

***Alchornea cordifolia* Aqueous Extract Relieves Adulthood Cognitive Disorders and Oxidative Damages Due to LPS-Induced Neuroinflammation in Neonatal Wistar Rats**

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Abstract

Alchornea cordifolia extract is widely used as ethnomedicine in Ivory coast to treat various ailments related to bacterial, fungal, parasitic and inflammatory disorders, infertility problems, among other. Here, the objective was to evaluate some long lasting neuroprotective properties of *Alchornea cordifolia* extract against a rat's model of LPS-induced early-life neuroinflammation. The neuroinflammation was induced with a single dose of LPS (1mg/kg, i.p) in neonatal rats (postnatal day 4) or PBS rats (1ml/100g, vehicle). Six hours later, some LPS rats were treated either with minocycline (50 mg/kg, i.p) or *Alchornea cordifolia* extract (200 or 400 mg/kg, p.o) for 14 consecutive days. Then, rats were followed carefully till adulthood (P 90) for the evaluation of cognitive behavioral status and oxidative damage level in hippocampus and prefrontal cortex. Results showed that the treatment with *Alchornea cordifolia* extract (200 or 400 mg/kg) attenuated LPS-induced deficits of spatial learning and memory (Y-maze and Morris water maze) and recognition memory (Object recognition test). However, only dose of 200 mg/kg of *Alchornea cordifolia* extract reduced significantly lipid peroxidation through MDA content and enhanced the enzymatic antioxidant SOD level in hippocampus and prefrontal cortex. On the whole, the threshold dose 200 mg/kg of *Alchornea cordifolia* demonstrated long lasting beneficial neuroprotective effects against LPS-induced early-life neuroinflammation incomparable to reference molecule minocycline. This findings suggest that *Alchornea cordifolia* rich in quercitrin bioactive need further biochemical and histopathology studies before promising as efficient therapeutic agent against neurodegenerative and psychiatric diseases.

Keywords: Neonatal neuroinflammation, oxidative stress, cognitive disorders; *Alchornea cordifolia*, lipopolysaccharide (LPS).

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INTRODUCTION

Early-life immune system activation is closely associated to neurodevelopmental disturbance « programming and sensitization » in progeny (Alirezaei *et al.*, 2008 ; Hood *et al.*, 2003), and long-term cognitive behavioral and affective disorders (Williamson *et al.*, 2011). Several rodent's model of neonatal inflammation due to infectious agents have demonstrated pathophysiological and delayed neurobehavioral alterations. In fact, an overactivation of microglia, a resident immune cells in the brain, during neonatal stage lead to significant proinflammatory cytokines release such as tumor necrosis factor alpha (TNF α), interleukin-

1 β (IL-1 β) and nitric oxide (NO) exacerbating the neurodevelopmental process and leading to long lasting detrimental cognitive functions (Yirmiya *et al.*, 2015; Williamson *et al.*, 2011). It has also found a strong relationship between neuroinflammation process and oxidative stress biomarkers generation including the ROS, malondialdehyde (MDA), non protein thiol (NP-SH), as well as antioxidant enzyme superoxide dismutase (SOD) in peripheral blood and brain tissue (Rossetti *et al.*, 2020). Regarding the neonatal rat's model of LPS-induced neuroinflammation, most of data reported neuronal damage and significant cognitive and affective disorders later at adolescence or adult stage (Berkiks *et al.*, 2018; Lei *et al.*, 2017). Moreover, microglia

activation by LPS are served as reference model to study the exacerbated pathophysiological mechanisms of Alzheimers disease and cognitive outcomes (Lee *et al.*, 2010; He *et al.*, 2018).

To date, the semi-synthetic second generation molecule namely minocycline remains the appropriate compound able to repress the neuroinflammatory process mediates by microglial cells activation and subsequent oxidative stress biomarkers generation (Morimoto *et al.*, 2005). Since, the synthetic chemical drugs present several controversial effects against neurological diseases, it worthwhile to promote neuroprotective actions of natural products.

