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Original Article

Evaluation of the Anti-inflammatory and Immunomodulatory Properties of *Jatropha multifida* Stem Sap

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ABSTRACT

Inflammatory diseases remain a significant public health issue, posing challenges in their management due to the adverse effects associated with conventional anti-inflammatory drugs. Exploring medicinal plants as a viable avenue for researching novel anti-inflammatory drugs offers a promising alternative. *Jatropha multifida* L. is a well-known traditional plant of the *Euphorbiaceae* family with undisputed medicinal properties, both haemostatic and antimicrobial. Very little data exists on its anti-inflammatory properties. This study aimed to assess the anti-inflammatory and immunomodulatory properties of *J. multifida* stem sap. The anti-inflammatory activity of the stem sap was evaluated *in vitro* by the anti-hemolytic activity assessment method, *in vivo* by the paw edema test in rats. The immunomodulatory potential was determined using a chemical mediator of inflammation, TNFα. Its antioxidant activity by the DPPH method was evaluated. The results of this study revealed that *J. multifida* sap with an LC_{50} of 0.604 mg/mL, is non-toxic and showed a 66.21% higher anti-hemolytic effect than Diclofenac (25.94%) and Ibuprofen (40%). It also has a high DPPH inhibitory power (86.61%) at 0.5 mg/mL similar to that of vitamin C. Sap at the concentration of 800 mg/Kg has overall the same effects on paw inflammation as Diclofenac and results in a greater decrease in TNFα (286.15) at values similar to those of normal control rats. The therapeutic use of this sap in a traditional setting is therefore once again justified due to its proven anti-inflammatory and immunomodulatory properties.

INTRODUCTION

Inflammation is a response triggered by the organism after tissue damage, stress, or infection, aimed at restoring its morphological and biological parameters [1]. As such, it involves a cascade of reactions from effector cells, plasma, and cellular mediators to regulate the inflammatory site and eliminate debris in the acute phase [2]. If resolution of the acute phase fails, it transitions into the chronic phase. Chronic inflammation is often associated with the development of various conditions, including diabetes, obesity, osteoarthritis, and rheumatic fever [3, 4]. The use of anti-inflammatory agents can be beneficial in the treatment of inflammatory disorders [1].

Non-steroidal anti-inflammatory drugs are widely prescribed due to their effectiveness against inflammation and infection [5]. These drugs inhibit the action of cyclooxygenase-1 (COX1), responsible for the synthesis of prostaglandins, the main chemical mediators of inflammation. However, their long-term therapeutic use is often associated with adverse effects such as epigastric distress, gastric ulceration, osteoporosis, and iatrogenic Cushing's syndrome [6]. Additionally, the difficulty of access linked to their cost has sparked interest in homeopathic solutions using plants for the treatment of inflammation, relying

on natural molecules that are effective and free of adverse effects. Traditional African medicine is generally the primary therapeutic option for meeting primary health care needs in Africa due to its affordability, accessibility, cultural and spiritual acceptance, and knowledge of its preparations and products [7, 8]. The efficacy of compounds derived from medicinal plants is increasingly reported worldwide, leading to a growing interest in their ability to treat and manage various diseases [9]. Several studies in Africa have demonstrated the anti-inflammatory activities of various medicinal plants from different geographical regions, each exhibiting unique modes of action [2].

Known to the public as an ornamental species [10], *Jatropha multifida* L. is a plant of the *Euphorbiaceae* family and its therapeutic use is justified by its multiple pharmacological properties, particularly its haemostatic and antibacterial effects [11]. Previous studies have confirmed the hemostatic and antibacterial properties of the plant's sap [12, 13]. Results of these studies revealed a mechanism of action independent of classical coagulation factors and demonstrated bactericidal activity. *J. multifida* has various therapeutic uses and is not limited to the Beninese pharmacopoeia. The stem sap of *J. multifida* widely used as a curative product with proven effectiveness [14], has never been studied for its anti-inflammatory properties. Anti-inflammatory properties have only been evaluated on its leaves in Togo [15, 16] and its roots in Nigeria [17]. The aim of the present study was to evaluate the *in vitro* and *in vivo* anti-inflammatory and immunomodulatory properties of *J. multifida* stem sap.

MATERIELS AND METHODS

Ethical Consideration

The experimental design was approved by the Ethical Committee of the Research Unit in Applied Microbiology and Pharmacology of natural substances at the University of Abomey-Calavi in Benin (Approval Number: 0025/2018/CE/URMAPha/UAC). All experiments were conducted in accordance with the guidelines for the care and use of laboratory animals established by the National Institute of Health (NIH).

Plant Material

It consists of the stem sap of *J. multifida*, directly collected in sterile plastic collection tubes after foliar cleavage of the plant, following the technique indicated by Klotoé *et al.* [14]. The samples were then stored in a refrigerator at 4 °C.

Animal Equipment

It consists of male Wistar albino rats aged 16-20 weeks and weighs between 200 g and 250 g. They were kept in the URMAPha animal facility at a constant temperature of 22 ± 1 °C with a cycle of 12 hours in the light and 12 hours in the dark. They had free access to food and water.

In vitro Anti-inflammatory Activity

Sap membrane stabilisation activity was assessed using the method described by several authors [18, 19]. Diclofenac (reference) and stem sap of *J. multifida* were concentrated at 2.5 mg/mL. A 2% (v/v) red cell suspension in physiological water was prepared from fresh bovine blood erythrocytes. The stability of the membrane was expressed as a percentage according to the following formula:

$$100 - \frac{(\text{DO tested drug} - \text{DO control drug}) * 100}{\text{DO control hematopoietic}}$$

Legend: Hematopoietic control OD corresponding to 100% lysis or 0% stability.

