

ANXIOLYTIC - LIKE PROPERTIES OF *Hallea ciliata* IN MICE

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Abstract

Background: The aim of the present study was to evaluate the anxiolytic properties of the decoction of stem bark of *Hallea ciliata* in mice. The decoction of *Hallea ciliata* is used in traditional medicine in Cameroon to treat diseases like anxiety disorders, fever, infantile convulsions and malaria.

Materials and Methods: Stress induced hyperthermia, elevated plus maze, open field and hole-board tests were used. Four different doses of the decoction were administered to mice and their effects were compared to the effects of diazepam and vehicle. Phytochemical characterization of the decoction revealed the presence of alkaloids, flavonoids, tannins and saponins.

Results: Administration of *Hallea ciliata* resulted in a significant decrease of stress induced hyperthermia in mice at the doses of 29.5, 59 and 118 mg/kg. In the elevated plus maze test, *Hallea ciliata* increased the number of entries and the percentages of entries and time into the open arms, and reduced the number of entries and the percentages of entries and time into the closed arms. In the hole-board test, *Hallea ciliata* increased the number of both head-dipping and crossing and decreased the latency to the first head-dips and rearing. The decoction of *Hallea ciliata* and diazepam increased locomotion in the open field test.

Conclusion: The number of rearing and the mass of fecal boli produced were decreased in mice treated with decoction and diazepam. In conclusion, the results indicated that decoction of *Hallea ciliata* has anxiolytic-like properties in mice and could potentially be used for anxiety treatment.

Key words: anxiety, herbs, pharmacology, diazepam.

Introduction

Anxiety and stress disorders are currently among the ten most important public health concerns, according to the World Health Organization and, have reached epidemic proportions (Thase, 2006). In Cameroon, anxiety disorders are among the top three mental diseases (Ministry of Health of Cameroon, 2006). These disorders are recognized as main risk factors for many other diseases, including cardiovascular, metabolic and neuropsychiatric diseases (Cryan and Holmes, 2005; Thase, 2006). Furthermore, anxiety is among the most prevalent mental disorder with very high comorbidity and severe impact on quality of life (Ngo Bum et al., 2011). In Africa, and particularly in Cameroon, patients mostly go to traditional healers because of the stigma of mental diseases and the side effects of anxiolytic drugs (Abdallah et al., 2014; Griffiths et al., 2014).

A growing number of herbal medicines are being introduced into psychiatric practice on the basis of their efficacy and low side effects for the treatment of psychiatric disorders, as severe depression, anxiety (Ji et al., 2006; Ngo Bum et al., 2009; Shaw et al., 2007; Taiwe et al., 2010). *Hallea ciliata* Aubrey & Pelegr. (Rubiaceae) (*H. ciliata*) is used in several conditions in African traditional medicine, particularly in the Northern Cameroon, to treat pain, infantile convulsions, anguish, inflammation, fever, arterial hypertension, diarrhea, diabetes, parasitic infection, mental impairment, and as local antiseptic. The decoction of this plant together with *piper guinense* and *Xylopi aetiopa* is effective for lung diseases, thus being nontoxic in human, (FAO, 1986). Previous studies showed that *H. ciliata* possess anti-inflammatory and analgesic properties (Dongmo et al., 2003); myorelaxant, antiplasmodial and antipyretic properties (Mibindzou, 2004); anti-trypanosomiase activity (Ogbunugofor et al., 2007) and is a good immunostimulator (Fofana, 2005). However, very little information is available on the effects of *H. ciliata* on the central nervous system. The present study was undertaken to verify the hypothesis that *H. ciliata* could exert anxiolytic properties because of its used in traditional medicine in Cameroon to treat anxieties disorders.

Material and Methods

Animals and Treatment

Adult male and female Swiss mice (*Mus musculus*) weighting 20-24 g were used. The animals were bred in the colony established at the department of Biological Sciences of the University of Ngaoundere (Cameroon). They were housed 5 or 6 per cage and were familiarized with the experimenters and the laboratory environment for at least 4 days before the experiments. Food and

water were available *ad libitum*.

