema was determined every hour for 4 hours. The trans metatarsal diameter, the ankle diameter and the circumference of the paw at metatarsal level (yaw) were determined every hour until the 4th hour using a "Dijite" electronic caliper with digital display. The percentage of œdema inhibition was then calculated using the formula:

Percentage (%) of inhibition = 100 x (C - C1)/C.

Where: C= average diameter of the paw of rats in the positive control lot

C1= average diameter of the paw of rats in the treated lot (diclofenac or *U. chamae* 400 mg/kg or 800 mg/kg).

Immunological parameters: TNF α assay

Five hours after the induction of inflammation, blood samples were taken by retro-orbital puncture from rats of the different lots for the determination of TNFα concentration on rat (Mohanty *et al.*, 2015)**.** This determination was made by ELISA technique, using the "Rat TNFα ELISA" kit from the Fischer Science laboratory. This kit exclusively recognizes rat TNFα in its natural or recombinant form.

DPPH antioxidant activity

The method adopted in this study is that of Klotoé *et al.* (2021). Thus, 100 µL of different concentrations of each extract is added to 1900 µL of the ethanolic solution of DPPH (0.4 mg/mL). The blank is prepared by mixing 100 µL of the extraction solvent with 1900 µL of the DPPH solution. After incubation in the dark for 1 hour at room temperature, absorbance readings were taken at 517 nm using a MINDRAY spectrophotometer (BA-88-A). The optical densities recorded were used to calculate the percentage of DPPH radical trapping, which is proportional to the antioxidant power of the sample. Vitamin C and BHT were used as reference standards. Samples were prepared in triplicate for each analysis. The percentage of DPPH radical scavenging was determined by the formula: P=(Ab-Ae)/Ab × 100

P: trapping percentage; Ab: absorbance of the blank; Ae: absorbance of the sample.

Larval toxicity of hydroalcoholic extract of U. chamae

Larval toxicity of the hydroalcoholic extract of *Uvaria Chamae* was assessed on brine shrimp larvae. This test was carried out on the *Artemia salina* model using the method described by Agbodjento *et al.* (2020). *Artemia salina* larvae were obtained by hatching 10 mg of *Artemia salina* eggs under continuous agitation in 1L of seawater for 72 h. Series of dilutions of order 2 of a stock extract solution with a concentration of 20 mg/mL were carried out. To 1 mL of each of these diluted solutions was added 1 mL of seawater containing 16 live larvae. A control solution without the extract was prepared under the same conditions. All solutions were incubated under agitation for 24 hours. The number of dead larvae in each solution was counted using an optical microscope to plot the number of surviving larvae as a function of extract concentration. The data (concentration-response) was log-transformed and the LC_{50} (mean lethal concentration) was determined. To assess the larval toxicity of the extract, the correlation grid associating the degree of toxicity with the LC_{50} was used (Mousseux, 1995).

Statistical analysis

Statistical analyses were performed using SPSS software version 26.0. For each parameter, the mean and standard deviations were calculated. For the study of anti-inflammatory activity in vivo, the results of the test lots were compared with the control lots. Univariate analysis of variance was performed to compare the diameters of the legs of the different lots of rats as a function of time. Student's t-test was used to compare the means of TNFα. A significance level of α = 0.05 was defined for all statistical tests used. The IC_{50} or 50% inhibitory concentration of the DPPH radical was determined using the probit function.

Results

In vitro anti-inflammatory activity by membrane stability of red blood cells

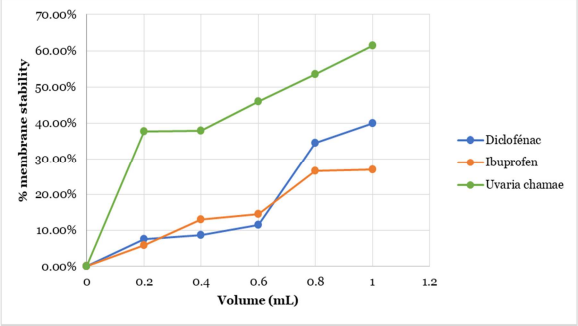

Fig 1. Membrane stabilization of the hydroalcoholic extract of *U. chamae* and reference drugs

The results obtained showed that the power of hydroalcoholic extracts of *U. chamae* root to stabilize red blood cell membranes increased in a dose-dependent manner. These extracts show a higher percentage of membrane stabilization than that obtained with the two reference drugs, whatever the dose used (Fig. 1). Indeed, for volumes of hydroalcoholic extract of *U. chamae* root, Diclofenac and Ibuprofen between 0 mL and 1 mL, the percentage of membrane stabilization increased from 0 % for 0 mL to 61.43 %, 40.01% and 26.94 % for 1 mL respectively.