Alchornea cordifolia, a perennial plant of west africa, is widely used alone or in mixture with other plants to solve many health problems (Olaleye *et al.*, 2006). In ivorian's traditional pharmacopeia, *Alchornea cordifolia* is helpful for the treatment of inflammatory disorders, malaria and fever, infertility, respiratory problems (Boniface *et al.*, 2016). Phytochemical informations indicate that *Alchornea cordifolia* is mainly rich in phenolic acids, alkaloids, terpenoid and polyphenol as flavonoid (Boniface *et al.*, 2016) and mainly a new isolated anti-inflammatory flavonoid called quercitrin (Manga *et al.*, 2004). However, no study hasn't been carry out regarding the neuroprotective effects of *Alchornea cordifolia* extract. In this context, we tested the long lasting cognitive behavioral outcomes related to rat's model of LPS-induced early-life neuroinflammation and the antioxidant reponses from crude extract of *Alchornea cordifolia* as therapeutic approach.

MATERIAL ET METHODS

Animals

Wistar rat strains were gifted by the *vivarium* of higher normal school (ENS), Abidjan, Ivory Coast, acclimated under controlled conditions of temperature (20-22°C), with good relative humidity (50-60%), submitted to good photoperiod condition, and with no food and water restriction. Virgin female rats were mated to males and followed carefully till parturition stage.

Experimental Design

At neonatal stage (PND 4), pups were separated from mothers and subjected to an intraperitoneal injection with fresh LPS dissolved in PBS (1 mg/kg, Merck, Morocco) or only PBS (vehicle). Six hours after the injections, the LPS rats were grouped in four batches (n = 6 per batch). Two groups of LPS rats received an oral treatment with crude extract of *Alchornea cordifolia* (200 or 400 mg/kg) for 14 days, a last one treated with minocycline (50 mg/kg, i.p). After the daily treatments, the pups were returned at home cage. At the end of all treatments, rats were carefully followed up PND 90 before beginning behavioural testing.

The plants aqueous extract of *Alchornea cordifolia* (AEAC) was kindly gifted by a next door phytochemistry laboratory of national floristic centre (CNF, Abidjan).

BEHAVIOURAL ANALYSIS

Y-maze Test

This test is useful to assess spontaneous alternation behavior as a measure of working spatial memory. The apparatus was made with in fine wood with three arms (A, B and C) measuring 40 in length 10cm in width and 13 cm in height) and painted in different colour patterns. A central platform is formed by triangles of 120° between each arm. Rats were allowed 5 min to explore the three arms of apparatus and is driven by an innate curiosity to explore previously unvisited areas. An entry is validated when the four paws are within the arms. The sequence of arms entries was monitored with a camera video. Alternation was taken as consecutive entries into the three arms on overlapping triplets (*i.e.* ABCACBAACB = 5 alternation). Spontaneous alternation behavior was calculated with the following equation: % alternation = 100 x (number of alternation/ total arm entries – 2).

The arms were cleaned using 10% ethanol to exclude the possible effect of odorous cues on animal's performance between sessions.

Novel object recognition task (NORT)

The object recognition test procedure was conducted as describe by Ennaceur and Delacour (1988) (Ennaceur *et al.*, 1988). The apparatus is an Open box with floor measurements 50 cm in length, 50 cm in width, and 40 cm in height walls. In the trial (familiarization session), rats were allowed 5 min to explore freely the box with two identical objects and return in their home cage. After 2 h delay, to evaluate short term recognition Memory (STM), rats were return in open field in which one object was switched by another one different for colour, shape and size, and experiment was repeated during 5 min with one novel object and one identical previously explored. To evaluate long -term memory (LTM) 24h later from the familiarization phase, rats were submitted to explore again two objects for 5 min, one identical and another novel one. Objects and box were cleaned with ethanol 70% during intertrial period. The exploration time of each object was recorded the video tracking, and the exploration feature is defined as the directing the nose at a distance less than 1 cm from the object. The ratio of preference of the novel object of each animal was calculated from the exploration frequency of novel object divided the total frequency spent for exploring both objects.

The working area and objects were cleaned with 10% ethanol between trials.

Morris Water Maze (MWM) Test

The goal of this behavioral test is to assess spatial learning and memory abilities in the rodents. The apparatus is an open circular tank (130 cm in diameter ; 50 cm high) filled to two-third with tepid water (24°C). A platform (50 cm in diameter ; 10 cm high) was placed in an inert off-center position inside the pool, but it submerges to 1 cm above the surface of water. To make sure that the platform is hidden the water was made opaque with small amount of pasteurised milk. Also, four allocentric hallmarks are located on the walls around the tank, which served as extra-maze visual cues. The training phase consisted to 4-trials per day in which the rat was allowed 60 s to find the visible platform, during 5 days. If rat failed to reach out the platform, it was guided to the platform and allowed to stay on it for approximately 10 s. A video tracking recorded the latency which is considered to the time spent to find the platform during each trial (Morris *et al.*, 1984). The latency is measure of spatial learning performance. On the sixth day, the probe test permitted to evaluate reference memory ability of rat through the exploration of the pool without no platform. The time spent in virtual position of the platform quadrant is taken account.

