International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
(A Peer Reviewed, Referred, Indexed and Open Access Journal)
DOI: 10.22192/ijarbs Coden: IJARQG (USA) Volume 11, Issue 2-2024

Research Article

DOI: http://dx.doi.org/10.22192/ijarbs.2024.11.02.006

Evaluation of anti-arthritic activity of aqueous extract of *Sarcocephalus latifolius* (SM.) bruce fruits on freund's adjuvant-induced arthritis in rats

DA Filkpièrè Léonard[*,1,2], TINDANO Basile[2], BALLO Mahamadou[3], KOUSSOUBE Ignace[1], SOUDRE Albert[1], BAYALA Balé[2], BELEMTOUGRI G. Raymond[2]

[1]Université Norbert ZONGO, Science and Technology Training and Research Unit, Laboratory of Life and Earth Sciences;
[2]Université Joseph KI ZERBO,Science and Technology Training Unit, Laboratory of Life and Earth Sciences, Department of Biology and Animal Physiology, Laboratory of Animal Physiology ;
[3]University of Sciences, Techniques and Technologies of Bamako, Mali - Faculty of Pharmacy
*Corresponding author: DA Filkpièrè Léonard.
University Norbert ZONGO, Laboratory of Life and Earth Sciences, BP: 376,Koudougou, Burkina Faso
E-mail: *dafilleo@yahoo.fr*

Abstract

Objective: Rheumatoid arthritis is a chronic inflammation of the joints that leads to severe bone destruction. Plants can help relieve these diseases. The aim of this study was to evaluate the anti-arthritic effect of aqueous extract of *Sarcocephalus latifolius* fruit on chronic Freund's adjuvant-induced arthritis in rats. **Materials and Methods:** Five groups of rats were injected with 0.1 ml of CFA. These different groups were orally treated with distilled water at 1 ml/kg, *Sarcocephalus latifolius* extract at 100, 250 and 500 mg/kg and prednisone (5 mg/kg) for 21 days. Normal rats served as controls. Inflammation diameter, hematological and biochemical analyses, tissue oxidative markers and paw histological sections were evaluated. **Results:** On day 21, the 500 mg/kg extract produced a 48 % inhibition compared with control rats. This effect was the maximum inhibition. Aqueous extract of *Sarcocephalus latifolius*, at doses of 250 and 500 mg/kg, significantly ($p<0.05$) stabilized some hematological (white and red blood cells, granulocytes, platelets and hemoglobin) and biochemical (transaminases, bilirubin, phosphatase, creatinine and urea) constants altered by CFA. Compared with arthritic rats (CFA), extract at 500 mg/kg induced a significant decrease ($p<0.05$) in

MDA levels and SOD activity in liver, kidney and spleen. At the doses of 100 and 500 mg/kg, extract induced a significant increase ($p<0.05$) in CAT activity in kidney and liver, while all extract doses induced a significant decrease ($p<0.01$) in SOD activity in spleen. The different doses of extract restored bone tissue that had been altered by the adjuvant. **Conclusion:** The results obtained revealed a potential anti-arthritic activity of the aqueous extract of *Sarcocephalus latifolius* fruits.

Keywords: *Sarcocephalus latifolius*, rats, Anti-arthritis, complete Freund's adjuvant, fruit

Introduction

Rheumatism is a term that covers all diseases of the joints and structures around the joint. Degenerative rheumatism, such as inflammatory rheumatism, includes rheumatoid arthritis. Rheumatoid arthritis is a systemic autoimmune inflammatory disease which affects almost 1% of the world's population. In Burkina Faso, the prevalence of degenerative rheumatism is 74.7% with 19.7% for arthrosis (Ouédraogo et *al.*, 2014). This inflammation results in synovial hyperplasia, swelling, joint deformity and bone destruction (Picerno et *al.*, 2015 ; Deane et *al.*, 2017). Rheumatoid arthritis negatively affects patient's quality of life, normal work capability, and life expectancy. Besides the consequences on the health status of individuals, it has a substantial economic impact on patients, their family and society. Arthritis can affect people of any age. However, the usual age of onset is between 25 and 50, with a peak in the 40s and 50s. (Shivanand et *al.*, 2010). Both sexes are affected but the females are more susceptible, approximately in the ratio 3/1 (Silman and Thomson 1993). Age, gender, obesity, eating habits, lifestyle, heredity and hormonal factors are just some of the risk factors for arthritis (Sunetra et *al.*, 2010). In the animal model, injection of CFA into rats induces arthritis. This test induces a systemic pathology with articular and visceral manifestations similar to human rheumatoid arthritis (Zhang et *al.*, 2002). Plantar injection of Freund's complete adjuvant in rats produces an immediate edematous reaction (primary inflammation) (Agathe et *al.*, 2009). The treatment of rheumatoid arthritis today involves four types of medication. These are non-steroidal anti-inflammatory drugs, steroid hormones, disease-modifying anti-rheumatic drugs and immunosuppressants. These treatments have limited efficacy, and many side effects are observed with prolonged administration. Alternative medicine is becoming a promising approach to anti-arthritic therapy. This medicine is based on natural plant extracts. Indeed, various medicinal plants have been studied in rheumatoid arthritis therapy, where a reduction in pain and inflammation has been clearly demonstrated. Many studies worldwide show the nutritional, anti-nephrotoxic and hepatoprotective properties of *Sarcocephalus latifolius* (Plassart et *al.*, 2015, Da et *al.*, 2023). However, few studies have been conducted on the anti-arthritic properties of *Sarcocephalus latifolius*. The aim of this study was to evaluate the anti-arthritic effect of aqueous extract of *Sarcocephalus latifolius* fruit on chronic Freund's adjuvant-induced arthritis in rats.