Table 1. Estimated marginal averages of leg diameter according to lots of rats

Lot	Average	Error std.	95% confidence interval	
			Lower terminal	Upper
Formol	7.666a	0.148	7.368	7.964
Diclofenac	5.873 b	0.148	5.574	6.171
U. chamae 400 mg/Kg	7.465 a	0.148	7.167	7.764
U. chamae 800 mg/Kg	5.895 b	0.148	5.596	6.193

Lots whose averages are marked by the same letters do not show any statistically significant difference.

Table 2. Estimated marginal means of leg diameter as a function of lots*hour factors

Lots		Means	Standard deviation std.	95% Confidence Interval	
				Lower Terminal	Upper Terminal
Formol	0H	5.247	0.300	4.641	5.852
	1H	7.353	0.300	6.748	7.959
	2H	7.737	0.300	7.131	8.342
	3H	8.417	0.300	7.811	9.022
	4H	9.577	0.300	8.971	10.182
Diclofenac	0H	5.017	0.300	4.411	5.622
	1H	5.573	0.300	4.968	6.179
	2H	5.797	0.300	5.191	6.402
	3H	6.420	0.300	5.815	7.025
	4H	6.557	0.300	5.951	7.162
U. chamae 400 mg/Kg	0H	5.047	0.271	4.529	5.565
	1H	7.976	0.271	6.951	9.001
	2H	8.447	0.271	7.956	8.938
	3H	7.720	0.271	6.942	8.498
	4H	8.137	0.271	7.626	8.648
U. chamae 800 mg/Kg	0H	5.174	0.271	4.501	5.841
	1H	5.560	0.271	4.910	6.211
	2H	5.797	0.271	4.716	6.878
	3H	6.270	0.271	5.082	7.458
	4H	6.674	0.300	4.523	8.825

In vivo anti-inflammatory activity

Effect of treatments on the Wistar rat model of induced œdema

The results (Table 1) showed that the estimated marginal mean diameter of the paws of rats in the reference lot (diclofenac) was 5.873 ± 0.148 mm and significantly reduced compared with that of the positive control lot (formalin), which was 7.666 ± 0.148 mm ($p<0.05$). Lots treated with *U. chamae* extract at doses of 400 mg/Kg and 800 mg/Kg also showed a reduction in rat paw diameter with a significant effect at the 800 mg/Kg dose ($p<0.05$).

Table 2 present estimated marginal means of leg diameter as a function of lot*hour factors. From this table, it is clear that in all lots, formalin induced an increase in the diameter of the rat's paw, indicating inflammation. However, a variation was observed between the different lots. For untreated rats, the diameter of the paw increased from 5.247 mm to 9.577 mm between 0 and 4 hours, an increase of 82.52 %. In contrast, *U. Chamae* extract at the doses tested induced a reduction in the variation in paw diameter compared with untreated rats. At 400 mg/kg, the extract induced a variation in paw diameter from 5.047 mm to 8.137 mm, an increase of 61.22 %. At 800 mg/kg, a variation in leg diameter from 5.174 mm to 6.674 mm, i.e. an increase of 28.99 %, was observed. The diclofenac lot showed a similar variation to that observed for the 800 mg/kg extract (30.69 %).

