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Leaves Aqueous Extract of *Boerhavia coccinea* Induces Antinociceptive Effect but Increases Nitric Oxide Production in Macrophages

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Abstract

Pain treatment is one of the most challenging situations of the modern medicine. To overcome the actual limitations, new strategies should be developed and phytotherapy represents a promising alternative. *Boerhavia coccinea* is a medicinal plant used for the treatment of pain. The present work was undertaken to evaluate the antinociceptive effects of crude aqueous extract of the leaves of *Boerhavia coccinea* (AE) on acute pain and examine its mechanism of action. The analgesic effect of AE was evaluated at doses 50, 100, 200 and 400 mg/kg using the formalin-induced nociception in mice. The specific analgesic effect of AE was verified by testing its effect of AE on the sleep induced by diazepam. The anti-inflammatory effects of AE were also tested *in vivo* (100 and 200 mg/kg) on CFA-induced inflammation and *in vitro* (1 to 300 µg/ml) on the production of NO by non-activated or activated (LPS, 1 µg/l) macrophages. The effect of AE in non-stimulated macrophages was evaluated in absence and in presence of L-NAME (100 µg/ml) or dexathetasone (30 µg/ml). AE administered orally significantly ($p < 0.001$) inhibited both neurogenic and inflammatory phases of the formalin pain with a maximum inhibition percentage of 81% at the 1st phase and 90% at the 2nd phase, but did not show any anti-inflammatory effect. AE at 50 mg/kg increased the latency to sleep and reduced sleep duration. AE drastically ($p < 0.001$) increased the NO production by non-activated or activated macrophages by up to 5654%. L-NAME and dexamethasone potentiated the activation effect of AE on NO production. In conclusion, AE possess potent antinociceptive effect that is not related to any anti-inflammatory activity. Instead, this extract increases the

nitric oxide production by an unknown mechanism.

Keywords

Boerhavia coccinea, Aqueous Extract, Analgesic, Macrophage, Nitric Oxide

1. Introduction

The most recent definition of pain considers it as "*An aversive sensory and emotional experience typically caused by, or resembling that caused by, actual or potential tissue injury*" [1]. The constant modification of this definition by the pain researchers or the International Association for the Study of Pain (IASP) demonstrates the complexity of the pathology that also underlines the difficulties and the limitations of the treatments. Indeed, the pathogenesis of pain includes many mechanisms and involves several chemical mediators [2] [3]. Nitric oxide (NO) is one of those chemical mediators highly involved in the process of pain transmission. The complex role of NO in inflammatory and neuroplasticity phenomena means that this NO can have either a beneficial effect or an adverse effect depending on its site of action and loco-regional conditions [4]. Indeed, NO is involved in the analgesic activity of non-steroidal anti-inflammatory drugs, opioids, and local anesthetics. NO is involved in the mechanisms of pain generation and transmission throughout the central and peripheral nervous systems and locally released pain mediators (including formation of inflammation and vascular edema) [5]. In the same way, NO is also a controversial molecule in inflammation. It plays a key role in the pathogenesis of inflammation but gives an anti-inflammatory effect under normal physiological conditions [6]. Therefore, it should be carefully managed in the treatment of pain and inflammation. However, it is noteworthy to indicate that whatever the case, the overproduction of NO will be harmful to the normal signaling process and the organism. Hence, drug discovery process targeting pain and inflammation should pay special attention to NO production.

One of the perspectives in finding alternative painkillers today is the use of medicinal plants. *Boerhavia coccinea* is an herbaceous plant of the Nygtaginaceae family. It is found in Western India, America, Australia and Africa. In Africa, *B. coccinea* is found in abundance in Cameroon [7] [8] where it grows in the East and North regions. The leaves and roots of *B. coccinea* are widely used in traditional medicine to treat liver, pain, urinary and gastroenteric diseases, prolapsed uterus, asthma, scabies, skin rashes, smallpox, oral candidiasis, aphthous ulcers, toothache and pneumonia [9] [10]. It is used by local people in the East region of Cameroon to treat stomach pain, toothache or to relieve pain in pregnant women. The present study was undertaken to evaluate the antinociceptive activity of the aqueous extract of *Boerhavia coccinea* and to determine their possible mechanism of action focusing on NO production.

