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THE NEUROPROTECTIVE EFFECT OF *CALOTROPIS PROCERA* (AITON) W.T.AITON AGAINST TOXICITY OF MERCURY CHLORIDS

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Abstract. Central nervous system intoxication can result from exposure to various toxins, including mercury chloride. Although several chelating agents are available for mercury chloride detoxification, their efficacy can diminish over time. *Calotropis procera* (Aiton) W.T.Aiton, a medicinal plant, has shown potential as a protective agent against mercury chloride-induced brain damage. This study aims to evaluate the protective effects of *C. procera* in mitigating mercury chloride toxicity. This study investigates the protective effects of *C. procera* against mercury chloride toxicity in Wistar albino rats. A total of 36 rats, comprising both males and females, were housed under controlled laboratory conditions and divided into two main groups based on five animal. Each group received certain nutrition: standard nutrition, *C. Procera*, mercury chloride and all together. Treatments were administered for 20 days. After the treatment period, the rats were euthanised, and brain tissues were collected for histopathological analysis. After the brain tissues were fixed in 10% saline-buffered formalin, they were processed through a series of ascending grades of ethanol to dehydrate them. The tissues were then cleared in xylene and embedded in paraffin. The paraffin-embedded brains were treated three times with pure paraffin to ensure proper infiltration and were subsequently moulded into blocks. Sections of 5 μm thickness were prepared using a Leica microtome and stained with haematoxylin and eosin (H&E) for histopathological examination.The study adhered to ethical guidelines and was approved by the relevant regulatory body. The results of this study demonstrated that mercury chloride caused significant cerebral toxicity, manifesting as inflammation and pyknosis of the nuclei. *C. procera* reduced mercury toxicity and preserved the nuclei in male rats. In female rats, *C. procera* completely preserved the brain tissue.

Key words: *Calotropis procera* (Aiton) W.T.Aiton, Mercury chloride, Brain, inflammation, rats.

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НЕЙРОПРОТЕКТОРНОЕ ДЕЙСТВИЕ *CALOTROPIS PROCERA* (AITON) W.T.AITON ПРОТИВ ТОКСИЧНОСТИ ХЛОРИДОВ РТУТИ

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Аннотация. Интоксикация центральной нервной системы может быть вызвана воздействием различных токсинов, включая хлорид ртути. Хотя для детоксикации хлоридом ртути существует несколько хелатообразующих средств, но их эффективность со временем может снижаться. Лекарственное растение (Aiton) W.T. Aiton продемонстрировало потенциал в качестве защитного средства от повреждения головного мозга,

вызванного хлоридом ртути. Цель данного исследования – оценить защитные эффекты *C. procera* в снижении токсичности хлорида ртути. В работе изучаются защитные эффекты *C. procera* против токсичности хлорида ртути у белых крыс линии Wistar. В общей сложности 36 крыс, как самцов, так и самок, содержались в контролируемых лабораторных условиях и были разделены на две основные группы по пять особей. Каждая группа получала определенное питание: стандартное, *C. Procera*, хлорид ртути и все вместе. Лечение проводилось в течение 20 дней. По истечении срока лечения крыс подвергли эвтаназии, а ткани головного мозга были собраны для гистопатологического анализа. После того, как ткани мозга были помещены в 10 %-ный формалин, забуференный физиологическим раствором, они были обработаны несколькими сортами этанола по возрастанию для их обезвоживания. Затем ткани были обработаны ксилолом и залиты в парафин. Залитые в парафин мозги трижды обрабатывали чистым парафином, чтобы обеспечить надлежащую пропитку, и затем формовали из них блоки. С помощью микротома Leica были получены срезы толщиной 5 мкм, окрашенные гематоксилином и эозином (H&E) для гистопатологического исследования. Исследование проводилось в соответствии с этическими нормами и было одобрено соответствующим регулирующим органом. Результаты этого исследования показали, что хлорид ртути вызывает значительную церебральную токсичность, проявляющуюся в виде воспаления и пикноза ядер. *C. procera* снижает токсичность ртути и сохраняет ядра у самцов крыс. У самок крыс *C. procera* полностью сохраняет мозговую ткань.

Ключевые слова: *Calotropis procera* (Aiton) W.T.Aiton, Хлорид ртути, мозг, воспаление, крысы.

