

Ethanol Extract of *Chrysophyllum albidum* Stem Bark Elicits Neurobehavioural Alterations via Oxidative Stress, Histological Changes, and Cholinergic Disruption in Wistar Rats

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Ethanol Extract of Chrysophyllum albidum Stem Bark Elicits Neurobehavioural Alterations via Oxidative Stress, Histological Changes, and Cholinergic Disruption in Wistar Rats

Chrysophyllum albidum Gövde Kabuğunun Etanol Ekstresi, Wistar Sıçanlarında Oksidatif Stres, Histolojik Değişiklikler ve Kolinerjik Bozulma Yoluyla Nörodavranışsal Değişikliklere Yol Açıyor

SUMMARY

Chrysophyllum albidum stem bark reportedly possesses antioxidant activity and ameliorates some markers of diabetes complications. However, limited data exist on its safety. Thus, this study reports the neurobehavioral effects of ethanol extract of *Chrysophyllum albidum* stem bark (ECASB) in male Wistar rats. A total of twenty Wistar rats were grouped into four ($n = 5$) and were administered ECASB at doses of 55.56, 166.67, 500 mg/kg, or distilled water (10 mL/kg) for 14 days. From the 11th day of treatment, the animals were subjected to neurobehavioral evaluations. On the 15th day, the animals were euthanized and brain tissues were harvested for Acetylcholinesterase activity, reduced glutathione, malondialdehyde level and histological analysis. At 500 mg/kg ECASB, a significant decrease in animals' body weight was recorded. In the Morris water maze test, learning improved during training, although retention was slightly impaired in experimental rats. In addition, reduced locomotor activity was observed in rats administered 166.67 and 500 mg/kg doses of ECASB. Interestingly, anxiety-like behaviour was reduced, as depicted by increased time spent in the light compartment of the light/dark box test in the two higher doses. Additionally, depressive-like behaviours were lessened. Administration of the extracts induces oxidative stress and increases acetylcholinesterase activity, especially at 500 mg/kg. The histological analysis showed a decrease in viable Purkinje neurons, especially in the animals treated with 500 mg/kg ECASB. The ethanol extract of *C. albidum* stem bark appears to offer neurobehavioral and cognitive benefits at lower doses, while higher doses could pose neurotoxic risks.

Keywords: *Chrysophyllum albidum*, neurotoxicity, behavioural activities, oxidative stress, toxicity.

ÖZ

Chrysophyllum albidum gövde kabuğunun antioksidan aktiviteye sahip olduğu ve diyabet komplikasyonlarına ilişkin bazı belirteçleri iyileştirebildiği bildirilmekle birlikte, güvenliliğine dair veriler sınırlıdır. Bu çalışmada, *Chrysophyllum albidum* gövde kabuğu etanol ekstresinin (ECASB) erkek Wistar sıçanlardaki nörodavranışsal etkileri değerlendirilmiştir. Toplam 20 sıçan, her grupta beş hayvan olacak şekilde dört gruba ayrılmış ve 14 gün boyunca 55,56, 166,67 veya 500 mg/kg ECASB ya da distile su (10 mL/kg) uygulanmıştır. Tedavinin 11. gününden itibaren nörodavranışsal testler gerçekleştirilmiş; 15. günde hayvanlar ötanazi edilerek beyin dokularında asetilkolinesteraz aktivitesi, indirgenmiş glutatyon ve malondialdehit düzeyleri ile histopatolojik değişiklikler incelenmiştir. ECASB'nin 500 mg/kg dozunda vücut ağırlığında anlamlı azalma gözlenmiştir. Morris su labirenti testinde öğrenme performansı eğitim süresince iyileşirken, deney gruplarında bellek kalıcılığının hafif düzeyde bozulduğu belirlenmiştir. Buna ek olarak, 166,67 ve 500 mg/kg dozlarında ECASB uygulanan sıçanlarda lokomotor aktivitenin azaldığı gözlenmiştir. İlginç olarak, bu iki yüksek doz grubunda aydınlık/karanlık kutu testinin aydınlık bölümünde geçirilen sürenin artması, anksiyete benzeri davranışların azaldığını göstermiştir. Ayrıca depresyon benzeri davranışlarda da azalma saptanmıştır. Bununla birlikte, özellikle 500 mg/kg dozunda oksidatif stresin ve asetilkolinesteraz aktivitesinin arttığı, histolojik incelemede ise canlı Purkinje hücrelerinin sayısının azaldığı belirlenmiştir. Sonuç olarak, *Chrysophyllum albidum* gövde kabuğu etanol ekstresi düşük dozlarda bazı nörodavranışsal ve bilişsel yararlar sağlayabilse de yüksek dozlarda oksidatif stres, kolinerjik sistem değişiklikleri ve Purkinje hücre kaybıyla ilişkili nörotoksik etkilere yol açabilir.

Anahtar Kelimeler: *Chrysophyllum albidum*, nörotoksisite, davranışsal aktiviteler, oksidatif stres, toksisite.

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INTRODUCTION

Chrysophyllum albidum (African star apple) is a medicinal plant with edible fruits that is widely distributed across the vegetation zones of Uganda, Nigeria, Niger Republic, and Cameroon (Olowoyeye et al., 2019). The fruit pulp, root, leaves, bark, and seeds have reportedly been used for various medicinal purposes. The bark is used to treat fibroid and malaria (Ihekwereme, Okoye, Agu, & Oli, 2017), the leaf is used to prepare an emollient used in the treatment of stomach-ache, diarrhoea and skin eruptions (Akinpelu et al., 2016), and the seed cotyledons are used in the treatment of skin infections in Western Nigeria and Cameroon (Olowoyeye et al., 2019). The stem bark of *C. albidum* has shown acetylcholinesterase inhibition in an *in vitro* study reported by Ezema et al. (2021). Also, studies have reported the presence of procyanidin, stigmasterol, epigallocatechin, and epicatechin in the stem bark (Idowu et al., 2016), which are known to have neuromodulatory effects (Chen et al., 2021; Li, Wang, Liu, & Chen, 2024). Likewise, phytochemical study of *C. albidum* stem bark shows the presence of secondary metabolites like flavonoids, saponins, and tannins with known neuromodulatory properties (Idowu et al., 2016). However, while the other parts of *C. albidum* have been proven to possess neuroprotective effects (Ibukun & Omoregie, 2022; Kumar et al., 2021), the neuroprotective and neurotoxic potential of the stem bark remains under-explored. This study thus seeks to explore the potential neurobehavioral effects of *C. albidum* stem bark in Wistar rats.