2. Materials and Methods

2.1. Preparation of the Aqueous Extract

The leaves of *Boerhavia coccinea* (Nyctaginaceae) were collected in Bertoua, East Region of Cameroon, in July 2015. The plant was authenticated at the Cameroon National Herbarium, Yaoundé, by Mr. Tadjouteu Fulbert in comparison to samples Letouzey R.6445. Vroumsia Tchinaye 151VT and Bounougou E09, preserved at this institute. The leaves of *B. coccinea* (Mill.) were dried and ground to obtain powder. The aqueous extract was obtained by maceration of 200 g of powder in 500 ml of distilled water for 24 hours, followed by filtration using the filter paper No. 3. The filtrate was then evaporated in a ventilated oven at 40˚C. The resulting aqueous extract was weighed and kept in the freezer until use. The mass of aqueous extract obtained was 15.2 g, for an extraction yield of 7.6%.

2.2. Animals and Peritoneal Macrophages Collection and Culture

Wistar rats (180 to 200 g) and Swiss mice (23 to 30 g) of both sex aged 3 months were used in this study. These animals were bred in the animal house of the Laboratory of Animal Physiology and Phytopharmacology of the University of Dschang with free access to standardized rodent diet and water. For each experiment, animals were grouped in equal number of male and female. The number of animals used was the minimum possible to determine the consistent effects of the drug treatments. All protocols were submitted and approved by the local Ethics Committee and conformed to the guidelines for the study of pain in awake animals established by the International Association for the Study of Pain. Part of the study was also performed *in vitro* on macrophages collected from mice peritoneum.

For in vitro studies with macrophages, the protocol previously described by Tseuguem *et al.* [11] was used. Mice were sacrificed by cervical dislocation, the abdomen cleaned with 70% ethanol and the skin cut opened. Five milliliters PBS-EDTA (5 mM EDTA) was injected into the peritoneal cavity and the abdomen massaged for about 15 seconds. The peritoneum liquid was removed, centrifuged at 1500 rpm for 5 minutes and the pellet washed with PBS by repeating the process. The obtained cells were suspended in RPMI-1640 without phenol red, supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 U/ml penicillin and 100 µg/ml streptomycin. Macrophages were incubated for 3 h at 37˚C in a humid atmosphere and 5% CO_2. The supernatant containing non-adherent cells was removed and the adherent cells were harvested, suspended in the same culture medium and plated in 96 wells plate at 10^5 cells per well for the experimental procedure.

2.3. Pharmacological Tests

2.3.1. Evaluation of the Antinociceptive Effects of *Boerhavia coccinea* Extract on Formalin-Induced Pain-Like Behavior in Mice

This experiment followed the procedure previously described by Piegang *et al.* [12].

B. coccinea extract was given orally at the doses of 50, 100, 200 and 400 mg/kg. These doses were obtained from the therapeutic doses of the traditional healer and our preliminary screening studies. Morphine administered by intraperitoneal route at the dose of 10 mg/kg was used as reference drug. The control group received distilled water. Pain was induced in all animals by injecting 20 µl of formalin (2.5% in saline) under the aponeurosis of the dorsal surface of the right hind paw of each animal, one hour after extract administration or 15 minutes after morphine injection. Mice were individually observed and pain evidenced by licking, flinching or biting the injected paw was quantified using an electronic timer, in two periods. The first period consisted of the first 5 min indicating neurogenic pain and second period (15th to 30th minute) corresponding to the tonic inflammatory pain.

2.3.2. Evaluation of Sedative Properties of the Aqueous Extract *Boerhavia coccinea*

This test was conducted to verify the specific analgesic activity of the *B. coccinea* extract. The aqueous extract was administered orally at the doses of 50, 100, 200 and 400 mg/kg and diazepam was injected 4 hours later through the intraperitoneal route at the dose of 50 mg/kg to induce sedation. Animals were later placed on their side and the latency and the duration of sleep were recorded with an electronic stopwatch [13].

2.3.3. Testing the Anti-Inflammatory Effects of *Boerhavia coccinea* Extract on the CFA Model in Rats

To evaluate the contribution of the anti-inflammatory process in the analgesic activity of aqueous extract of *B. coccinea*, the extract was tested on CFA-induced inflammation. Rats were randomly distributed in 5 groups of 6 animals each. The paw diameter of each rat was recorded as baseline and inflammation were induced as follow: rats were lightly anesthetized by ether moisten on a hydrophilic cotton in a jar Plexiglas, the left hind paw skin was sterilized with 75% ethyl alcohol, and 100 µl CFA (100%, Sigma-Aldrich, Germany) was injected in the subplantar region of the left hind paw using an insulin syringe needle. The sham group was injected with the same volume of saline [11]. Forty eight hours after CFA injection animals were treated as follows: the sham group received no treatment, the negative control group was treated with 5% DMSO (vehicle), group 3 considered as positive control was treated with indomethacin (5 mg/kg/day dissolved in NaCl 0.9%), the two others groups received AE at the doses of 100 and 200 mg/kg/day of body weight. Using an electronic caliper (Fine Science, Heidelberg, Germany), the paw volume was measured at the 1st, 3rd, 5th, 7th and 24th hours post treatment.