In vivo Anti-inflammatory Activity

The *in vivo* anti-inflammatory activity of the sap was assessed in adult Wistar albino rats. Rats of similar weight were divided into five batches, each comprising four rats. The batches were treated as follows: batch 1 served as the negative control (no induction of inflammation), batch 2 as the positive control (induction of inflammation without treatment), batch 3 as the reference batch (induction of inflammation with diclofenac treatment), and batches 4 and 5 as the test batches (induction of inflammation with sap treatment at concentrations of 400 and 800 mg/mL). Treatment was administered one hour prior to inflammation induction, achieved by injecting 0.05 ml of a 2% formalin solution under the plantar pad of the right hind paw [20].

The progression of inflammation was assessed based on morphological and immunological parameters. Morphological data were gathered by measuring the circumference (Co) of the right hind leg using a digital display plethysmometer before inducing inflammation and one hour afterward, over a four-hour period. Immunological parameters were determined by assaying TNFα, a pro-inflammatory mediator, before inflammation induction and again five hours later [21].

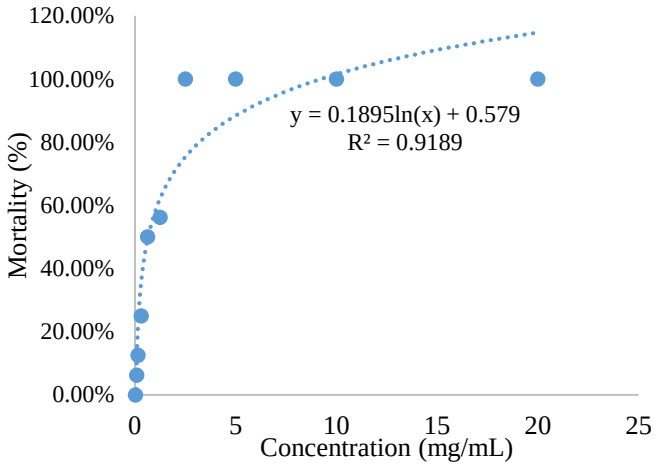

Fig. 1 Mortality rate of *Artemia salina* larvae in relation to *J. multifida* stem sap concentrations

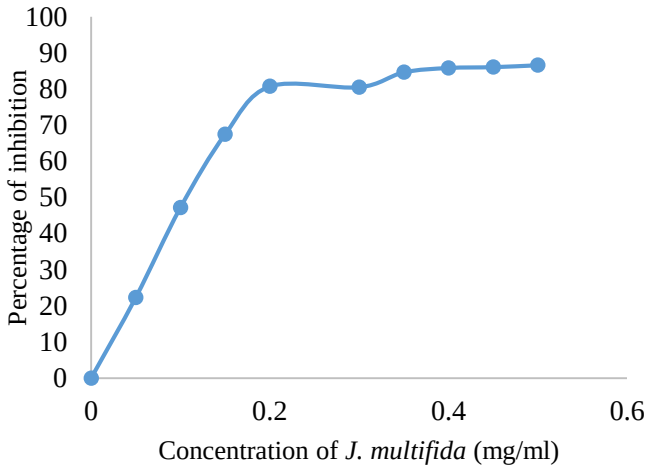

Fig. 2 Percentage of DPPH inhibition as a function of *J. multifida* sap concentrations

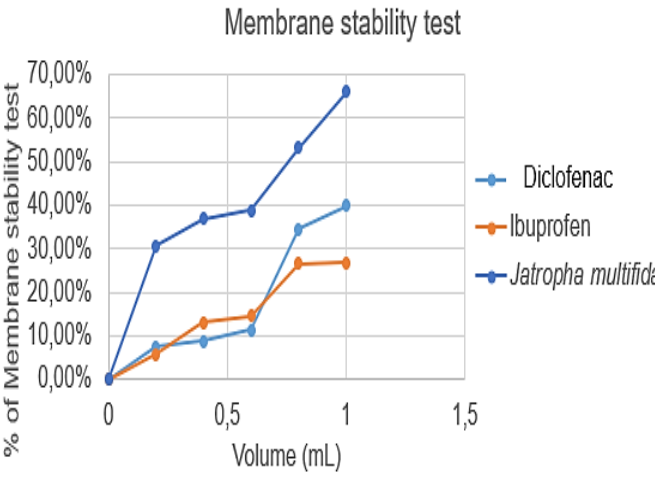

Fig. 3 Membrane stabilization of *J. multifida* sap and reference drugs

Antioxidant Activity at DPPH (2,2 diphenyl 1-picrylhydrazyl)

The test was carried out according to the methodology described by Trabsa [22]. In this test, antioxidants reduce diphenyl picrylhydrazyl to a yellow compound, with the intensity being inversely proportional to the proton-donating capacity of the antioxidants in the medium. The anti-free radical activity is determined using the following equation:

Percentage of inhibition = [(Ac – At) / Ac] x 100.