MATERIALS AND METHODS

Sample collection and identification

Chrysophyllum albidum stem bark sample was collected from Aroro Beri village, Igbo Oloyin, Ibadan, Oyo State. Identification and authentication (FIH number: 112937) were done at the herbarium of the Forest Research Institute of Nigeria (FRIN), Jericho, Ibadan, Oyo State.

Preparation of ethanol extract of *Chrysophyllum albidum* stem bark

The collected stem bark of *C. albidum* was air-dried at room temperature and coarsely ground. The grounded stem bark (500 g) was then macerated in 2 L of 70% ethanol (plant: solvent ratio 1:4 w/v) for 72 hours with intermittent shaking. The maceration was repeated three times for maximum yield. A rotary evaporator was used to concentrate the filtrate at 40°C under reduced pressure, after which the yield was stored at -40 °C.

Experimental animals

Healthy male Wistar rats weighing 120 - 150 g used in this experiment were purchased from the breeding unit of the animal house at the University of Ibadan. The animals were housed in standard cages, fed rat cubes, and given water *ad libitum*. The experimental animals were allowed to acclimatise to standard laboratory conditions for one week in the Department of Pharmacology and Toxicology's animal holding facility at the Faculty of Pharmaceutical Sciences, University of Ibadan, before the commencement of the study. Ethical approval for the care and use of animals was obtained from the University of Ibadan Animal Care and Use Research Ethics Committee (UI-ACUREC) with approval number UI-ACUREC/003-0124/09. The procedures for the use and care of animals were in accordance with the National Institutes of Health (NIH) guidelines for the care and use of laboratory animals (National Research Council (US) Committee, 2011).

Experimental design

Before the main study, a preliminary study was conducted in mice to determine the acute toxicity of the ECASB at doses of 5000, 2000, and 1000 mg/kg, following the Organization for Economic Co-operation and Development (OECD) 423 guideline (2022). The highest dose, 5000 mg/kg, gave a 33.33% death on day 3 post-treatment, while no death or overt sign of mortality was recorded in 2000 and 1000 mg/kg doses. Thus, the LD₅₀ is greater than

5000 mg/kg. Based on this submission, high, middle, and low doses of 55.56, 166.67, and 500 mg/kg were selected, enabling the evaluation of the effects of sub-acute exposure to ECASB at doses safely below the lethal range.

A sub-acute toxicity study was carried out according to the method described in OECD 407 (2025). Twenty healthy male Wistar rats were randomly assigned to four groups ($n = 5$). The animals in the control group received distilled water, and those in the other groups received graded oral doses of ECASB extract (55.56, 166.67, or 500 mg/kg) daily. Fresh extracts were prepared daily by dissolving in distilled water. Water and feed intake were measured on a daily basis. From the 11th day of treatment, animals were subjected to neurobehavioral evaluations.

Neurobehavioral assessments

All behavioural studies were carried out under standardized conditions and at the same time each day. Also, the apparatus used for the experiment was cleaned with 70% ethanol between animals to remove olfactory cues.

Test for cognitive function using Morris Water Maze (MWM)

Partial memory in the rodents, assessed by their ability to navigate the water-filled area to locate the elevated escape platform, was assessed using the Morris Water Maze (Vorhees & Williams, 2006). The protocol followed was as described by Ezurike et al. (2024).

Test for spontaneous locomotor activity (Open Field Test)

The test was performed using the method reported by Ezurike et al. (2023). Briefly, the animals are placed in the centre of the open field box (one at a time) and left there for 5 minutes to explore the box ($50 \times 50 \times 40$ cm). An overhead camera captured this whole process. The videos were analysed using ANY-maze (Stoelting Co., Wood Dale, IL) video tracking software, and data, including the total distance travelled within the box, the number of lines crossed, as well as the rearing and grooming frequency, were generated.

Test for anxiety-like behaviour (Light and Dark Box (LDB) Test)

Unconditioned anxiety response in rodents is popularly assayed using the light and dark box test (LDB; Michel & Martine, 2003). The animals were individually placed in LDB (light compartment: $27 \times 18 \times 27$ cm; dark compartment: $18 \times 18 \times 27$ cm), and the overhead video camera was positioned above the LDB to capture a clear view of the entire box. Each animal was placed at the centre of the light compartment, facing the compartment wall. The duration for the test for each animal was 5 minutes, and after each test, the box was wiped clean and dry with 70% ethanol and allowed to dry before the next animal was placed in the box.

Depression-like behaviour test (Sucrose Splash Test)

The sucrose splash test, as described by da Costa et al. (2023), was used to assess depression-like behaviour in the rats. Briefly, each rat was sprayed on their dorsal coat with 10% sucrose solution. After spraying the animal, the frequency of grooming and time spent grooming were recorded for 5 minutes.