2.3.4. Determination of Effects of the Aqueous Extract of *Boerhavia coccinea* on Nitric Oxide Production by Macrophages

To further understand the mechanism of action of the aqueous extract of *B. coccinea*, it was evaluated on nitric oxide production and cytotoxicity in non-stimulated and LPS-stimulated macrophages.

In the first experiment, macrophages were plated in a 96 well plate at the concentration of 1×10^5 cells/well in the absence or presence of the aqueous extract at the concentration of 1, 3, 10, 30, 100 and 300 µg/ml. They were cultured for 8h at 37˚C in a 5% CO_2 humidified incubator. At the end of the incubation period, the supernatant was collected to measure nitric oxide. The attached cells were used to evaluate cytotoxicity by the MTT assay [9]. Each tested concentration was repeated 5 times in a plate and the experiment was done twice.

To determine the effect of the extract in inflammatory conditions, another experiment was carried out, where macrophages were stimulated with LPS. Macrophages were incubated for 1h at a density of 10^5 cells/well, with aqueous extract (1 - 300 µg/ml) or dexamethasone (30 µg/ml). Then, lipopolysaccharide was added to each well for a final concentration of 1 µg/ml. The plate was incubated for 8 h in the same conditions as described above. At the end of the incubation, the supernatant was collected for NO assay and the cells used for the evaluation of cytotoxicity.

The last set of experiments L-NAME (an inhibitor of nitric oxide synthase, 100 µg/ml) and dexamethasone (an inhibitor of inducible NO synthase, 30 µg/ml) were used in attempt to evaluate the mechanism of action of AE. Macrophages (10^5 cells/well) were pre-incubated with L-NAME or dexamethasone for one hour, treated with AE (3, 10 or 30 µg/ml) and then incubated for 8 hours. Reference wells (extract alone) were also introduced. After the incubation period, the supernatant was collected for NO assay and the cells used for the evaluation of cytotoxicity.

The level of NO production was assessed in culture media supernatant by the Griess method as described by [14]. Percentage of increase in NO production was calculated as follow: % of increase = $([NO]_T - [NO]_C) \times 100/[NO]_C$; where $[NO]_C$ is the nitric oxide concentration in non-treated cells and $[NO]_T$ the concentration in treated cells.

After harvesting the supernatant to assess NO, the wells were emptied and washed two times with PBS (300 µl). Then, 120 µl of MTT prepared at 0.03% in supplemented RPMI medium was added to each well. The plate was incubation at 37˚C and 5% CO_2 for 3 h. MTT reagent was then replaced with DMSO (150 µl/well) and the cellular viability was measured at 620 nm using a microplate reader [15].

2.4. Statistical Analysis

Results are expressed as Mean ± SEM (Standard Error of the Mean). One-way ANOVA (Analysis Of Variance) followed by the Tukey's posttest was used to compare the averages for the various groups in the antinociceptive tests in mice and in NO production by macrophages. Two-way ANOVA repeated measures followed by the Bonferroni posttest was used to analyze data on inflammation. The Student "t" test was used when comparing the effect of the plant extract in present of an antagonist. These analyses were performed using Graph Pad Prism

software version 5.0.

3. Results

3.1. Antinociceptive Effect of the Aqueous Extract of *Boerhavia coccinea* on Formalin-Induced Pain in Mice

The aqueous extract of *Boerhavia coccinea* reduced the pain induced by formalin as shown in Figure 1. This extract exhibited significant activities ($p < 0.001$) compared to the control. During neurogenic phase, the doses 100 and 200 mg/kg showed the best activities with respective inhibitory percentages of 78 and 81%, while the dose of 50 mg/kg did not prove any antinociceptive property. In the inflammatory phase of formalin pain the plant extract exhibited a significant ($p < 0.001$) and dose-dependent antinociceptive effect with inhibitory percentage ranging from 45% to 90%. Morphine used as reference drug at the dose of 10 mg/kg inhibited the neurogenic phase by 84% ($p < 0.001$) and completely abolished ($p < 0.001$) the second phase of formalin-induced pain.

3.2. The Aqueous Extract of *B. coccinea* Reduces Sedation in Diazepam Model

The results in Figure 2 show that the intraperitoneal administration of diazepam induced a sleep with a latency period of 529 seconds and a total duration of sleep of 8876 seconds. Although all the doses of the aqueous extract of *Boerhavia coccinea* tended to increase the latency and reduce the duration of sleep, only the dose of 50 mg/kg aqueous extract exhibited ($p < 0.01$) significant effects, increasing the sleep latency by 50 fold (Figure 2(A)) and reducing the sleep duration by 76% (Figure 2(B)).

