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Original paper

Studying some neuroprotective effects of Calotropis procera extracts against scopolamine- induced neuropschiatric comorbidities in a rodent model of epilepsy

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Abstract Many plants are largely used in alternative medicine of Burkina Faso for neuropsychiatric disorders treatment. However, their neuro-pharmacological properties are less evaluated through scientific studies. The present study aims to evaluate the neuroprotective effect of *Calotropis procera* leaves and root-bark aqueous extract, focusing on a scopolamine-induced model of epilepsy in rodents. In this study, we evaluated this plant extracts possible protective effects on the central nervous system, through the behavioral tests and the enzymes activity assays. Thus, elevated plus-maze test and Y-maze task were used to evaluate animals behavioral and UV/visible spectrophotometer methods were used to evaluate the enzyme's activities in brain's supernatant. Our results are showing no significant protective effects of leaves extract, but it revealed a significant neuroprotective effect of root-bark aqueous extract, as well as in the behavioral tests and the brain's oxidative enzymes specific activity evaluation. Indeed, anti-amnesic and anxiolytic activities were observed through Y maze task and elevated plus maze tests for the groups of animals receiving root-bark extract (100 mg/kg b.w.). In these test, inhibition of disturbances of Time spent in Open Arms, Spontaneous Alternation, and Transfer Latency induced after scopolamine administration were recorded with animals received root-bark extract. Likewise, the superoxide dismutase and catalase activity disturbance induced by scopolamine were also inhibited in root-bark extract pre-administered group. Thus, our study provides biochemical and neuro-pharmacological data for traditional use of *C. procera* for neuropsychiatric disorders treatment, including scopolamine-induced epilepsy symptoms (mainly referring to the psychiatric comorbidities of this disorder).

Keywords *Calotropis procera*, anti-amnesic, antioxidant, behavioral; neuroprotective

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Introduction

Medicinal plants are diversely used in traditional therapy in the world to treat various diseases. These plants are very important for alternative medicine in Burkina Faso because most of rural populations use them to ensure their primary healthcare [1]. The neuropsychiatric diseases which are characterized by anomalies of the thought, emotions, behavior and relationship with others, increasingly affect population in Burkina Faso [2]. Epilepsy is reported as one of the most common neurological disorders through the world. It affects around 1% of people in the world, including 80% in developing countries with a high prevalence in Africa [3,4]. According to the previous studies, most symptoms of this disorders include anxiety, depression, amnesia and oxidative damage [5,6,7].

Calotropis procera is one of species of Asclepiadaceae family, which is reported to have therapeutic potential. This plant is used in traditional medicine to treat many diseases such as epilepsy, madness, hallucination, Insomnia, nerves diseases, otitis, tumors, drepanocytosis and liver diseases [8,9,10]. This plant is widely found in urban and rural areas and it is reported to contain important natural substances such as cardenolides (calotropin, calactin, uscharin), alkaloids (α-amyrine, β-amyrine), phenolic acid (ellagic, chlorogenic, caffeic and coumaric acids) and flavonoids (rutin, quercetin, kaempferol) [8,11,12]. Some of these chemical contents are well known to act on the nervous system [13,14].

Scopolamine was previously used, besides its amnesic effects [15,16,17,18,19], on inducing some epilepsy-like manifestations in rodents [20,21,22,23].

Thus, in the present study, based on the aspects mentioned above, we decided to Study the possible neuroprotective effects of *Calotropis procera* extracts against scopolamine-induced neuropschiatric comorbidities in a rodent model of epilepsy.

Materials and Methods

Plant collection and preparation of extracts

Calotropis procera leaves and root-bark were collected in Gampela, a village from central area of Burkina Faso. Plant was identified by botanists of the Plants Department of University Joseph Ki-ZERBO/Burkina Faso. Voucher specimens were deposited at the herbarium of this university having the identity number 16972.

Twenty-five grams (25 g) of dried powder were extracted with 500 mL of distilled water at 100°C for 30 min. It was filtered using muslin cloth and centrifuged at 4000 rpm for 10 min. The supernatant was collected and lyophilized to dryness. The residue was weighed to obtain the extracted yield and it was kept at 4°C.

Chemical

Scopolamine, monobasic sodium phosphate (NaH_2PO_4), dibasic sodium phosphate (Na2HPO4), ethylenediamine tetraacetic acid (EDTA), all solvents were analytical grades and purchased from Sigma-Aldrich (Germany). Diazepam was purchased from a local pharmacy.