Biochemical and histological assessment

After the whole experiment, the animals were anesthetized using ketamine/diazepam and euthanized. Brain samples were collected, weighed, and processed for biochemical assays. Some of the animals were perfused with 10% formalin in normal saline, and their brains were harvested and fixed in 10% formalin for histological assessment. The brain tissues for biochemical assays were placed on ice and homogenized in 0.1 M phosphate buffer, pH 7.4 (1:10 w/v). Centrifugation of the brain homogenates was carried out at 10,000 rpm for 15 minutes at a temperature of 4 °C. The supernatant was thereafter separated into different portions in freshly chilled plain bottles and stored at -80 °C for various assays. Reduced glutathi-

one (GSH) was quantified in the tissue homogenates as described by Jollow, Mitchell, Zampaglione, & Gillette (1974), while the level of lipid peroxidation was quantified by measuring the malondialdehyde (MDA) level (Varshney & Kale, 1990). Ellman's method was used to quantify acetylcholinesterase activity in brain tissue (Ellman, Courtney, Andres, & Featherstone, 1961). As described by Romanucci et al. (2018), the brain samples harvested for histological assessment were dehydrated in ethanol and clarified in xylene. Thereafter, they were processed by the routine method (hematoxylin and eosin staining). Photo micrographs (x100 and x400) of the hippocampus and cerebellum region of the brain samples were taken using a digital camera and Stream Basic imaging software attached to a binocular microscope (Olympus CH, Japan).

Statistical analysis

Values were presented as mean $\pm$ SEM. One-way analysis of variance (ANOVA) was used for group comparison followed by post-hoc test (Dunnet's) for multiple comparisons. The significance level was set at $p < 0.05$ for all statistical tests. Data were analysed using GraphPad Prism 8.0.4 (GraphPad Software Incorporated, San Diego, CA).

RESULTS

Extract yield (%) of *Chrysophyllum albidum*

The percentage yield of ethanol extract of *Chrysophyllum albidum* stem bark (ECASB) was 16.7%.

Effects of ECASB on some physiological parameters

Effect of ECASB on mortality rate

Table 1 shows the effect of ECASB on the mortality rate in male Wistar rats. At the end of the 14 days sub-acute toxicity study, no mortality was observed following repeated oral administration of ECASB at the selected doses.

Effect of ECASB on the body weight of male Wistar rats

A significant decrease in the body weight of the animals that received the highest dose, 500 mg/kg (24.14 ± 0.51 g), relative to the control animals (27.83 ± 1.21 g) was observed (Figure 1A.).

Effects of ECASB on relative brain weight, feed, and water intake

Oral administration of ECASB for 14 days reduced the average daily feed intake in all the treatment groups (12.89 ± 1.82 - 14.99 ± 0.89 g) compared to the animals in the control group (16.79 ± 1.18 g), as shown in Figure 1B. A significant decrease in water intake of animals administered 166.67 and 500 mg/kg (23.09 ± 4.04 and 22.15 ± 3.85 mL, respectively) was recorded relative to the animals in the control group (35.10 ± 3.19 mL; Figure 1C.).

Additionally, the relative brain weight of the experimental animals treated with ECASB and control animals was similar (Figure 1D.).

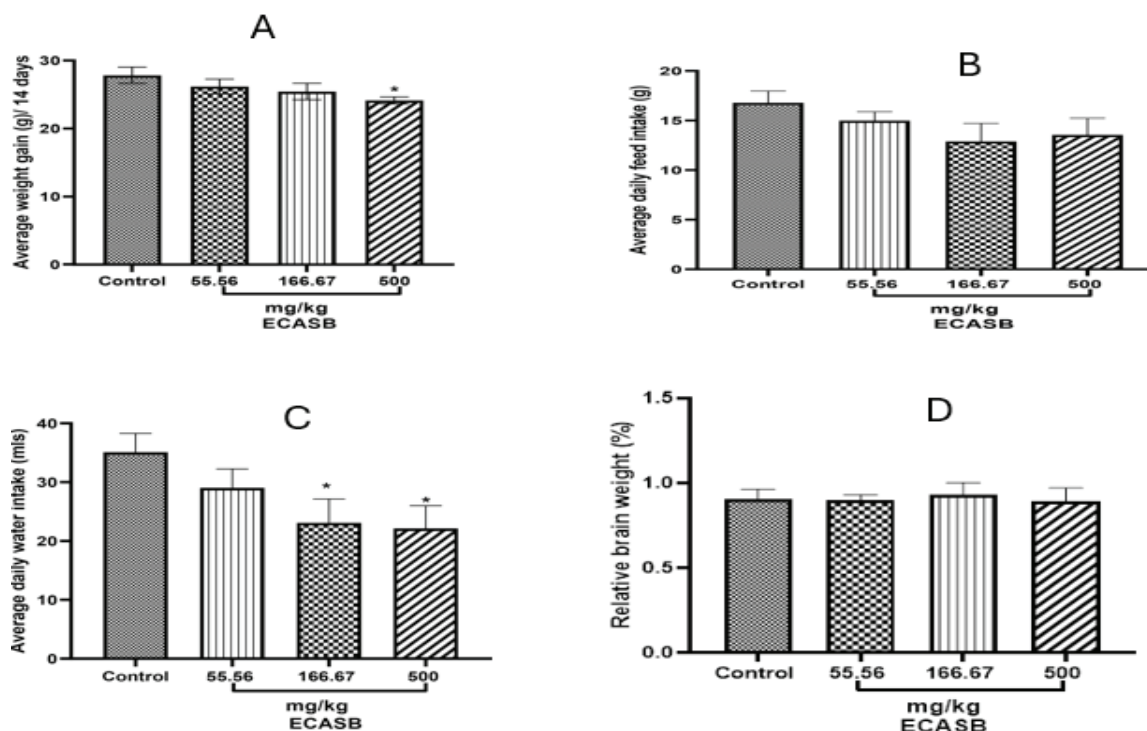


Figure 1. Effect of ECASB on physiological parameters

The values are expressed as mean $\pm$ SEM (One-way ANOVA followed by Dunnett's Post hoc test) $n=5$; * $p < 0.05$, vs control

Effects of ethanol extract of *Chrysophyllum albidum* on cognitive behaviour (learning and memory) in male Wistar rats

The escape latency is the time taken by the animal to locate the submerged platform and escape from the pool. On the 11th, 12th, and 13th days of treatment, the first, second, and third training sessions for the Morris Water Maze task were performed. There was a sig-

nificant decrease in the escape latency in all groups, with 166.67 mg/kg producing the most significant effect (Figure 2A.). Memory retention was evaluated on the 14th day of the treatment without the platform in the pool. The time spent in the platform zone in ECASB-treated rats at 55.56 mg/kg and 500 mg/kg was reduced, whereas it was similar to that of the control in rats treated with 166.67 mg/kg ECASB (Figure 2B.).

