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Alcohol-induced neurotoxicity and mitigating effect of *Trichocereus macrogonus* stem-sap on the amygdala of Wistar rats

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ABSTRACT

Alcohol is known to have neurotoxic effects on the brain, particularly on the amygdala, a pertinent brain structure for emotional processing, learning, memory, and social behavior. This study was to assess the mitigating effects of *T. macrogonus* (TM) stem-sap on the amygdala of alcohol-induced neurotoxicity in Wistar rats. Twenty-five adult male Wistar rats (190-240 g) were grouped into five groups of five rats each; Group A served as the control and was administered with 10 mL/kg body weight (b.w.) of distilled water; Groups B-E received 88 mg/kg of TM stem-sap, 1.34 mL/kg of alcohol, 0.13 mL/kg of alcohol + 88 mg/kg of TM stem-sap, and 1.34 mL/kg of alcohol + 264 mg/kg TM stem-sap, respectively. The administrations were oral and lasted for 21 days. A day later, the animals were sacrificed immediately after ketamine hydrochloride intraperitoneal anesthesia. Animals were perfusion-fixed with phosphate-buffered saline, the brains excised, fixed in 10% buffered formalin, processed, and stained with hematoxylin and eosin (H & E) and Cresyl fast violet (CFV). The result showed there was no significant difference in body weight in all groups. Histologically, mild and moderate histomorphological alterations of the amygdala in groups D and E compared to A and C groups, respectively. CFV stain showed highly and moderately stained Nissl bodies in groups D and E, respectively. In conclusion, *T. macrogonus* step-sap as a neuroprotective agent was able to mitigate the damaging effect of alcohol-induced neurotoxicity but was dosage-dependent.

Keywords: *Trichocereus macrogonus*, alcohol, Nissl substance, amygdala

1. INTRODUCTION

Neurotoxicity is a function of exposure to many substances, such as alcohol, prescription pharmaceuticals, heavy metals, pesticides, and drugs (Liddelow and Barres, 2017). It causes illnesses and the aging of the brain. According to Sosna et al., (2018) environmental factors such as exposure to medications, poisons, and other

factors can affect epigenetic modifications such as mitochondrial malfunction, leading to brain toxicity and neurological disorders. Deficits in motor and cognitive functions result from gradually losing of neuronal structure and function in the central nervous system. This results in a hallmark of neurodegenerative disorders such as epilepsy, Parkinson's disease, Alzheimer's disease, and Huntington's disease (Sosna et al., 2018). Shortly, soon, neurotoxicity might be a devastating disease for our society. Many people are ignorant about both this condition and the way that toxins in our surroundings gradually damage our brains and neurological systems.

Numerous industrial and environmental toxins have shown to have adverse effects on the immune system in research involving animals and humans (Dasgupta, 2018). With an average yearly intake of 6.2 L of pure alcohol per capita or 13.5 g of pure alcohol per day, alcohol is the most widely used social drug in the world (WHO, 2014). After smoking and hypertension, alcohol intake is the third most significant preventable cause of any disease (Singal and Anand, 2013). The World Health Organization (WHO) states that alcohol use is associated with over 200 diseases and injury-related health conditions (WHO, 2014). The alcohol contained in wines and beers is known as ethanol. The primary alcoholic ingredient in wines, spirits, and beers is ethanol (Griffith and France, 2018). Ethanol's chemical makeup and weight facilitate its simple diffusion into the bloodstream and central nervous system, inducing a sense of intoxication in the participant.

According to disability-adjusted life years (DALYs), alcohol consumption is responsible for 4.2% of the world's disease burden. This burden is highest among those in their prime, between the ages of 15 and 59 (Gakidou et al., 2017). The alcohol content of different alcoholic beverages varies. For instance, fruit juice has an alcohol content of 0.1% (v/v), beer has an alcohol content of about 4%–7%, champagne has an alcohol content of 12%–13%, and table wine has an alcohol content of 7%–8%. However, average vodka, whiskey, rum, and brandy can have an alcohol content of 40% or more, depending on the brand (Griffith and France, 2018). The most often abused substance is alcohol. Most organ systems are adversely affected by binge drinking, particularly the parts of the brain in charge of memory, coordination, and emotional processing (Jacobus and Tapert, 2013; Lopez-Caneda et al., 2019). Medicinal plants have been used for centuries to treat various ailments, including neurological disorders.

The use of medicinal plants to prevent or lessen the consequences of neurotoxicity has gained popularity in recent years. Cactus is one of such valuable medicinal plants. *Trichocereus macrogonus* (*Echinopsis macrogonus*) is a genus of plants in the Cactaceae family. Usually coming from Bolivia, Chile, Ecuador, Peru, and southeast Argentina, it is of Andean descent and reaches the Atlantic coast. In conventional medicine, *Trichocereus macrogonus* is used to treat several conditions, including fever, coughing, and inflammation. It is also believed to have analgesic properties and has been used to alleviate pain. Its large, white flowers bloom at night and are highly fragrant. Phytochemical analysis of this plant has revealed the presence of various compounds, including alkaloids, flavonoids, and phenolic compounds.