3.3. The Aqueous Extract of *B. coccinea* Lacks Acute Antiinflammatory Effects on CFA Model

The subplantar administration of CFA resulted in a significant ($p < 0.001$) and

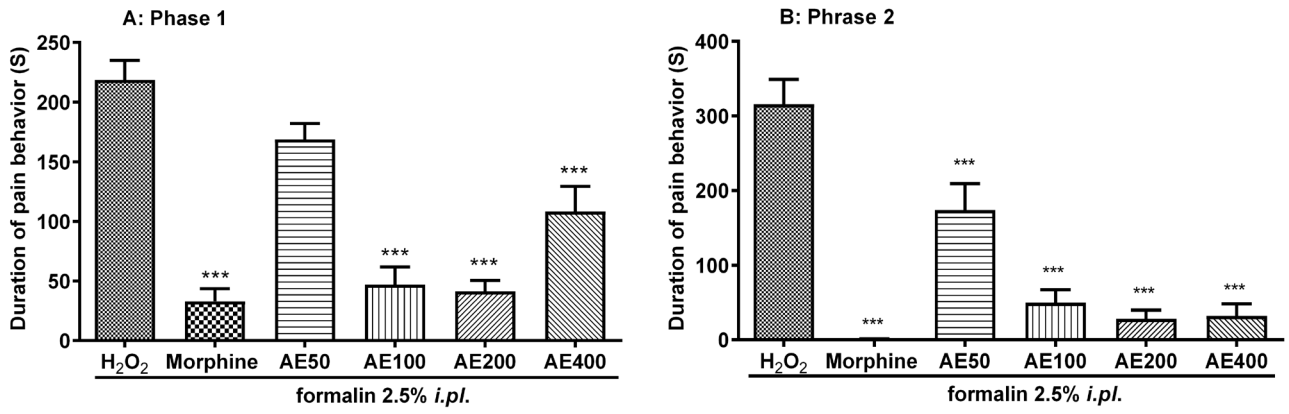

Figure 1. The aqueous extract of *Boerhavia coccinea* reduced the neurogenic (A: phase 1) and inflammatory (B: phase 2) phases of pain induced by the intraplantar injection of formalin in mice. Each bar represents the mean ± SEM of 6 animals; ***$p < 0.001$ statistically significant compared to control (H_2O_2), using one-way ANOVA followed by the Tukey posttest. AE = aqueous extract.

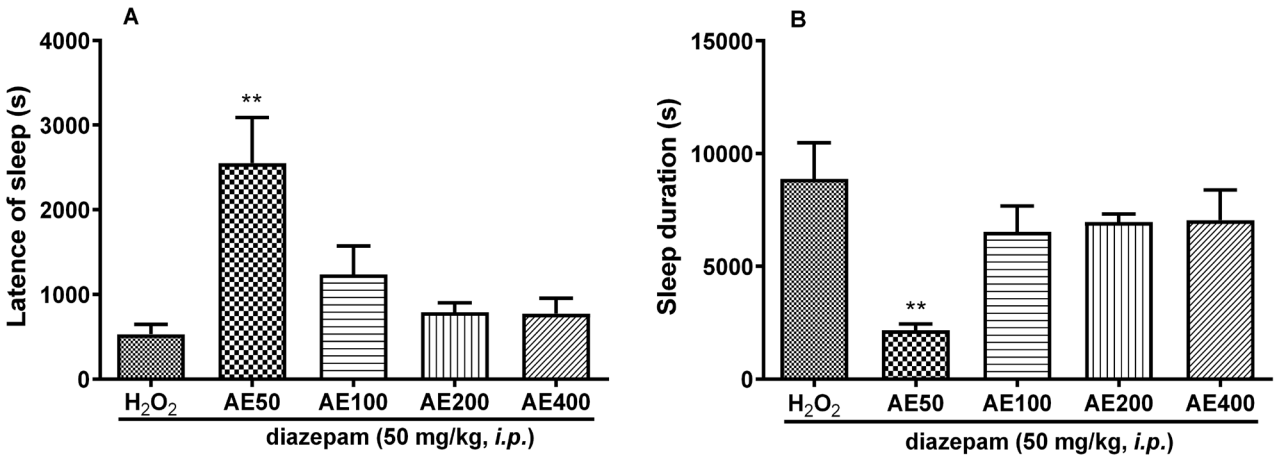

Figure 2. The aqueous extract of *Boerhavia coccinea* increases sleep latency and reduces the duration of sleep induced by intraperitoneal injection of Diazepam in mice. Each bar represents the mean ± SEM of 6 animals; $^{**}P < 0.01$ statistically significant compared to control (H_2O_2), using one-way ANOVA followed by the Tukey posttest. AE = aqueous extract.