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Introduction

The use of mercury has a long history, including its notorious rôle in poisoning Agnès Sorel, the mistress of Louis XIV. Historical analyses have also indicated that Isaac Newton suffered from mercury exposure, as evidenced by bone examinations [4]. A major mercury poisoning crisis occurred in Minamata, Japan, where mercury discharge from a chemical factory led to severe health consequences. This tragedy marked the onset of new neurological diseases, the mechanisms of which remained poorly understood for a long time. However, recent studies have provided new insights into the mechanisms of mercury-induced damage to the brain, with a particular focus on Minamata Bay. Advanced analytical techniques, such as high-resolution synchrotron X-ray absorption spectroscopy [3], have revealed that mercury preferentially binds to sulphur groups. This binding action leads to cerebral lesions by disrupting cellular functions, as mercury's interaction with sulphur interferes with normal biological processes. The Minamata tragedy not only exposed the severe neurological diseases caused by mercury poisoning but also marked the beginning of our understanding of the neuropathological mechanisms associated with mercury exposure. This awareness has been crucial in understanding similar mechanisms in other contexts. Investigating the properties of medicinal plants in relation to mercury toxicity could open new avenues for treating these neurological conditions. Detoxifying mercury from the brain can eliminate this toxic substance, but the resulting damage is challenging to repair with chemical chelators. However, there are natural ways that can help in repairing neurotoxic lesions caused by mercury chloride. To detoxify the mercury chloride, more toxic plants similar to it can be used but in a bénéficial way. Among these plants there is *Calotropis procera* (Aiton) W.T.Aiton which is able to import new voices of detoxification of the mercury chloride. *C. procera* is a plant known in the Algerian Tuareg population by "Torha". This plants characterized by the presence of a white liquid which circulates in all parts of the plant. This liquid is the latex of *C. procera*. It contains cysteine proteins, rich in thiol groups, which have been widely implicated in the detoxification of heavy metals. It is a plant characterized by strong odor, giant green leaves and filled inside with white milk. *C. procera* is characterized by the presence of several antioxydant components. It contains sweet components like Calotropin and Calotoxin both helping protect neuronal cells from inflammatory damage. This anti-inflammatory action is complemented by rutin and quercetin, which provide strong antioxidant benefits. Oleanolic Acid contributes by stabilizing cell membranes, which

minimizes oxidative stress and inhibits neuroinflammation, enhancing the protective effects of the other compound [1]. The objective of this study is to explore new natural methods to reduce mercury chloride toxicity in the brain, potentially offering new therapeutic strategies for neurological conditions associated with mercury exposure.

Material and Methods

Male and female albino rats Wistar weighting 250 g obtained from Pasteur Institute (Algiers) were reared in the animal house of University of Badji Mokhtar-Annaba.There were kept in the laboratory under constant conditions of temperature (24 ± 2 °C) at one month before and through the experimental work, being maintained on a standard diet and water were ready ad-libitum.

The experiment involved 30 rats, divided into two main groups, each consisting of 15 males and 15 females. Each main group was further divided into five subgroups, with each subgroup containing three rats. The first group Healthy control group received distilled water for 20 days, while the second group the animals received (200mg/Kg) of the *C. procera* by gavage. The third groupe received mercury chlorids at dose of 0,2mg/Kg by gavage and forthy group received (200 mg/kg) of *C. procera* and (0,2 mg/kg) by gavage and the fourth groups were respectively plants *C. Procera* and mercury chlorid treated with 1 g/kg/day (Eth1) and 2 g/kg/day (Eth2) of ethanol. The rats of the fifth (Eth1 + SMI) and the sixth (Eth2 + SMI) groups were firstly treated respectively with 1g/kg/day and 2g/kg/day of ethanol, after one hour, animals were given SMI (200 mg/kg/day). After fixation of brain tissues in 10% saline buffered formalin, the brain tissues were dried in ascending grades of ethanol, cleared in xylol, and then immersed in paraffin. Impregnated brain was treated three times in pure paraffin to be established in blocks. Sections (5 µm thick) were preparatory using Leica microtome and stained by hematoxylin and eosin (H&E) for histopathological investigation [2].

Discussion of Result

The histological analysis of this study revealed the protective role of *C. procera* in mitigating the toxic effects of mercury chloride on the brain (see fig. 1, 2). Mercury chloride is a potent neurotoxin that induces severe cerebral toxicity, manifested by significant cytoplasmic and nuclear alterations, such as the observed densified cellular nuclei with laminated chromatin in male