BIOCHEMICAL ASSAY

Tissues Preparation

After the behavioural tests, the rats were anesthetized with 7% chloral (300 mg/kg, i.p). Then, hippocampal and prefrontal cortex (PFC) tissues from each rat were isolated and homogenized in 500 µL of ice-cool buffer (50 mM Tris-HCl, pH 7.4). The homogenate was centrifuged at 3000 rpm/min for 10 min at 4°C. The supernatant (S1) was collected and kept at -20°C till the assay day.

Acetylcholinesterase (AChE) level

AChE level was assayed according to Ellman's method (Ellman *et al.*, 1961). Reaction mixture was made with 100 µL of S1 supernatant, 25µL (75nM), 100 µL Ellman's reagent DTNB (100 mM) and 2.6 ml phosphate buffer (0.1M ; pH 8). The reactional middle was thoroughly mixed and the rate of Acetylthiocholine iodide hydrolysis was measured at 412 nm for 10 min. The AChE level in tissue was expressed as percent of inhibition from vehicle rats group.

Oxidative Stress Biomarkers

Malondialdehyde level

Malondialdehyde (MDA), a biomarker of lipid membrane peroxidation, was assayed according method described elsewhere (Satoh *et al.*, 1978). Briefly, supernatant S1 was mixed with 1.5 ml of trichloroacetic acid (10%), vortexed, and incubated at room temperature for 10 min. Then it was added to the mixture 1,5 ml of thiobarbituric acid (0.67%) and heated in boiling bath water for 15 min. After cooling, 1.5 ml of n- butanol was mixed into the solution and strictly vortexed. The sample

was centrifuged at 800 rpm for 5 min, and the supernatant S2 was collected. The absorbance was determined spectrophotometrically at 532 nm. The results were expressed as MDA level in nmol/g of tissue.

Superoxide dismutase (SOD) level

The SOD activity was assessed in brain tissues according to the method described by Winterbourn *et al.*, (Winterbourn *et al.*, 1975). The principle is based on the ability of SOD to inhibit the reduction of nitroblue tetrazolium (NBT). The reagent medium was made with 1 mL of a solution containing 50 mM potassium phosphate buffer (pH 7.4), 1.5mM of methionine, 0.1 mM EDTA, 0.025% Triton X 100, and 300 µL of the supernatant. The reaction was initiated by adding 0.12 mM riboflavin to each sample. After 10 min of incubation, the illumination of riboflavin in the presence of O₂ and electron donor as methionine is a factor of generation of superoxide anions and SOD activity assay. The reduction of superoxide anion radical by NBT develops a specific colour that is monitored at 560 nm. The amount of enzyme needed to induce 50% inhibition of NBT reduction was termed 1 U of SOD, and expressed as U/mL of supernatant.

Analysis of non-protein thiols (NP-SH) level

NP-SH level was assessed according to the method described by Ellman (Ellman *et al.*, 1959). The supernatant was treated with 10% of trichloroacetic acid to precipitate proteins, and the sample was centrifuged at 2,000 rpm for 10 min. To the supernatant, was added 1 M of potassium buffer (pH 7.4) and 1mM of Ellman's reagent (5,5-dithiobis-2-nitrobenzoic acid). The NP-SH level was determined at 412 nm and expressed in µmol/g of tissue.

Statistical analysis

GraphPad Prism 6.0 version was used to record data and their analysis. The experimental data were expressed as mean ± S.E.M (Standard Error of Mean). Statistical analysis was done one-way analyses of variance followed by Tukey post-hoc test for multiple comparisons. $p < 0.05$ was considered statistically significant.

RESULTS

Behavioural Tests

Y-maze task

Rats exposed at neonatal stage to LPS showed a significant reduction of the percent of alternation behaviour ($p < 0,05$). The treatment of LPS rats with 200 mg/kg of AEAC improved the level of alternation behavior in the same way the reference molecule minocycline. Surprisingly, the AEAC at 400 mg/kg didn't improve the alternation behavior as represented by the lowest percent ($p < 0,05$).