Materials and Methods

Plant

Collection and authentication of plant

The fruits of *Sarcocephalus latifolius* (Smith) Bruce were collected in Gaoua, in southwest of Burkina Faso. This harvest occurred in august 2023 between 6 a.m. and 8 a.m. The plant was identified and a sample was deposited at the "Laboratoire de Biologie et Écologie Végétale" of Université Joseph KI-ZERBO under number 18028.

Plant extraction

These fruits were washed, cut into small pieces, dried without sunlight and pulverized. Four hundred grams (400 g) of powder were macerated in 1000 ml of distilled water for 24 hours. The

filtrate was centrifuged at 2000 rpm for 10 minutes. The supernatant was frozen at -23˚C and lyophilized. Thus the aqueous extract of *Sarcocephalus latifolius* fruits (EASL) obtained was stored at -4˚C until the use. The extraction yield was 24.23%.

Animals

Female Wistar rats from Université Joseph KI-ZERBO weighing 150-170 g were used. Rats were subjected to breeding conditions of 22 ± 3 ◦C, 12-h light-dark cycle and 50 ± 10% humidity. They were fed regularly and provided with water *ad libitum*. All methods used in the present study were approved by Animal Experiment Ethics Committee of Université Joseph KI-ZERBO.

Adjuvant-induced chronic arthritis

The effects of aqueous extract of *Sarcocephalus latifolius* fruit were assessed on chronic inflammation induced by Freund's complete adjuvant (FCA) according to the methods described by Pearson et *al.*, (1963), Fotio et *al.*, (2009). Twenty-five rats were injected with 0.1 ml of Freund's complete adjuvant in the paw. On the ninth day of induction, the rats were divided into five groups. The different groups received distilled water at 1 ml/kg, *Sarcocephalus latifolius* extract at 100, 250 and 500 mg/kg and prednisone (5 mg/kg). Treatments were daily and the road of administration was oral. A neutral control group of rats received no induction or treatment of inflammation. Inflamed paw volumes were measured before adjuvant injection (Vo) and on day ninth using the UGO BASILE plethysmometer. Paw volumes were measured every two days until day 21. Edema inhibition was calculated using the following formula :

Edema inhibition (%) = (Vc – Vt/Vc) x 100

Vc is the mean edema volume in the negative control group (no treatment) and Vt is the mean edema volume in the treatment groups (prednisone or extracts).

Biochemical studies

On day 21, rats were anesthetized by dichlormethane inhalation, sacrificed and blood collected in dry tubes and centrifuged at 3000 rpm for 15 min. The serum obtained was collected and stored at -4˚C. Aspartate amino transferase (ASAT), alanine amino transferase (ALAT), alkaline phosphatase (PAL), total bilirubin (TB), creatinine and urea levels were determined. Atlas diagnostic kits were used for these investigations.

Histopathology studies

Three inflamed bone articulations per rat group were collected and fixed in formalin (10%) at the end of sacrifice. Forty-eight hours later, these bones were decalcified in nitric acid (15%) for one week. After immersion in alcohol and xylene baths, the paws were embedded in paraffin. Histological sections of the bones were cut and spread on glass slides. After drying, the prepared slides are stained with hematoxylin and eosin (H&E) for morphological observation under a optical microscope.

Preparation of liver, kidney and spleen homogenates

An equivalent mass of 0.2 g of liver, kidney and spleen was crushed in a porcelain mortar. One (1) millilitre (ml) TrisHCl (50 mM) was added to the crushed tissues. The homogenates were centrifuged at 3600 rpm for 15 minutes. The supernatants were used to determine oxidative stress parameters.

Determination of lipid peroxidation

Tissue MDA content was analyzed to evaluate lipid peroxidation in the different rat groups. This analysis was performed according to Guemmaz et al. (2018) with some modifications. To 0.5 mL homogenate were added 0.5 mL TCA (20%) and 1 ml TBA (0.67%). The mixture was incubated at 95°C for one hour. After incubation, the samples were cooled. The mixture was centrifuged at 3000 rpm for 15 min. The supernatant was collected and the absorbance was read at 532 nm.

Determination of catalase

Homogenate (50 µL) and phosphate buffer (0.1 M ; pH 7.5) (750 µL) were introduced into test tubes. It was added hydrogen peroxide (50 mM) (200 µL) and 1 minute later dichromate/acetic acid solution (2 mL) was added too. In the control tube, homogenate (50 µL) and phosphate buffer (0.1M, pH 7.5) (800 µL) were added. The mixture was heated at 100°C for 10 minutes. After cooling, the optical density was read at 570 nm (Sinha, 1972).