The IC_{50} represents the concentration of the test sample required to reduce 50% of the DPPH radical. The IC_{50} values are calculated graphically through linear regressions of plotted graphs, showing the percentage of inhibition as a function of different concentrations of the tested sample

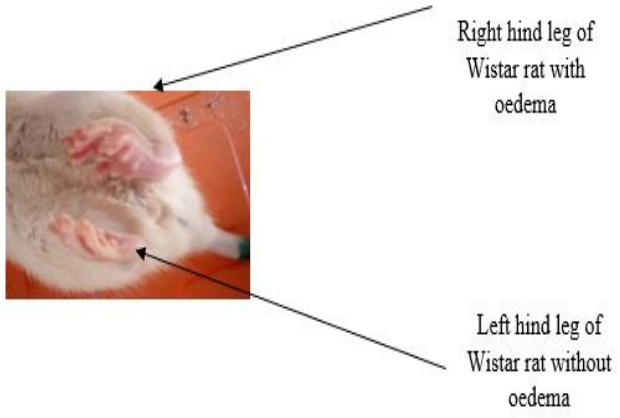

Fig. 4 Image of the rat paw after induction of inflammation

Toxicity Test

The toxic effect of the stem sap of J. *multifida* was evaluated using *Artemia salina* larvae (ARTEMIO JBL GmbH D-67141 Neuhofem) as described by Nguta *et al.* [23]. *Artemia salina* larvae were obtained by hatching 10 mg of eggs placed under continuous agitation in 1 L of seawater for 72 hours. A half serial dilution was made from stem sap of *J. multifida* concentrated to 20 mg/mL (determined by the quantitative measurement method using the mass and volume of the sap). To 1 mL of each of these dilutions, contained in haemolysis tubes, was added 1 mL of seawater containing 16 live larvae counted using a 1000 µL micropipette. The concentration range tested was 10 mg/mL to 0.02 mg/mL. A control solution without sap was prepared under the same conditions. All solutions were incubated under agitation for 24 hours. From the number of dead larvae counted under an optical microscope, a curve representing the number of surviving larvae as a function of the different sap concentrations was generated. The LC_{50} was determined from the regression line obtained on the curve representing the number of surviving larvae as a function of the

concentration of the extract. Each test was performed in duplicate

Statistical Analysis

The statistical analyses were conducted using SPSS version 17 software. Mean and standard deviations were calculated for each parameter. The results of the test batches were compared with those of the control batch using the paired samples t-test. For the assessment of *in vivo* anti-inflammatory activity, a univariate analysis of variance was employed to compare the paw diameters of different batches of rats over time. Student's t-test was utilized to compare TNFα means. A significance level of $\alpha = 0.05$ was established for all statistical tests employed.

RESULTS

Larval Toxicity

Based on the trend curve illustrating the number of deceased larvae in relation to sap concentration, it can be inferred that larval mortality follows a dose-response relationship. The quantity of surviving larvae is thus dependent on the sap concentration. The sap concentration resulting in the demise of half (8) of the 16 initially introduced larvae (LC_{50}), as determined by the regression equation $Y= aX + b$, is presented in the following Figure 1. The LC_{50} of *J. multifida* sap is 0.604 mg/mL. This value is higher than 0.1 mg/mL and allows us to conclude according to the Mousseux scale that *J. multifida* sap is not toxic.

Antioxydant Activity of DPPH

The antioxidant activity of the sap was investigated by assessing the inhibitory potency of *J. multifida* stem sap at various concentrations. Figure 2 illustrates the percentage of DPPH inhibition relative to the concentrations of *J. multifida* stem sap. The graph indicates a rise in free radical inhibition with increasing sap concentration in the medium, reaching a maximum threshold of 86.61% at 0.5 mg/mL. The determined IC_{50} is 0.102 mg/mL, a value comparable to the LC_{50} of vitamin C, which was employed as a reference molecule.

Anti-inflammatory Activity *in vitro*

Red Cell Membrane Stability Test

The results of the red cell membrane stabilization test are depicted in Figure 3. Analysis of the figure reveals that the anti-hemolytic activity of the reference drugs and the sap varies with the volume of these substances in the medium, reaching its maximum threshold at a volume of 1 mL. Specifically, Diclofenac (2.5 mg/mL) and Ibuprofen (2.5 mg/mL) exhibit anti-hemolytic effects of 25.94% and 40%, respectively. *J. multifida* sap demonstrates a noteworthy anti-hemolytic effect, surpassing that of the two reference drugs, starting at a volume of 0.6 mL (40%) and peaking at 1 mL (66.21%). A comparative analysis of the sap and the two reference drugs indicates that the sap achieves a higher inhibition of hemolysis than that obtained with Diclofenac and Ibuprofen at the volumes tested (Figure 3)

Effect of *J. multifida* sap on Induced Inflammation in Rats

The in vivo anti-inflammatory effect of *J. multifida* sap in rats was initially evaluated by observing the impact of sap treatment on paw diameter following the induction of inflammation. Figure 6 depicts an image of the rat paw taken after the induction of inflammation (refer to figure 4).

Table 1 gives an estimate of the diameter of the legs according to the batches of rats. According to the data presented in this table, rats treated with sap at 400 mg/kg exhibited a comparable effect to untreated rats (Formol batch). In contrast, both Diclofenac and sap treatments at 800 mg/kg led to a significant decrease in paw inflammation. The 800 mg/kg sap demonstrated an overall effect on paw inflammation similar to that of Diclofenac.

Table 2 shows a comparison of leg diameters according to batch and time. From this table, it is evident that the diameter of the legs increased with time in all batches, indicating that formalin-induced inflammation increases over time. However, the increase in paw edema varied between batches of rats. In the non-formalin-treated batch, there was a change in diameter from 5.25 mm at 0 hours to 9.58 mm at 4 hours, reflecting an increase of 82.47%. For the batch treated with Diclofenac, there was a 31.08% increase observed between 0 hours and 4 hours after formalin injection. In the batches treated with 400 mg/kg and 800 mg/kg of *J. multifida* sap, the paw diameters increased by 61.34% and 55.39%, respectively.