steady paw inflammation as compared to the sham group. A single administration of indomethacin at the dose of 5 mg/kg induced a significant reduction of the paw edema with the maximal inhibitory effect of 48%, as compared to the CFA group. Surprisingly, the aqueous extract of *Boerhavia coccinea* at all the doses used, did not show any anti-inflammatory effect during the 24 hours of observation (Table 1).

3.4. The Aqueous Extract of *Boerhavia coccinea* Increases the Nitric Production in Non-Stimulated Macrophages with No Effect on Cell Viability

As depicted in Figure 3(A), incubation of macrophages with the aqueous extract of *Boerhavia coccinea* concentration-dependently and drastically ($p < 0.001$) increased the production of nitric oxide by non-stimulated macrophages. The extract at the concentration of 300 µg/ml was able to increase the production by up to 90-fold as compared to the control untreated cells (TN). In addition, this extract at concentrations of 10, 100 and 300 µg/ml, significantly ($p < 0.05$ and $p < 0.001$) increased cell viability (Figure 3(B)).

3.5. The Aqueous Extract of *Boerhavia coccinea* Potentiates the Nitric Oxide Production in LPS-Stimulated Macrophages

The effect of the aqueous extract of *Boerhavia coccinea* was also evaluated in inflammatory conditions, mimic here by the stimulation of macrophages with LPS. *B. coccinea* extract at the concentration of 1 µg/ml as dexamethaxone (30 µg/ml) decreased the NO production by 66% and 63% respectively, in stimulated macrophages. From the concentration of 10 mg/ml, the aqueous extract of *B. coccinea* drastically ($p < 0.01$ and $p < 0.001$) increased the nitric oxide production in this experimental conditions. The effect of the plant extract was concentration-dependent, with a maximal increase of 5654% at the concentration of 300 µg/ml (Figure 4(A)).

Table 1. Effects of the aqueous extract of *Boerhavia coccinea* on CFA-induced inflammation in rats.

Treatment	Paw inflammation (mm) over the time after treatment (hour)				
	1	3	5	7	24
Sham	0.01 ± 0.00$^{\$\$\$}$	−0.03 ± 0.01$^{\$\$\$}$	−0.02 ± 0.00$^{\$\$\$}$	−0.03 ± 0.01$^{\$\$\$}$	−0.01 ± 0.01$^{\$\$\$}$
CFA	2.84 ± 0.14	2.63 ± 0.19	2.63 ± 0,23	2.87 ± 0.12	2.58 ± 0.16
CFA + Indo 5 mg/kg	2.27 ± 0.17	1.70 ± 0.10**	1.79 ± 0.13**	1.50 ± 0.19***	1.70 ± 0.12**
CFA + AE 100 mg/Kg	3.19 ± 0.30	3.02 ± 0.22	2.63 ± 0.24	3.07 ± 0.31	2.74 ± 0.21
CFA + AE 200 mg/kg	2.73 ± 0.17	2.70 ± 0.25	2.89 ± 0.31	2.83 ± 0.32	2.70 ± 0.28

Data are presented the mean ± SEM of 6 animals; $^{\$\$\$}p < 0.001$ significant difference as compared to CFA control; $^{**}p < 0.01$, $^{***}p < 0.001$ statistically significant compared to sham group, using two-way ANOVA followed by the Bonferroni posttest. AE = aqueous extract.

