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Histological Appearance And Effect Of Aqueous Leaf Extract Of Morinda lucida BENTH In Balb/c Mice In An LPS-Induced Neuroinflammatory Model.

MBON OBAMI Rex Dassaut[1*], MIGUEL Landry Martial[1, 2], ATAKA Modeste[3], AKPO TAYO ONIODJE Wilfried[3], ABENA Ange Antoine[1, 2].

Author's details :

1 Laboratory of Biochemistry and Pharmacology, Faculty of Health Sciences, Marien Ngouabi University, Brazzaville (Congo).

2 Denis Sassou Nguessso University, Kintélé (Congo).

3 Biopathology Department of the medical analysis and morphology laboratory of the Edith Lucie BONGO ODIBA general hospital, OYO (Congo).

Author's department: health human biology ; faculty of Health Sciences, Marien N'GOUABI University

Author's City and Country: Brazzaville, Republic of Congo.

Abstract:

Objectives: The objective of the present study was to evaluate the effects of aqueous leaf extract of Morinda lucida BENTH (M.l BENTH) in Balb/c mice in an LPS-induced neuroinflammatory model. **Methods:** Twenty-five male and female Balb/c mice were divided into five (5) groups of five (5) Balb/c mice each and treated for 7 days (Per os) then with LPS (i.p) for 3 days in groups. (B, C, D, E): group A, distilled water 10 ml/kg; group B, distilled water then LPS; group C, Donépezil 5 mg/kg then LPS; group D, aqueous extract of leaves of M.l BENTH 200mg/kg then with LPS; and group E, aqueous extract of leaves of M.l BENTH 400mg/kg then with LPS. LPS has been used to induce neuroinflammation. Serum levels of p.TAU, β-amyloid, TNF-α, and IL-1β were assessed by Sandwich-ELISA. Histological sections of the brain were taken and stained by the standard HE (hemalun-eosin) method. **Results:** The effects of aqueous extract of M.l BENTH leaves in Balb/c mice in an LPS-induced neuroinflammatory model showed a non-significant increase ($p>0.05$) in the level of β –amyloid concentration in the Balb/c mouse treated with donepezil 5 mg/kg and aqueous extract of M.l BENTH leaves at doses of 200 and 400 mg/kg compared to controls. Significantly higher p.TAU concentration levels in LPS-induced Balb/c mice than in control mice: B: $**p<0.006$, C: $*p<0.01$, D: *p<0, 03, E: $**p<0.004$. Furthermore, a significant increase ($*p<0.01$) in the level of TNF-α in Balb/c mice treated with the aqueous extract of M.l BENTH leaves at a dose of 400 mg/kg and high levels not significant in Balb/c mice treated with donepezil 5 mg/kg and aqueous extract of M.l BENTH leaves at a dose of 200 mg/kg compared to controls. In addition, a non-significant increase ($p > 0.05$) in IL-1β levels in mice treated with donepezil 5 mg/kg and aqueous extract of M.l BENTH leaves at doses of 200 and 400 mg/kg compared to controls. The aqueous extract of M.l BENTH leaves has a dose-dependent positive impact on the inflammatory infiltration of the cerebral cortex of treated Balb/c mice, and on the synthesis of immune cells of the central nervous system. **Conclusion:** The aqueous extract of M.l BENTH leaves appears to regulate the LPS-induced neuroinflammatory process in Balb/c mice by dose-dependently influencing

positively the inflammatory infiltration in the cerebral cortex of Balb/c mice, by stimulating cell synthesis immune system of the central nervous system while fighting against the onset of TAU pathology.

Keywords: Leaves, Morinda lucida BENTH, Balb/c mouse, LPS, Donepezil.

INTRODUCTION

The management of dementia pathologies such as Alzheimer's disease is a crucial issue to slow or even stop the progression of the disease; Faced with this, man resorts to conventional medications. However, these available and recommended conventional treatments do not act on the progression of the disease. Many initially promising molecules have failed during clinical trials in recent years, due to the absence of a therapeutic target and treatment of the disease at too late a stage results from the failure of current therapies [1,2].

To this end, the use of direct use of medicinal plants and animal models to evaluate the effects of these medicinal plants remains promising avenues of research to achieve more effective therapeutic solutions. Note that natural rodents do not develop amyloid plaques even at a very advanced age, there is no access to non-transgenic models of AD. The most used method being the intracerebral (intra-cerebro-ventricular or intrahippocampal) injection of the Aβ peptide or the Tau protein, the interest of these models is to study the impact of an acute injection of biomarkers of the AD on the cellular environment such as neuro-inflammatory processes or synaptic losses.