Table 1 Estimated marginal means of paw diameters by rat batch

Batches	Mean	Error std.	Confidence interval at 95%	
			Lower terminal	Upper terminal
Formol	7.666 a	0.121	7.421	7.911
Diclofenac	5.873 b	0.121	5.628	6.118
J. multifida 400 mg/Kg	7.582 a	0.121	7.337	7.827
J. multifida 800 mg/Kg	6.236 b	0.121	5.991	6.481

Table 2 Estimated marginal means of leg diameter as a function of batch*time factors

Batches		Mean	Error std.	Confidence interval at 95%	
				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
J. multifida 400 mg/Kg	0H	5.017	0.271	4.469	5.565
	1H	8.453	0.271	7.905	9.001
	2H	8.390	0.271	7.842	8.938
	3H	7.950	0.271	7.402	8.498
	4H	8.100	0.271	7.552	8.648
J. multifida 800 mg/Kg	0H	5.293	0.271	4.745	5.841
	1H	5.663	0.271	5.115	6.211
	2H	6.330	0.271	5.782	6.878
	3H	6.910	0.271	6.362	7.458
	4H	8.220	0.300	7.615	8.825

Legend: 0H: zero hour; 1H: One hours; 2H: two hours; 3H: three hours; 4H: four hours

Consequently, treatment of rats with Diclofenac and *J. multifida* sap at 800 mg/kg induced an inhibition of inflammation by 62.31% and 32.84%, respectively.

Table 3 presents the results of paired (independent) linear comparisons among the estimated marginal means. The data in this table indicates that, prior to the induction of inflammation (at 0 hours), there are no significant differences in mean leg diameters among the various experimental batches. However, one hour after the initiation of inflammation, notable differences emerge between the mean leg diameters of batches that received only formalin (i.e., untreated) and those that were exposed to either Diclofenac or *J. multifida* sap at 400 mg/Kg and 800 mg/Kg.

Table 3 Pairwise (independent) linear comparisons between estimated marginal means

Hour	Batches (I)	Batches (J)	Mean difference (I-J)	Error std.	Significance b	Confidence interval at 95%	
						Lower terminal	Upper terminal
0H	Formol	Diclo	0.230	0.383	0.552	-0.545	1.005
	Formol	*J. multifida* 400 mg	0.230	0.383	0.552	-0.545	1.005
	Formol	*J. multifida* 800 mg	-0.047	0.383	0.904	-0.822	0.728
	Diclo	*J. multifida* 400 mg	8.882E-16	0.383	1.000	-0.775	0.775
	Diclo	*J. multifida* 800 mg	-0.277	0.383	0.475	-1.052	0.498
	J. multifida 400 mg	*J. multifida* 800 mg	-0.277	0.383	0.475	-1.052	0.498
1H	Formol	Diclo	1.780 *	0.383	0.000	1.005	2.555
	Formol	*J. multifida* 400 mg	-1.100 *	0.383	0.007	-1.875	-0.325
	Formol	*J. multifida* 800 mg	1.690 *	0.383	0.000	0.915	2.465
	Diclo	*J. multifida* 400 mg	-2.880 *	0.383	0.000	-3.655	-2.105
	Diclo	*J. multifida* 800 mg	-0.090	0.383	0.816	-0.865	0.685
	J. multifida 400 mg	*J. multifida* 800 mg	2.790 *	0.383	0.000	2.015	3.565
2H	Formol	Diclo	1.940 *	0.383	0.000	1.165	2.715
	Formol	*J. multifida* 400 mg	-0.653	0.383	0.096	-1.428	0.122
	Formol	*J. multifida* 800 mg	1.407 *	0.383	0.001	0.632	2.182
	Diclo	*J. multifida* 400 mg	-2.593 *	0.383	0.000	-3.368	-1.818
	Diclo	*J. multifida* 800 mg	-0.533	0.383	0.172	-1.308	0.242
	J. multifida 400mg	*J. multifida* 800 mg	2.060 *	0.383	0.000	1.285	2.835
3H	Formol	Diclo	1.997 *	0.383	0.000	1.222	2.772
	Formol	*J. multifida* 400mg	0.467	0.383	0.231	-0.308	1.242
	Formol	*J. multifida* 800 mg	1.507 *	0.383	0.000	0.732	2.282
	Diclo	*J. multifida* 400 mg	-1.530 *	0.383	0.000	-2.305	-0.755
	Diclo	*J. multifida* 800 mg	0-.490	0.383	0.209	-1.265	0.285
	J. multifida 400 mg	*J. multifida* 800 mg	1.040 *	0.383	0.010	0.265	1.815
4H	Formol	Diclo	3.020 *	0.383	0.000	2.245	3.795
	Formol	*J. multifida* 400 mg	1.477 *	0.383	0.000	0.702	2.252
	Formol	*J. multifida* 800 mg	2.593 *	0.383	0.000	1.818	3.368
	Diclo	*J. multifida* 400mg	-1.543 *	0.383	0.000	-2.318	-0.768
	Diclo	*J. multifida* 800mg	-0.427	0.383	0.272	-1.202	0.348
	J. multifida 400mg	*J. multifida* 800mg	1.117 *	0.383	0.006	0.342	1.892

***** The mean difference is significant at the 0.05 level.

Table 4.a TNFα values between different batches of rats.

Rats batches	Mean	N	Standard deviation	Mean standard error
Control	286.15	4	20.15234	10.07617
Formol	401.20	4	70.14138	35.07069
Diclofenac	327.45	4	26.16480	13.08240
J. multifida	289.10	4	15.23373	7.61687

Table 4.b T-test for comparison of mean TNFα between different batches of rats.