Determination of SOD

The test tube contained 33.5 µL of homogenate and 416.5 µL of carbonate buffer (0.05 M, pH 10.2). In the control tube, 450 µL of carbonate buffer (0.05 M, pH 10.2) was added. The reaction was started by adding 50 µL of adrenaline (0.3 mM) to each tube. The mixture was homogenized. Absorbance was read at 480 nm at times 20 and 80 seconds (Friedewald et al., 1972).

Data analysis

All values are presented as means ± SEM. Differences between the drug-treated groups and the control group were evaluated by independent unpaired sample t-tests using the program Graph pad Prism 5.03. $P < 0.05$ was considered significant.

Results and Discussion

Results

Effect of aqueous extract of *Sarcocephalus latifolius* fruits on rats paw oedema

Injection of Freund's complete adjuvant (FCA) into the rat paw induced inflammation. This injection induced a significant ($p<0.001$) increase in paw volume on day 9, to 0.75 ± 0.05 ml. On day 21, the highest dose of extract produced a maximum inhibition of 48.98% compared with control animals.

Table I : Effect of aqueous extract of *Sarcocephalus latifolius* fruits on rat paw oedema

Treatments	Days				
	9	12	15	18	21
DW 1 mg/kg	0.75 ± 0.05	0.85 ± 0.07	0.62 ± 0.03	0.60 ± 0.02	0.56 ± 0.05
Prednisone 5 mg/kg	0.34 ± 0.02 (54.19) ***	0.35 ± 0.06 (59.64) ***	0.22 ± 0.02 (65.29) ***	0.21 ± 0.02 (64.74) ***	0.24 ± 0.03 (57.47) ***
EASL 100 mg/kg	0.57 ± 0.03 (23.32) *	0.58 ± 0.04 (30.82) *	0.43 ± 0.02 (29.76) ***	0.42 ± 0.01 (30.71) ***	0.37 ± 0.02 (33.31) *
EASL 250 mg/kg	0.48 ± 0.02 (35.73) ***	0.52 ± 0.03 (38.86) **	0.37 ± 0.02 (39.83) ***	0.38 ± 0.03 (38) ***	0.35 ± 0.03 (37.54) **
EASL 500 mg/kg	0.43 ± 0.04 (43.08) ***	0.45 ± 0.06 (47.19) ***	0.34 ± 0.01 (43.39) ***	0.34 ± 0.02 (43.25) ***	0.28 ± 0.03 (48.98) ***

DW : Distilled water. The table shows the volumes of inflamed paws. Values in parenthesis show percent inhibition. (*) indicates comparison between DW and other groups. n = 5

Effect of aqueous extract of *Sarcocephalus latifolius* fruits on rat blood cell counts

Table II shows the effects of aqueous extract of *Sarcocephalus latifolius* fruit on haematological parameters. The analysis showed a significant increase ($p<0.001$) in white blood cells, lymphocytes and granulocytes, and a significant decrease ($p<0.001$) in red blood cells, hemoglobin and platelets in the negative control group (CFA). At the doses of 250 and 500 mg/kg, white blood cell counts were significantly decreased ($p<0.01$) compared with the negative control group. At the same doses, the extract caused a significant

increase in red blood cells ($p<0.05$) compared with the negative control group. At all doses, the extract caused a significant decrease in granulocytes ($p<0.001$). At the dose of 500 mg/kg, the extract produced a significant increase in platelets and hemoglobin ($p<0.001$) compared with the negative control group.

Tableau II: Effect of aqueous extract of *sarcocephalus latifolius* fruits on rat blood cell counts

	DW 1 mg/kg	**CFA**	Prednisone **5 mg/kg**	EASL **100 mg/kg**	EASL **250 mg/kg**	EASL **500 mg/kg**
White Blood Cell (10^3/**µL**)	4.93 ± 0.16 ≠≠≠	8.05 ± 0.06 ***	5.71 ± 0.1 ≠≠≠	7.04 ± 0.56 **	6.26 ± 0.37 ≠≠	6.27 ± 0.31 ≠≠
Lymphocytes (%)	42.16 ± 0.52 ≠≠≠	64.15 ± 1.32 ***	49.73 ± 4.12	59.88 ± 1.65 ***	53 ± 0.94 * ≠	51.38 ± 1.15 *≠≠
Granulocytes (%)	30.8 ± 1.43 ≠≠≠	58.85 ± 2.40 ***	34.01 ± 1.47 ≠≠≠	36.21 ± 0.96 ≠≠≠	34.07 ± 1.08 ≠≠≠	36.84 ± 1.13 ≠≠≠
Platelet (10^3/**µL**)	1039 ± 5.40 ≠≠≠	710.39 ± 79.2 ***	980.31 ± 13.02 ≠≠≠	819.5 ± 5.10 **	920.25 ± 21.69 ≠≠	995.5 ± 29.95 ≠≠≠
Red Blood Count (10^6/**µL**)	9.47 ± 0.08 ≠≠≠	7.67 ± 0.29 ***	8.80 ± 0.29 ≠≠	8.31 ± 0.05 **	8.6 ± 0.15 ≠	8.58 ± 0.21 ≠
Hemoglobin (g/dL)	16.84 ± 0.50 ≠≠≠	11.98 ± 0.50 ***	15.2 ± 0.38 ≠≠≠	14.26 ± 0.3 ** ≠≠	14.46 ± 0.3 ** ≠≠	14.79 ± 0.47 *≠≠≠

