

RESEARCH ARTICLE

Assessment of *Voacanga africana* effect on cognition and motor ability in a mouse model of Parkinson's disease

Houndjo DS¹, Loubano-Voumbi G^{2,3}, Gnahore Eric⁴, Camara Abdou Aziz⁵, Diop Doudou⁶, Sarr Abdou⁷, Diaw Mor¹, Sow Abdou K¹, Seck Aissatou¹, Toure Maimouna¹, Coly Mame Saloum⁸, BA Awa⁹, Gaye Magaye¹⁰, Mbengue Arame⁸, BA Abdoulaye¹, Sar Fatou Bintou⁸, Samb Abdoulaye¹

¹Laboratory of Physiology and Functional Explorations, FMPO/UCAD, Dakar, Senegal, ²Laboratory of Physiology Sciences, Marien Ngouabi University, Brazzaville, Congo – Brazzaville, ³Unit of Traditional Pharmacology and Physiology, National Institute in Health Sciences, Congo-Brazzaville, ⁴UFR Natural Sciences, Nangui Abrogoua University, Abidjan, Côte d'Ivoire, ⁵Faculty of Technical Sciences: Plant Biology Option, Université Cheikh Anta Diop de Dakar, Dakar-Fann, Senegal, ⁶Botanical Laboratory, UCAD Institut Fondamental d'Afrique Noire, Dakar, Dakar-Fann, Senegal, ⁷Pharmacognosy Laboratory, FMPO/UCAD, Dakar, Senegal, ⁸Laboratory of Physiology, UFR of Health Sciences of Thies, Thies, Senegal, ⁹UFR Health and Sustainable Development, Alioune Diop University of Bambey, Bambey, Senegal, ¹⁰Laboratory of Anatomy and Organogenesis FMPO/UCAD, Dakar, Senegal

Correspondence to: Houndjo DS, E-mail: salimatadiagne.houndjo@ucad.edu.sn

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ABSTRACT

Background: Parkinson disease (PD) is a neurodegenerative affection. At the pre-symptomatic stage, the clinical manifestations are mainly cognitive. **Aim and Objective:** Our study explores the effects of *Voacanga africana* (VOC) on cognitive disorders, more importantly on depressive troubles, in a mouse model (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; MPTP) of PD. **Materials and Methods:** We used albinos mice ($n = 39$) taken from the “Institut de Recherche et Développement” based in Dakar. These albinos mice were divided into two groups each composed of three lots. The first group was treated over 15 days (D) with VOC, water (W), and MPTP on D1 (L1: Control [no MPTP/W/VOC]; L2: [MPTP D1 + W D1]; and L3: [MPTP D1 + VOC D1]). The second group was also treated over a period of 30 days. Mice were pretreated with distilled water (W) and VOC 15 days before induction of the disease [L4: Control (no MPTP/W/VOC); L5: (W D1+ MPTP D15); and L6: (VOC D1+ MPTP D15)]. After different protocols of treatment, we used tests to assess motor skills and depression among the selected animals. Tests were carried out weekly and the ANOVA test and the post-test Bonferroni allowed us to compare the average of the different groups. **Results:** MPTP + VOC subjects were less depressed ($P < 0.0001$; D15) ($P < 0.0001$; D30) and had better sensorimotor coordination ($P < 0.001$; D15) than MPTP + W subjects regardless of the period. **Conclusion:** These preliminary results could confirm our hypothesis that the VOC has antidepressant effect and potentially a positive effect on motor performance in the pre-symptomatic stage of PD.

KEY WORDS: *Voacanga africana*; Parkinson Disease; Neurodegenerative

INTRODUCTION

Parkinson disease (PD) is essentially characterized by motor symptoms which constitute the parkinsonian triad: Akinesia, rest tremor, and stiffness.^[1,2] The symptomatology of PD is not only limited to motor disorders but also includes cognitive and psychiatric disorders^[3] (Charcot, 1872).