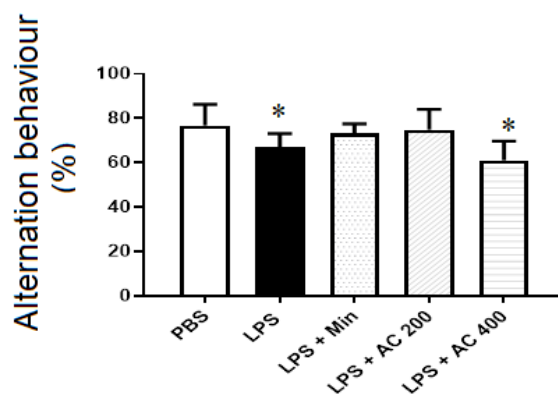


Figure 1: Effects of *Alchornea cordifolia* extract on long term alternation behaviour changes in LPS-induced rats neonatal neuroinflammation

Data represented mean of % of alternation behaviour \pm S.E.M. One-way ANOVA / Tukey post-hoc analysis (n= 6 rats per group). * $p < 0.05$ (LPS or LPS + AC 400 versus other experimental groups)

Novel object recognition test

As shown on Fig 2A, the neonatal administration of LPS (1mg/kg, i.p) alone reduced STM recognition through their index below threshold of recognition (50%). The treatment of LPS rats with both doses of AEAC 200 and 400 mg/kg improved the value of the recognition index, and more higher than that of minocycline ($p < 0,05$).

Regarding LTM recognition, we observed that treatment with AEAC at 200 and 400 mg/kg helped to increase the recognition index comparable to that of LPS-treated minocycline rats, but that remained statistically non-significant when compared to the performance of LPS rats (Fig 2B).

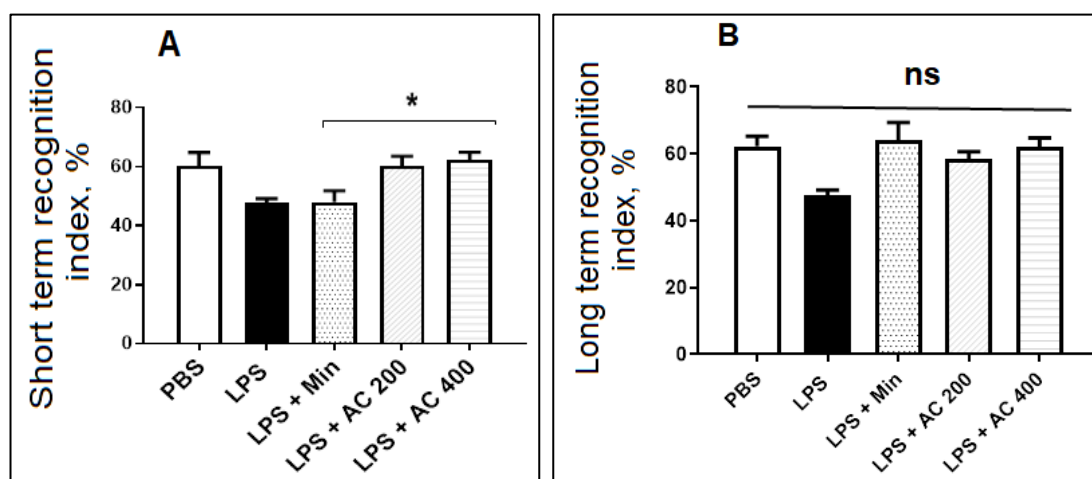


Figure 2 : Effects of *Alchornea cordifolia* extract on long term recognition memory abilities in LPS-induced rats neonatal neuroinflammation. A, Short term memory recognition phase ; B, Long term memory recognition phase

Data represented mean of recognition index \pm S.E.M. One-way ANOVA / Tukey post-hoc analysis (n = 6 rats per group). * $p < 0.05$ (LPS + Min versus LPS + AC 200 or LPS + AC 400); ns, non-significant.

Morris Water Maze

During training phase as depicted in Fig 3, the level of latency time to find the hidden platform was higher in LPS rats (up day five, $p < 0,01$) in comparison to other experimental groups. However, the treatment of LPS rats with an AEAC at 200 mg/kg significantly improved their performance, they spent the least of time to locate the platform (from day one to five, $p < 0,01$), when compared to the group LPS rat treated with AEAC 400 mg/kg and minocycline.