Table 3. Paired comparisons of the dependent variable diameter as a function of the factors time and Lots

Times			Difference of average	Error std.	Signification	95 % Confidence interval Lower terminal	Upper terminal
	Formol	Diclo	0.23	0.467	0.625	-0.714	1.174
	Formol	*U. chamae* 400 mg	0.2	0.467	0.671	-0.744	1.144
	Formol	*U. chamae* 800 mg	0.073	0.467	0.876	-0.87	1.017
0H	Diclo	*U. chamae* 400 mg	-0.03	0.467	0.949	-0.974	0.914
	Diclo	*U. chamae* 800 mg	-0.157	0.467	0.739	-1.1	0.787
	U. chamae 400 mg	*U. chamae* 800 mg	-0.127	0.467	0.788	-1.07	0.817
	Formol	Diclo	1.780*	0.467	0	0.836	2.724
	Formol	*U. chamae* 400 mg	-0.623	0.467	0.189	-1.567	0.32
	Formol	*U. chamae* 800 mg	1.793*	0.467	0	0.85	2.737
1H	Diclo	U. *chamae* 400 mg	-2.403*	0.467	0	-3.347	-1.46
	Diclo	*U. chamae* 800 mg	0.013	0.467	0.977	-0.93	0.957
	U. chamae 400 mg	*U. chamae* 800 mg	2.417*	0.467	0	1.473	3.36
	Formol	Diclo	1.940*	0.467	0	0.996	2.884
	Formol	*U. chamae* 400 mg	-0.71	0.467	0.136	-1.654	0.234
	Formol	*U. chamae* 800 mg	1.940*	0.467	0	0.996	2.884
2H	Diclo	*U. chamae* 400 mg	-2.650*	0.467	0	-3.594	-1.706
	Diclo	*U. chamae* 800 mg	4.46E-15	0.467	1	-0.944	0.944
	U. chamae 400 mg	*U. chamae* 800 mg	2.650*	0.467	0	1.706	3.594
	Formol	Diclo	1.997*	0.467	0	1.053	2.94
	Formol	*U. chamae* 400 mg	0.697	0.467	0.144	-0.247	1.64
	Formol	*U. chamae* 800 mg	2.147*	0.467	0	1.203	3.09
3H	Diclo	*U. chamae* 400 mg	-1.300*	0.467	0.008	-2.244	-0.356
	Diclo	*U. chamae* 800 mg	0.15	0.467	0.75	-0.794	1.094
	U. chamae 400 mg	*U. chamae* 800 mg	1.450*	0.467	0.003	0.506	2.394
	Formol	Diclo	3.020*	0.467	0	2.076	3.964
	Formol	*U. chamae* 400 mg	1.440*	0.467	0.004	0.496	2.384
	Formol	*U. chamae* 800 mg	2.903*	0.467	0	1.96	3.847
4H	Diclo	*U. chamae* 400 mg	-1.580*	0.467	0.002	-2.524	-0.636
	Diclo	*U. chamae* 800 mg	-0.117	0.467	0.804	-1.06	0.827
	U. chamae 400 mg	*U. chamae* 800 mg	1.463*	0.467	0.003	0.52	2.407

* The average difference is significant at 0.05.

Table 3 shows the pairwise comparisons of the dependent variable "diameter" as a function of the factors "time" and "lots". From this table, it noted that before induction of inflammation, the mean paw diameters of Wistar rats between the different lots did not differ significantly. This average varied from 5.017 mm to 5.560 mm. This means that before the experiment, untreated and treated rats did not show a significant difference ($p > 0.05$) in the diameter of their paws.

During the first and two hours after induction of inflammation, the diclofenac group showed a significant reduction in paw diameter compared with untreated animals. The same observation was made for *U. chamae* extract at 800 mg/kg ($p < 0.05$). Furthermore, at this dose, the effect of the extract was similar to that of the reference molecule (diclofenac) ($p > 0.05$) during the first few hours. However, at 400 mg/kg, there was no significant difference between the mean diameters of treated and untreated rats.

3 and 4 hours after induction of inflammation, the 800 mg/kg extract and diclofenac continued to produce an anti-inflammatory effect by inhibiting œdema in the rats' paws.

TNF-alpha assay

Analysis of the data revealed a significant increase in the mean concentrations of TNFα in the formol lot compared with the normal control. Similarly, the lot treated with *U.chamae* extract showed a significant decrease in mean TNFα concentration compared with the formol lot (Table 4). However, there was no

statistically significant difference between the mean TNFα concentrations of the formalin and Diclofenac lots, or between Diclofenac and *U. chamae*.