The animal room was maintained at 25 ± 1 °C with a 12/12h light/dark cycle (light on at 6.00 a.m). All experiments were carried out in a quiet room under controlled light conditions between 10:00 a.m. and 3:00 p.m. Mice were divided randomly in 6 groups: one negative control group received distilled water (vehicle), one positive control group received a well-known anxiolytic drug (diazepam or phenobarbital) depending on the test, and four test groups received different doses of the decoction of *H. ciliata*.

All experiments were performed in accordance with the National Ethical Committee (N°.FWA-IRB00001954) and the internationally accepted Principles for Laboratory Animal Use and Care presented in the US guidelines. All efforts were done to minimize suffering and number of animals used.

Diazepam ampoules (HOFFMANN LA ROCHE, BASEL, SWITZERLAND) and phenobarbital (SIGMA-ALDRICH CO., ST. LOUIS, MO, USA) were used as reference drugs. All solutions were prepared freshly in distilled water on the test days and administered intraperitoneally (i. p) in a volume of 10 mL/kg body weight.

Diazepam, phenobarbital and the different doses of the decoction of *H. ciliata* were administered orally (p.o) once, 1 hour prior to the beginning of all behavioral tests.

Preparation of the Decoction

Stem bark of *H. ciliata* were collected in Dang, locality of Ngaoundere (Adamaoua, Cameroon). Voucher specimen of *H. ciliata* was deposited at the National Herbarium of Cameroon in Yaounde under the number 2101HNC. For the preparation of the decoction, 5 g of *H. ciliata* stem bark were macerated in 50 mL of distilled water and boiled to 100 °C for 20 minutes. After cooling, the mixture was filtered with wathman paper n° 1 and used for animal experiments. In order to quantify the dried extract of the decoction, the obtained decoction was dried and the residue was weighed. The doses of the decoction were expressed in mg of dried extract per kg of body weight. The decoction was prepared each day of the experiment. The following doses were used: 11.8, 29.5, 59 and 118 mg/kg.

Phytochemical Screening

Preliminary qualitative chemical screening of the decoction of the stem bark of *H. ciliata* was done using the methods described for the determination of flavonoids, alkaloids, saponins, tannins, anthraquinones and polyphenols (Harbone, 1976).

Behavioral Tests

Behavioral recording was done blindly.

Stress Induced Hyperthermia (SIH) Test

Experiments were performed according to the methodology described by Borsini et al., (1989) with small modifications (Ngo Bum et al., 2009). The animals (10 per group) were weighed and marked on the tail. Phenobarbital (20 mg/kg, i.p) was given to positive control group, the four doses of the plant were given to the four test groups and distilled water as vehicle was given to the negative control group. 1 hour after each treatment, the rectal temperature was recorded by introducing the rectal borer of thermometer (2 mm of diameter and 2 cm of length) in the rectum of the mice. Rectal borer was put into saline solution (NaCl 9%) before each temperature measurement. SIH was determined as the difference between the mean temperature of three last mice and the mean temperature of the three first mice in each group.

Elevated Plus Maze (EPM) Test

This test is based on the natural empty space fear of rodents (Ollat and Pirot, 2003). The maze made of wooden consisted of two open arm (15 cm x 45 cm) and two closed arms (15 cm x 45 cm x 20 cm), extending from a central platform (5 cm x 5 cm) and elevated to a height of 50 cm above the floor (Ngo Bum et al., 2009). Here, diazepam (3 mg/kg, i.p.) was given as positive control. One hour after treatment, each animal was placed individually in the center of the maze facing an open arm, away from the observer. The behaviors of the animals were recorded for 5 minutes. The number of entries and the time spent into the open and closed arms were registered. An entry was defined as entry of head and at least the half of the body of animal into an arm. The percentage of time spent into open or closed arms (open or closed arm time/5 min x 100) and percentage of entries (open or closed arm entries/total entries x 100) were calculated for each animal. The number of rearing, head-dipping and grooming were recorded (Augustsson, 2004). The maze was cleaned with ethylic alcohol 70° after each mouse test.