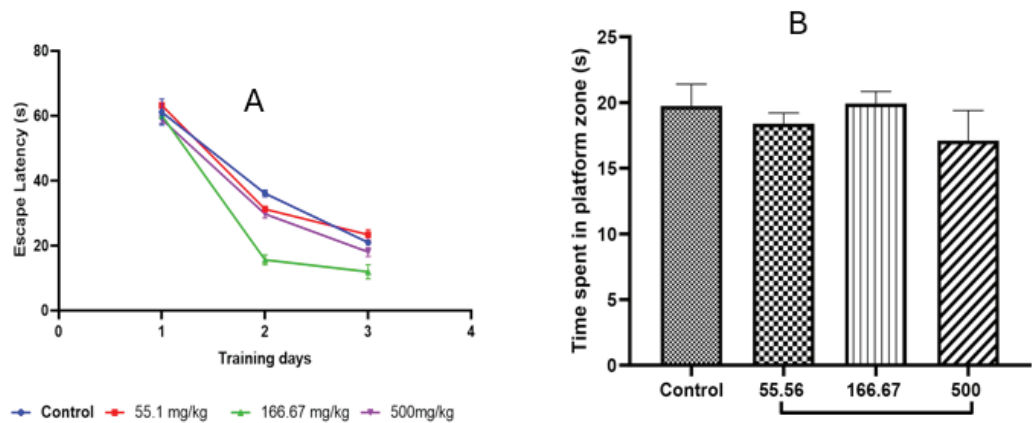


Figure 2. Effects of ECASB on Cognitive behaviour in male Wistar rats

The values are expressed as mean $\pm$ SEM (One-way ANOVA followed by Dunnett's Post hoc test) $n=5$

Effects of ECASB on spontaneous locomotor activity in male Wistar rats

Figure 3A shows the effect of ECASB on total distance travelled within the open field. The total distance travelled was lowered significantly in all the treatment groups compared with the control group. Similarly, the number of lines crossed reduced significantly at 166.67 mg/kg and 500 mg/kg relative to the control group, as shown in Figure 3B. Rearing frequency was significantly reduced in the treatment group that received a 500 mg/kg dose and an increase in the grooming frequency of the animals in the treatment groups 55.56, 166.67, and 500 mg/kg when compared with the control (Figure 3C and Figure 3D, respectively).

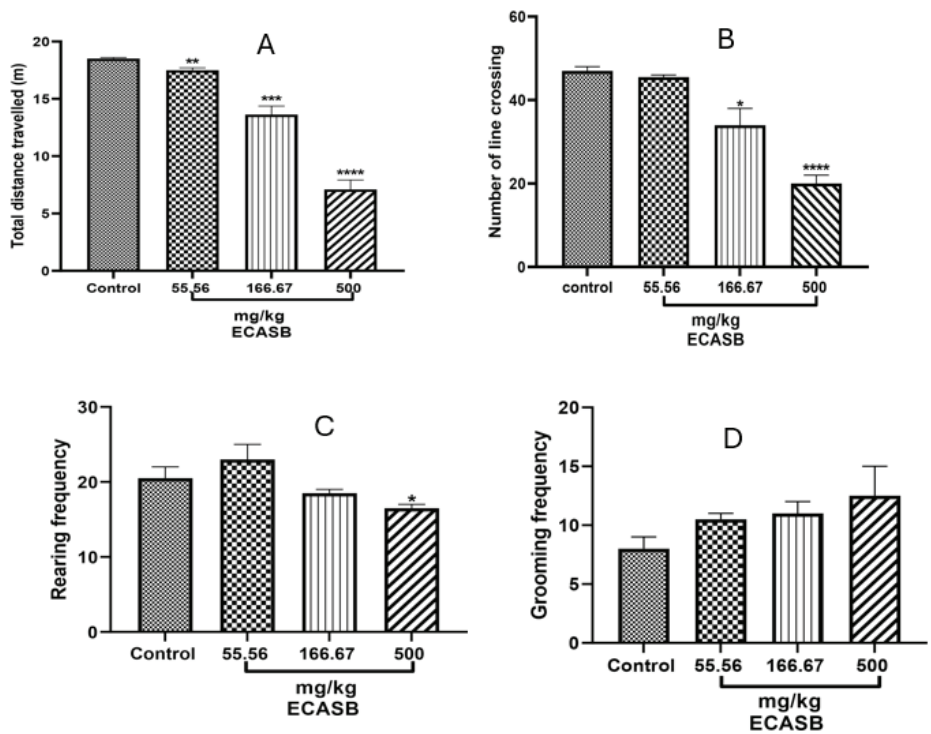


Figure 3. Effects of ECASB on Spontaneous locomotor activity in male Wistar rats

The values are expressed as mean $\pm$ SEM (One-way ANOVA followed by Dunnett's Post hoc test) $n=5$; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ (significance difference) when compared with control

Effects of ECASB on depressive-like behaviours in a sucrose splash test in male Wistar rats

Administration of ECASB caused a significant in-

crease in the grooming time in experimental rats in a sucrose splash test compared to animals in the control group (Figure 4.).