Batches of rats	Mean	Ecart type	Mean standard error	t	ddl	Sig. (bilateral)
Control - Formol	115.0500	60.45701	30.22850	-3.806	3	.032
Formol - Diclofenac	73.7500	35.76462	34.77130	2.030	3	.036
Formol - *J. multifida*	112.1000	55.76462	27.88231	4.020	3	.028
Diclofenac - *J. multifida*	38.3500	35.23573	17.61786	2.177	3	.118

Significant distinctions are also observed between the mean leg diameter of rats in the 400 mg/Kg *J. multifida* sap batch and both the Diclofenac and 800 mg/Kg *J. multifida* sap batches. Conversely, no significant difference was observed between the batch treated with Diclofenac and the one treated with sap at 800 mg/kg from two hours after inflammation. No difference in paw diameter was observed between the rats treated with *J. multifida* sap at 400 mg/kg and the untreated rats (formalin batch) from two hours after inflammation. However, differences persisted between the untreated batch (formalin batch) and both the Diclofenac and *J. multifida* sap 800 mg/kg batches on one hand, and between the *J. multifida* sap 400 mg/kg batch and the Diclofenac and *J. multifida* sap 800 mg/kg batches on the other hand. Nevertheless, the mean difference between the batch treated with Diclofenac and the one treated with sap at 800 mg/kg was not significant. Thus, Diclofenac and *J. multifida* sap at 800 mg/kg exhibited approximately the same effect on formalin-induced inflammation in the rat hind paw. However, at 400 mg/kg, *J. multifida* sap showed no significant effect on the reduction of paw diameter from the second hour after inflammation.

Effect of *J. multifida* sap on TNF Alpha

Table 4.a presents the TNFα values of rats from various batches. The table indicates a significant increase in TNF alpha values for the Formol batch (401.20) compared to the control batch (286.15). Furthermore, the TNF alpha values for the Diclofenac batch (327.45) are lower than those of the Formol batch. It is noteworthy that the Jatropha lot exhibits TNFα values (289.1) closest to those observed in the control batch (286.15). Thus, it should be emphasized that the *J. multifida* sap induces a decrease in TNFα levels, which is nearly equivalent to the levels found in a normal control.

Furthermore, we observe a significant difference in the means of TNFα between the control and formalin batches, as indicated in Table 4.b. This signifies a notable increase in TNFα production following formalin injection. Both diclofenac and *J. multifida* sap pre-treatments lead to a significant reduction in TNFα production. However, there is no significant difference between the effects of these two treatments on the concentration of the pro-inflammatory mediator.

TNFα, a chemical mediator of inflammation. Before conducting the sap membrane stabilization test, a toxicity test is necessary to determine the concentrations to be used. By analyzing the trend curve of the number of dead larvae as a function of the sap concentration, it can be inferred that larval mortality follows a dose-response relationship. Referring to the toxicity scale established by Moshi *et al*. [26] allows for a comprehensive assessment. The LC_{50} value of the sap (0.604 mg/mL) is higher than 0.1 mg/mL, above which the sap is considered non-toxic. These results indicate that the sap of *J. multifida* is not toxic. These findings are consistent with those of Meyer *et al*. [27]. Indeed, in their study on several species of the genus Euphorbia with the larva *Artemia salina*, these authors demonstrated that these plants are not toxic.

DISCUSSION

The inflammatory response is characterized at the tissue level by increased vascular permeability, protein denaturation, and alteration of cell membranes [24, 25].Therefore, to assess the anti-inflammatory potential of substances extracted from plants, the simplest approaches would be to evaluate their red blood cell membrane-stabilizing activity in vitro and their ability *in vivo* to reduce paw diameter and exhibit immunomodulatory potential through

The maintenance of plasma membrane fluidity is an essential prerequisite for cell function, viability, growth, and reproduction [28]. Consequently, various methods have been developed and applied to confirm the anti-inflammatory efficacy of plant extracts in vitro, including the red cell membrane stabilization test. This test exposes erythrocytes to a hypotonic solution at an elevated temperature, given the similarity of the lysosome membrane to that of the red cell [29, 30]. In parallel, the sap of *Jatropha multifida* was tested as an anti-hemolytic agent,

where the activity was found to be very high and even superior to the synthetic drug, Diclofenac. Diclofenac was used as a reference molecule due to its analgesic, antipyretic, and anti-inflammatory properties. The latter are related to its inhibition of prostaglandin and thromboxane synthesis by inhibiting the action of the two isoforms of the membrane enzyme cyclooxygenase (COX-1 and COX-2), thereby impairing platelet function by inhibiting their aggregation [31].

In fact, the sap presents an anti-hemolytic effect of 66.21% with a volume of 1 mL, whereas Diclofenac and Ibuprofen present an effect of 25.94% and 40%, respectively, with the same volume. Thus, from a comparative study of the effect of the sap and that presented by the two reference drugs, it is found that the treatment of erythrocytes with the sap presents an inhibition of hemolysis clearly superior to that obtained with Diclofenac and Ibuprofen at the volumes tested. This can be justified by the richness of *J. multifida* sap in phenolic compounds, especially flavonoids.

The results of the phytochemical screening of *J. multifida* indicated that the sap is very rich in flavonoids. Several studies have reported the effect of some molecules belonging to the flavonoid family on the inhibition of cyclooxygenase (COX) and lipooxygenase (LOX) (quercetin and myricetin), TNF-α and NF-κB (luteolin), STAT-1, NF-Kb. They affect the control of MAP kinase and/or COX-2 and the expression of iNOS, as well as the production of NO (genistein) [32–34].