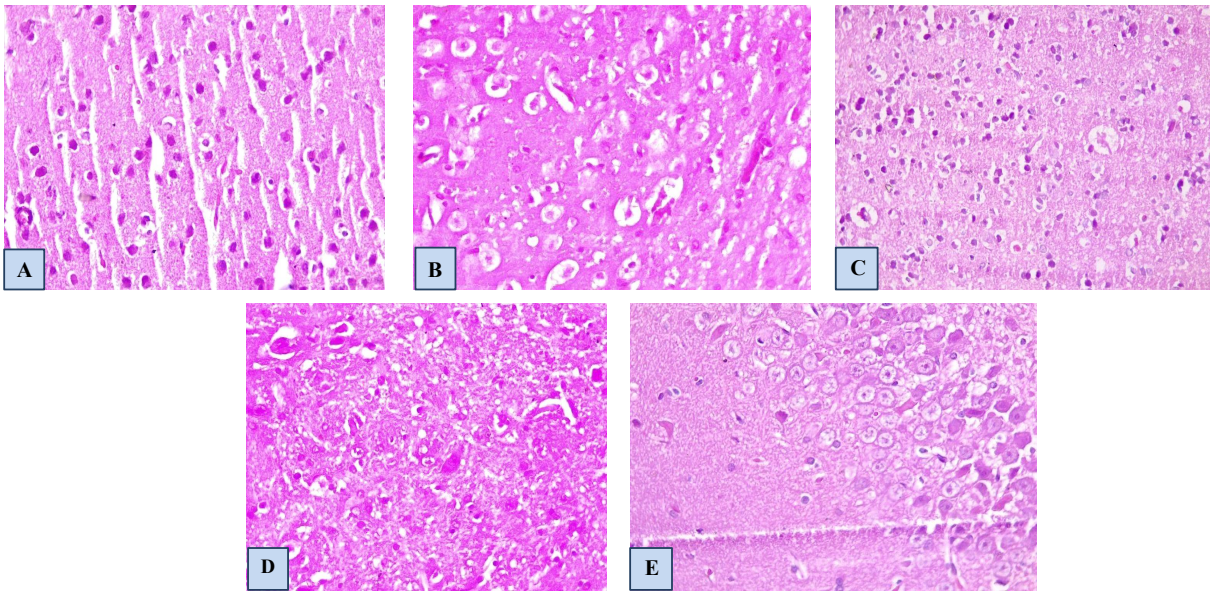

Fig. 1. Male rat histological study of brain:

A – Histological section of male rat treated with mercury chloride; *B* – Histological section of male rat treated with mercury chloride and *C. procera*; *C* – Histological section of male rat treated with plants *C. procera* and mercury chlorids; *D* – Histological section of male rat treated with *C. procera*; *E* – Histological section of male rat control

rats (see fig. 1,*A*). Histological results from male rats treated with both mercury chloride and *C. procera* demonstrated complete preservation of brain tissue against mercury-induced toxicity (see fig. 1,*B*,*D*). Examination of brain tissue sections from male rats treated with *C. procera* and mercury chloride revealed occasional images of karyolysis associated with edema, particularly within the plexiform or molecular layers. However, the cytoplasmic contours were preserved, and cellular nuclei remained intact (see fig. 1,*C*). No pathological processes were detected in the control group (see fig. 1,*E*).

Histological analysis of brain tissue from female rats treated with mercury chloride showed scattered nuclear pyknosis and increased nuclear density, along with laminated chromatin (fig. 2,*A*). Conversely, tissue sections from female rats treated with both *C. procera* and mercury chloride exhibited well-preserved nuclei (fig. 2,*B*). Microscopic examination of the brain tissue from female rats treated with mercury chloride and *C. procera* also revealed signs of pyknosis and nuclear densification, with well-preserved nuclei in other regions of the brain tissue (fig. 2,*C*). Brain tissue from control female rats exhibited subnormal cerebral morphology (fig. 2,*D*). No pathological processes were detected in the control group (fig. 2,*E*).

Our study, which involved both male and female rats, investigated the protective effect of *C. procera* against the neurotoxic effects of mercury chloride. We found that mercury chloride induced severe cerebral toxicity in both sexes, characterized by significant cytoplasmic and nuclear alterations. Previous research has shown that mercury chloride causes necrosis of nerve cells affecting the cerebral cortex, hypothalamus, and cerebellum [10]. Other studies have also reported that mercury chloride induces necrosis and apoptosis of neurons and astrocytes in the motor cortex [9]. These studies indicate that even low exposure to mercury chloride can impair nerve cells. Our results also demonstrated that within the plexiform layer of brain tissue from male rats treated with both *C. procera* and mercury chloride, there were instances of karyolysis associated with edema, while cytoplasmic contours and cellular nuclei were conserved. Similar effects were observed during the Minamata incident in Japan, where mercury chloride was found to preferentially affect the plexiform molecular layer of the brain [5]. Autopsy studies of mercury-intoxicated animals revealed