Hole-Board (HB) Test

The hole-board apparatus consisted of a wooden box (40 cm x 40 cm, 1.8 cm height). The floor was divided in 16

equidistant holes of 3 cm in diameter (Lourenzo et al., 2001). The positive control group received diazepam (0.5 mg/kg, i.p.) and four tests groups receive different doses of *H. ciliata* decoction (11.8, 29.5, 59 and 118 mg/kg, p.o.). One hour after treatment each animal was placed individually in the center of the board facing away the observer. And the number of rearing (when the animal stand on its back paws, raises its forepaws off the ground, extending its body vertically), head-dipping (when the animal places its head into one of the holes), crossing (when the animal enter a new area with all four paw) were recorded for 5 min. The latency to the first head-dipping was measured using a stopwatch (Taiwe et al., 2010). The floor of the apparatus was cleaned with 70 % alcohol solution between each trial.

Open Field (OF) Test

This method is used to evaluate locomotor activity, level of exploration and emotional reaction of animals (Crawley, 1985). Open field consisted of a surrounding square (40 cm x 40 cm) divided in 16 small square and 1 center field (10 cm x 10 cm) with a wall of 19 cm high (Brown et al., 2007; Jenck et al., 1997). The positive control group received diazepam (0.3 mg/kg, i.p.). One hour after treatment, each mouse was placed individually in the center of the arena and the number of crossing, grooming, rearing, the time spent in the center and fecal boli weight were recorded for 5 min. Recording were measured manually with a stopwatch.

Data Analysis

Comparisons of the results were performed using computerized linear regression analysis, of GraphPad Prism (version 4.00, GraphPad Software Inc., San Diego, CA, USA). The statistical analysis of the data was performed in each graph by one-way analysis of variance (ANOVA) followed by Dunnet’s bilateral or multiple comparison test. In all cases differences were considered significant at $p \leq 0.05$.

Results

Phytochemical Screening

Chemical screening of the decoction of stem bark of *H. ciliata* showed the presence of polyphenols (flavonoids, tannins, phlobotannins), alkaloids, saponins, and anthraquinons.

Effect of *H. Ciliata* on Stress Induced Hyperthermia Test

The decoction of *H. ciliata* reduced dose dependently the SIH from 2.27 °C in the control group to -0.4° C at the dose of 118 mg/kg [F (6, 60) = 0.45; P<0.01]. HIS was also reduced by phenobarbital (Fig 1). In the contrary, *H. ciliata* had no effect on the body temperature of mice (Fig 2).

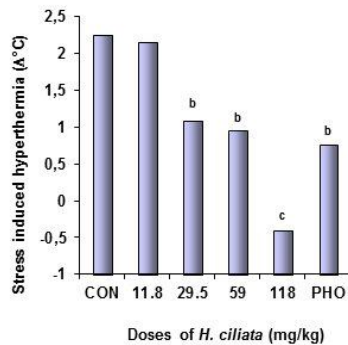


Figure 1: Effect of the decoction of *H. ciliata* on the stress induced hyperthermia

Data are Δ°C (the difference between the mean temperature of the three last mice and the mean temperature of the three first mice). N = 6, $p \leq 0.01$, $p \leq 0.001$; one-way analysis of variance (ANOVA) followed by Dunnet’s bilateral comparison test. CON = distilled water (vehicle), PHO = phenobarbital 20 mg/kg. All other groups were compared to CON, the negative control group

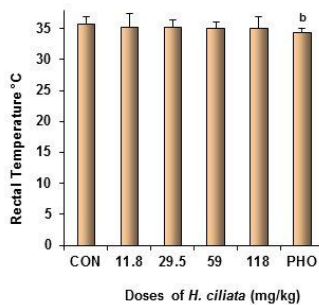


Figure 2: Effect of the decoction of *H. ciliata* on the body temperature on stress induced hyperthermia in mice.

Data are temperature (°C). N = 10, $p \leq 0.01$; one-way analysis of variance (ANOVA) followed by Dunnet’s bilateral comparison test. CON = distilled water (vehicle), PHO = phenobarbital 20 mg/kg. All other groups were compared to CON, the negative control

group.

