

ANTI-INFLAMMATORY EFFECT OF THE HYDROALCOHOLIC EXTRACT OF *Muehlenbeckia tamnifolia* (Kunth) Meins LEAVES IN A RAT PAW MODEL

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Abstract

Inflammation is the local or systemic process by which a vascularized tissue defends itself against different situations through cellular and molecular mediators (references of molecules and inflammatory cells) that play a primordial role in the maintenance of the organism homeostasis. Classically its treatment has required the intervention of different agents like Anti-inflammatory Non-Steroidal (NSAIDs), Corticosteroids and biological therapy; these drugs, despite being effective, have many side effects. The purpose of this research is to evaluate the anti-inflammatory activity of the hydroalcoholic extract of leaves of the plant species *Muehlenbeckia tamnifolia* (Kunth) Meins in a rat model. 20 female Wistar rats were randomly distributed in 4 groups of 5 animals each; inflammation was induced by the injection of 0.1 ml of a 1% lambda-carrageenan solution into the plantar aponeurosis of the right paw of the rats. Group I and II were treated with the complete *Muehlenbeckia*, at different doses, Group III was positive control (Diclofenac) and Group IV was negative control (water). With the lowest dose of the extract had a lower net volume of inflammation as with diclofenac; same result was observed with the percentage inhibition of inflammation.

Keywords: *Muehlenbeckia tamnifolia*, anti-inflammatory activity, carrageenan, medicinal plants.

Introduction

Inflammation is the local or systemic process by which a vascularized tissue defends itself against different situations through cellular and molecular mediators (references of molecules and inflammatory cells) that play a primordial role in the maintenance of the organism homeostasis [1-2]. Acutely, the inflammatory process allows the elimination of infectious type noxas, tissue repairing through local mechanisms or favoring the migration of substances circulating in the blood; However, this initially physiological process may not be self-limiting or it may not be effective in its purpose giving way to a pathological outcome such as autoimmune diseases, some metabolic or degenerative disorders and hypersensitivity reactions [2-4].

The identification of the inflammatory response is subject to different tools depending on the manifestation and evolution time of the same, because according to the latter, it is classified as acute (related to an innate immunity response) or chronic (it is accompanied by adaptive immunity) in which the processes that occur in each one, allow the intervention of different cellular and molecular mechanisms [2].

Classically the treatment of inflammation has required the intervention of different agents among these groups the most used are Anti-inflammatory Non-Steroidal (NSAIDs), Corticosteroids and biological therapy, with different level of complexity for its use, effectiveness and indications in the Inflammatory diseases, However, its adverse effects are well known which vary with each group, including increased cardiovascular risk or spoilage of a pre-existing condition, metabolic syndrome, diabetes mellitus, immunosuppression, tendency to bleeding, liver and renal damage, in addition to the high costs associated with the consumption of these medicines [5-8].

Considering these antecedents, to know in detail the pathophysiology of the inflammatory diseases, allows the exploration of alternative managements; And this is how in the literature, through different methodological designs, it has attempted to demonstrate the anti-inflammatory potential of various plants or their derivatives as *Withania somnifera*, *Gastrodia elata*, *Ziziphus spina-christi*, *Geranium wilfordii*, *Piptadeniastrum africanum*, *Aloe emodin*, *Cannabis sativa*, *Altaea officinalis* L, *Arctium lappa* L, *Artemisia absinthium* L, *Citrus medica*, *Uncaria tomentosa*, *Rosa canina*, *Andrographis paniculata*, *Ginger* among others. Often without sufficient scientific evidence to support its use, as is especially the case with *Muehlenbeckia tamnifolia* (Kunth) Meins (Polygonaceae), whose species are frequently, used through various preparations, for the treatment of gastric ulcers, kidney affections, joint pains, attributing to it an important component against inflammation [12-25].

In spite of the above, and to the specific location of the species *Muehlenbeckia tamnifolia* (Kunth) Meins in South America, there are very few studies on this species that allows to go beyond the traditional indigenous knowledge of the areas where it concentrates, concentrating more towards Pain relief, wound repair, and ultimately antidiabetogenic activity [25-26], however, none of these studies have been developed in Colombia and have not scientifically demonstrated the anti-inflammatory activity of the plant that explains its action in the different entities, The purpose of this research is to evaluate the anti-inflammatory activity of the hydroalcoholic extract of leaves of the plant species *Muehlenbeckia tamnifolia* (Kunth) Meins in a rat model.