Experimental animals

Wistar rats weighing 340 ± 8 g were used. They were obtained from the animal house of University Joseph KI-Zerbo, and housed for a week under controlled conditions for acclimatization before the experiments. Animals were kept in plastic cages under identical animal house conditions and provided with standard pellet and water ad libitum. Twelve-hour light and dark alternate cycles (started at 6:00 a.m.) were provided, temperature was maintained at 22 ± 3°C and relative humidity was 50 ± 10%. Rats were treated in accordance with the guidelines of animal bioethics from the Act on Animal Experimentation and Animal Health and Welfare Act from Burkina Faso (Ethics committee acceptance CE-UOI-2018-03) and all procedures were in compliance with the European Council Directive of 24 November 1986 (86/609/EEC). All evaluations were performed between 9 a.m. and 4 p.m.

Drug administrations

This study was conducted according to previously described method [24]. Drugs were daily prepared and administered by oral route during 14 consecutive days. The rats were randomized into five groups (n=6). The 1st group (Control) and the 2nd group (Sco) received saline (NaCl 0.9%). The group 3 (CpL+Sco) and 4 (CpR+Sco) received respectively *C. procera* leaves extract and root-bark extract (100 mg/kg). The last group (Dzp+Sco) received diazepam (1,5mg/kg). The 14th day, all groups excepted control received scopolamine hydrobromide (0,7mg/kg, intraperitoneal route) 1 h after drug administration. Behavioral tests were performed 30 min after Scopolamine administration. Then, the anxiolytic, anti-amnesic and antioxidant effects of extracts were estimated.

The anxiolytic evaluation

Elevated plus-maze test

Resting on the natural fear of rodents on height and open spaces, the elevated plus-maze is a general search tool used for the neurobiological evaluation of drugs such as anxiety, exploration, motor [25]. This test was used in the present study to evaluate anxiety effect of extracts. The plus-maze consists of four arms, 49 cm long and 10 cm wide, elevated 50 cm above the ground. Two arms were enclosed by walls 30 cm high and the other two arms were exposed. Rats were individually placed at the juncture of arms and time spent on the open arms was recorded during 5 min. Time spent in the closed arms is an index of anxiety.

The anti-amnesic evaluation

Elevated plus-maze test

A second model of Elevated plus-maze test was used to evaluate memory according to procedure previously described [26]. The used elevated plus maze consisted of two open arms (49 cm × 10 cm) and two covered arms (49cm × 10 cm × 30 cm), elevated to a height of 50 cm from the floor. On the first day, each rat was placed at the end of an open arm, facing away from the central platform. Transfer latency (TL) was defined as the time taken by the animal to move from the open arm into one of the covered arms with all its four legs. TL was recorded on the first day (i.e., 14th day of drug administration) for each animal. The rat was allowed to explore the maze for 2 minutes and then returned to its home cage. Retention of this learned-task (memory) was estimated 24 h (15th day) after the first day trial. Significant reduction in TL value of retention indicated improvement in memory.

Y-maze task

This test estimates the short-term memory through the spontaneous alternation behavior [24]. The used Y-maze consisted of three arms (35 cm long, 25 cm high and 10 cm wide) and an equilateral triangular central area. Rats were placed at the end of one arm and allowed to move freely through the maze for 8 min. An arm entry was counted

when the hind paws of the rat were completely within the arm. Spontaneous alternation behavior was defined as entry into all three arms on consecutive choices. The number of maximum spontaneous alternation behaviors was then the total number of arms entered minus 2. The spontaneous alternation (%) was calculated as (actual alternations/ maximum alternations)×100. Spontaneous alternation behavior is considered to reflect spatial working memory, which is a form of short-term memory.

Brain's enzymes activity assays

Supernatant preparation

After the Y-maze behavioral test, rats were decapitated and whole brains were removed. The hippocampal and cortical regions of each rat were carefully excised, weighted and homogenized (10%) in ice-cold potassium phosphate buffer (0,1M, pH 7.4). The homogenate was centrifuged at 1000 x g for 15 min and the supernatant was used for AChE, SOD and CAT activity assays.

Acetylcholinesterase (AChE) activity assays

The AChE activity of rat brain homogenate was evaluated according to the method of Ellman [27] slightly adapted. In the principle of the reaction, acetylcholinesterase hydrolyzes the substrate ATCI (acetylthiocholine iodide) to thiocholine and acetate, unstained products. Thiocholine in the presence of DTNB (5,5'-dithiobis-2-nitrobenzoic acid) gives a yellow product (5-thio-2-nitrobenzoate), which allows to follow the kinetics spectrophotometer. For this test, 20 µl of homogenate was introduced into each microplate well containing 150 µl of phosphate buffer (0.1 M, pH 8), 10 µl of ATCI (14 mM) and 10 µl of DTNB (10 mM). The appearance of the yellow color is measured at 412 nm every minute intervals for 5 min using UV/visible spectrophotometer.