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Parkinson patients develop mood changes and personality disorders,^[4] depressive symptoms,^[5] and dementia. The PD is characterized by a set of neuronal degenerations which affect not only the dopaminergic system but also the noradrenergic, serotonergic, and cholinergic systems.^[6]

During the evolution of the PD, the topographic distribution of the brain regions is affected by the degenerative process, initially asymptomatic, spreads, and gradually leads to the appearance of motor symptoms.^[7] The motor and limbic systems were initially segregated into parallel and independent entities.^[8]

As a matter of fact, many authors such as Joel and Weiner^[9] have formulated the hypothesis of a circuit including a limbic/motor interface. According to them, this communication is carried out not only by the striatomesencephalic loops but also by the striatocortical loops.^[9] Thus, we set out to assess the effect of *Voacanga africana* (VOC) on cognitive and PD motor disorders. That plant from the Apocynaceae family has anti-inflammatory and neuroprotective properties. VOC is traditionally used to treat a wide range of conditions in Africa, including leprosy, diarrhea, generalized edema, mental disorders, and as an analgesic and anti-inflammatory.^[10-12] In our countries, traditional medicine is widely used and vernacular names are assigned to it in almost several languages across the subregion.

In Senegal, Ivory Coast, and all around in many African countries, VOC is also used to reduce body aches and trauma.^[13,14] These effects are thought to be due to a very important component, voacamine, a powerful alkaloid found in the bark of this plant, which could preserve the brain from the neuronal degeneration observed in neurodegenerative diseases,^[15] hence a positive consequence on behavioral performance in an animal model. Our study was carried out with the help of a dozen traditional healers in Ivory Coast and in Senegal and also with the help of botanical specialists for the identification of the plant.

MATERIALS AND METHODS

Plant Materials

Fresh root bark was harvested in February 2018 in Abidjan, Ivory Coast (CI) and was identified by researcher in Biodiversity and Sustainable Ecosystem Management (plant ecology-Forestry) from Nangui Abrogoua University (CI) and by a Botanical Engineer, from the Plant Biology Laboratory of the Faculty of Science and Technology of Cheikh Anta Diop University of Dakar (Forced Swim Test [FST] – UCAD); where the voucher specimen (DPB-16-10) was deposited.

The root barks were cut and dried at 25°C for 3 weeks. A brown powder was obtained after spraying.

Animal Material

We used 2-month-old Albinos mice from the “Institut de Recherche et Développement” based in Dakar ($n = 39$) with weights around 30–40 g. On arrival, mice had free access to food and water during the breeding and experimental period. Animal houses were kept at a constant temperature of 22–28°C and lit on a light-dark cycle from 12/12 h. Animals had a week of acclimatization before the start of the experiments.

Experimental Procedure

All the experiments were carried out according to the recommendations of the ethics committee of Cheikh Anta Diop University of Dakar (015/2020/CER/UCAD).

Extraction

The fresh root barks were rinsed, air dried, out of direct sunlight, and ground into powder. About 500 g of powdered root bark of *Voacanga africana* (Stapt et Scoot-Elliot) (Apocynaceae) was extracted from a moderate decoction under reflux with extract in 2 L of a hydroethanolic mixture (20 v/80 v) for 30 min. After filtration, the hydroethanolic extract obtained was evaporated using a rotary evaporator (“IKA” RV10) until a pasty residue was obtained. This residue was then dried in a desiccator and reduced to powder using a mortar and pestle.

Parkinsonian Model Induction

Our animals received two injections spaced from 2 h of 12 mg/kg 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP; Sigma-Aldrich) intraperitoneally (ip). This protocol induces the pre-symptomatic phase of Parkinson’s disease.^[16] These MPTP injections were carried out first in groups of animals at D1 of experimentation in the batches (L1-L2-L3) and second in others at D15 in the batches (L4 -L5-L6).

Treatments

Our animals were divided into two groups of three lots each. For the first group (G1), the animals were treated over a period of 15 days divided as follows: L1: Control (no MPTP/W/VOC) ($n = 9$); L2: (MPTP D1 + W D1) ($n = 6$); and L3: (MPTP D1 + VOC D1) ($n = 6$). The second group (G2) was treated over a period of 30 days: L4: Control (no MPTP/W/VOC) ($n = 6$), L5: (W D1 + MPTP D15) ($n = 6$); and L6: (VOC D1 + MPTP D15) ($n = 6$). VOC or distilled water (W) was administered to our mice daily by injections. In subjects treated over 15 days, distilled water or *Voacanga* was administered immediately the same day after the second injection of MPTP. For the subjects treated over 30 days, distilled water or *Voacanga* was administered on D1 (experimental pretreatment) and MPTP on D15 [Figure 1].

Behavioral Tests

Our tests were selected on the basis of the search for cognitive signs such as depression with the forced swimming test which sometimes appears in the pre-symptomatic phase, and motor skills (rotarod) usually reduced in the context of the onset of PD.