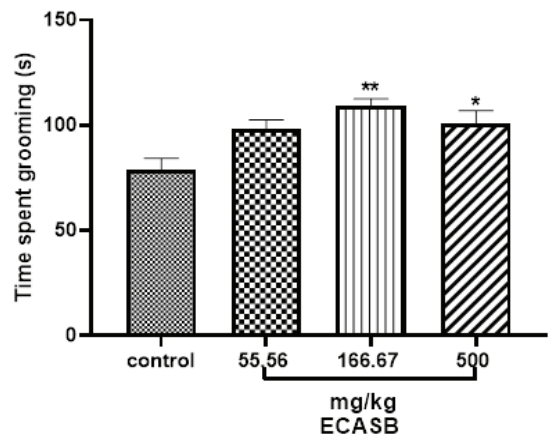


Figure 4. Effect of ECASB on time spent grooming by male Wistar rats in the sucrose splash test
The values are expressed as mean $\pm$ SEM (One-way ANOVA followed by Dunnett's Post hoc test), n=5; *p < 0.05, **p < 0.01 (significant difference) when compared with control

Effects of ECASB on anxiety-like behaviour in male Wistar rats

The time spent in the light compartment signifi-

cantly increased in the rats treated with 166.67 and 500 mg/kg compared to the control group (Figure 5.).

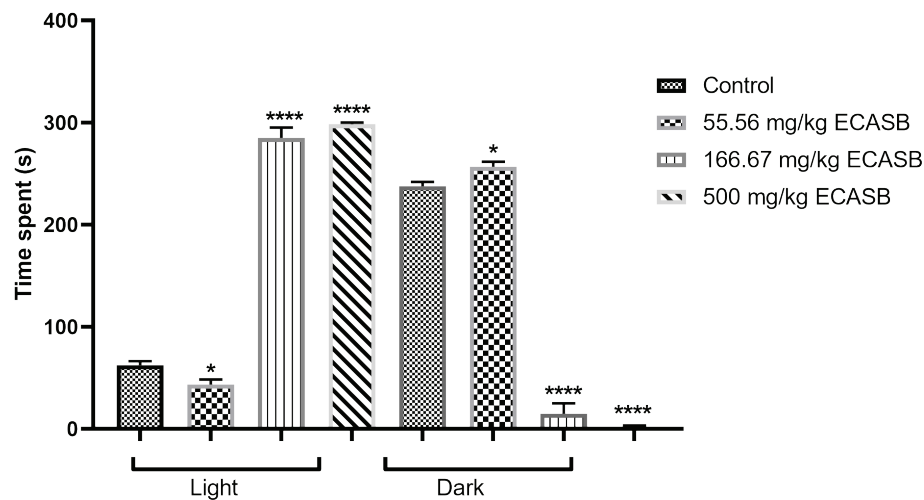


Figure 5. Effect of ECASB on time spent in the L/D compartment by male Wistar rats
The values are expressed as mean $\pm$ SEM (One-way ANOVA followed by Dunnett's Post hoc test) n=5; *p < 0.05, ****p < 0.0001 (significance difference) when compared with control

Biochemical assessment of the effects of ECASB in male Wistar rats

Brain AChE activity reduced in the rats treated with 55.56 and 166.67 mg/kg ECASB, while treatment with 500 mg/kg ECASB produced significantly increased activity compared to the control group (Fig-

ure 5A.). Furthermore, reduced glutathione level was significantly low in rats treated with 500 mg/kg when compared to the control group (Figure 5B.). Malondialdehyde an indicator of lipid peroxidation was elevated in all the treatment groups, although 500 mg/kg had the most significant elevation compared to the control group (Figure 5C.).

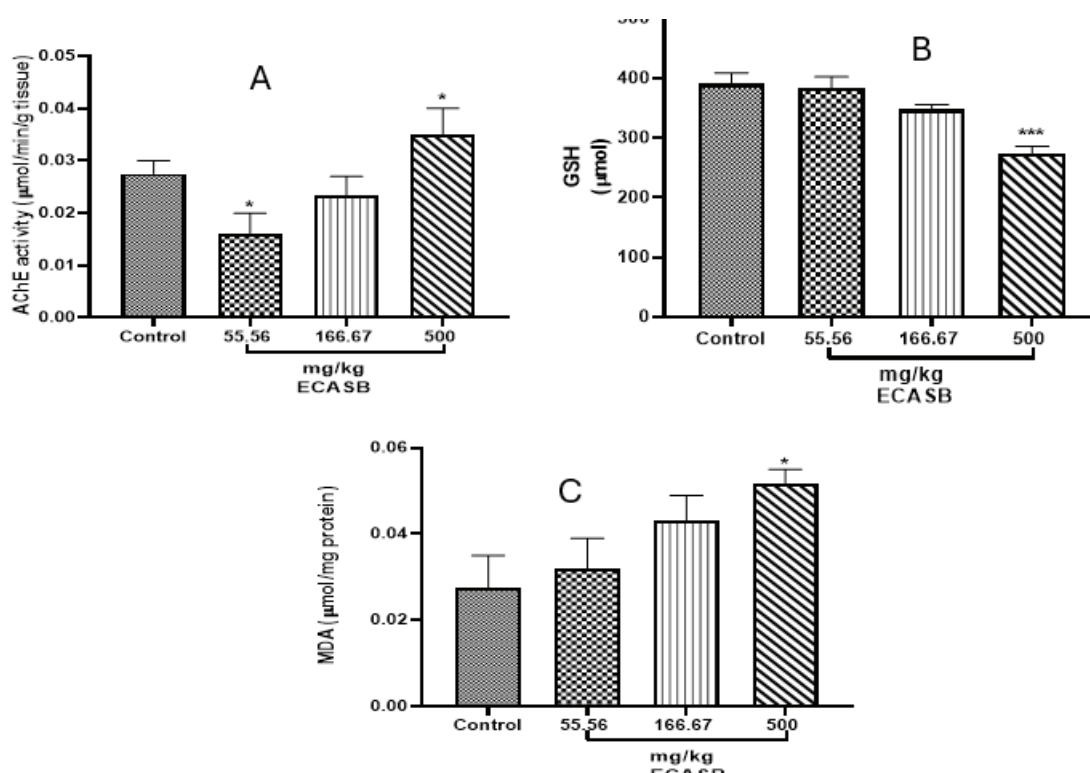


Figure 6. Effect of ECASB on biochemical parameters in the brain sample homogenates of male Wistar rats