Flavonoids are renowned for their antioxidant activity, attributed to their highly reactive hydroxyl groups. They exert this activity by preventing the increase in microviscosity of erythrocyte membranes induced by lipid peroxidation [35]. Indeed, they can react with most free radicals, removing a hydrogen from the (C-H) group situated between the two double bonds of polyunsaturated fatty acids in the membrane through trapping, inactivating, and stabilizing free radicals (hydroxyl radicals (OH-), superoxide anions (O-), and peroxylipid radicals). Consequently, they form intermediate radical species with low reactivity. Additionally, they can chelate metal ions (released from their binding or transport proteins), which might otherwise enhance deleterious effects by producing hydroxyl radicals (OH-) [36–38]. This justifies the high DPPH inhibition power of 86.61% observed in the sap of *J. multifida* at a concentration of 0.5 mg/ml. Graphically calculated, the concentration of the test sample required to reduce 50% of the DPPH radical (IC_{50}) is 0.102 mg/mL, while that of vitamin C is 0.104 mg/mL. Therefore, the inhibitory power of DPPH by the sap is comparable to that of vitamin C. Consequently, these natural antioxidants also exhibit an anti-hemolytic effect, stabilizing the membrane of red blood cells against hypotonic lysis and maintaining their membrane integrity (the asymmetric distribution of phospholipids) [36]. This attribute confers anti-inflammatory properties to the sap *in vitro*.

The *in vivo* anti-inflammatory effect of *J. multifida* sap in rats was assessed by observing the impact of sap treatment on paw diameter following the induction of inflammation. The edema induced after the injection of 2% formalin into the paw of the mouse consists of three distinct phases. The first phase involves histamine and 5-hydroxytryptamine, which promote vasodilation, plasma transudation, and edema. The second phase involves kinins, which increase vascular permeability. The final phase is characterized by the secretion of prostaglandins and the migration of leukocytes into the inflamed area. Prostaglandins play a role in both acute and chronic inflammatory processes [39]. Generally, anti-inflammatory drugs work by counteracting the effects of these chemical mediators (histamine, serotonin, kinins, and prostaglandins), and the anti-inflammatory property of flavonoids may be attributed to the presence of certain active molecules in the sap [40].

However, from the results of the assessment of the reducing potential of the sap leg diameter. it is clear that the leg diameter increased over time in all batches. This indicates that formalin-induced inflammation increases over time [41]. However, the increase in paw oedema varied between batches of rats. For example, in the formulated batch that received no treatment, a variation in diameter from 5.25 mm at 0 hour to 9.58 mm at 4 hours was observed. an increase of 82.47%. For the batch treated with Diclofenac. an increase of 31.08% was observed between 0 Hour and 4 Hours after formalin injection. For the batches treated with 400 mg/Kg and 800 mg/Kg of *J. multifida* sap. the paw diameters increased by 61.34% and 55.39% respectively. Accordingly, treatment of rats with

Diclofenac. *J. multifida* sap 800 mg/Kg induced inhibition of inflammation by 62.31% and 32.84% respectively. Thus. the rats treated with the sap at 400 mg/Kg showed approximately the same result as the untreated rats (Formol batch). In contrast. the Diclofenac and 800 mg/Kg sap treatments resulted in a significant decrease in paw inflammation. Sap at 800 mg/Kg had the same overall effect on paw inflammation as Diclofenac. A better activity was noted for the hydroalcoholic extract of *Apium graveolens* leaves which significantly reduced the increase in paw volume with a percentage of inhibition of 80.17%. 86.50% and 89.66%. respectively at 200. 300 and 500 mg/kg body weight [42]. This difference may be due to the fact that formalin-induced inflammation. which is the case in our study. seems to be less controllable than carrageenan-induced inflammation [43].

Finally, pretreatment with Diclofenac and *J. multifida* sap significantly reduced TNFα production and there was no significant difference between the effects of these two treatments on the concentration of the pro-inflammatory mediator. However, the increase in TNFα production in the batch treated with *J. multifida* sap (289.1) is lower than in the batch of rats treated with Diclofenac (327.45). These values are closest to those found in the control batch (286.15). As in the present study. the inhibition of the production of pro-inflammatory molecules such as TNF alpha by extracts or derivatives of medicinal plants is widely reported [35, 44].

CONCLUSION

Inflammation is a reaction of the organism associated with several pathologies that must be controlled by anti-inflammatory drugs. In the present work, the study of the anti-inflammatory effect of sap yielded positive results, partially due to its significant role in limiting the inflammatory response by stabilizing and protecting the lysosomal membrane. The sap also demonstrated a strong antioxidant capacity, inhibition of pro-inflammatory factors, including TNFα, and reduction in leg diameter after the induction of inflammation. Based on these findings, further pharmacological studies are necessary to determine the molecular and cellular mechanisms of the active compounds in the sap and to assess their effects on the inflammatory process, as well as on the enzymes involved in the production of reactive oxygen specie.

ABBREVIATIONS AND ACRONYMS

LC_{50}: Lethal Concentration at 50
IC_{50}: Inhibitory Concentration at 50
TNFα: Tumor Necrosis Factor α
NF-κB: Nuclear Factor-kappa B
DPPH: 2,2 diphenyl 1-picrylhydrazyl
COX-1: cyclooxygenase-1
COX-2: cyclooxygenase-2
Co: Circumference
J. multifida: J. multifida
OD: Optical Density
Diclo: Diclofenac
LOX: Lipooxygenase
iNOS: Inducible Nitric Oxide Synthase
NO: Genisteine
STAT-1: Signal Transducer and Activator of Transcription 1