Table 4. TNFα values between different lots of rats

Lots of rats	Average	N	Standard dev.	Average Error standard
Control	286.1500	4	20.15234	10.07617
Formol	401.2000[a]	4	70.14138	35.07069
Diclofenac	327.4500	4	26.16480	13.08240
U. chamae	312.7000[b]	4	28.08962	14.04481

[a]: significantly different from the control lot; [b]: significantly different from the formol lot

Antioxidant activity

The results obtained showed that the IC_{50} of the hydroalcoholic extract of *U. chamae* is 0.669 mg/mL, compared with 0.104 mg/mL obtained for vitamin C, which is used as the reference drug.

Larvae toxicity

The sensitivity of *Artemia salina* larvae to different concentrations of the hydroalcoholic extract *U. Chamae* is shown in Fig. 2. This shows increasing mortality of *Artemia salina* larvae as the concentration of the plant extracts studied increases. The LC_{50} obtained was 0.033 mg/mL. This LC_{50} value compared to the Mousseux scale allows us to conclude that, at the concentrations tested, the hydroalcoholic extract of *U. Chamae* is only slightly toxic to *Artemia salina* larvae ($LC_{50} \geq 0.05$ mg/mL).

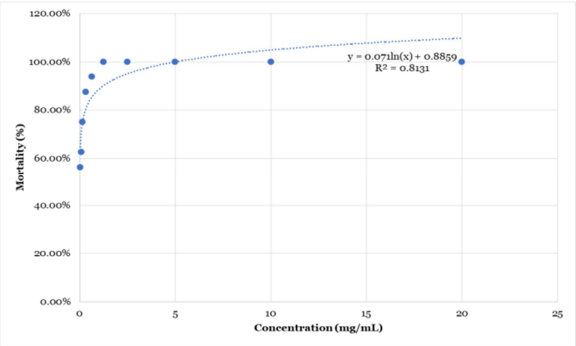

Fig. 2. Mortality rate of *Artemia salina* larvae as a function of concentrations of hydroalcoholic extract of *Uvaria chamae*

Discussion

In the present study, the biological properties of the hydroalcoholic extract of the roots of *Uvaria chamae* were evaluated using *in vitro* and *in vivo* approaches. In the *in vivo* approach, formol was used to experimentally induce inflammation in rats to study the effects of *U. chamae* extracts on œdema and TNFα production. Although there are other methods of inducing inflammation, the use of formalin is perfectly justified. Indeed, formol induces local inflammation, due to tissue damage, when injected into the fascia of the sole of the rat foot (Singh *et al.*, 1997). Under experimental conditions, formalin induced paw œdema, the maximum volume of which was obtained after three hours (Singla and Pathak, 1990). The development of rat paw œdema induced by formalin injection is a biphasic phenomenon. The initial phase, which occurs between 0 h and 2 h 30 min after formalin injection, corresponds to the release of serotonin, histamine and bradykinin, as well as their actions on vascular permeability. The second phase corresponds to the massive biosynthesis and release of substances such as prostaglandins and leukotrienes into the tissues (Peters-Golden and Henderson, 2007). This biosynthesis can continue for more than 5 hours after formalin injection and is mediated by cyclooxygenases (COX) and 5-lipooxygenase, which act on arachidonic acid (Pérez-Guerrero *et al.*, 2001). Tissue damage also induces the release of platelet-activating factor (PAF), nitric oxide (NO) and cytokines including TNFα (Azab *et al.*, 2016). This scientific informations supports the results obtained of this study on the induction of œdema and the production of TNFα levels by formol.

Our results showed that the hydroalcoholic extract of the roots of *Uvaria chamae* reduced œdema induced by formalin in a dose-dependent and time-dependent manner (400 mg/kg and 800 mg/kg). The inhibition of œdema by hydroalcoholic extract of *Uvaria chamae* root 800mg/kg is comparable to that of diclofenac. However, a variation in the inhibitory effect was observed according to the doses tested. At 400 mg/kg, inhibition of paw œdema by the extract began 3 hours after induction of œdema (9.09%) and increased to 18.78% at 4 hours. At a dose of 800 mg/kg, the hydroalcoholic extract of the roots of *Uvaria chamae* not only reduced œdema during the first three hours, but also TNFα production at 4 h after induction of inflammation. These data suggest