FST

Mice were put in a transparent cylinder (diameter: 13 cm, height 25 cm). The water was kept at a temperature of $21^{\circ}\text{C} \pm 4$, and animals were observed over a period of 10 min by a digital camera DCR-TRV110E (Sony Co., Tokyo, Japan). The measured parameter was the immobility time which is defined as the lack of activity other than that required to keep the animal's head out of the water.^[17] At the end of the test, the animals were removed from the water and dried using absorbent paper, then placed under a red heating light for 30 min. This test was performed on D7-D15 for G1 (L1-L2-L3) and D7-D15-D23-D30 for G2 (L4-L5-L6).

Rotarod (R)

Rotarod highlights the difficulties of motor coordination in rodents. To highlight the motor problems of the animals, we made them move on a rotating cylinder (rotarod; Bioseb, Chaville, Fr) at constant speed (14 rpm) for 5 min. Following this forced walking exercise repeated 3 times, the mouse falling on a pedal stops the stopwatch. The average of the time of the three exercises will be retained as a score. This test was performed on D7-D15 for G1 (L1-L2-L3) and D7-D15-D23-D30 for G2 (L4-L5-L6).

Statistical Analyses

The mean of the parameters evaluated in the behavioral tests was calculated for each batch and the differences between

the treatment groups were determined by the ANOVA test. The post-test Bonferroni allowed us to identify the difference between the group means.

RESULTS

In batches (L1-L2-L3) at D15, we noticed a significant difference when it comes to the forced swimming test between L1 (control) and L2 subjects (MPTP D1 + W D1) ($P < 0.0001$) compared to L3 subjects (MPTP D1 + VOC D1). This significant difference was observed from the 15th day post-treatment ($P < 0.0006$). The L1 and Parkinsonian subjects treated with distilled water (L2) were significantly more depressed than the Parkinsonian subjects treated with VOC (L3) after 15 days of treatment [Figure 2].

For the rotarod test, a significant difference was noted on D0 between the control group L1 and the parkinsonian subjects treated with water (L2) ($P < 0.001$) and with VOC (L3) ($P < 0.001$), however, on D15, this difference was less significant ($P < 0.047$) and concerned only parkinsonian subjects treated with water (L2).

This could mean that motor performance would decrease overtime in people with PD with a predominance in people treated with water. For the subjects (L4-L5-L6) treated over 30 days [Figure 3], in the forced swimming test, a very significant difference ($P < 0.001$) was noted between the controls (L1) and the parkinsonian subjects treated with water (L2) compared to parkinsonian subjects treated with VOC (L3) ($P < 0.01$) on D23. This difference seems to be attenuated on D30 and concerned only the parkinsonian subjects treated with water (L2) ($P < 0.049$). Thus, in the parkinsonian subjects, we observed a depression not dependent on the pre-treatment (W or VOC) compared to the control subjects where the depression was less.