REFERENCES

1. Borges R.S., Ortiz B.L.S., Pereira A.C.M., Keita H., Carvalho J.C.T. Rosmarinus officinalis essential oil: A review of its phytochemistry, anti-inflammatory activity, and mechanisms of action involved. J. Ethnopharmacology. 2019;229:29–45.
2. Oguntibeju O.O. Medicinal plants with anti-inflammatory activities from selected countries and regions of Africa. J Inflamm Res. 2018; 11: 307–317.
3. Ahmed A.U. An overview of inflammation: mechanism and consequences. Frontiers in Biology. 2011;6:274–281.
4. Leelaprakash G., Dass S.M. Invitro anti-inflammatory activity of methanol extract of Enicostemma axillare. International J. Drug Development and Res. 2011;3: 189-196.
5. Assayad O. Enjeux de l'automedication par les ains en periode COVID-19. PhD Thesis 2022.
6. Meriem B.A., Rania B. Contribution à l'étude des effets indésirable De l'automédication, PhD Thesis. Université Larbi Tébessi-Tébessa. 2022.
7. Abdullahi A. A. Trends and challenges of traditional medicine in Africa, African Journal of Traditional. Complementary and Alternative Medicines. 2011;8.
8. Maroyi A. Traditional use of medicinal plants in south-central Zimbabwe: review and perspectives. J. Ethnobiology and Ethnomedicine. 2013;9:1–18.
9. Khan A., Hassan S.M., Mughal S.S. Biological Evaluation of a Herbal Plant: Cichrorium intybus. Sci. Technol. 2022;6:26–38.
10. Akoègninou A., Burg W.J. van der, Maesen L.J.G. van der, Flore analytique du Bénin. Backhuys Publishers, Leiden, 2006.
11. Klotoé J. Ethnopharmacological Survey on Antihemorrhagic Medicinal Plants in South of Benin, EJMP, 2013; 3: 40–51.

12. Klotoe J.R., Ategbo J.M., Dougnon V., Loko F., Dramane K. Hemostatic Effect of *Jatropha multifida* L. (Euphorbiaceae) in Rats Having Coagulation Disorders, J App Biol Biotech, 2017;5:26–29.
13. Victorien D., Toussaint S., Jerrold A., Alidah A., Afoussatou A., Jacques D., *et al*. Evaluation of the Antibacterial Activity of *Jatropha multifida* sap and Artemisia annua Extract on some Clinical Strains Responsible of Urinary Tract Infections. Indian J. Sci. Technol. 2019;12:1–10.
14. Klotoé J., Dougnon V., Dougnon T., Agbodjento E., Ategbo J., Loko F. *Jatropha multifida* linn (euphorbiaceae): exploration des proprietes antibacteriennes et du pouvoir cicatrisant de la seve de cette plante chez le rat albinos de souche wistar, remise: Revue de Microbiologie Industrielle Sanitaire et Environnementale. 2014; 8:120–132.
15. Anani K., Adjrah Y., Améyapoh Y., Karou S.D., Agbonon A., de Souza C., *et al*. Antimicrobial, Anti-inflammatory and Antioxidant Activities of *Jatropha multifida* L. (Euphorbiaceae), Pharmacognosy Res. 2016;8:142–146
16. Moharram F., Marzouk, Haggag E., El-Batran S., Ibrahim R. Biological examination and novel biflavone di-C-glycosides from *Jatropha multifida* L. leaves. Planta Medica - PLANTA MED. 2007; 73.
17. Falodun A., Igbe I., Erharuyi O., Agbanyim O.J. Chemical Characterization, Anti-inflammatory and Analgesic Properties of *Jatropha multifida* Root Bark. Journal of Applied Sciences and Environmental Management. 2013;17:357–362.
18. M. Nguta J., M. Mbaria J., W. Gakuya D., K. Gathumbi P., D. Kabasa J., G. Kiama S. Evaluation of Acute Toxicity of Crude Plant Extracts from Kenyan Biodi-versity using Brine Shrimp, Artemia salina L. (Artemiidae). The Open Conference Proceedings J. 2012;3.
19. Trabsa H. Activité antioxydante et anti-inflammatoire des fractions des plantes médicinales: Sedum sediforme et Lycium arabicum. PhD Thesis. 2018.
20. Falade O.S., Otemuyiwa I.O., Oladipo A., Oyedapo O.O., Akinpelu B.A., Adewusi S.R.A. The chemical composition and membrane stability activity of some herbs used in local therapy for anemia. J Ethnopharmacol. 2005;102:15–22.
21. Sangeetha G., Vidhya R. In vitro anti-inflammatory activity of different parts of Pedalium murex (L.). Int. J. Herb. Med. 2016;4:31–36.
22. Smahia R., Nasser B., Khaled S., Abdelkrim C. Evaluation de l'activité anti-inflammatoire d'extraits aqueux de feuilles Limoniastrum feei (PLUMBAGINACEA), Université Kasdi Merbah Ouargla. 2018.
23. Mohanty S.K., Swamy M.K., Middha S.K., Prakash L., Subbanarashiman B., Maniyam A. Analgesic, Anti- inflammatory, Anti- lipoxygenase Activity and Characterization of Three Bioactive Compounds in the Most Active Fraction of Leptadenia reticulata (Retz.) Wight & Arn. - A Valuable Medicinal Plant, Iran J Pharm Res. 2015; 14: 933–942.
24. Scott P.M., Bilodeau P.S., Zhdankina O., Winistorfer S.C., Hauglund M.J., Allaman M.M., *et al.* GGA proteins bind ubiquitin to facilitate sorting at the trans-Golgi network. Nat Cell Biol. 2004; 6: 252–259.
25. Stankov S. Definition of Inflammation, Causes of Inflammation and Possible Anti-inflammatory Strategies. The Open Inflammation J. 2012; 5: 1–9.
26. Moshi M.J., van den Beukel C.J., Hamza O.J., Mbwambo Z.H., Nondo R.O., Masimba P.J., *et al.* Brine Shrimp Toxicity Evaluation of Some Tanzanian Plants Used Traditionally for the Treatment of Fungal Infections. Afr J Tradit Complement Altern Med. 2006; 4: 219–225.
27. Meyer B.N., Ferrigni N.R., Putnam J.E., Jacobsen L.B., Nichols D.E., McLaughlin J.L. Brine shrimp: a convenient general bioassay for active plant constituents. Planta Med. 1982; 45: 31–34.
28. Amani A., Loubna B. Etude phytochimique et Evaluation de l'activité anti-inflammatoire d'une plante médicinale: Ephédra alata alenda. 2018.
29. Oyedapo O.O., Makinde A.M., Ilesanmi G.M., Abimbola E.O., Akinwunmi K.F., Akinpelu B.A. Biological activities (anti-inflammatory and anti-oxidant) of fractions and methanolic extract of Philonotis hastate (Duby Wijk & MargaDant). African Journal of Traditional. Complementary and Alternative Medicines. 2015;12:50–55.
30. Reshma, Kp A., P B. In vitro anti-inflammatory, antioxidant and nephroprotective studies on leaves of aegle marmelos and ocimum sanctum. Asian J Pharmaceutical and Clinical Res. 2014;121–129.
31. Ahmad T., Ohlsson C., Sääf M., Ostenson C.-G., Kreicbergs A. Skeletal changes in type-2 diabetic Goto-Kakizaki rats. J Endocrinol. 2003; 178: 111–116.
32. Hämäläinen M., Nieminen R., Vuorela P., Heinonen M., Moilanen E. Anti-inflammatory effects of flavonoids: genistein, kaempferol, quercetin, and daidzein inhibit STAT-1 and NF-kappaB activations, whereas flavone, isorhamnetin, naringenin, and pelargonidin inhibit only NF-kappaB activation along with their inhibitory effect on iNOS expression and NO production in activated macrophages. Mediators Inflamm. 2007;456730.
33. Hwang J.-T., Lee Y.-K., Shin J.-I., Park O.J. Anti-inflammatory and anticarcinogenic effect of genistein alone or in combination with capsaicin in TPA-treated rat mammary glands or mammary cancer cell line. Ann N Y Acad Sci. 2009;1171:415–420.
34. Xagorari A., Papapetropoulos A., Mauromatis A., Economou M., Fotsis T., Roussos C. Luteolin inhibits an endotoxin-stimulated phosphorylation cascade and