Materials and Methods

Plant material

The plant material of *M. tamnifolia*, was collected in the municipality of La Calera (Cundinamarca, Colombia). The species was identified in the National Herbarium of Colombia with the voucher number COL550147.

Extraction procedure

The dried and milled plant material (600 g) was extracted with ethanol at room temperature for 30 days (maceration). The dried ethanolic extract (89 g) was dissolved with an ethanol / water (1: 1) mixture; Subsequently, the mixture was concentrated under reduced pressure at 40 ° C to yield 0.945 g of hydroalcoholic fraction.

Acute toxicity test

The bioassay was performed in the laboratory of the Juan N. Corpas University Foundation with Swiss albino ICR-CD1 mice distributed in 4 groups of 5 animals each. For determination of lethal dose 50 (LD50) of the hydroalcoholic fraction of *M. tamnifolia*, all groups of animals were placed in standard cages at room temperature (20 ± 2 ° C) and 12-hour light / dark cycles, With balanced feed (LabDiet) and water (ad libitum). Prior to dose administration, the animals were fasted for 12 hours with free access to water. Then, 175, 550 and 2000 mg / kg of the hydroalcohol extract were administered intraperitoneally to each group, respectively.

Anti-inflammatory activity in a rat paw edema model induced by carrageenan

From the 3,5 mg/ml solution (170 mg/kg dose) two serial dilutions with 1/5 dilution factor were prepared, giving concentrations of 0,7 mg/ml and 0,14 mg/ml. These concentrations were evaluated in 20 female Wistar rats of 4-6 weeks of age weighing between 130 and 170 g, randomly distributed in 4 groups of 5 animals each, which were subjected to 12 hours of fasting with limitation in consumption of water during the time of the experiment, in order to guarantee uniformity in the hydration and to minimize the variability in edematous response.

Inflammation was induced by the injection of 0.1 ml of a 1% lambda-carrageenan solution into the plantar aponeurosis of the right paw of the rats. Group I was treated with the complete *Muehlebeckia* extract at a dose of 4.66 mg/kg. Group II was given the complete *Muehlebeckia* extract at a dose of 0.93 mg/kg. Group III was used as a positive control and given diclofenac at a dose of 100 mg/kg. Group IV was the negative control and only received saline solution. All treatments were administered intraperitoneally. Measurement of the volume of the inflamed paw was performed by immersion using a Ugo Basile model 7140 plethysmometer. The measurements were made in duplicate and recorded at times of 1, 3 and 5 hours after the start of the experiment.

The inflammation inhibition percentage was calculated as follow:

$$\text{inflammation inhibition (\%)} = \frac{\bar{X}_t - \bar{X}_c}{\bar{X}_c} \times 100$$

\bar{X}_t : Mean of inflammation of treated group

\bar{X}_c : Mean of inflammation of control group

Histopathological study

Histological section

Subsequent to the application of the ethanolic extract of *M. tamnifolia*, histological sections were made to the lower extremities of the animal, which were preserved in a 10% formaldehyde solution. The samples were then fixed with paraffin, cut to a thickness of 15 microns and placed on a slide. Subsequently the corresponding staining was performed. These slides were sent to the pathology department to evaluate the lesions.

The histopathological process was performed in four stages: 1. Fixation of the tissue with 10% formaldehyde, 2. Dehydration of the tissues, 3. Impregnation in paraffin, cut, staining (Hematoxylin-Eosin) and assembly, 4. Observation of material in Microscope (enlargements 40, 100 and 400).

The parameters associated with the normal inflammatory response were evaluated: local erythema, edema, vasodilatation and inflammatory cell infiltration (2).

Statistical analysis

The results were expressed as the mean \pm SEM (standard error of the mean). To determine the differences between the different groups, a two-way ANOVA analysis and the significance test for multiple Bonferroni comparisons were performed. A $p < 0.05$ was considered significant. For the analysis, the statistical packages GraphPad Prism Version 6.0 and Minitab Version 15 were used.