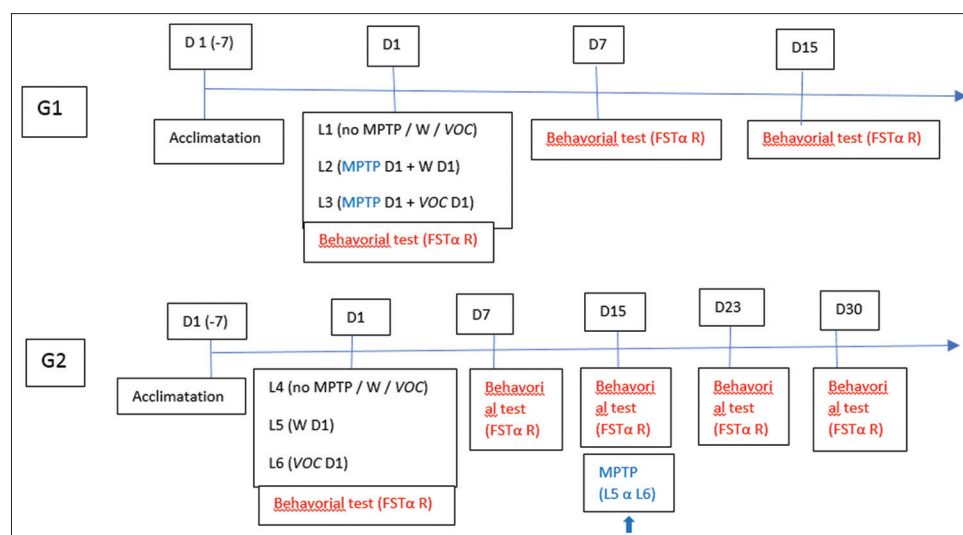


Figure 1: Summary of the experimental protocol for G1 and G2

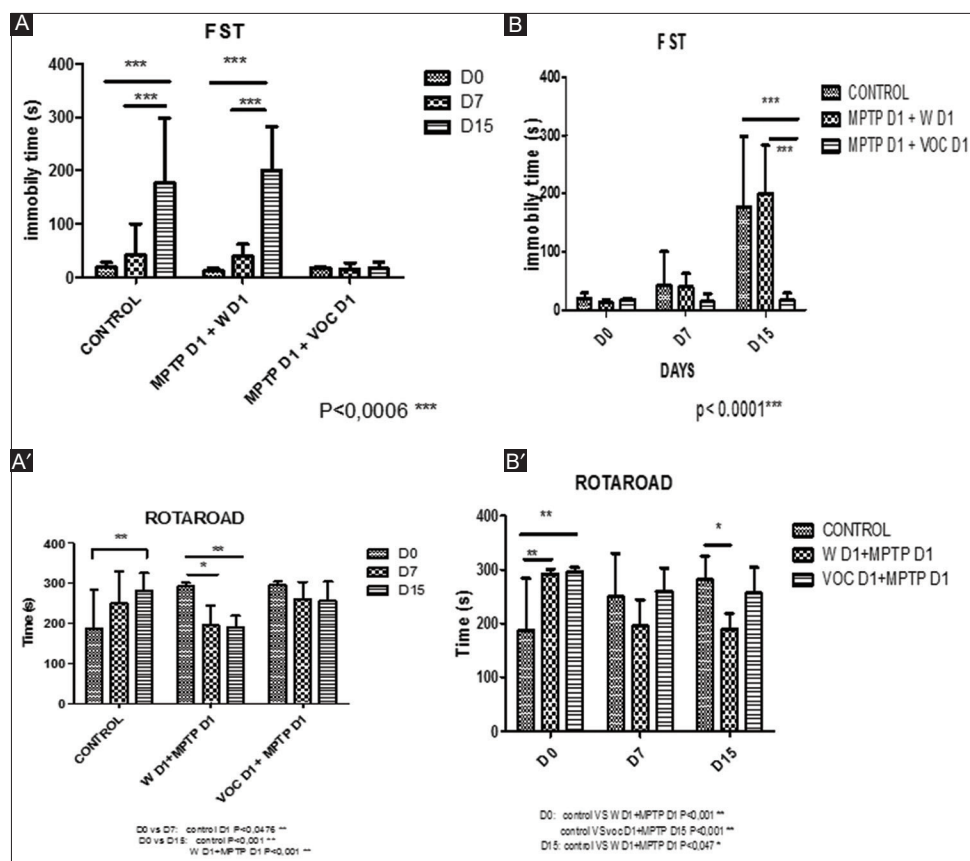


Figure 2: Charts representing behavioral tests: The forced swimming test A; B and rotarod A'; B' performed in mice over a period of 15 days. Figure A; A' represents the intragroup comparisons and B; B' intergroup comparisons