The values are expressed as mean $\pm$ SEM (One-way ANOVA followed by Dunnett's Post hoc test) $n=5$; * $p < 0.05$, *** $p < 0.001$ (significance difference) when compared with control

Effect of ECASB on histology of the hippocampus and cerebellum

Figure 7A-D is a representation of the histology of the hippocampus of the control rats treated with distilled water and rats treated with selected doses of ECASB. The insert box in Figure 7A shows the area magnified in A1, B2, C3, and D4. An increased number of neurons in treatment groups (55.56, 166.67,

and 500 mg/kg) were observed in the area magnified when compared to the control group. However, there was decreased viability in the neurons of rats in the treatment groups. Figure 8A-8D is a representation of the cerebellum of the rats in the control group and the treatment groups. Viable Purkinje neurons are abundant (black arrows) in 8A (the control rats) than in rats treated with the highest dose 8D.

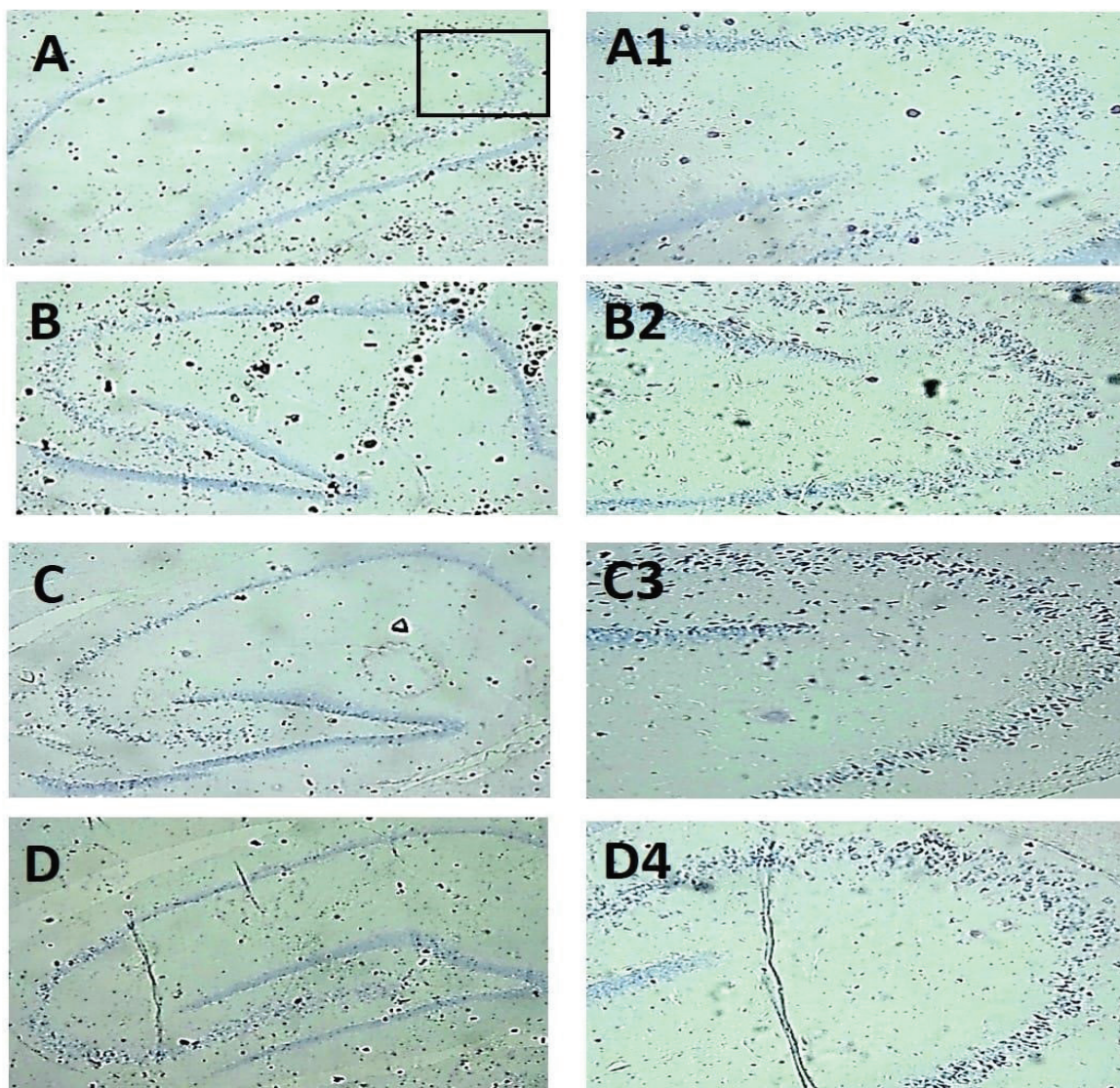


Figure 7. Representative photomicrograph of histological examination of rats' hippocampus stained with cresyl at the end of the experimental period. X400

A= Control group, B= 55.56 mg/kg ECASB, C= 166.67 mg/kg ECASB, D= 500 mg/kg ECASB