The calculation of the LD50 was performed using the Probit function of the Graphpad Prism 6.0 software.

Ethical aspects

The project was evaluated by the ethics committee of the university to ensure the welfare of experimental animals

Results

Following the design of experiments to evaluate the acute toxicity and calculate the lethal dose 50 (LD50) with a Probit regression, a value of 7.5 mg/kg was obtained; with this value, according to the Williams scale, the extract is classified as moderately toxic [27-28].

The hydroalcoholic extract of the leaves of *M. tamnifolia*, at doses of 4.6 and 0.93 mg/kg, shows anti-inflammatory activity in rat paw edema model induced with lambda carrageenan, when comparing the effect with the negative control. Animals treated with the different doses of *M. tamnifolia*, at different experimental times (1, 3 and 5 h), exhibit lower net volumes of inflammation compared to animals given distilled water (Figure 1).

The highest inhibition inflammation percentage is evidenced for the hydroalcoholic extract of *M. tamnifolia* at a dose of 0.93 mg/kg, at the third hour of experimentation. At the fifth hour a slight decrease in anti-inflammatory effect is observed (Figure 2).

Histopathological description

Histological section of Wistar rats / weight 150 g / dose 0,93 mg/kg (Image 1 and 2).

Sample 1: Stratified keratinized squamous epithelium, stroma with edema, leukocyte migration and medium vessel with mild acute inflammation infiltrate.

Sample 2: Stratified keratinized squamous epithelium, stroma with edema and mild acute inflammation infiltrate.

Sample 3: Stratified keratinized squamous epithelium, stroma with edema and mild acute inflammation infiltrate.

Sample 4: Stratified keratinized squamous epithelium, stroma with edema, leukocyte migration and medium vessel with mild and moderate acute inflammation infiltrate.

Sample 5: Stratified keratinized squamous epithelium, stroma with edema and mild acute inflammation infiltrate represented by neutrophil.

Histological section of Wistar rats / weight 150 g / dose 4,66 mg / kg (Image 3 and 4).

Sample 1: Stratified keratinized squamous epithelium, stroma with edema, leukocyte migration and medium vessel with moderate acute inflammation infiltrate.

Sample 2: Stratified keratinized squamous epithelium, stroma with edema and medium vessel with moderate acute inflammation infiltrate.

Sample 3: Stratified keratinized squamous epithelium with moderate acute inflammation infiltrate.

Sample 4: Stratified keratinized squamous epithelium, stroma with edema and moderate acute inflammation infiltrate.

Sample 5: Stratified keratinized squamous epithelium, stroma with edema and moderate acute inflammation infiltrate.

Histological section of Wistar rats / weight 150 g / Negative control (water) (Image 5 and 6).

Sample 1: Stratified keratinized squamous epithelium, stroma with edema, leukocyte migration and small vessel with acute inflammation infiltrate. Striated muscle with usual appearance.

Sample 2: Epitelio escamoso estratificado queratinizado, estroma de tejido fibroso edematizado, infiltrado inflamatorio agudo, neutrófilos, vaso de pequeño calibre con diapédesis, glándulas sebáceas de aspecto usual.

Sample 3: Stratified keratinized squamous epithelium, stroma with edema, leukocyte migration and small vessel with acute inflammation infiltrate represented by neutrophils, Sebaceous glands, striated muscle, adipose tissue of usual appearance.

Sample 4: Stratified keratinized squamous epithelium, stroma with edema, leukocyte migration and small vessel with acute inflammation infiltrate.

Sample 5: Stratified keratinized squamous epithelium, stroma with edema, leukocyte migration and small vessel with moderate acute inflammation infiltrate. Sweat glands, striated muscle, peripheral nerve of usual appearance.

Histological section of Wistar rats / weight 150 g / Positive control (Diclofenac) (Image 7).

Sample 1: Stratified keratinized squamous epithelium, stroma with edema and mild acute inflammation infiltrate. Sebaceous glands and striated muscle of usual appearance.

Sample 2: Stratified keratinized squamous epithelium, stroma with edema and mild acute inflammation infiltrate.