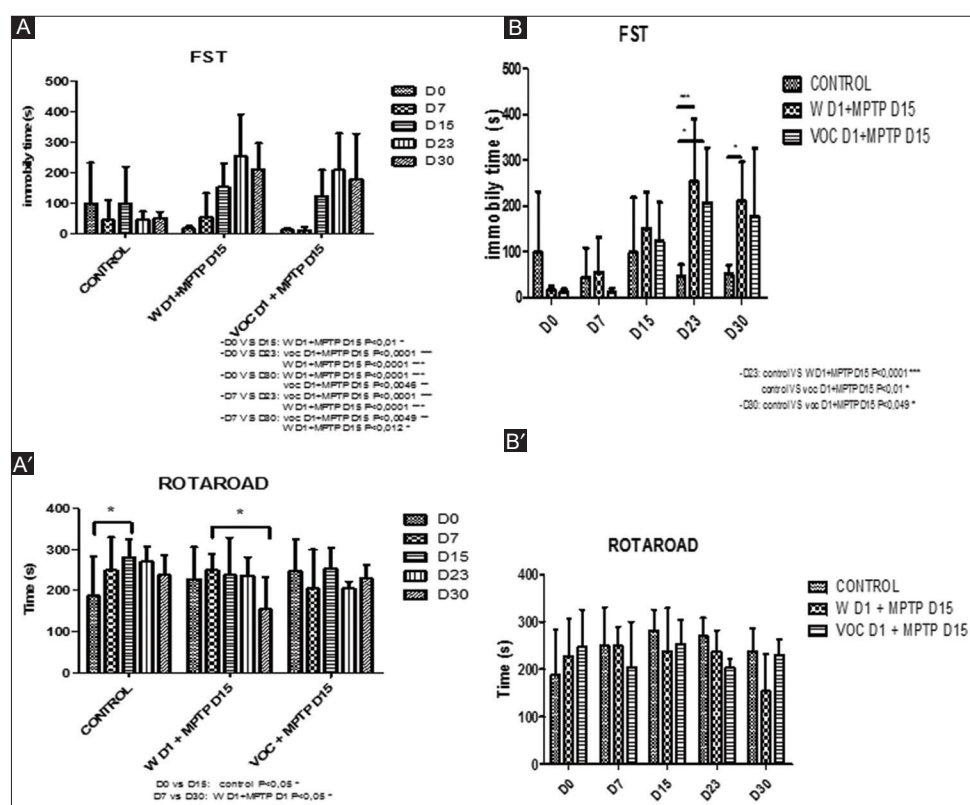


Figure 3: Charts representing behavioral tests: The forced swimming test A; B and rotarod A'; B' performed in mice over a period of 30 days. Figures A; A' represents the intragroup comparisons and B; B' intergroup comparisons

The rotarod test did not show any significant difference between the batches; however, a significant improvement in the motor performance of the control subjects was noticed on D15. In subjects treated with water, a decrease in motor performance was noted D30.

DISCUSSION

In our study, we have observed decreasing depressive disorders in subjects treated with VOC and an improvement in motor performance despite the progressive onset of the PD. We also have observed that preventive VOC treatment did not prevent the onset from depressive disorders in our Parkinsonian subjects. These results are similar to those found by several authors including Akunne *et al.*, 2017^[18]

This author used the FST who is one of the approved animal neuropharmacological models to study the antidepressant effect of extracts. He has shown an abolition of depressive behavior with a dose-dependent response in depressed animals treated with VOC. Currais *et al.*, 2014,^[15] have shown the neuroprotective effect of VOC extract on Alzheimer's disease by isolating voacamine as a major alkaloid of VOC and probably implicated in this neuroprotection.^[10] Indeed, the VOC according to several studies is made up with flavonoids, tannins, terpenoids, steroids, and alkaloids,^[19,20] the latter would have according Koroch *et al.* potent antidepressant-like effects on the nervous system.^[12] Martínez-Vázquez *et al.*, 2012,^[21] isolated certain alkaloids from *Annona cherimola*, including 1,2-dimethoxy-5,6,6a,7-tetrahydro-4H-dibenzoquinoline-3,8,9,10-tetraol, anonaine, lirioidenine, and nornuciferine. The results showed that repeated treatment with this plant produced an antidepressant-like action in mice.^[21] The β -carboline alkaloids such as harmaline, norharmaline, and harmine dose dependently reduced the immobility time in the mouse FST and thus produced an antidepressant-like effect.^[22] The specific bioactive compound responsible for the antidepressant effect of VOC cannot be identified at this stage of the study. However, Akunne *et al.*^[18] tried to explain the antidepressant power of the VOC. According to them, the mechanism through which the VOC exhibited the antidepressant effect might be related to the general mechanism of antidepressants action which is inhibition of metabolism of neurochemicals such as serotonin and norepinephrine.^[23]

Kaul *et al.*, 2015,^[24] in the preliminary reports suggested that amisulpride may have antidepressant effects. The presumed selectivity of amisulpride for D2 and D3 dopamine receptors has led to the prevailing hypothesis that modulation of dopaminergic signaling is responsible for its antidepressant efficacy. Thus, all of this work seem to highlight a hypofunction of the dopaminergic and serotonergic system in the onset of depressive disorders. The degeneration of dopaminergic neurons and Lewy intraneuronal bodies

in the substantia nigra pars compacta is the signature neuropathological lesions of PD. However, it is well known that in PD, neurological disease extends beyond the average brain, and also involves the discrete loss of noradrenergic and serotonergic neurons. Together, these neural systems are associated with the regulation of mood and reward systems as well as mood disorders in patients with PD and in the general population.

The study we carried out is the first to have studied VOC in Parkinson's disease with an *in vivo* model. However, we met some limitations such as the death of animals a few days before the end of the study, which reduced the size of our sample.

CONCLUSION

These preliminary results could confirm our hypothesis that the VOC has antidepressant effect and potentially a positive effect on motor performance in the pre-symptomatic stage of PD.

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