Superoxide Dismutase (SOD) activity assays

The activity of superoxide dismutase (SOD) was assayed using the method of Misra and Fridovich [28] with slight modification. It is based on the inhibition of autoxidation of epinephrine to adrenochrome. This oxidation has a very complex chemical mechanism, but several of its steps are $O_2^{\cdot-}$ dependent, and thus it can be used for SOD measurement. The rat's brain supernatant 0.5 ml was added to distilled water 0.8 ml, ice cold ethanol 0.25 ml and chloroform 0.15 ml. The mixture was shaken for 5 minutes at 4°C and then centrifuged. 0.2 ml of EDTA (0.6 mM), 0.4 ml of Na_2CO_3 (0.25 M) and 0.2 ml of epinephrine (3 mM) were added to the reaction mixture and the absorbance was measured at 420 nm.

Catalase (CAT) activity assays

The CAT activity was measured according to the method of Beers and Siezer [29]. For this trial, 50 µl of rat brain homogenate is introduced into a tube containing 950 µl of phosphate buffer (50 mM, pH 7.4). The reaction is initiated by the addition of 500 µl of hydrogen peroxide (H_2O_2, 30 mM). The control consisted to 1 ml of phosphate buffer and 500 µl of hydrogen peroxide (H_2O_2). The reduction of the optical density (OD) due to the decomposition of the hydrogen peroxide is measured after one minute of incubation at 240 nm. The enzyme activity is expressed as µmol of H_2O_2 consumed/min/mg of protein.

Statistical analysis

Statistical analysis was performed with GraphPad Prism 5.03 for Windows (Graph Pad Software, Inc., California USA), using One-way ANOVA. All results were expressed as the mean ±S.E.M. Statistical differences were determined by Tukey's post hoc test for multiple intergroup comparisons. Differences were considered significant when the p value was less than 0.05 ($p < 0.05$).

Results and discussion

Anxiolytic effect of C. procera extracts

The behavior in the elevated plus-maze is mainly used to estimate the anxiety effect of drug. From results, scopolamine treated group (Sco) showed significant decrease ($p<0.05$) of time spent in open arms as compared to the control group (figure 1A). Likewise, the frequency of entries in open arms also has decreased in scopolamine group (figure 1B). These results indicate an anxiety effect induced by scopolamine on rats. However, animals pre-administered with *C. procera* root-bark extract showed significant difference ($p<0.05$) of time spent in open arms as compared to the rats of scopolamine treated group (figure 1A). The same profile was observed for the frequency of entries in open arms (figure 1B). These results suggest that the extract inhibited the anxiety effect induced by scopolamine. This assumes that *C. procera* root-bark has an anxiolytic property.

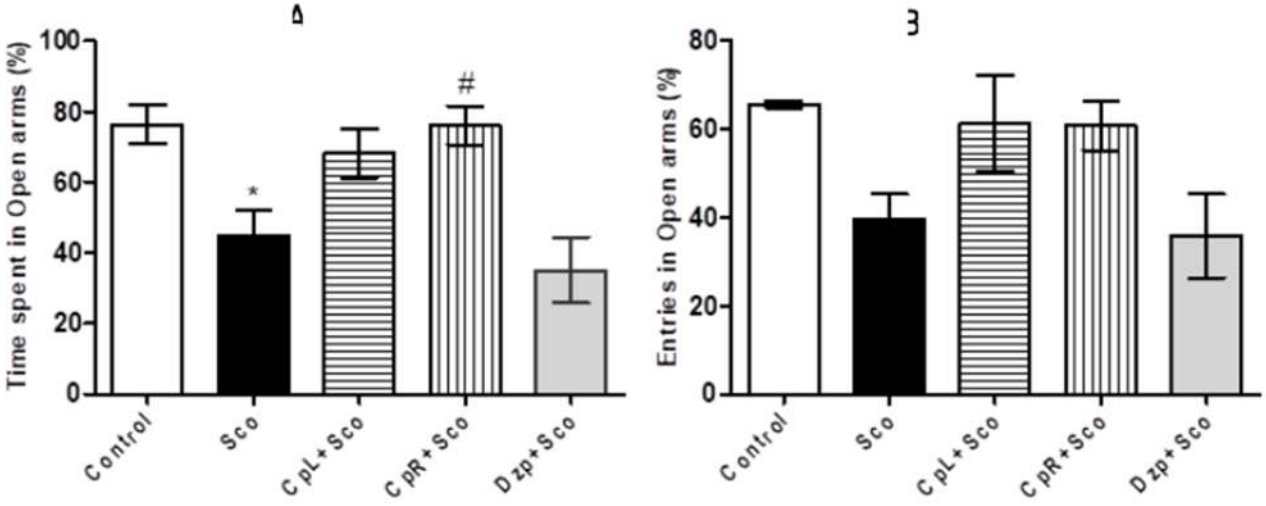

Figure 1. Effect of *C. procera* leaves and root-bark extracts on the Time spent in Open Arms [**A**], and the frequency of Entries in Open Arms [**B**] of rats. *$p<0.05$ compared with control group. #$p<0.05$ compared with scopolamine group.