Sample 3: Stratified keratinized squamous epithelium, stroma with edema and mild acute inflammation infiltrate.

Sample 4: Stratified keratinized squamous epithelium, stroma with edema and moderate acute inflammation infiltrate.

Sample 5: Stratified keratinized squamous epithelium, stroma with edema and mild acute inflammation infiltrate.

Discussion:

The results of the research, aimed at evaluating the anti-inflammatory activity of *M. tamnifolia* hydroalcoholic extract, in a rat paw inflammation model with lambda carrageenan, showed that animals treated with the extract, especially at the dose of 0.93 mg/kg,

had a lower net volume of inflammation compared to those who did not receive this treatment; this effect was also observed with previous administration of diclofenac, however, this result is expected given that the latter is part of the group of NSAIDs, drugs recognized and endorsed commercially throughout the world for treatment among other pathologies of inflammation, particularly diclofenac with demonstrated efficacy, safety and rapid response to inflammation and acute pain by having action described on prostaglandins, Leukotrienes and arachidonic acid [29].

Observing the percentage inhibition of inflammation, animals receiving 0.93 mg / kg compared to the negative control or the higher *M. tamnifolia* dose of 4.66 mg / kg had a similar response to the positive control (diclofenac), specifically at the third hour after administration. It is also observed that at the fifth hour, the anti-inflammatory effect achieved decreases; however, this effect is also obtained in the group of rats treated with the NSAID described. This is important considering that the effect of the inflammation induced with carrageenan after being injected into the rat's paw is evidenced as an edema that, despite being localized, presents a biphasic behavior. The initial phase, between the first and the fifth hour, with an exaggerated response of inflammatory expression by the immediate release of various mediators such as histamine, bradykinin, platelet activating factor and serotonin; After this, in the second phase the characteristic cellularity of acute inflammation is observed, which is mainly polymorphonuclear [30-31].

This is consistent with the histopathological findings of the study, which is also consistent with the literature concerning the acute inflammatory process (2), after administration of carrageenan, especially in animals that do not received treatment, given a rapid transient lesion (seconds) with vasoconstriction and later vasodilation, which mainly affects the arterioles and leads to the opening of vascular beds in the area of the lesion, resulting in an increase in blood flow, which is the cause of erythema and the local increase of temperature in the acute phase; this effect was further counteracted when the dose of 0.93 mg/kg of *M. tamnifolia* was given with mild acute inflammatory edema and infiltrate, in contrast to the dose of 4.66 mg/kg for which both Edema as the inflammatory infiltrate in the different samples was moderate.

The anti-inflammatory effect is not dose-dependent, on the contrary an inversely proportional relationship is observed between the effect and the dose used. This fact is explained, taking into account that hydroalcoholic extracts are rich in high polarity degradation compounds such as polymerization products or degradation of lignans, glycosides, polysaccharides, among others, that increasing the concentration of the extract increases the

amount of these Inert substances, decreasing the anti-inflammatory effect [32].

Some phytochemical studies of *M. tamnifolia* have described the presence of polyphenols such as flavonoids, compounds known to show effects that counteract inflammatory processes through direct actions on different cytokines, chemokines and their receptors, nuclear factor Kappa B, type receptors Toll and prostaglandins, even after oral administration, with positive effects on pathologies of high mortality worldwide such as hypertension, intervening directly on endothelial dysfunction, metabolic syndrome, type 2 diabetes and obesity, through Of its antioxidant action improving liver function and in turn exerting a beneficial effect on the metabolism of lipids. Thus, the above could explain the results of the evident anti-inflammatory response observed in this research [25,33-35].