that the reduction in œdema by hydroalcoholic extract of *U. chamae* root at 800 mg/ml is due to inhibition of the release of early cellular mediators such as histamine, serotonin and bradykinin. The significant reduction in œdema after 3 h, by the hydroalcoholic extracts of *U. chamae* root (400 mg/kg and 800 mg/kg) would probably be due to the inhibition of cyclo-oxygenases and 5-lipooxygenase, thus preventing the release of prostaglandins and leukotrienes. In addition, the hydroalcoholic extract of the roots of *Uvaria chamae* protects against destabilization of the membrane of red blood cells subjected to osmotic and thermal shock, with a better result (61.43%) compared to the reference drugs (Ibuprofen (26.94%) and Diclofenac (40.01%)). The membrane of red blood cells has the same constitution as that of lysosomes. This result could therefore indicate the existence of compounds that oppose the formation of lysosomes and proteases in the inflammatory zone (Oteiza *et al.*, 2005). Several phytochemical studies carried out on *U. chamae* root extract have revealed that it contains: tannins, terpenoids, polyphenols, flavonoids, alkaloids, reducing sugars and essential oil (Okwuosa *et al.*, 2012; Monon *et al.*, 2015; Oluremi *et al.*, 2010). These phytochemical groups are involved in the anti-inflammatory activities of medicinal plants. The composition of the ethanolic extract of *U. chamae* root in these different chemical constituents could explain the membrane stabilization of red blood cells produced by this extract and therefore its anti-inflammatory activity.

On the another hand, luteolin or 3',4',5,7-tetrahydroxyflavone, is a chemical compound of the flavonoid family (Imran *et al.*, 2019). It is one of the most common flavones found in medicinal plants (Aziz *et al.*, 2018; Nabavi *et al.*, 2015). It has a powerful anti-inflammatory effect *in vitro* and *in vivo*. Luteolin inhibits the production of NO and active oxygen species. It also inhibits the production of TNFα by macrophages (Chen *et al.*, 2014). However, the biological mechanisms by which luteolin assumes these functions are not yet well defined (Chen *et al.*, 2014).

Our results showed a reduction in TNFα production in the presence of the hydroalcoholic extract of *U. chamae* root, with a better result compared to that obtained with diclofenac. This could be explained by the presence of large quantities of flavonoids, specifically luteolin, in the plant extract (Nwaehujor *et al.*, 2013; Santos and Salatino, 2000; Wiya *et al.*, 2018).

The results obtained during our study concerning antioxidant activity showed that the hydroalcoholic extract of *U. chamae* has an antioxidant effect on the DPPH radical, as the IC_{50} of the hydroalcoholic extract of *U. chamae* is 0.669 mg/ml. However, this antioxidant effect is lower than that of vitamin C (the reference drug for DPPH activity), whose IC_{50} is 0.104 mg/mL. This antioxidant power could be due to the flavonoids and phenolic compounds present in the extract. These compounds are known to be antioxidant substances capable of trapping reactive forms of oxygen and radical species whose action amplifies the inflammatory response (Kumar and Pandey, 2013). This would have reinforced the anti-inflammatory activity of this plant.

Finally, the toxicity of the hydroalcoholic extract of *Uvaria chamae* was assessed on the basis of the toxicity test using *Artemia salina* larvae. This is a preliminary exploratory test designed to assess the cytotoxicity of the natural products studied (Solis *et al.*, 1993). Our results showed that the LC_{50} of the hydroalcoholic extract of *U. chamae* showed low toxicity. These results are comparable to those obtained by Thomas and Essien (2018) who reported that the ethanolic extract of *U. chamae* root exhibited larval toxicity with an LC_{50} of 0.025 mg/mL

Conclusion

The present study investigated the anti-inflammatory, immunomodulatory and antioxidant effects of the ethanolic extract of *U. chamae* roots used in traditional medicine to treat various diseases. The results of this study showed that the extract has anti-inflammatory and antioxidant properties. These results suggest that the ethanolic extract of *U. chamae* roots possesses pharmacological active ingredients that give it the anti-inflammatory, immunomodulating and antioxidant properties observed.

Author contributions

JRK, FL and MG participated at the conception of the study. GM, SK, DE and AE participed at the collect and analysis of the Data. JRK, EA wrote the draft of the manuscript. VD and YA provided the scientific direction of the study. All the authors have read and approved the manuscript

Declaration of interest

There is no conflict of interest.

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