Anti-amnesic effect of C. procera extracts

This activity was assessed in the Y-maze task and the elevated plus maze test. The first trial evaluates the short-term memory of the animals. From results obtained, the scopolamine group (Sco) showed significant decrease ($p < 0.05$) of the spontaneous alternation (SA) as compared to the control group. This result indicates a difficulty for animals to remember the arm immediately explored before the current arm (arm from which it is going out), which suggested a short-term memory deficit (amnesia). For the rats pre-administration with *C. procera* root-bark extract (CpR+Sco), the SA was not changed compared to control group (Figure 1A). This result suggests the root-bark extract inhibit the scopolamine effect on rats.

The memory of rats 24 hours after scopolamine administration was evaluated in the elevated plus maze. The Latency Time (TL) was recorded on the 14th day (30 min after scopolamine treatment) and the 15th day (24 h after scopolamine treatment). Animals received scopolamine alone (Sco) showed a significantly increase ($p < 0.01$) of the TL at the 14th and the 15th day compared to the control group (Figure 1B). These results indicate a memory deficit in these animals the 14th day and persisted after 24 hours. Rats pre-administered with root-bark extract significantly ($p<0.05$) inhibited TL increase the 15th day.

Results of these two tests suggest root-bark extract have anti-amnesic effect against memory deficit induced by scopolamine. While, leaves extract and diazepam did not influence significantly scopolamine effect.

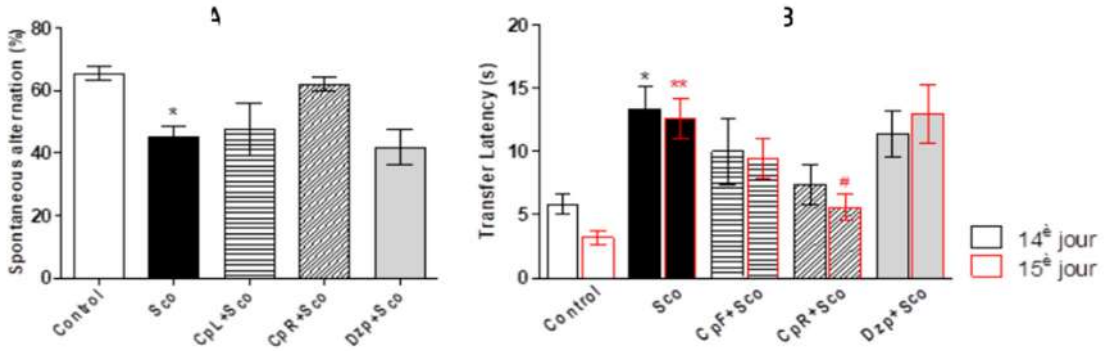

Figure 2. Effect of *C. procera* leaves and root-bark extracts on Spontaneous Alternation [**A**], and Transfer Latency [**B**] of rats. *p<0.05, **p<0.01 compared with control group. #p<0.05 compared with scopolamine group.

C. procera extracts effects on Brain's enzymes activities

The AChE specific activity estimated in the rat brain homogenates, revealed no statistical difference ($p > 0.05$) in scopolamine treated groups as compared to control group. Likewise, no difference was observed between profiles of leaves extract group, diazepam group and scopolamine group. However, AChE activity in root-bark treated group kept the same profile as the control group (Figure 2a).

SOD and CAT are very important enzyme able to protect cells and tissues biological integrity against harmful effects of free radicals [30]. Previous studies showed that memory deficit induced by scopolamine affects the stress oxidative parameters and reduce SOD and CAT specific activities [31,32]. In the oxidative evaluation, all animals of groups treated with scopolamine registered SOD and CAT activity lightly decreasing compared to control, except root-bark group. The root-bark extract pretreated group like the control group, presents an opposite profile to that of scopolamine group (Figure 2b, 2c). This observation suggests the extract prevents scopolamine oxidative effect.