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Conflicts of interest:

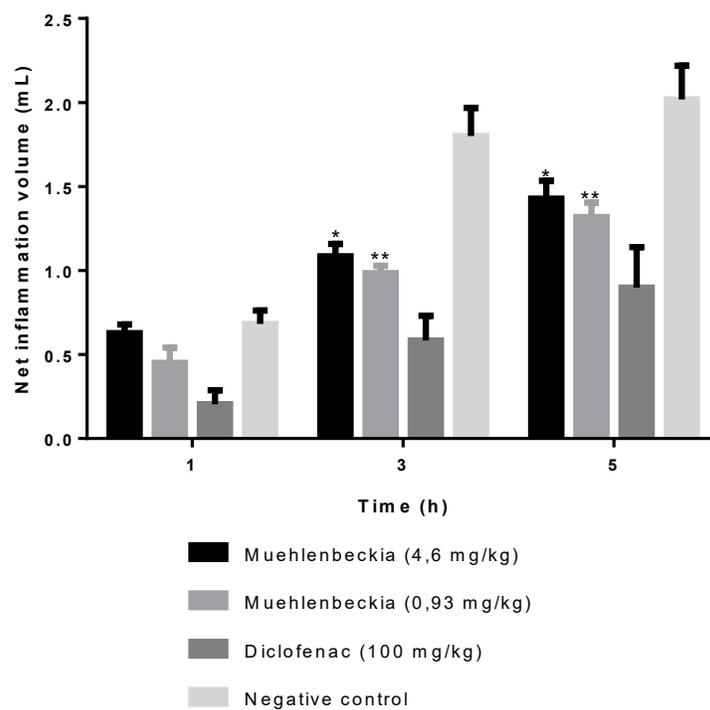
The authors declare that they do not face any conflicts of interest in this article

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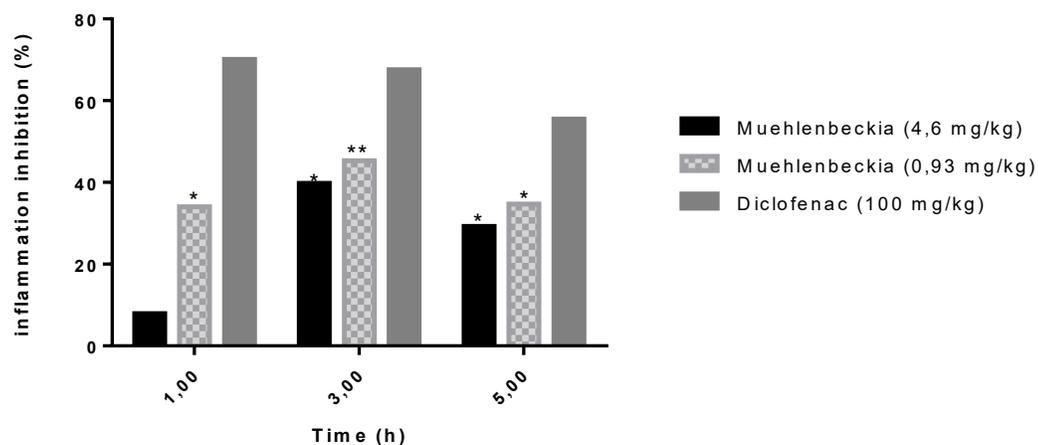
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Figure 1. Anti-inflammatory effect of the leaves of *Muehlenbeckia tamnifolia*, measured as net volume of inflammation.



*,** p<0,05 two-way ANOVA analysis and Bonferroni test for multiple comparisons

Figure 2. Inflammation inhibition (%) of the leaves of *Muehlenbeckia tamnifolia*.



*,** p<0,05 two-way ANOVA analysis and Bonferroni test for multiple comparisons with respect to the negative control.

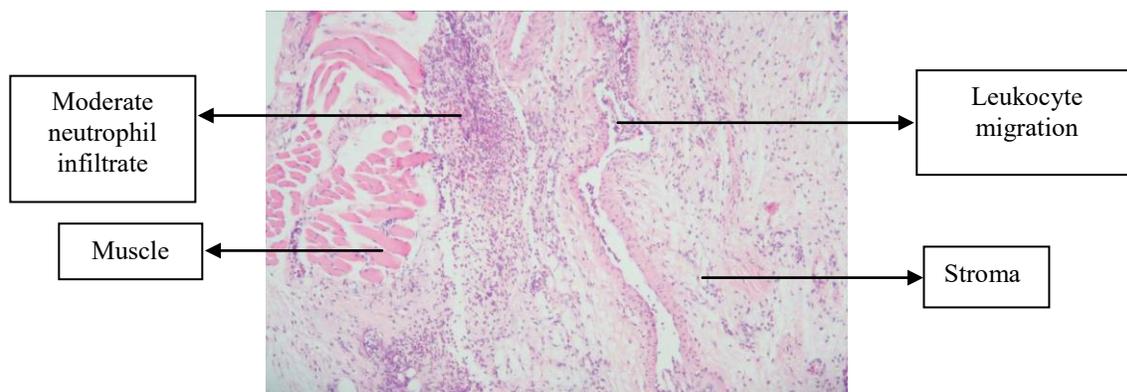


Image 1. Sample No 1 of histological section of right lower limb of Wistar rats, dose 0,93 mg / kg.