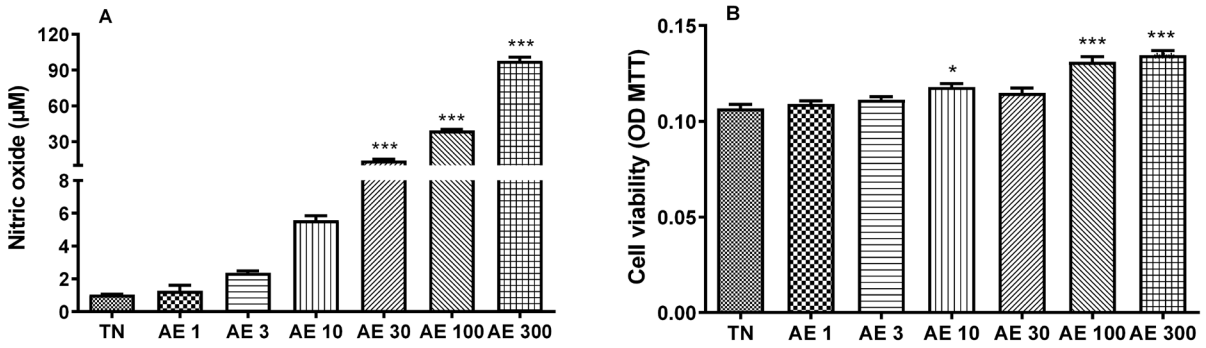

Figure 3. The aqueous extract of *Boerhavia coccinea* increases the production of nitric oxide and cell viability in non-stimulated macrophages. Data are presented as mean ± standard error on the mean and each bar represents the average of 5 repetitions. $^{*}p < 0.05$; $^{***}p < 0.001$ significant difference compared to the control (TN), using one-way ANOVA followed by the Tukey posttest. AE = aqueous extract.

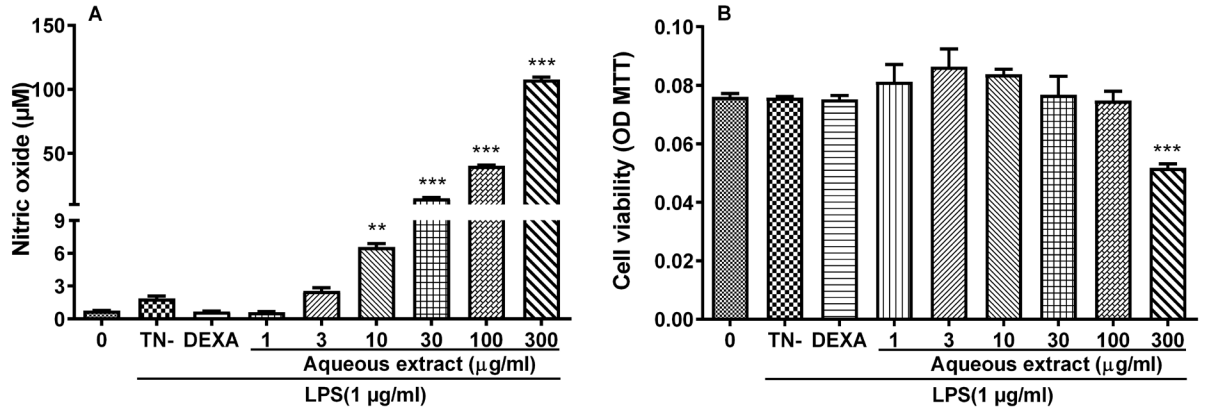

Figure 4. Effects of aqueous extract of *Boerhavia coccinea* on the production of NO and cell viability in LPS stimulated macrophages. The data are presented in the form of mean ± standard error on the mean and each bar represents the Average of 5 repetitions. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ significant difference compared to the control (TN-), using one-way ANOVA followed by the Tukey posttest.

This extract showed no effect on cell viability except at the concentration of 300 µg/ml where a significant ($p < 0.001$) reduction was observed (**Figure 4(B)**).

3.6. Effects of Dexamethasone on NO Production Induced by Aqueous Extract of *Boerhavia coccinea* on Non-Stimulated Macrophages

The aqueous extract administered at concentrations of 10, 30 and 100 µg/ml stimulated the production of NO by macrophages. Dexamethasone at a concentration of 30 µg/ml potentiated the stimulatory effect of the extract. In fact, the NO production induced by the different concentrations of the extract in the presence of dexamethasone was significantly ($p < 0.001$) greater than in the presence of the extract alone. At a concentration of 100 µg/ml, the activity of the aqueous extract increased by 66% compared to the effect of the extract when tested alone (Figure 5(A)). No significant variation in cell viability was observed with these various treatments (Figure 5(B)).

3.7. Effects of L-NAME on NO Production Induced by the Aqueous Extract of *Boerhavia coccinea* on Non-Stimulated Macrophages

As shown in Figure 6(A), L-NAME by itself did not affected the basal production of nitric oxide in quiescent macrophages. The concentration dependent increase in nitric oxide production induced by the aqueous extract of *B. coccinea* at the concentrations of 10 and 30 µg/ml was not affected by the L-NAME pretreatment. However, at the concentration of 100 µg/ml, L-NAME potentiated ($p < 0.001$) this production by 192%. As observed in Figure 6(B), no significant variations in cell viability was induced by the different treatments.