These non-transgenic models facilitate the specific study of the impact of the Aβ peptide and not of the many other peptides present in transgenic models such as the different forms of APP. Although transgenic and non-transgenic models of AD involve many players that are difficult to control, there is currently no perfect model of AD [3,4].

Besides intracerebral injection of Aβ peptide or Tau protein, injection of LPS or carrageenan are classical and similar models used to evaluate the cascade of acute inflammatory responses. The inflammatory reaction being a natural defense mechanism, constitutes an important process that helps the body respond to harmful attacks [5], in these classic models, this inflammatory can be differentiated into a two-phase reaction. The first phase is called early, develops after the induction of inflammation and is characterized by a release of pre-synthesized inflammatory mediators such as serotonin, histamine and others. The second so-called delayed phase begins one hour after the process of activation of the mediators of the early phase leads to the infiltration of neutrophils as well as the release of prostaglandins resulting from the action of cyclooxygenases (COX). Neutrophil-derived free radicals, nitric oxide (NO), and proinflammatory cytokines such as interleukin-1b (IL-1b) and tumor necrosis factor-α (TNF-α) are other characteristic mediators of this delayed phase [6,7]. Additionally, LPS induces cellular responses that extremely stimulate natural (macrophage) immunity; this stimulation of macrophages by PLS constitutes an effective means of studying the inflammatory reaction via the inflammatory mediators activated by these macrophages [8].

In AD, the simultaneous presence of Aβ peptide and Tau neurofibrils results in chronic inflammation that causes neuroinflammation. This neuroinflammation induces the phenomenon of microglia priming [9,10] which rapidly differentiates towards a state of M1 activation [11]. This activated state allows microglia to recognize the Aβ peptide and Tau neurofibrils via different receptors: SCARA1 (scavenger Receptor Class A Member 1), CD14, CD36, CD47 and TLR. This first role of microglia is beneficial and consists of eliminating these residues. However, the binding of the Aβ peptide to CD36, TLR4 or TLR6 leads to the activation of microglia with triggering of neuroinflammation, production of pro-inflammatory cytokines and chemokines and reactive oxygen species (or ROS for Reactive Oxygen Species).) which are associated with the increase in the concentration of the Aβ peptide. [12,13].

This neuroinflammation makes it possible to obtain an effective response against any damage to the CNS; the implementation of this process could be chronic, thus leading to numerous deleterious modifications for the cerebral environment. The chronicity of neuroinflammation would above all lead to significant synaptic dysfunction, a reduction in the synaptic connection associated with the presence of IL-1β [14].

In Congo, the use of medicinal plants is a common practice anchored in the lifestyle of the population to seek solutions to various bacterial, viral and fungal pathologies. Morinda lucida BENTH (Rubiaceae) is one of these medicinal plants used and known under the names of ossika or musika in the north of the country and musiku or lidungu in the south of the country [15]. This study aims to evaluate the immunomodulatory profile and the histological appearance of nervous tissues in Balb/c mice exposed to the aqueous extract of M.l BENTH leaves in a model of neuroinflammation induced by LPS.

Material and methods

Plant material and preparation of the aqueous extract

Fresh leaves of M.l BENTH, manufactured in the commune of Makoua (Cuvette department) on May 3, 2018 at 4 p.m. They were identified and registered under number N°: 8.014 on June 6, 2018 at the National Herbarium of Congo. These leaves were reduced to powder using the electronic crusher; 50g of powder obtained were macerated in 500ml of distilled water with magnetic stirring for 48 hours. The macerated obtained was filtered through carded cotton then concentrated to a quarter (1/4) of its initial volume at 65°C in an oven. The pure extract obtained was transferred to a sterile plastic container; hermetically sealed and stored in the refrigerator at 4°C for psychopharmacological analyses. [16].

Animal equipment and treatment

We used Balb/c mice, male and female, weighing 16 to 28 g respectively (age between 7 and 10 weeks). They came from the animal store of the Faculty of Health Sciences. These animals were maintained in their natural habitat and subjected to a 12/12 hour light/dark cycle, with free access to water and food. These animals were divided into five (5) groups of five (5) Balb/c mice per group and treated for 7 days orally then with LPS for groups (B,C,D,E) for 3 days by IP (intraperitoneal injection). As follows: group A, treated with distilled water 10 ml/kg; group B, treated with distilled water then with LPS; group C, treated with Donepezil 5 mg/kg then with LPS; group D, treated with aqueous extract of M.l BENTH leaves 200 mg/kg then with LPS; and group E, treated with aqueous extract of leaves of M.l BENTH 400 mg/kg then with LPS.