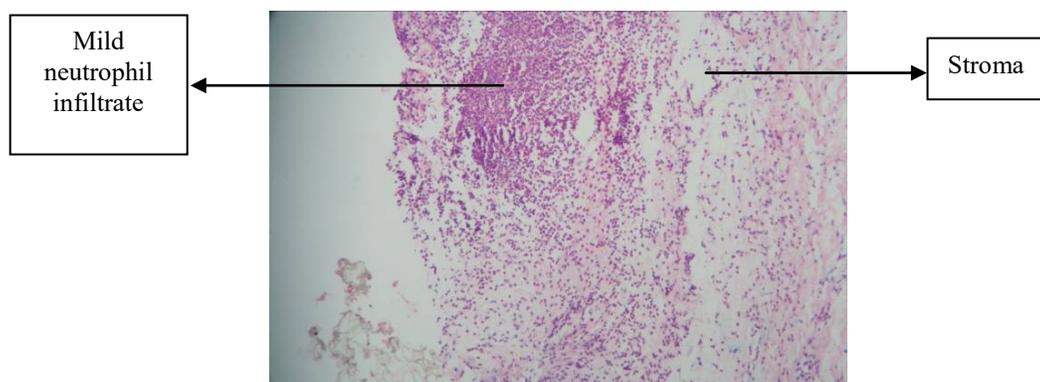


Image 2. Sample No 5 of histological section of right lower limb of Wistar rats, dose 0,93 mg / kg.

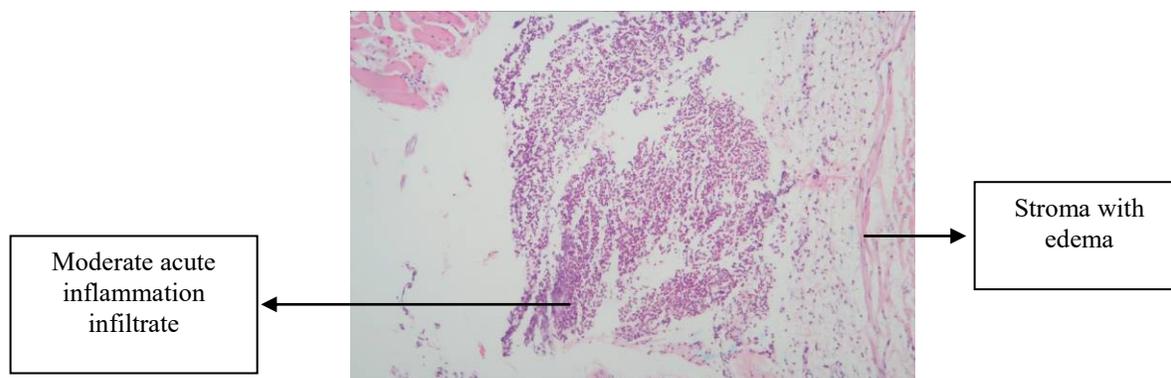


Image 3. Sample No 2 of histological section of right lower limb of Wistar rats, dose 4,66 mg/kg.