On probe phase (6th day), the rats treated with LPS alone showed the most of difficulty to recall the virtual place of the platform, they spent less time in the target quadrant ($p < 0,05$). However, the treatment of LPS rats with 200 mg/kg of AEAC significantly increased the time in target quadrant at the same manner minocycline (50 mg/kg) when compared to those treated with AEAC 400 mg/kg.

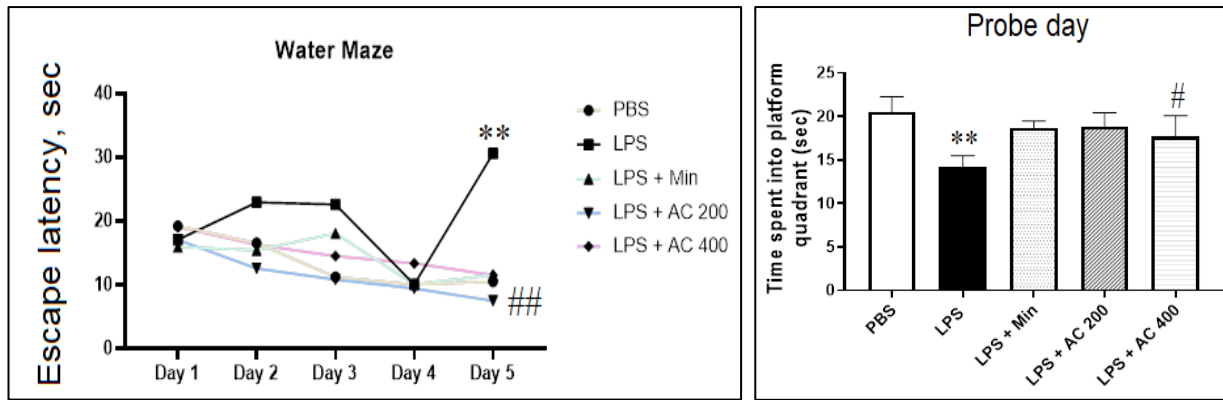


Figure 3: Effects of *Alchornea cordifolia* extract on long term spatial memory alterations in LPS-induced rats neonatal neuroinflammation

Data represented by mean of escape latency and time spent in target quadrant ± SEM One-way ANOVA / Tukey post-hoc analysis (n = 6 rats per group)

** $p < 0.01$ (LPS versus other experimental groups); ## $p < 0.01$ (LPS + AC 200 versus LPS + AC 400); # $p < 0.05$ (LPS + AC 200 versus LPS + AC 400)

Effects of AEAC on oxidative stress biomarkers and AChE level in the hippocampus and PFC

In hippocampus (**table 1**): we observed a significant inhibition of membrane lipid peroxidation through the lowest level of MDA in LPS rats treated with 200 mg/kg of AEAC ($p < 0,05$) when compared to the rats received LPS alone. Interestingly, LPS caused the highest level of MDA. Furthermore, the level of MDA was significantly reduced in LPS rats treated with 200 mg/kg of AEAC ($p < 0,05$) in comparison with those treated extract at 400 mg/kg and minocycline (50 mg/kg). We noted also that the treatment of LPS rats with 200 mg/kg of AEAC significantly promoted enzymatic antioxidant SOD production ($p < 0,001$) compared to other experimental groups. In contrast, no significant change were observed in the content of NP-SH. Regarding the AChE level, the inhibition rate was

increased to +8% above the baseline value of PBS rats ($p < 0,05$) in comparison to other groups.

In PFC (**table 2**), the level of MDA content was markedly increased in reponse to neonatal exposure to LPS-induced neuroinflammation ($p < 0,05$) compared to other experimental groups. We revealed also a the highest level of SOD in the LPS rats treated with AEAC at 200 mg/kg ($p < 0,001$). However, the treatment of LPS rats with minocycline (50 mg/kg) improved significantly SOD production ($p < 0,01$) in comparison to that of 400 mg/kg of AEAC. With respect to the NP-SH level, we found only that only the LPS rats treated with minocycline and AEAC at 400 mg/kg showed a significant increase ($p < 0,01$). Finally, the inhibition rate of AChE increased to + 15% above the baseline value ($p < 0,05$) in LPS rats without treatment and + 8% for LPS rats treated 400 mg/kg of extract ($p < 0,05$).