Blood sampling

The animals were anesthetized with chloroform “Ether Cooper bottle 125 ml” one hour after the last dose of treatment. Blood was collected from the orbital level using capillary micropipettes and from the jugular level. Samples were centrifuged at 3000 rpm for 15 minutes and supernatants/serum were collected in 2 ml tubes, stored in a refrigerator at –4°C for biochemical analyses.

Biomarker assay

The concentrations of biomarkers studied (p.TAU, β –amyloid, TNF-α and IL-1β) in the sera of the samples taken were measured by the Sandwich-ELISA method, using ELISA kits (Sunlongbiotech; www. Sunlongbiotech.com). according to the supplier's recommendations. The sera from the treated animals were diluted with the standard dilution samples present in the ELISA kits. The O.D. was read at 450 nm using an Autobio PHOmo brand microtiter plate reader. The results obtained were multiplied by the dilution factor: x 5.

Histological Analysis

Each batch of Balb/c mice was euthanized, the brains were removed, and stored in flasks containing 10% PBS. Histological sections of the brains were prepared and stained using the standard HE (Hemalun-Eosin) method. After mounting, the slides were read under a light microscope (Leica) using objectives (x10, x40, and x100).

Statistical analysis

Results are expressed as mean ± SEM. The significance level was at $P < 0.05$; and it was calculated by student's t-test using IBM SPSS Statisticts 23 software.

Results

3.1. Biomarkers analyses

Analysis of the results shows a non-significant increase ($p>0.05$) in β –amyloid concentration levels in LPS-induced Balb/c mice treated with donepezil 5 mg/kg and the aqueous leaf extract. of M.l BENTH at doses of 200 and 400 mg/kg compared to controls. p.TAU concentrations in LPS-induced Balb/c mice are significantly higher than in control mice: B: **$p<0.006$, C: *$p<0.01$, D: *$p<0$, 03, E: **$p< 0.004$. Furthermore, a significant increase (*$p<0.01$) in the level of TNF-α in Balb/c mice treated with the aqueous extract of M.l BENTH leaves at a dose of 400 mg/kg and high levels not significant in Balb/c mice treated with donepezil 5 mg/kg and aqueous extract of M.l BENTH leaves at a dose of 200 mg/kg compared to controls. In addition, a non-significant increase ($p > 0.05$) in IL-1β levels in mice treated with donepezil 5 mg/kg and aqueous extract of M.l BENTH leaves at doses of 200 and 400 mg/ kg compared to controls. These results are illustrated in Table I.

Table 1: Effect of aqueous extract of M.l BENTH leaves on neuroinflammation parameters in Balb/c mice (n = 5).

Parameters	Water Dist.10ml/Kg	LPS + Water Dist.10ml/Kg	LPS+Donépezil 5mg/kg	LPS + M.l BENTH 200mg/Kg	LPS + M.l BENTH 400mg/Kg
p.TAU	45.20±1.01	53.00±1.41**	54.00±1.64*	49.00±0.70*	55.20±1.06**
β –amyloïde	56.60±0.74	56.20±4.97	60.80±2.97	63.20±3.08	91.60±26.05
TNF-α	16.60±1.43	17.40±3.52	19.40±0.40	20.80±1.62	22.20±0.58*
IL-1β	77.80±1.88	78.00±5.64	71.00±3.17	98.00±19.57	75.00±3.94

3.2- Histological analyses

Microscopic observation of histological sections of the brain at the mouse cortex revealed : (A), the presence of a series of homogeneous cellular necrosis of the nervous tissue ; (B), presence of areas of homogeneous cellular necrosis, dissociating significant foci of polymorphic inflammatory infiltrates, made up of lymphocytic and polynuclear inflammatory elements ; (C), presence of areas of homogeneous cellular necrosis and significant reduction in foci of polymorphic inflammatory infiltrates ; (D), presence of areas of homogeneous cellular necrosis, dissociating from large areas of slightly altered inflammatory infiltrates ; (E), presence of areas of homogeneous cellular necrosis, dissociated from foci of moderate and polymorphic inflammatory infiltrates, made up of altered lymphocytic and polynuclear inflammatory elements; as well as nerve cells (glial cells).