proinflammatory cytokine production in macrophages. J Pharmacol Exp Ther. 2001; 296: 181–187.

35. Tsai Y.-C., Wang S.-L., Wu M.-Y., Liao C.-H., Lin C.-H., Chen J.-J., *et al.* Pilloin, A Flavonoid Isolated from Aquilaria sinensis, Exhibits Anti-Inflammatory Activity In Vitro and In Vivo. Molecules. 2018; 23: 3177.
36. Chaudhuri S., Banerjee A., Basu K., Sengupta B., Sengupta P.K. Interaction of flavonoids with red blood cell membrane lipids and proteins: antioxidant and antihemolytic effects. Int J Biol Macromol. 2007; 41: 42–48.
37. Ghedira K. Les flavonoïdes: structure, propriétés biologiques, rôle prophylactique et emplois en thérapeutique. Phytotherapy. 2005; 3:162–169.
38. Mladěnka P., Macáková K., Filipskỳ T., Zatloukalová L., Jahodář L., Bovicelli P., *et al.* In vitro analysis of iron chelating activity of flavonoids. Journal of Inorganic Biochemistry. 2011; 105: 693–701.
39. Sanogo R., Diallo D., Diarra S., Ekoumou C., Bougoudogo F. Activité antibactérienne et antalgique de deux recettes traditionnelles utilisées dans le traitement des infections urinaires et la cystite au Mali. Med. Afr. noire (En ligne), 2006;18–24.
40. Rai A.K., Singh S.P., Pandey A.R., Ansari A., Ahmad S., Sashidhara K.V., *et al.* Flavonoids from Polyalthia longifolia prevents advanced glycation end products formation and protein oxidation aligned with fructose-induced protein glycation. Natural Product Research. 2021; 35: 2921–2925.
41. Ho C.-L., Li L.-H., Weng Y.-C., Hua K.-F., Ju T.-C. Eucalyptus essential oils inhibit the lipopolysaccharide-induced inflammatory response in RAW264. 7 macrophages through reducing MAPK and NF-κB pathways. BMC Complementary Medicine and Therapies. 2020;20: 1–11.
42. Dellal A., Toumi-Benali F., Dif M.M., Bouazza S., Brikhou S., Mekhfi N. Anti-inflammatory, Analgesic and Antioxidant Activities of the Hydroalcoholic Extract from Celery (Apium graveolens) Leaves, Phytothérapie. 2018;16:S237–S244.
43. Basit A., Shutian T., Khan A., Khan S.M., Shahzad R., Khan A., *et al.* Anti-inflammatory and analgesic potential of leaf extract of Justicia adhatoda L. (Acanthaceae) in Carrageenan and Formalin-induced models by targeting oxidative stress. Biomedicine & Pharmacotherapy, 2022;153:113322.
44. Gierlikowska B., Gierlikowski W., Bekier K., Skalicka-Woźniak K., Czerwińska M.E., Kiss A.K. Inula helenium and Grindelia squarrosa as a source of compounds with anti-inflammatory activity in human neutrophils and cultured human respiratory epithelium. J Ethnopharma. 2020;249:112311.