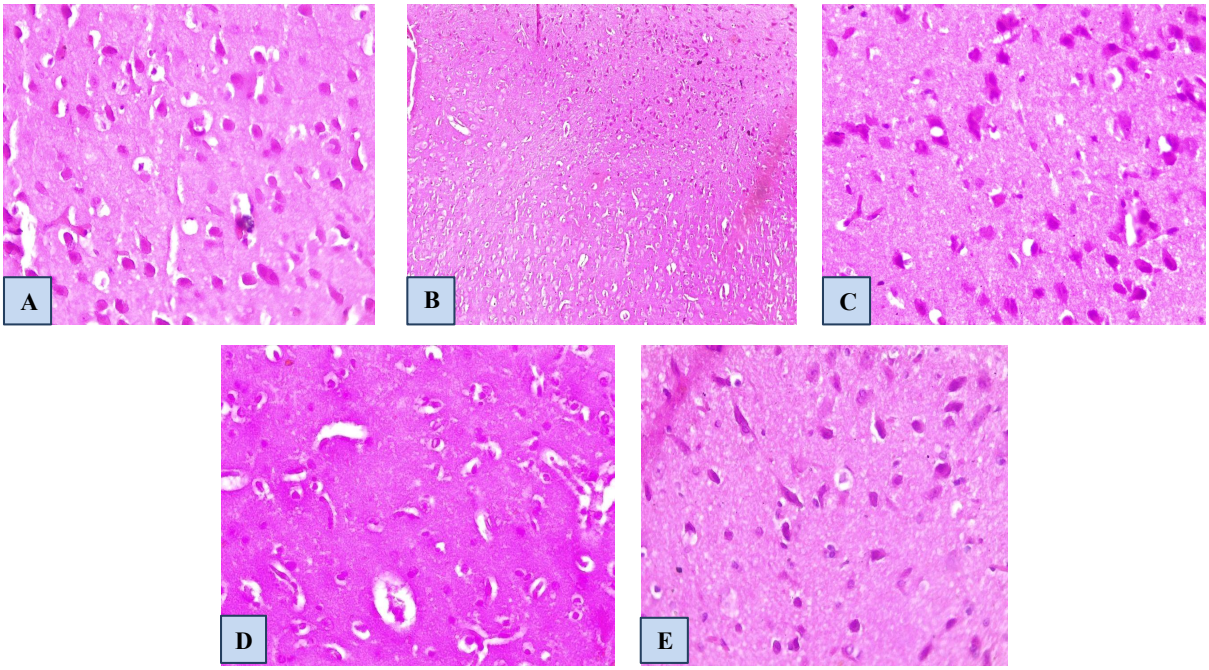

Fig. 2. Female rat histological study of brain:

A – Histological section of female rat treated with mercury chloride; *B* – Histological section of female rat treated with mercury chloride and plants *C. procera*; *C* – Histological section of female rat treated with mercury chlorids and *C. Procera*; *D* – Histological section of female rat treated with *C. Procera*; *E* – Histological Section of female Rat control

karyolysis of nuclei, as well as vascular damage in subcortical brain regions, including edema, vessel damage, widening of perivascular spaces, and infiltration of pale red fluid. Other studies have also identified karyolysis leading to neuronal damage and a decrease in neuron count [8].

Our findings indicate that *C. procera* reduced the toxic inflammatory effects caused by mercury chloride and protected cellular integrity by preserving cytoplasmic contours and cellular nuclei within brain tissue. Phytochemical investigations of this Saharan plant have highlighted its richness in flavonoids, such as rutin [6]. Rutin has been shown to reduce brain lesions observed in cancer [7], which may explain its neuroprotective role against mercury chloride-induced toxicity observed in our study. Additionally, stilbene, another compound in *C. procera*, can halt the cascade of cerebral inflammatory reactions. Stilbene also protects against brain lesions and inflammation by stimulating the expression of the enzyme heme oxygenase. Histological results from male rats treated with both mercury chloride and *C. procera* demonstrated that the plant completely protected brain tissue from mercury chloride toxicity. This protective effect of *C. procera* is attributed to its wealth of molecules that target toxic brain proteins. For example, oleandrin, a neuroprotective molecule, inhibits the expression of the toxic alpha-synuclein protein, thereby preventing brain lesions associated with this protein's toxicity.

Conclusion

This study investigates a plant called *C. procera* and its effect to reduce the effect of mercury chloride. The result demonstrates that *C. procera* can reduce the inflammatory reaction in both male and female rats. *C. procera* has been shown to preserve cell integrity by protecting the cellular nuclei despite the toxic effects of mercury chloride. This effect is due to the antioxidants in *C. procera*, which were synthesised under high heat conditions in the Sahara Desert of Algeria. This harsh climate has led to the formation of molecules that have been able to penetrate the brain and counteract the effects of mercury chloride.Although the toxicity of mercury chloride is well-known, this study suggests that *C. procera* could open new avenues for the treatment of inflammatory brain diseases.

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