DW : Distilled water. (*) indicates comparison between CFA and other groups ; (≠) indicates comparison between DW and other groups. n = 5

Effect of aqueous extract of *Sarcocephalus latifolius* fruits on rats liver and kidney markers

The figures 1 and 2 shows the effects of aqueous extract of *Sarcocephalus latifolius* fruit on levels of ASAT, ALAT, total biluribin, creatinine, urea and alkaline phosphatase activity in blood. ASAT, ALAT and creatinine levels were highly significantly ($p<0.001$) increased in negative control rats compared with normal controls. Total biluribin and alkaline phosphatase activity were not significantly ($p>0.05$) increased in negative control rats compared with normal control group. Urea levels were significantly ($p<0.05$) increased in negative control rats compared with normal control group. At doses of 250 and 500 mg/kg, the extract induced a highly significant ($p<0.001$) decrease in ALAT, ASAT and creatinine levels compared with negative control rats. At 500 mg/kg, the extract induced a significant decrease ($p<0.05$) in urea levels compared with negative control rats. Total biluribin and alkaline phosphatase activity were not significantly reduced ($p>0.05$) compared with negative control rats.

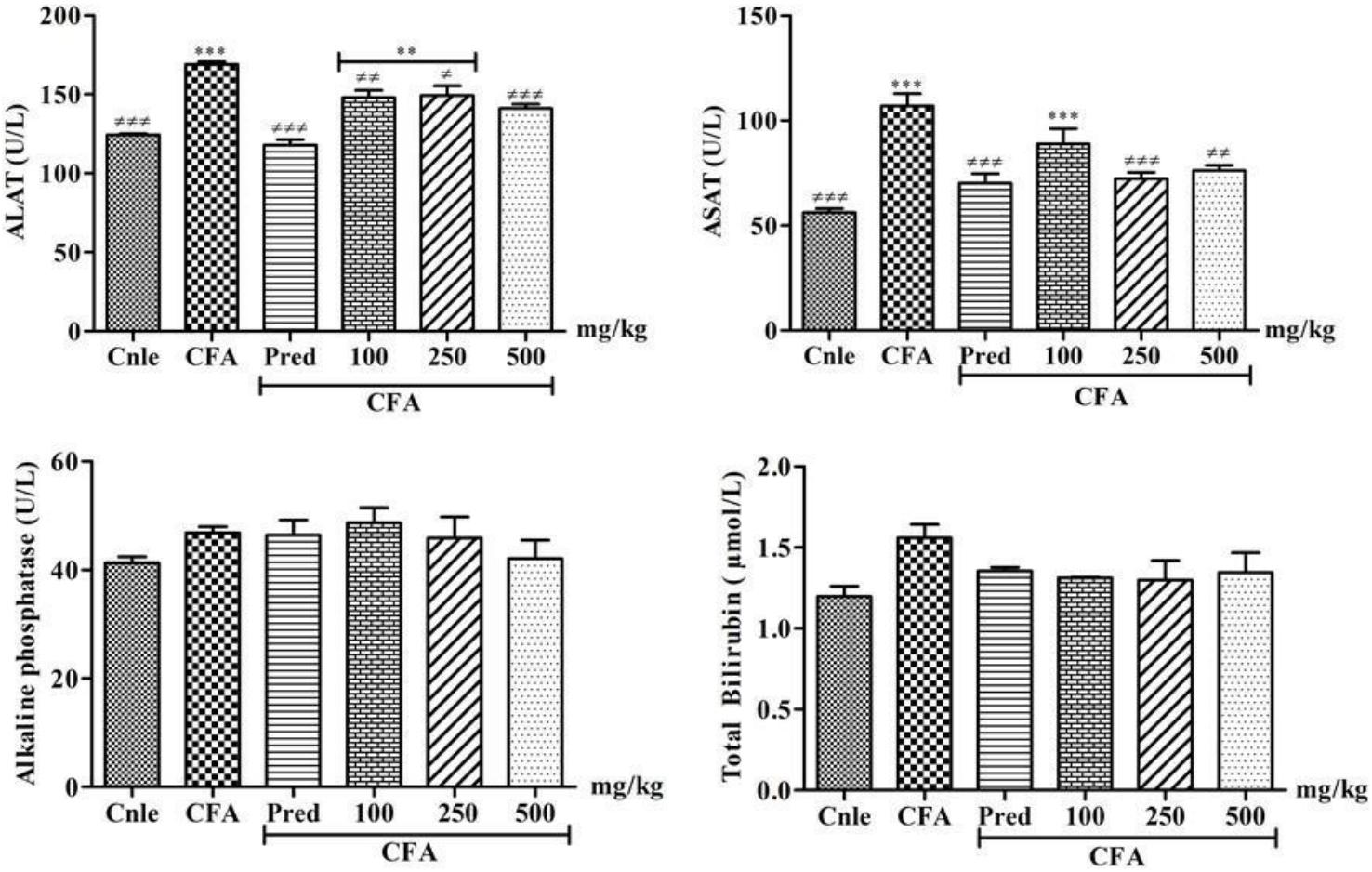

Figure 1: Effect of aqueous extract of *Sarcocephalus latifolius* fruits on rats liver markers