Table 1: Effects of AEAC on oxidative stress biomarkers and AChE level in the hippocampus

	PBS	LPS	LPS + Min	LPS + AC 200	LPS + AC 400
MDA (nmol / g)	45,42 ± 1,79	49,7 ± 1,66*	43,87 ± 1,79	40,82 ± 1,79	45,2 ± 1,4
SOD (Unit / ml)	0,1633 ± 0,0534	0,1433 ± 0,0534	0,16 ± 0,0568	0,51 ± 0,29***	0,141 ± 0,075
NP-SH (µmol / g)	431,05 ± 18,67	440,04 ± 18,45	442,06 ± 18,33	430,45 ± 18,54	452,64 ± 18,76
AChE (% of inhibition)	100,3 ± 1,4	101,7 ± 2,3	101,2 ± 1,45#	102,06 ± 2,45	108,05 ± 2,00*#

All data were expressed as means ± SE (n=6/group). Data comparison was performed using two way ANOVA followed by Tukey’s Multiple Comparison Test. * $p < 0,05$, *** $p < 0,001$ (versus vehicle); # $p < 0,05$.

Table 2: Effects of AEAC on oxidative stress biomarkers and AChE level in the PFC

	PBS	LPS	LPS + Min	LPS + AC 200	LPS + AC 400
MDA (nmol / g)	47,71 ± 2,34	54,90 ± 2,34*	47,33 ± 2,34	47,76 ± 2,34	47,71 ± 2,34
SOD (Unit / ml)	0,06 ± 0,02	0,04 ± 0,02	0,16 ± 0,12**##	0,36 ± 0,23***###	0,04 ± 0,02
NP-SH (µmol / g)	417,91 ± 18,76	425,02 ± 18,56	481,92 ± 18,76**	425,03 ± 18,44	481,91 ± 18,76**
AChE (% of inhibition)	100,01 ± 4,60	115,92 ± 4,51*	100,20 ± 4,02#	102,21 ± 4,18#	107,52 ± 4,54

All data were expressed as means ± SE (n=6/group). Data comparison was performed using two way ANOVA followed by Tukey’s Multiple Comparison Test. * $p < 0,05$, ** $p < 0,01$, *** $p < 0,001$ (versus vehicle); # $p < 0,05$, ## $p < 0,01$ (versus LPS).

DISCUSSION

Since long time, medicinal plants show much of interest in healthcare practices in several parts of the world for treating or alleviating diverse ailments conditions. Among them, *Alchornea cordifolia* is widely used as Ivoirian's traditional pharmacopeia to cure tropical diseases (Handa *et al.*, 2006; Zerbo *et al.*, 2012), but little is known about the long-term neuroprotective actions, cognitive and oxidative stress status after early-life immune system activation in the brain. In the present study, the rats exposed alone to LPS at neonatal stage (P4) showed significant deficits of spontaneous alternation as measure of spatial working memory (Y-maze), recognition memory (object recognition task) and spatial memory impairment as well (Morris pool) at young adulthood stage. Those cognitive outcomes are notably accompanied by inappropriate level of oxidative stress biomarkers. The therapeutic assay using crude extract of *Alchornea cordifolia* against LPS-induced early-life neuroinflammation revealed cognitive health benefits. The finding from present study might be explained by the neuroinflammation altering neurodevelopmental events and cytoarchitectural processes in the brain due to an imbalance of microglia homeostasis (Stevens *et al.*, 2007; Boulanger *et al.*, 2009; Garay *et al.*, 2010), and as consequence cognitive behavioral changes in adulthood of LPS rats (Batista *et al.*, 2019; Berkiks *et al.*, 2018; Bilbo *et al.*, 2008; Bilbo *et al.*, 2005). The anti-inflammatory compounds from *Alchornea cordifolia* extract are thought to repress neuroinflammation mechanisms and enhance cognitive behavioral deficits found in LPS rats. In fact, this specie contains some phytochemicals of flavonoid family which are well-known for their neuroprotection properties as reported elsewhere (N'Go *et al.*, 2021; Berkiks *et al.*, 2018). More interesting, the treatment of neonatal LPS-exposed rats with *Thymelaea lytrhoides* rich in bioactive compounds Kaempferol and quercetin (flavonoids) reduced adulthood psychoaffective disorders including depression and anxiety (Berkiks *et al.*, 2018). Regarding the material plant (*Alchornea cordifolia*) used in present study, it has been demonstrated *in vivo* anti-inflammatory flavonoids, but also first time identified compounds like quercitrin and hyperoside (Manga *et al.*, 2004). Those isolated compounds are suggested to be responsible of anti-neuroinflammation (Berkiks *et al.*, 2018). They found significant production of cytokines as TNF- α and IL-1 β by microglia hyper stimulated in LPS-rats compared to those with a plant extract treatment. Past studies have established a strong relationship between an increase of pro-inflammatory cytokines release during post-natal brain developmental and later change on cognitive as well as emotional behaviour status (Berkiks *et al.*, 2018; Dias *et al.*, 2019). LPS-induced cytokines release reportedly causes a persistent reduction of BDNF expression which is a key factor for supporting and maintaining neuronal circuits involved in synaptic plasticity, learning and memory functions (Zagrebelsky *et al.*, 2014). A key issue of current work is to get an insight of some downstream processes of