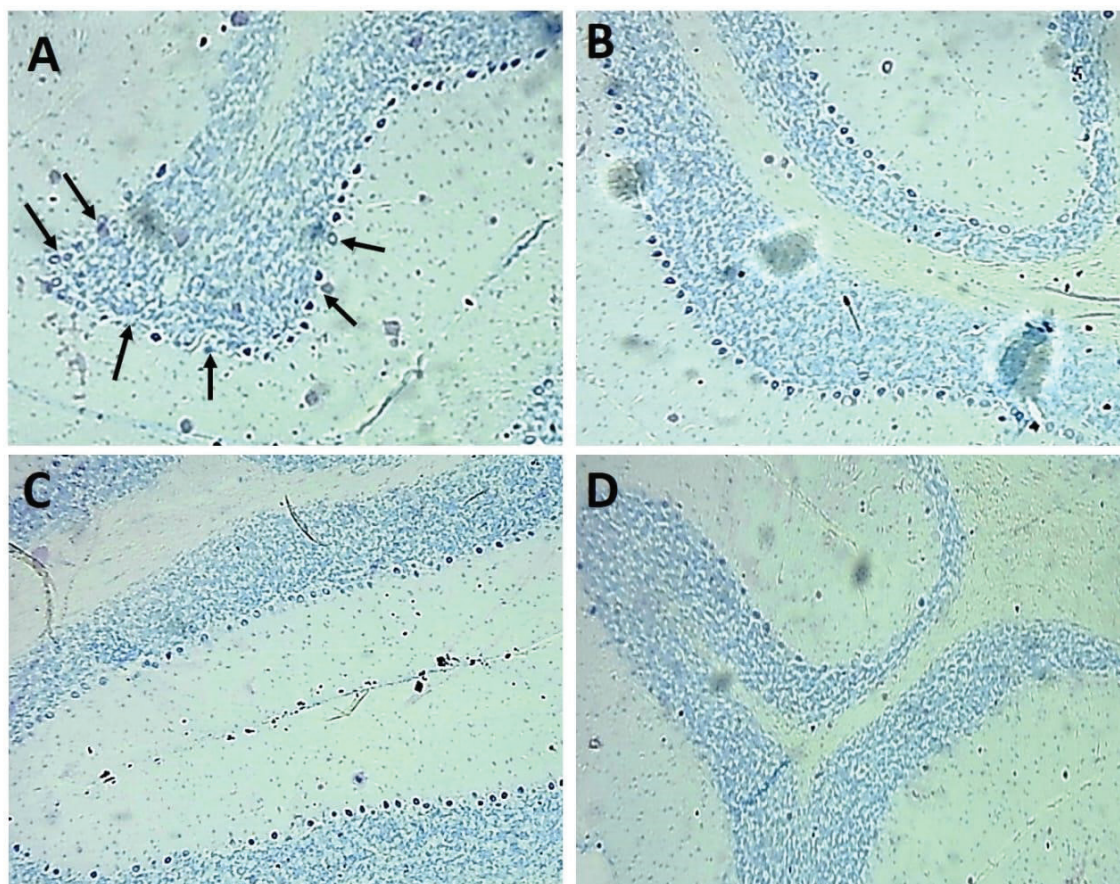


Figure 8. Representative photomicrograph of histological examination of rats' cerebellum stained with cresyl at the end of the experimental period x400

A= Control group, B= 55.56 mg/kg ECASB, C= 166.67 mg/kg ECASB, D= 500 mg/kg ECASB

DISCUSSION

The leaf, fruit, and stem bark of *Chrysophyllum albidum* have been reported to have very significant pharmacological activities, including anti-plasmodial, anti-diabetic, antioxidant, and antimicrobial activities (Ajayi, Chidebe, Ben-Azu, & Umukoro, 2020; Darko et al., 2021; Odewande, Mukhtar, & Ibrahim, 2023; Ogunleye, Fapohunda, & Nwangwu, 2020). The fruit has also been reported to be useful in managing stress and inflammation-related neuropsychiatric disorders (Ajayi, Oyetayo, & Akomolafe, 2022). However, detailed information on the safety profile of the stem bark is limited. This study evaluated the sub-acute oral toxicity profile of the ethanol extract of *C. albidum* stem bark (ECASB) and its effect on neurobehavioral patterns in Wistar rats.

No death was recorded during the 14-day oral administration of ethanol extract of *C. albidum* stem bark (ECASB) at the doses tested. Body weight was significantly reduced at the highest dose (500 mg/kg). Feed and water consumption are true indicators of experimental animals' growth rate (Todic, Bakic, Begovic, Krosnjak, & Zulic, 2023). Their determination is also an essential component in assessing the safety of any therapeutic agent. In this study, feed and water intake were reduced in the treatment groups compared with the control group, suggesting a metabolic or physiological disturbance. Aversion to the feed, potentially due to the extract's taste, smell, or post-ingestive effects, is an important determinant of food perception and appreciation (Chambers, 2018), and it may explain the observed low food intake. It

could also indicate mild toxicity or metabolic changes that reduce appetite or thirst (McCrickerd & Forde, 2016). It may also be that ECASB possesses anti-obesity activity. Furthermore, organ weight evaluation is an essential component of toxicological investigation, providing information on the organ-specific effects of the test compound/sample (Abebe, 2023). In this study, the extract did not produce any significant difference in the relative brain weight compared with the control group.

Behavioural assessment is an essential tool for evaluating nervous system function and potential dysfunction (Calabrese & Baldwin, 2023; Sanchlemente-Alaman, Jimenez, Martin, Guiu, & Pinedo, 2020). Functional consequences of neuronal communication disruption can be assessed using neurobehavioural and physiological methods (Moser, Aschner, Richardson, & Philbert, 2010). The neurobehaviors evaluated in this study were cognitive function, depression, anxiety and locomotor activity.