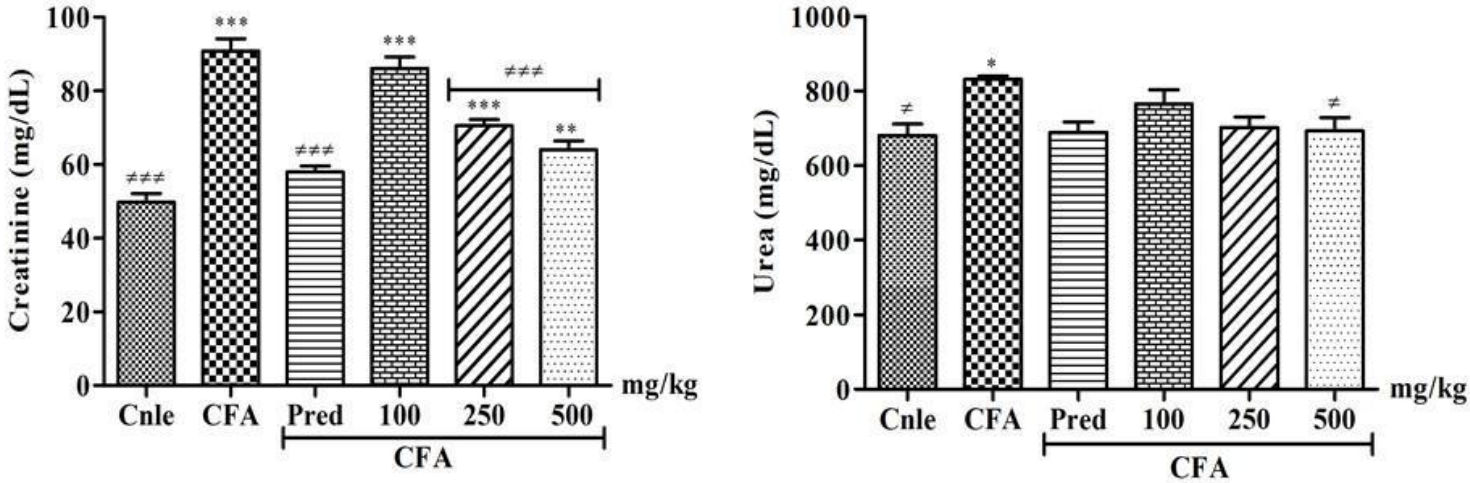

Figure 2: Effect of aqueous extract of *Sarcocephalus latifolius* fruits on rats renal markers

Antioxidant effects of aqueous extract of *Sarcocephalus latifolius* fruits on liver, kidneys and spleen

The figures 3 show the effects of aqueous extract of *Sarcocephalus latifolius* fruit on malondialdehyde (MDA) levels, catalase (CAT) and superoxide dismutase (SOD) activities in liver, kidney and spleen. MDA levels and SOD activity were significantly increased ($p<0.01$) in the livers, kidneys and spleens of negative control rats compared with normal control group. CAT activity, on however, decreased significantly ($p<0.01$) in the livers, kidneys and spleen of negative control group compared with normal control group. At the dose of 500 mg/kg, the extract induced a significant decrease ($p<0.05$) in MDA levels and SOD activity in the livers, kidneys and spleens of negative control rats. At the same dose, the extract induced a significant increase ($p<0.05$) in CAT activity compared of the liver of negative control rats. At the dose of 100 mg/kg the extract induced a significant increase ($p<0.05$) in CAT activity of kidneys compared with negative control rats kidneys. All doses of extract caused a significant decrease in SOD activity of spleen ($p<0.01$) compared with negative control group spleen (Figure 3).