Effect of *H. Ciliata* on Elevated plus Maze

The administration of the decoction resulted in a significant increase in the number of entries into open arms from 1.67 in the negative control group to 9.67 at the dose of 118 mg/ kg of *H. ciliata* [F (6, 35) = 9.13; p < 0.001] (Table 1). The percentage of open entries was therefore highly significantly increased from 24.54% in the control group to 76.82% at the dose of 118 mg/ kg of *H. ciliata* [F (6, 35) = 19.82; p < 0.001]. The percentage of time spent in the open arms increased also from 7.17% in the control group to 58.00% [F (6, 35) = 101.46; p < 0.001] at the dose of 118 mg/kg of *H. ciliata*. As expected, in the positive control group 3 which received diazepam (3 mg/kg, i.p.), the percentage of entries and time spent into the open arms increased (Fig 3). The time spent in the closed arms was as well reduced by both the decoction and diazepam. Diazepam and the decoction (dose 118 mg/kg) also induced a significant reduction of percentage of entries in the closed arms, from 73.94% in the vehicle-treated group to 23.18% and 16.59% [F (6, 35) = 19.78; p < 0.001], respectively (Fig 4). The number of rearing [F (6, 35) = 8.55; p < 0.001], head-dipping [F (6, 35) = 16.85; p < 0.0001] and stretched attend posture [F (6, 35) = 3.93; p < 0.008] were also reduced by diazepam and the decoction while the number of grooming increased (Table 1).

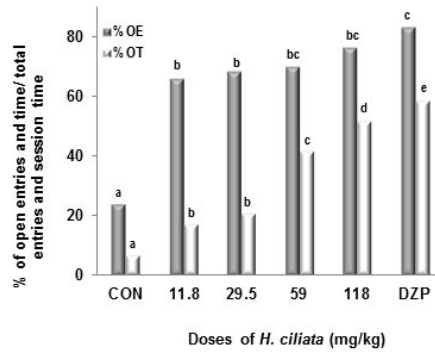


Figure 3: Effect of the decoction of *H. ciliata* on EPM in mice.

Data are the percentage of open arms entries and the percentage of open arms time. N = 6; one-way analysis of variance (ANOVA) followed by Dunnett’s multiple comparison test. %OE = Percentage of open arm entries, %OT = Percentage of time spent in open arm, CON = distilled water (vehicle), DZP = diazepam 3 mg/kg.

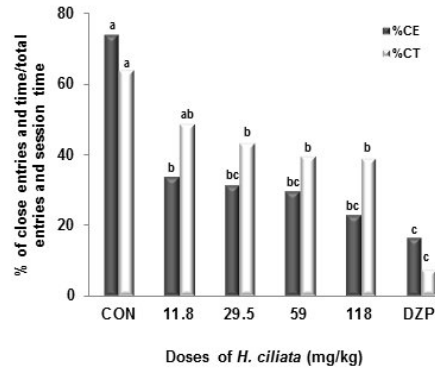


Figure 4: Effect of the decoction of *H. ciliata* on EPM in mice

Data are the percentage of closed arms entries and the percentage of closed arms time. N = 6; one-way analysis of variance (ANOVA) followed by Dunnett’s multiple comparison test. %CO = Percentage of closed arms entries, %CT: Percentage of time spent in closed arms, CON =distilled water (vehicle), DZP = diazepam 3 mg/kg.

Table 1: The number of open arms entries, closed arms entries, rearing, head- dipping and grooming on Elevated Plus Maze

Doses of <i>H. ciliate</i> (mg/kg)	Distilled water	11.8	29.5	59	118	Diazepam
Open arms entries	1.67 ± 0.44	7.83 ± 0.89 ^c	8.00 ± 0.67 ^c	9.00 ± 2.67 ^c	9.67 ± 2.67 ^c	10.00 ± 1.33 ^c
Closed arms entries	6.00 ± 2.67	4.00 ± 0.67	3.83 ± 1.5 ^a	3.16 ± 0.55 ^b	2.67 ± 0.67 ^b	2.00 ± 0.33 ^c
Total arms entries	7.67 ± 2.83	11.83 ± 1.16 ^a	11.83 ± 1.67 ^a	12.17 ± 2.83 ^a	12.33 ± 2.33 ^a	12.00 ± 1.33 ^a
Rearing	11.67 ± 2.09	6.50 ± 1.28 ^c	5.50 ± 1.50 ^c	6.83 ± 1.89 ^c	5.50 ± 2.17 ^c	5.83 ± 0.89 ^c
Head- dipping	12.17 ± 1.22	5.33 ± 1.17 ^c	4.83 ± 1.83 ^c	4.50 ± 1.00 ^c	3.17 ± 0.56 ^c	3.67 ± 1.00 ^c
Grooming	1.67 ± 0.27	2.00 ± 0.67	2.16 ± 0.61	1.67 ± 0.89	3.00 ± 1.00 ^a	4.17 ± 1.60 ^c
SAP	8.17 ± 4.22	8.16 ± 2.14	5.00 ± 2.67	4.67 ± 2.44	3.00 ± 3.00 ^a	0.83 ± 0.56 ^b

Data are mean ± S.E.M., n = 6, ^ap < 0.05, ^bp < 0.01, ^cp < 0.001, ANOVA followed by Dunnett (bilateral) comparison test.