These results confirm those observed in behavioral evaluation and certify that root-bark extract has neuro-protective activity. Results of this study corroborate those of Kumar's group who reported the antioxidant and membrane protective activities of *C. procera* root-bark extract [33].

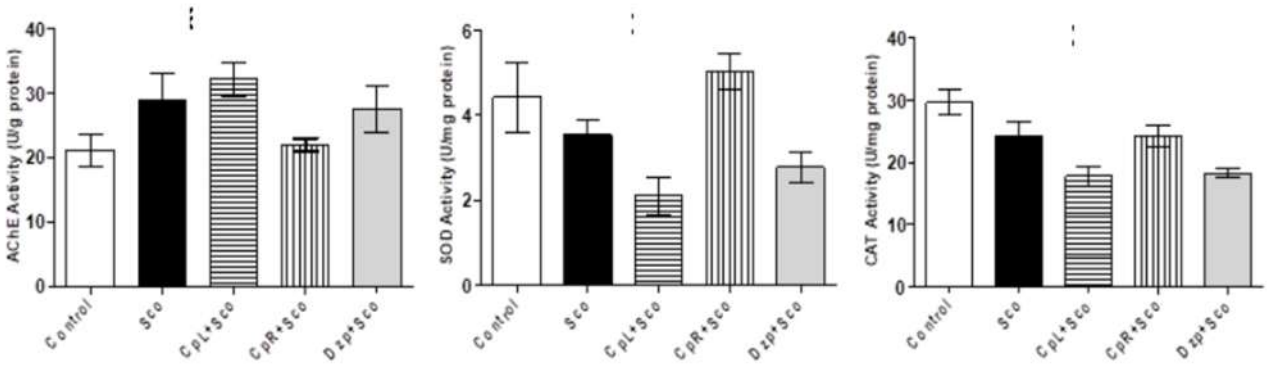

Figure 3. Effect of *C. procera* leaves and root-bark extracts on AChE activity [**a**], SOD activity [**b**] and CAT activity [**c**] in rat's brain.

Phytochemical compounds have the potential to modulate human metabolism. This potential has various beneficial effects including neurotransmitters modulation and antioxidant properties. These activities step in the prevention of chronic and degenerative diseases [33].

In the anxiolytic assay, results obtained suggest that *C. procera* root-bark extract inhibit the anxiety effect induced by scopolamine. Likewise, the root-bark extract shown anti-amnesic effect against memory impairment induced by scopolamine in rats. This anxiolytic and anti-amnesic potential is a good information and might to explain the wide use of this plant for neuropsychiatric diseases treatment in traditional medicine [9,34]. Indeed, some common manifestations of neuropsychiatric and degenerative disorders are anxiety, depression and amnesia [35,36], then all drug which protect against these symptoms contribute necessarily to improve the patient health.

In anti-oxidative tests, *C. procera* root-bark extract presented a profile which suggests that extract prevents scopolamine oxidative effect. Plant-derived antioxidants such as phenolic acids, flavonoids and terpenoids have the potential to delay or protection living organisms from the damage caused by uncontrolled production of free radicals, because of their redox properties [37,38]. *C. procera* was reported to possess these chemicals [39,40], which could support the obtained results.

Epilepsy is a serious neurological disorder which involves the abnormal discharges of electrical activity in the brain cells. The main symptoms of this disease include memory impairment and anxiety [5,41]. Likewise, it was reported that oxidative stress resulting from excessive free-radical generation is implicated in the initiation, development and progression of epilepsy [42].

Previous study recorded that in electroencephalographic evaluation of scopolamine-induced convulsions in fasted mice after food intake, scopolamine administration caused a series of high-voltage polyspikes and fast activity that represents a typical epileptiform manifestation [23]. The study of the relationship between amygdaloid brain-stimulation reward and the evolution of seizure activity showed that administration of scopolamine increase the intracranial self-stimulation rate. This result is consistent with the excitatory function of acetylcholine in epileptogenesis [21].

All results of the present study support a protective effect of *C. procera* root-bark extract against behavioral and oxidative disturbance induced by scopolamine in animals. This property could scientifically explain the *C. procera* use for epilepsy treatment in traditional medicine.

As a limitation of the present study, we can mention the lack of a clar validation/verification of the scopolamine-induced model of epilepsy, as this was done before by some previous research groups [20,21,22,23].

Conclusion

This study reports suggest that *C. procera* provide multiple benefits by reducing the negative health impact of a scopolamine-induced model of epilepsy in rodents, such as anxiety, memory loss and oxidative effect. The root-bark extract showed significant anxiolytic and anti-amnesic effect. While, further investigation is necessary to identify the active compounds responsible of these properties.

Conflicts of Interest

The authors declare no conflict of interest

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