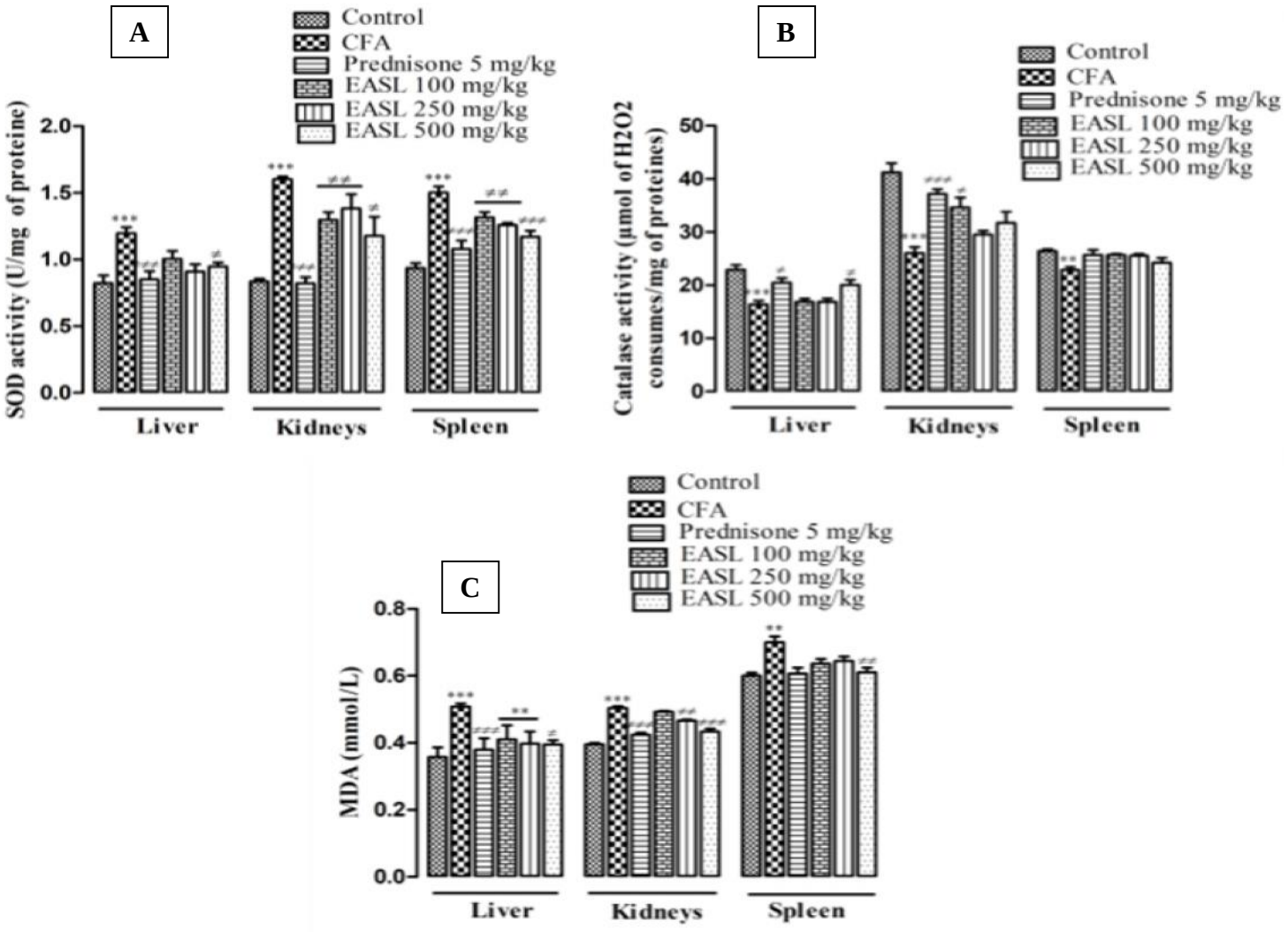

Figure 3: Antioxidant effects of aqueous extract of *Sarcocephalus latifolius* fruits on liver, kidneys and spleen. A : Superoxide dismutase activity ; B : Catalase activity ; C : Malondialdehyde level.

Effects of aqueous extract of *Sarcocephalus latifolius* fruits on ankle joint histopathology

CFA caused joint space erosion, granuloma formation and synovial expension. The different treatments with aqueous extract of *Sarcocephalus latifolius* showed a reduction in erosion characterized by a decrease in joint space, granuloma formation and synovial pannus (figure 4).