Open Field

As in the elevated plus maze, the number of rearing was decreased both by diazepam and the decoction from 24.4 for the vehicle-treated group to 12.0 at the dose 118 mg/kg of *H. ciliata* and to 4.4 for diazepam [F(5,29) = 9.82; p < 0.001] (Table 2). The decoction and diazepam also decreased fecal boli [F (5, 29) = 9.81; p < 0.001]. Contrarily, the decoction increased the number of crossing and center time from 20.8 and 3.4 in the vehicle-treated group to 72.4, and 16.6 at the dose of 118 mg/kg of *H. ciliata*, [F(5,29) = 12.00; p < 0.001] and [F (5,29) = 39.023; p < 0.001], respectively. Increase was also noted in grooming (Table 2).

Table 2: Number of crossing, rearing, grooming, center time and fecal boli on Open Field

Doses of <i>H. ciliata</i> (mg/kg)	Distilled water	11.8	29.5	59	118	Diazepam
Crossing	20.80 ± 2.24	58.40 ± 18.24 ^b	80.40 ± 4.88 ^c	84.20 ± 6.24 ^c	72.40 ± 10.32 ^c	78.40 ± 9.68 ^c
Rearing	24.40 ± 7.92	13.60 ± 4.88 ^b	13.00 ± 4.00 ^c	15.60 ± 5.12 ^b	12.00 ± 4.40 ^c	4.40 ± 1.92 ^c
Grooming	0.60 ± 0.48	2.00 ± 0.80	2.60 ± 1.52	2.60 ± 1.12	3.00 ± 1.20 ^a	3.60 ± 0.88 ^b
Center time (s)	3.40 ± 1.84	6.60 ± 5.52	10.40 ± 3.12	14.00 ± 10.00 ^a	16.60 ± 3.52 ^a	82.00 ± 10.00 ^c
Fecal boli (g)	0.27 ± 0.22	0.001 ± 0.002 ^c	0.001 ± 0.001 ^c	0.018 ± 0.003 ^c	0.00 ± 0.00 ^c	0.00 ± 0.00 ^c

Data are mean ± S.E.M., n = 6, ^ap < 0.05, ^bp < 0.01, ^cp < 0.001, ANOVA followed by Dunnett (bilateral) comparison test.

Hole-Board

The number of rearing was significantly reduced by *H. ciliata*, from 7.8 in the vehicle treated-group to 3.0 and 2.8 at the doses 29.5 and 59 mg/kg of the decoction, respectively. The latency time to the first head-dipping was also significantly reduced by *H. ciliata*, from 8.6 in the vehicle-treated group [(F (5, 29) = 8.99; p < 0.001] to 4.0 (dose of 118 mg/kg of the plant) and to 2.0 (diazepam). In the contrary, the number of head-dipping increased from 13.2 in the vehicle-treated group to 40.4 at the dose of 118 mg/kg of the decoction and to 33.6 for the diazepam-treated group [F (5, 29) = 4.52; p < 0.005]. The number of grooming was also increased by the decoction [F (5, 29) = 0.36; p < 0.90] and diazepam (Table 3).

Table 3: Number of crossing, rearing, grooming, head-dipping and first head - dipping time on Hole Board

Doses of <i>H. ciliata</i> (mg/kg)	Distilled water	11.8	29.5	59	118	Diazepam
Crossing	28.00 ± 6.40	28.00 ± 6.40	42.80 ± 9.36	38.20 ± 7.28	19.00 ± 6.40	31.80 ± 15.78
Rearing	7.80 ± 2.64	3.00 ± 0.80 ^a	3.00 ± 2.40 ^a	2.80 ± 0.72 ^a	3.20 ± 1.76 ^a	5.80 ± 2.56
Grooming	0.60 ± 0.48	2.00 ± 0.80	2.60 ± 1.52	2.60 ± 1.12	3.00 ± 1.20 ^a	3.60 ± 0.88 ^b
Head-dipping	13.20 ± 3.76	17.80 ± 4.56	20.00 ± 7.20	26.80 ± 3.84	40.40 ± 16.32 ^b	33.60 ± 9.92 ^a
Latency to first head-dipping (s)	8.60 ± 2.07	8.00 ± 1.00	7.20 ± 1.48	5.00 ± 1.87 ^a	4.00 ± 3.16 ^b	2.00 ± 1.00 ^c