4. Discussion

The purpose of this study was to evaluate the antinociceptive and anti-inflammatory effects of the acute administration of the aqueous extract of the leaves of *Boerhavia coccinea*, using *in vivo* and *in vitro* approaches. In the *in*

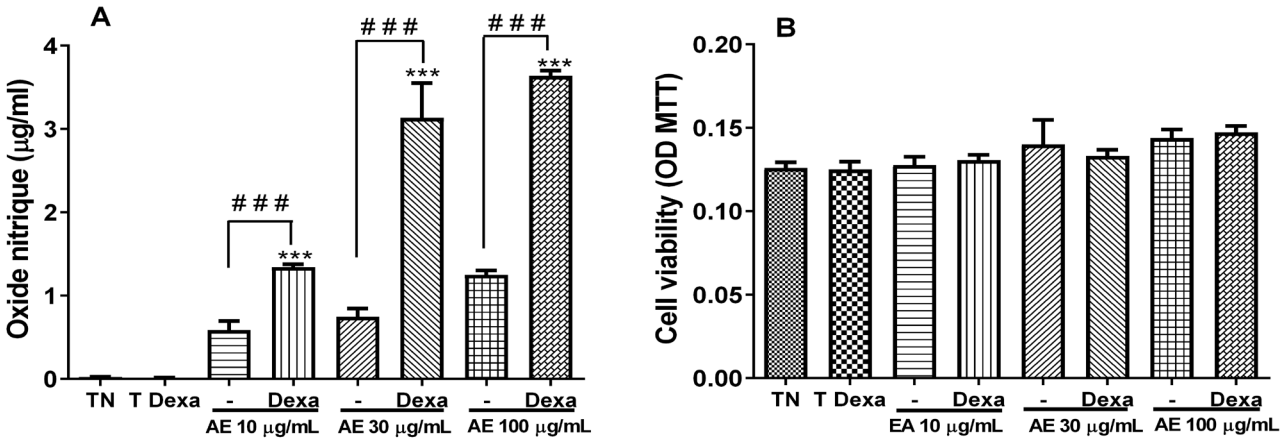

Figure 5. Effects of Dexamethasone on cell viability and NO production induced by aqueous extract of *Boerhavia coccinea* on non-stimulated (LPS) macrophages. Data is presented as the mean ± standard error of the mean and each bar represents the mean of 5 replicates. ***$p < 0.001$ significantly different from the control (TN) using one-way ANOVA followed by the Tukey's posttest. ###$p < 0.001$ significant difference between extract alone and extract + Dexamethasone using Student "t" test. AE = aqueous extract.

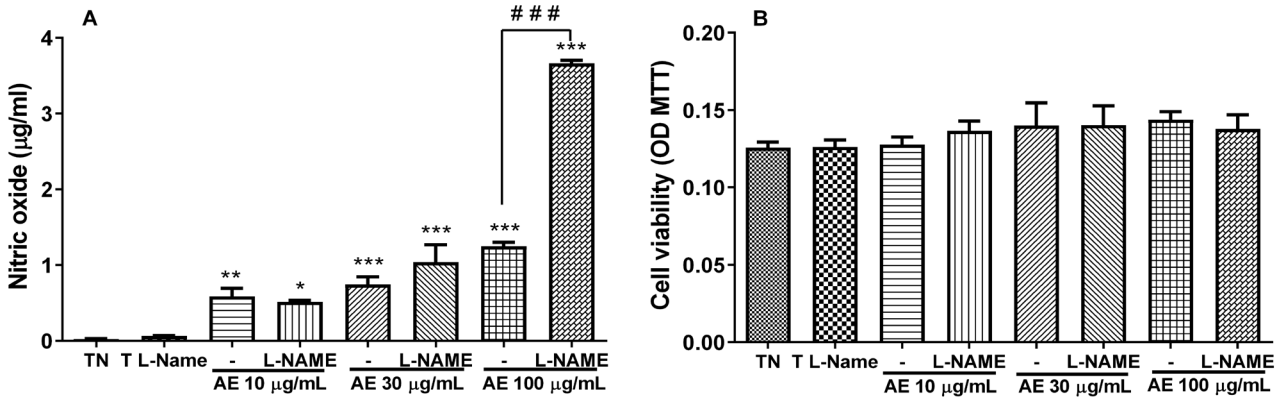

Figure 6. Antagonistic effects of aqueous extract of Boerhavia coccinea on inhibition of NO production (A) and cell viability (B) induced by L-Name in non-stimulated (LPS) macrophages. Data are presented as the mean ± standard error of the mean and each bar represents the mean of 5 replicates. $^{**}p < 0.01$; $^{***}p < 0.001$ significant difference compared to the neutral control (TN) using one-way ANOVA followed by the Tukey's posttest. $^{###}p < 0.001$ significant difference between extract alone and extract + L-Name using Student "t" test.

vivo animal models, the plant extract showed a potent antinociceptive effect on formalin-induced acute pain but lacked anti-inflammatory activity in CFA model. The plant extract also concentration-dependently increased the NO production both in non-stimulated and in LPS-stimulated macrophages.