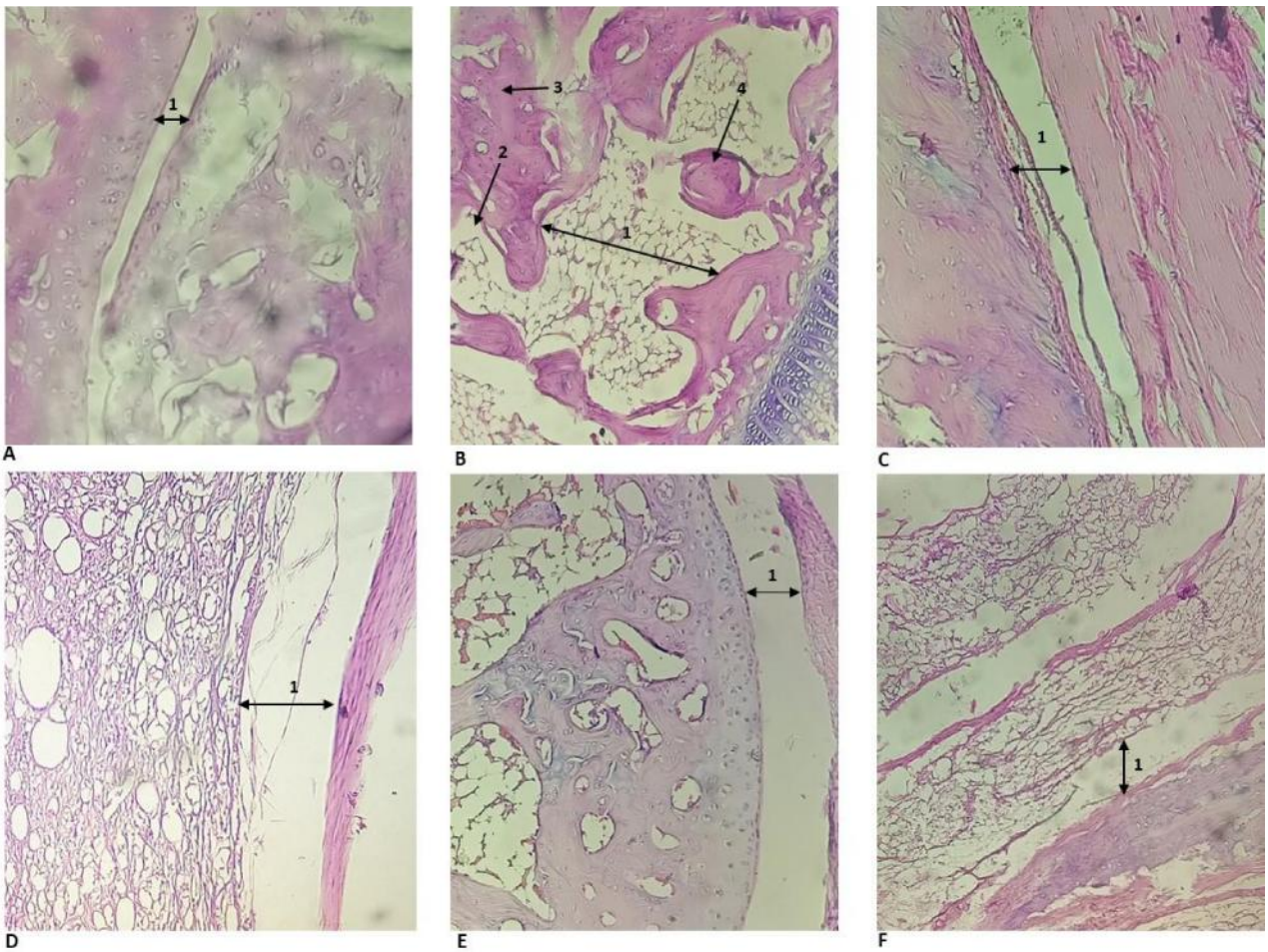

Figure 4 : Histopathology of ankle joint of CFA-injected arthritic rats (H&E x 100).
A : Healthy control show normal structure with small joint space (1) ; B : Arthritic control which shows very large joint space (1), pannus formation (2), synovial infiltration of mononuclear cells (3) and granuloma formation (4) ; C : Prednisone 5 mg/kg ; D : EASL 100 mg/kg ; E : EASL 250 mg/kg ; F : EASL 500 mg/kg.

Discussion

Freund's Complete Adjuvant is a bacterial component made from water-oil emulsion and complete mycobacteria. This adjuvant contains bacterial-specific molecular motifs able to activate non-specific defense mechanisms (O'Hagan, 2001). These components mimic the pro-inflammatory signals received from the immune system during a bacterial infection. These signals will determine the type of specific response initiated. Freund's Complete Adjuvant combines a dual effect. The first is linked to the water-oil emulsion, and the second to the activation of non-specific immunity in attenuated bacteria. These properties make CFA a powerful activator of both humoral and cellular responses (Comoy et al., 1997). The humoral response is

due to the oil-in-water emulsion. The cellular response is attributable to mycobacterial extracts (Billiau and Matthys, 2001). The strong pro-inflammatory properties of CFA are responsible for its many adverse effects. CFA causes abscesses, granulomas and skin necrosis. It is pyrogenic and can cause polyarthritis and various lesions due to autoimmune reactions (Murray et al., 1972).

ATPase- Calcium-dependent degrades ATP to produce energy to facilitate calcium incorporation into cell membranes. This promotes the release of histamine stored in vesicles. Anti-edema effects are attributed to the influence of flavonoids on histamine production. Indeed, flavonoids found in the aqueous extract inhibit the enzymes responsible for histamine release that cause rat paw edema. Polyphenols such as flavonoids have been shown to inhibit lipoxygenase. Some flavonoids are also powerful inhibitors of arachidonic acid, phospholipase A2, cyclooxygenase and NOS. This reduces the production of prostaglandins, leukotrienes and NO, which are key inflammatory substances. Flavonoids also reduce chemokine release, thus reducing leukocyte infiltration and edema (Serafini et al., 2010 ; García-Lafuente et al., 2009 ; Read et al., 1995).

The phagocytosis that accompanies a viral or bacterial infection is followed by the production of reactive oxygen species (ROS) by neutrophils, which will promote inflammation. The balance between free radical production and antioxidant defenses helps preserve cell integrity (Aitken and Fisher, 1994). ROS pathological effects are usually mediated by ion channel opening, lipid peroxidation, protein modifications, and DNA oxidation. The pro-inflammatory molecules generated in stressful situations through ROS cause inflammation, which plays a major role in aging and the development of autoimmune diseases (rheumatoid arthritis, inflammatory bowel disease) (Srivastava et al., 2015 ; Glennon-Alty et al., 2018). Mechanisms of antioxidant action can include suppression of ROS formation either by inhibition of enzymes or by chelating trace elements involved in free radical generation; scavenging ROS ; and upregulation or protection of antioxidant defenses (Halliwell et al., 1999 ; Mishra, et al., 2013). Lipid peroxidation is a common consequence of oxidative stress. Flavonoid protect lipids against oxidative damage (Kumar et al., 2013). Numerous flavonoids have shown to be antiinflammatory by binding free radicals, and decreasing radical production, consequently, being ideal compounds to target inflammation (Ciz et al., 2012, Ribeiro et al., 2015)