Data are mean ± S.E.M., n = 6, ^ap < 0.05, ^bp < 0.01, ^cp < 0.001, ANOVA followed by Dunnett (bilateral) comparison test.

Discussion

The anxiolytic properties of the decoction of *H. ciliata* were investigated for the first time using behavioral testing in mice. The present results showed that the decoction of the stem bark of *H. ciliata* reduced hyperthermia induced by stress. This decrease similar to that induced by phenobarbital, suggested anxiolytic properties of the plant, as hyperthermia induced by stress is reversed by anxiolytics (Berend and al., 2003; Borsini et al., 2002; Lecci et al., 1990; Ngo Bum et al., 2009). Anxiolytic properties of the decoction were confirmed in the elevated plus maze, the open field and hole board tests. The experiment on the elevated plus maze clearly showed that vehicle-treated mice had a strong preference for the closed arms compared to those treated with the decoction of *H. ciliata*. The number of entries and the time spent into open arms and their percentage; the main behavioral parameters of the evaluation of the anxiolytic effect on this maze (Fakeye et al., 2008; Grundmann et al., 2007; Pollyanna et al., 2007; Rabbani et al., 2008; Reginatto et al., 2006) were increased by the decoction of *H. ciliata*. Significant decrease in the number of "rearing" associated both to the decrease of closed arms entries and the increase of open arms entries revealed that the increase in open arms entries was not due to the decoction of *H. ciliata* induced locomotion but rather to the exploration confirming the decrease of anxiety in mice treated with the decoction (Ngo Bum et al., 2009; 2011). In addition, the plant induced a significant increase in the number of grooming in mice treated with the highest dose of *H. ciliata* (118 mg/kg) and diazepam. Grooming is a behavior that reflects the removal of stress in animals (Augustsson, 2004).

In open field test, *H. ciliata* and diazepam increased the number of "crossing". The increase in the number of crossing (exploratory activity) is a sign of intrinsic inhibition of anxiety induction and not an increase in locomotion since rearing which is a locomotion indicator in this test was reduced (Ngo Bum et al., 2009; Pitchaiah et al., 2008; Pollyanna and al., 2007). Reduction of defecation in mice treated with diazepam and the extract of *H. ciliata* also suggested an anxiolytic activity (Shaw et al., 2007). In hole-board test, the decoction of *H. ciliata* increased the number of crossing and head-dipping (exploratory activity) and decreased its latency of onset. Since increasing the number of crossing and head-dipping in this test is a sign of anxiolysis (Crawley, 1985; File and Wardill., 1975; Li Min et al., 2005; Lourenzo et al., 2001; Tsuji et al., 1998), these results also indicated the anxiolytic properties of *H. ciliata*. *H. ciliata* could have exerted anxiolytic properties through its flavonoids, alkaloids and tannins contain. For instance flavonoids selectively

bind with high affinity to benzodiazepine receptors and induce a significant anxiolytic activity (Grundmann et al., 2007). As anxiolytic activities could be mediated by different mechanism of action, we hypothesized that the anxiolytic properties of the decoction *H. ciliata* could be due to the interaction of its contained compounds with receptors in the central nervous system such as benzodiazepine and GABA sites of GABA-A receptor complex as agonists (EPM test is very sensitive to benzodiazepine receptor agonists), 5-HT1 receptor as agonists (Belzung, 1999; Garcia-Garcia et al., 2014; Lolli et al., 2007; Rodgers et al., 1997) or 5-HT-2 and 5-HT-3 receptors as antagonists (Brown et al., 2007; Ferrero et al., 1999; Rodgers, 2001).

Conclusion

The decoction of *H. ciliata* showed anxiolytic like-effects that are used in traditional medicine in Cameroon to treat anxieties disorders.

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