early immune system activation and long lasting cognitive behavioral changes, in order to promote its natural bioactives as promising therapeutic strategy for brain diseases. In fact, neuronal oxidative stress remains a consequence of chronic activation of microglia which could lead to brain disorders. In this regard, previous authors reported significant microglia response in LPS-treated rats and an increase of oxidative damage in hippocampus (Berkiks *et al.*, 2018). In agreement with these results, we showed a significant level of lipid peroxidation through MDA content in hippocampus and prefrontal cortex of LPS-rats. However, the treatment with *Alchornea cordifolia* extract of LPS rats attenuated LPS-induced oxidative stress and reinforced in the same time the antioxidant status such seen with SOD and NP-SH levels. Such results can be attributed to phytochemicals polyphenol flavonoids contained in *Alchornea cordifolia* crude extract. This imply that *Alchornea cordifolia* also improving cognitive and behaviour performances in LPS rats might be used to promote therapeutic strategies against some neurodegenerative diseases. This suggestion is in line with a previous findings of a work using antioxidant plant *Capparis spinosa* as neuroprotective effects against a LPS model miming symptoms of neurodegenerative diseases (Rahimi *et al.*, 2020). In addition, it is well-known that the antioxidant flavonoid compounds have a great neuroprotective action by interacting with neuronal/glial intracellular signaling pathways involved in neuronal resistance to oxidants (Rinwa *et al.*, 2013; Levites *et al.*, 2001).

Interestingly, the effects of *Alchornea cordifolia* as therapeutic approach were compared to reference minocycline drug commonly used in animal models of neuroinflammatory conditions, neurodegenerative diseases or ischemic injury (Fan *et al.*, 2005; Garrido-Mesa *et al.*, 2013; Cai *et al.*, 2006). We noted globally regarding the treatment of LPS rats that *Alchornea cordifolia* extract demonstrated significant pharmacological and clinical pertinence when compared to minocycline. Both at cognitive and biomarker levels, *Alchornea cordifolia* treatment was beneficial compared to minocycline. The advantageous effects of quercetin compound from a folk plant (*Thymelaea lytrhoides*) compared to minocycline were also revealed using an early-life rat's model of LPS-induce neuroinflammation (Berkiks *et al.*, 2018). These authors have suggested that the bioactive molecules could be involved in similar intracellular pathway as minocycline which acts as microglia inhibitor and potent anti-inflammatory agent. As aforementioned, the phytochemicals study identified mainly the presence of anti-inflammatory quercitrin in *Alchornea cordifolia* extract; a derived bioactive of quercetin (Manga *et al.*, 2004).

Remarkably, we found that the dose 200 mg/kg (p.o) of *Alchornea cordifolia* extract exerts better neuroprotective effects regarding cognitive behavioral

and antioxidant outcomes, compared to 400 mg /kg. Consequently, 200 mg/kg might be considered as therapeutic dose and need further experimental studies leading to discovery of new drug against neurological and psychiatric diseases.

As our study limitation, we didn't tackle the level of pro-inflammation cytokines, as well the histopathological analysis in order to show the neuronal degeneration. Also, the sexe dependent effects need to be investigated. Thus, that could be the next research topics to delve.

Conflict of Interest: None

Financial Support: None

Ethical Approval: All experimental protocols were carried out according to NIH guide for the care and use of laboratory animals for minimizing the number of animals used and their suffering.

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