Formalin-induced pain is a well know and validated model for study of antinociceptive substances. Its intraplantar injection produces a distinct two-phase response. The first phase called neurogenic or non-inflammatory pain results from immediate activation nociceptive receptors including transient receptor potential ankyrin 1 (TRPA1) and transient receptor potential vaniloid 1 (TRPV1) [16] [17] [18]. In this early phase of formalin-induced pain behavior, surface nociceptors can also be activated by the bradykinin, serotonin and substances released by resident cells. In addition, there is an increase in impulses transmission from C afferent fibers. During the second and last phase called inflammatory pain, pro-inflammatory mediators such as prostanoids, nitric oxide and cytokines are strongly implicated [19] [20] [21]. The aqueous extract of *B. coccinea* inhibited both phases of formalin-induced pain behavior, demonstrating its antinociceptive effects. In view of the foregoing, it may be thought that the *B. coccinea* extract could be active against the direct activation of nociceptors, the C fiber transmission and/or the inflammatory mediators' pathways. Interestingly, *B. coccinea* extract was more efficient on the inflammatory pain that on the neurogenic pain, suggesting the important contribution of the anti-inflammatory activity of this extract on its antinociceptive properties.

To ascertain this hypothesis, the anti-inflammatory effect of the extract was evaluated in steady inflammation induced by subplantar injection of CFA. Surprisingly, the extract was unable to exhibit any anti-inflammatory effect. This results suggest that the antinociceptive effect of the aqueous extract of *B. coccinea* is not dependent to any inflammatory effect, inferring therefore, a direct effect of this extract on the nervous system.

To verify that the observed antinociceptive effect is not related to a non-specific depression of the nervous system, the extract was tested on the sleep induced by diazepam. The lowest dose of *B. coccinea* extract (50 mg/kg) significantly increased the latency of sleep and reduced the duration of sleep, demonstrating the specific analgesic effect of this plant extract, which may be acting specifically on the pain transmission pathways either at the peripheral level or at the central level.

Nitric oxide is one of the main neuroinflammatory mediators implicated both in the initiation and maintenance in the inflammation process as well as in the neurotransmission of pain. In fact, it has been demonstrated that Nitric oxide synthase expression is up regulated in the spinal cord after formalin injection, and that nitric oxide (NO) plays important roles in the central mechanism of inflammatory algesia [22] [23]. To roll out the implication of the anti-inflammatory process in the antinociceptive effect of *B. coccinea* extract and further understand the lack of the anti-inflammatory activity of this extract, it was tested on the nitric oxide production by non-stimulated and LPS-stimulated macrophages. Indeed, LPS is an endotoxin derived from the wall of gram-negative bacteria that activates macrophages through Toll-like receptors and induces secretion and pro-inflammatory cytokines and NO [24].

The aqueous extract of *Boerhavia coccinea* significantly increased NO production by both non-stimulated and LPS-stimulated macrophages. These results suggest that the analgesic mechanisms of this extract would not be associated with inhibition of NO production.

For more insight in the mechanism of this effect, the AE was tested on non-stimulated macrophages in presence of L-NAME, an antagonist of NO synthase [25] [26] or dexamethasone, an antagonist of inducible nitric oxide [11] [27] Surprisingly, the two antagonists potentiated the stimulatory effect of the aqueous extract on the production of NO by macrophages. These results need additional data to be understandable. The MTT test on cell viability shows that the aqueous extract of *B. coccinea* did not cause the death of macrophages after exposure to the different doses tested for 8 hours, except at the concentration of 300 µg/ml, when co-applied with LPS. These results reflect a very low toxicity of this extract. The apparent cytotoxicity observed at the concentration of 300 µg/ml in presence of LPS might not be due to the direct effect of the extract but rather to the cytotoxicity of the NO which was drastically increased by more than 100-folds in this experimental condition. Indeed, the cytotoxicity of NO in macrophages stimulated by LPS is well documented [28].

5. Conclusion

In conclusion, the aqueous extract from the leaves of *Boerhavia coccinea* possesses potent antinociceptive effect that is not related to any anti-inflammatory activity. Instead, this extract increases the nitric oxide production in non-stimulated and LPS-stimulated macrophages by an unknown mechanism.

Acknowledgements

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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