Aqueous extract of *Sarcocephalus latifolius* fruit inhibits the proliferation of lymphocytes. Many studies have shown that polyphenols possess anti-inflammatory properties **(**Recio et al., 2012 **;** Vauzour et al., 2010**)** and are able to modulate the immune system through the modification in cytokines production, immune cell populations, and pro-inflammatory gene expression (Karasawa et al., 2011 ; John, et al., 2011). Polyphenols are powerful inhibitors of B and T lymphocytes proliferation (Hachimura et al., 2018). Blood analysis provides information to assess an organism's state of health (Marieb, 1999). A decrease in red blood cell count was due to kidney or bone marrow dysfunction. Tissue necrosis, stress or leukemia can cause a decrease in white blood cell levels (Young, 2010). Total cholesterol and triglyceride levels provide an indication of liver health (Guyton and Hall, 2003). Cholesterol is mostly produced by the liver (Semenkovich, 2007). Creatinine is produced by the breakdown of muscle creatine and eliminated by the kidneys (Perrone et al., 1992). An increase in its serum level indicates renal failure and is therefore a very good indicator of glomerular function (Delanaye, 2010). Higher-than-normal levels are sometimes indicative of acute tubular necrosis. Lower-than-normal levels may indicate muscular dystrophy (Bazari, 2007). Bilirubin is the final metabolite of heme oxidative catabolism. A marked increase in bilirubin levels is due to hemolysis. A moderate increase in bilirubin may be caused by hepatitis. However, a moderate level of bilirubin protects tissues against the oxidative effect of free radicals and other oxidants (Baranano et al., 2002).

Aqueous extract of *Sarcocephalus latifolius* stabilizes the hematological and biochemical constants modified by the adjuvant.

TNF- α activates inflammatory signaling pathway in vascular cells, and regulates the expression of cell adhesion molecules on endothelial cells, thus playing an important role in various inflammatory diseases (Garg et al., 2022 ; Rahman et al., 1998). Aqueous extract of *Sarcocephalus latifolius* fruit reduces edema, inflammatory cell migration, cartilage damage and bone erosion in the rat arthritis model. Our results are comparable to those of Rosillo et al. (2014) who showed that extra-virgin olive oil, rich in polyphenols produces the same effects on mice. These effects are the inhibition of NF-κB, which regulates the production of inflammatory mediators such as IL-1β, TNF, IL-6 and PGE2. Aqueous extract of *Sarcocephalus latifolius* fruit exerts a protective action on synovial expansion and reduces histological lesions in rat paws. Similar studies have shown that emodin, a polyphenolic compound obtained from Rhubarb, inactivates enzymes involved in epigenetic mechanisms, such as histone deacetylase, and reduces the production of pro-inflammatory mediators (Ha et al., 2011). Secondary metabolites contained in *Sarcocephalus latifolius* extract inhibit bone resorption by acting on osteoclasts. Rhubarb polyphenols inhibit osteoclast differentiation and the classical inflammatory pathways of arthritic collagen induction in mice (Hwang et al., 2013).

Conclusion

From these current experimental results on haematological, biochemical and oxidative parameters, it is concluded that at the dose of 500 mg/kg, the aqueous extract of *Sarcocephalus latifolius* fruit has the best anti-arthritic activity. *Sarcocephalus latifolius* fruits have anti-arthritic potential and could therefore be used in the treatment of chronic inflammatory diseases. Their anti-arthritic activity may be linked to their bioactive phytoconstituents. In the future, phytochemical screening is therefore needed to determine the metabolites of *Sarcocephalus latifolius* fruits.

Acknowledgments

The authors acknowledge the « Laboratoire de Toxicologie, Environnement et Santé, Ouagadougou, Burkina Faso » for its technical support during the research period.

Conflicts of interest

All authors declared no conflicting interests.

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ARTICLE INFO

Article History:

Received 7th January, 2024
Received in revised form 9th February, 2024
Accepted 13th February, 2024
Published online 29th February, 2024

Access this Article in Online

Website: www.ijarbs.com

Subject: Pharmacology

Quick Response Code

DOI:10.22192/ijarbs.2024.11.02.006

How to cite this article:
DA Filkpièrè Léonard, TINDANO Basile, BALLO Mahamadou, KOUSSOUBE Ignace, SOUDRE Albert, BAYALA Balé, BELEMTOUGRI G. Raymond. (2024). Evaluation of anti-arthritic activity of aqueous extract of *Sarcocephalus latifolius* (SM.) bruce fruits on freund's adjuvant-induced arthritis in rats. Int. J. Adv. Res. Biol. Sci. 11(2): 48-60.
DOI: http://dx.doi.org/10.22192/ijarbs.2024.11.02.006