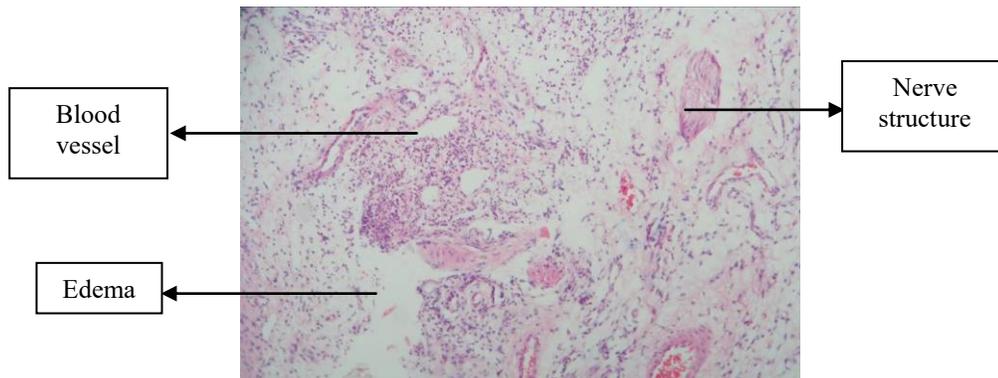


Image 4. Sample No 4 of histological section of right lower limb of Wistar rats, dose 4,66 mg/kg.

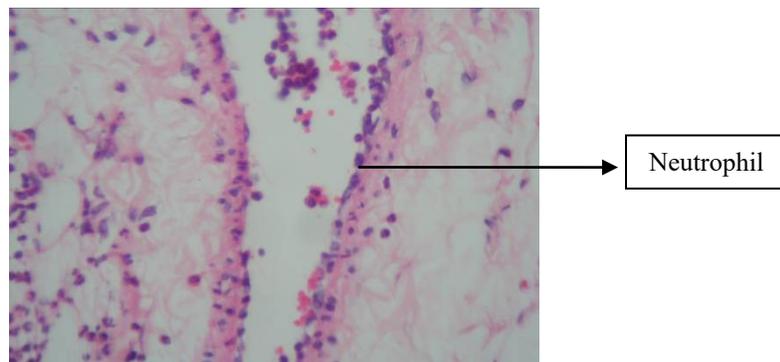


Image 5. Sample No 1 of histological section of right lower limb of Wistar rats, negative control.

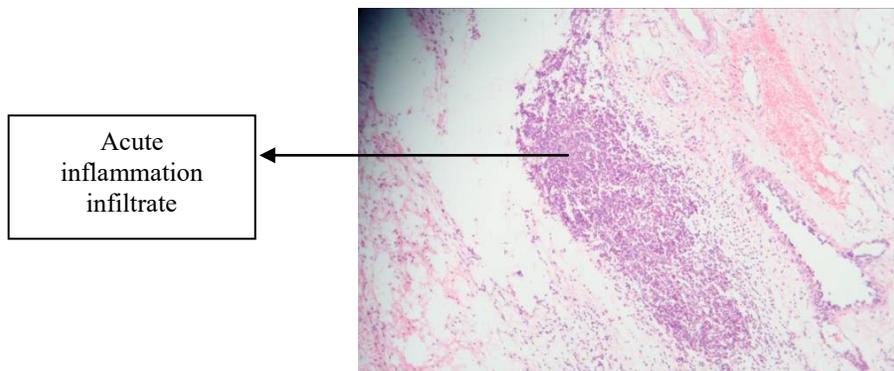


Image 6. Sample No 4 of histological section of right lower limb of Wistar rats, negative control.

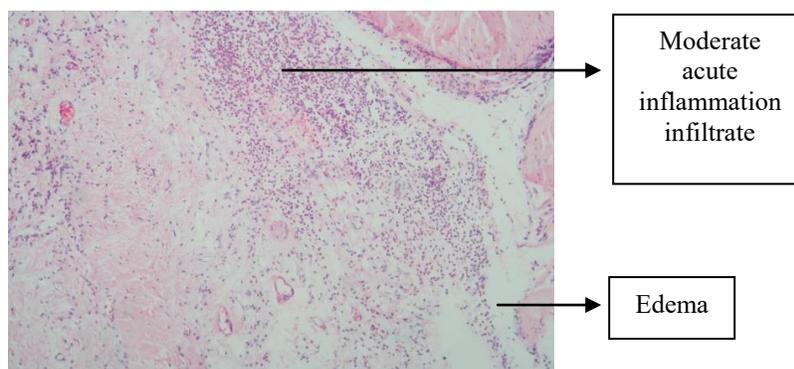


Image 7. Sample No 4 of histological section of right lower limb of Wistar rats, positive control.