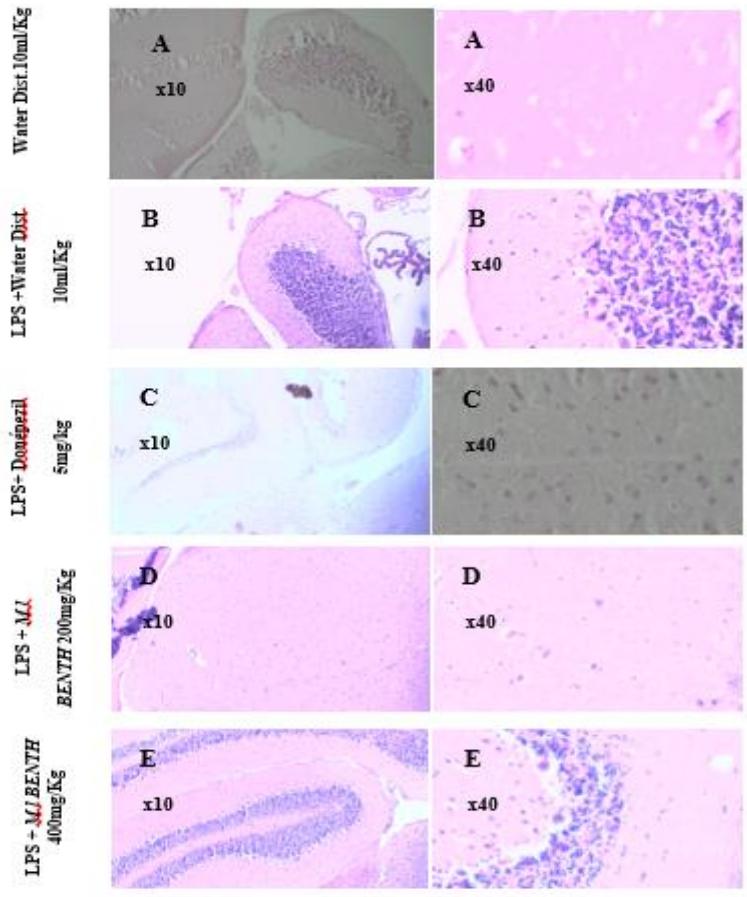

Figure 1: Histological aspects of the effect of the aqueous extract of M.l BENTH leaves on nervous tissues in Balb/c mice (n = 5).

Discussion

Neuroinflammation is an extremely complex multicellular process. It causes vascular changes as well as molecular modifications of the environment, to respond and resolve a CNS crisis [17]. These anatomical changes lead to important changes such as a modification of the BBB [18], a synaptic loss at the level of Long-Term Potentiation which is impaired in mutant mice [19]. Increasing the brain environment of pro-inflammatory cytokines such as TNF-α, IL-6 or IL-1β triggered the increase in BBB permeability [20]. The i.p. or intrahippocampal injection of LPS in an animal model causes learning disabilities and cognitive dysfunction [21,22]. Furthermore, in the animal model of LPS-induced systemic inflammation, elevated levels of β-amyloid 1-42 proteins are demonstrated in the brains of LPS-treated mice. The increase in levels of β-amyloid protein 1-42 explains the activation and stimulation of microglia at the origin of neuroinflammation [23]. Before the appearance of TAU pathology and clinical symptoms, the mechanism of development of Alzheimer's disease would be associated with the accumulation of β-amyloid protein 1-42 [24]. To prevent complications of the disease, donepezil is one of the drugs used; used to treat symptoms of certain forms of Alzheimer's disease [25].

Donepezil being an inhibitor of cholinesterase E, we used (donepezil 5 mg/kg) as a positive control in comparison with doses (200 and 400 mg/kg) of the aqueous extract of M.l BENTH leaves. In the present study, the induction of neuroinflammation by i.p. LPS and treatment of different groups made it possible to observe the variation in the levels of pro-inflammatory cytokines (TNF-α and IL-1β) as well as the characteristic markers of neurodegenerative diseases (p.TAU and β-amyloid protein) analyzed by the Sandwich ELISA technique.