Spatial learning and memory (cognitive function) were assessed using the Morris Water Maze. Spatial acquisition/learning involves the animal learning to navigate a direct path to the immersed platform using cues, starting from different perimeters or locations within the tank. The four locations used in this study were South, North, West and East. At the end of the training, a significant decrease in escape latency was observed, indicating that the animals showed spatial learning. However, the time spent in the platform zone by animals in the group treated with the highest dose was reduced compared with the control group. This suggests that ECASB may impair memory function in rats at the highest dose tested (Ren et al., 2022). The pronounced reduction in food and water intake in the animals, leading to weight loss, especially at the highest dose, might also be responsible for the behavioural alterations observed.

The open field test is used to evaluate locomotor activity and anxiety-like behaviour in experimental rodents (Himanshu, Dharmila, Sarkar, & Nutan

2020). In this study, across all ECASB doses, there was a significant decline in total distance travelled. Also, at the 166.67 and 500 mg/kg doses of ECASB, there was a reduction in line crossings, with rearing frequency significantly lower in the 500 mg/kg group than in the control group. This shows that ECASB reduces the exploratory behaviour of the animals, an indication of a loss of interest since parameters like rearing frequency, total distance travelled, and number of line-crossing give information about the exploratory behaviours of the animals (Hu et al., 2017; Seibenhenner & Wooten, 2015). This might mean that ECASB has a sedative-like effect, causes muscle relaxation, or has a CNS-depressant impact.

The groups administered ECASB also showed increased grooming frequency in the open field test, suggesting an anxiogenic-like effect of the extract, as excessive grooming can indicate depression (Karbowska et al., 2020). Anxiety-like behaviour in rodents can be measured using the light and dark box. It works on the principle that rodents often avoid light and spend more time in the dark. Thus, the longer the rat spends in the light compartment, the less anxious it is (Lee, Coelho, Sem, & Szumlinski, 2018). A significant increase in time spent in the light compartment by animals administered 166.67 and 500 mg/kg ECASB suggests anxiolytic potential of the extract. However, this contradicts the anxiolytic-like test result obtained from the open field box. Thus, the results from the light and dark boxes may be due to decreased locomotor and exploratory activity, rather than to an anxiolytic effect of the extract.

The sucrose splash test was used to test for depressive-like behaviour in this study. When splashed, the animals are expected to groom as a form of self-care. A reduction in the time spent grooming is viewed as a depressive-like behaviour (Becker, Pinhasov, & Ornoy, 2021; Planchez, Surget, & Belzung, 2019). Administration of ECASB significantly increased the animals' grooming time, although the increase was not linear with dose. Interestingly, although the highest dose (500 mg/kg) produced a significant effect, it

was less pronounced than that of the mid-dose. This pattern indicates a non-linear (inverted U-shaped) dose-response relationship, which is often observed in neuropharmacological and behavioural studies (Cools & D'Esposito, 2011). This phenomenon may arise from receptor saturation, activation of opposing or compensatory signalling pathways, or the emergence of sedative or adverse effects at higher doses that counteract behavioural stimulation (Arnsten, 2015; Calabrese & Baldwin, 2003). In particular, inverted U-shaped dose-response curves have been well documented in dopaminergic and serotonergic systems, both of which play key roles in motivation and self-directed behaviours (Albert, Vahid-Ansari, & Luckhart, 2014). Similar biphasic dose-response patterns have also been reported for plant-derived compounds and psychoactive agents in rodent models of affective behaviour, emphasising the importance of identifying an optimal therapeutic window when evaluating potential antidepressant-like effects (Zuardi et al., 2017).

Acetylcholinesterase (AChE) is an enzyme that plays a crucial role in cholinergic neurotransmission. It breaks down acetylcholine, a neurochemical implicated in learning, memory and involuntary movement (Liao et al., 2023; Sallam, El-Magd, Ahmed, Ghamy, & Mohammad, 2022). There was a significant increase in AChE activity in the brain of the group that received 500 mg/kg of ECASB compared to the control group. This may be responsible for the impaired cognitive function observed in the Morris water maze, causing the animals to spend lesser time in the platform zone since increased AChE activity leads to more breakdown of acetylcholine (Shelar, Nanaware, Arulmozhi, Lohidasan, & Mahadik, 2018). Likewise, the improved learning and memory observed in the group administered the intermediate dose may result from reduced AChE activity at that dose. This suggests enhanced cholinergic neurotransmission, a key mechanism underlying cognitive enhancement.

In addition, administration of ECASB at 500 mg/

kg resulted in a significant reduction in reduced glutathione and a concomitant increase in malondialdehyde levels. Reduced glutathione is a key antioxidant responsible for maintaining cellular redox balance (Aoyama, 2021), while malondialdehyde is a marker of lipid peroxidation arising from excessive free radical production (Cordiano et al., 2023). This imbalance in oxidative status may underlie cerebellar neuronal damage and contribute to the locomotor deficits observed at the highest dose. Furthermore, histology of the cerebellum revealed a reduced number of Purkinje neurones in the groups administered the highest dose of ECASB. This shows neuronal degeneration, which might be a result of the oxidative stress that resulted from the decreased antioxidant capacity within the system, as observed.

CONCLUSION

Overall, *Chrysophyllum albidum* stem bark demonstrates a narrow therapeutic window, offering cognitive benefits at low doses but causing neurotoxicity at higher doses via oxidative and cholinergic mechanisms. This underscores the importance of dose optimization in any prospective therapeutic application.

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AUTHOR CONTRIBUTION STATEMENT

O.O.S.: Data acquisition, analysis and manuscript writing, A.A.A.: Data interpretation and manuscript writing, J.O.L.: Data acquisition and analysis, P.A.A.: Data acquisition and analysis, I.V.A.: Data acquisition and analysis, O.F.-A.: Data acquisition and analysis, O.O.A.: Conception, study design and manuscript revision. The final manuscript was proofread and approved by all authors.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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