We found that p.TAU levels in LPS-induced Balb/c mice were higher, with significant differences, than in control mice. Unlike the significant difference of donepezil 5mg/kg, the significant reduction in p.TAU levels by the aqueous extract of leaves of M.l BENTH at doses of 200 and 400mg/kg would illustrate the effectiveness of the aqueous extract of leaves of M.l BENTH at these doses alongside donepezil 5mg/kg to fight against TAU pathology. However, no significance for β-amyloid levels in LPS-induced Balb/c mice compared to control mice; but an increase in β –amyloid levels for mice treated with donepezil 5 mg/kg and aqueous extract of M.l BENTH leaves at doses of 200 and 400 mg/kg compared to controls. Additionally, TNF-α levels in LPS-induced Balb/c mice were higher than in control mice, which was significant for M.l BENTH leaf aqueous extract at a dose of 400 mg/kg. Furthermore, no significance for IL-1β levels in LPS-induced Balb/c mice compared to control mice; but an increase in IL-1β levels for mice treated with donepezil 5 mg/kg and the aqueous extract of M.l BENTH leaves at doses of 200 and 400 mg/kg compared to controls. These results would justify that the aqueous extract of M.l BENTH leaves would have a potential influence on the synthesis of the pro-inflammatory cytokines (TNF-α and IL-1β) analyzed; and that it could be a potential candidate to fight against the establishment of TAU pathology. Unlike our animal model studied, the results of our study agree with those of Gomaa AA et al., in 2021, who demonstrated that donepezil did not have a significant effect on the regulation of proinflammatory cytokines (TNF-α and IL-1β) in an animal model of type 2 diabetes. Likewise, our results are consistent with those of Jiayi Zhao et al., 2019 who found high levels of β –amyloid proteins 1-42 in LPS-treated brains; which explains the activation and stimulation of microglia at the origin of neuroinflammation. [26,23]

To evaluate the impact of LPS and the different treatments in the Balb/c mice in our study, we analyzed the histological sections of the cerebral cortex of the animals from each experimental batch. As shown in Figure 1, the presence of areas of homogeneous cellular necrosis of the cerebral cortex is associated with significant foci of inflammatory infiltrates in the LPS-induced groups (B, C, D and E) in contrast to the negative control group (A). The comparison of the groups treated with the aqueous extract of leaves of M.l BENTH 200 and 400 mg/kg and of group C, treated with Donépezil 5 mg/kg shows a significant reduction in the foci of polymorphic inflammatory infiltrates in the cortex of mice Balb/c treated with Donepezil 5 mg/kg; a significant dissociation of foci of slightly altered inflammatory infiltrates in the cortices of Balb/c mice with the aqueous extract of treated leaves of M.l BENTH 200 mg/kg and a dissociation of foci of moderate and polymorphic inflammatory infiltrates, consisting of altered lymphocytic elements and polymorphonuclear inflammatory cells as well as nerve cells (glial cells) from the cortex of Balb/c mice treated with the aqueous extract of leaves of M.l BENTH 200 and 400mg/kg.

These results illustrate that the aqueous extract of M.l BENTH leaves would have a dose-dependent positive impact on the inflammatory infiltration in the cerebral cortex of treated Balb/c mice. And that the aqueous extract of M.l BENTH leaves would stimulate the synthesis of immune cells of the central nervous system. These results agree with those of Jiayi Zhao et al., 2019 who demonstrated the existence of an accumulation of β-amyloid 1-42 proteins accompanied by neuronal cell death. [23]

Conclusion

Overall, we demonstrated that i.p. LPS injection induced the neuroinflammation process in Balb/c mice. The regularization of this neuroinflammatory process by the aqueous extract of M.l BENTH leaves showed that the aqueous extract of M.l BENTH leaves would have a dose-dependent positive impact on the inflammatory infiltration in the cerebral cortex of treated Balb/c mice. . And that the aqueous extract of M.l BENTH leaves would stimulate the synthesis of immune cells of the central nervous system, in particular a potential influence on the synthesis of the pro-inflammatory cytokines (TNF-α and IL-1β) detected; and that it could be a potential candidate to fight against the implantation of TAU pathology.

Contribution of the authors

Mbon Obami Rex Dassaut and Miguel Landry Martial designed this work under the coordination of Abena Ange Antoine. Mbon Obami Rex Dassaut carried out the experimental studies and statistical analyzes with the assistance of Ataka Modeste and Akpo Tayo Oniodje Wilfried. Mbon Obami Rex Dassaut and Miguel Landry Martial wrote the draft of the manuscript. All of these authors have reviewed this manuscript.

Conflict of interest

We have no conflict of interest.

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