These substances have demonstrated to exhibit bioactive qualities such as antibacterial and antioxidant effects. Due to the reported beneficial properties of this plant, it may influence the function of specific brain areas -such as the amygdala. The adverse effects of neurotoxicity can vary depending on the type of chemical exposure and the duration of exposure. As a result, pharmacological regulation of neurotoxicity is emphasized as a potential therapeutic approach (Galluzzi et al., 2017; Iniodu et al., 2019). The consequences of neurotoxicity extend to a variety of clinical conditions, including cancer, neurodegeneration, and cardiac problems. It is to this concern that this research was to assess if this *T. macrogonus* stem-sap has effects on the amygdala of alcohol-induced neurotoxicity in Wistar rats.

2. METHODOLOGY

Materials

The cactus (*T. macrogonus*) was obtained from a local plant garden in Uyo Local Government Area of Akwa Ibom State. A brand of alcohol, St. Remy, was purchased from a supermarket in Uyo.

Source and Maintenance of Animals

A total of twenty-five adult male Wistar rats of body weight 190-240 g were used for the study. The rats were purchased from the Faculty of Basic Medical Sciences' Animal House, University of Uyo. After that, they were randomly divided into five groups (Groups A–E) and housed in well-ventilated standard animal cages. The animals were maintained at room temperature of 25-28 °C and a 12:12

light/dark cycle with free access to standard chow pellets and water *ad libitum*. Each group consists of five animals. Group A served as the control, while groups B-E served as the test group.

Test Solution and Administration

The median lethal dose (LD50) of 13.42 mL of alcohol was determined using Lorke's method. For alcohol, groups received 1% of 13.42 (0.13 mL/kg) and 10% of 13.42 (1.34 mL/kg). The dosages for *T. macrogonus* stem-sap were obtained from an already established study, according to Buckingham, (2014), using the lethal dose of 880 mg/kg of *T. macrogonus*: 10% and 30% of the score were low and high doses. Therefore, low dose = 88 mg/kg and high dose = 264 mg/kg. The administrations were oral for 21 days.

Experimental Design

The animals were divided into five groups (five rats per group): Group A served as the control; Group B received 88 mg/kg of *T. macrogonus* stem-sap for 21 days at 48 hours' interval; group C received 10% of alcohol (1.34 mL/kg) for 21 days at 48 hours interval; Group D received 1% (0.13 mL/kg) of alcohol daily for the first ten days followed by 88 mg/kg (low dose) of *T. macrogonus* stem-sap; and group E received 10% (1.34 mL/kg) of alcohol daily for the first ten days followed by 264 mg/kg (high dose) *T. macrogonus* stem-sap for the next ten days, as shown in (Table 1).

Table 1 Schedule of treatments of animals in the control and test groups

Groups (n = 5)	Treatment/Dosage	Duration
A	Distilled water (10 mL)	Three weeks
B	TM stem-sap (88 mg/kg)	Three weeks
C	Alcohol 10% (1.34 mL/kg)	Three weeks
D	Alcohol 1% (0.13 mL/kg) + 88 mg/kg of TM stem-sap	Three weeks
E	Alcohol 10% (1.34 mL/kg) + 264 mg/kg of TM stem-sap	Three weeks

TM – *Trichocereus macrogonus*

Termination of the Experiment

A day after the last administration, the animals were weighed and sacrificed after anesthetizing with ketamine hydrochloride (RotexMedica, Germany, 50 mg/kg body weight) intraperitoneally. The animals were perfused through the left ventricle of the heart using cold phosphate-buffered saline. The brains were excised and fixed in 10% buffered formalin for histological tissue processing.

Ethics Issues

Experimental procedures involving animal use and care were conducted following international guidelines for the care and use of laboratory animals. Ethical approval with number for this study was obtained from the Faculty of Basic Medical Sciences Research and Ethical Committee, University of Uyo, Akwa Ibom State, Nigeria.

3. RESULTS

The result showed (Table 2) there was no significant difference on the body weight in all groups. Result from histological sections of the amygdala in Group A (distilled water), Group B (88 mg/kg) of TM stem-sap, Group C (1.34 mL/kg) of alcohol, Group D (0.13 mL/kg) of alcohol + (88 mg/kg) of TM stem-sap, and Group E (1.34 mL/kg) of alcohol + (264 mg/kg) of TM stem-sap. Photomicrographs of the sagittal section of the amygdala with Haematoxylin and Eosin staining (H & E. Mag x 400) are presented in (Figure 1). In contrast, photomicrographs of the sagittal section of the amygdala with Crestyl Fast Violet staining (CFV. Mag x 400) are represented in (Figure 2).

Table 2 Effects of *T. macrogonus* and Alcohol on Body Weight of Wistar Rats

Groups	Before Administration(g)	After Administration(g)	Weight difference (g)
A= Normal Control	230.6±10.28	238.4±9.53	7.8±0.7
B= 88 mg/kg of TM stem-sap	222.2±10.11	230.8±9.60	8.6±0.51
C= Alcohol (1.34 mL/kg)	207.0±5.44	218.6±5.59	11.6±0.15
D= Alcohol (0.13 mL/kg) + 88 mg/kg of TM stem-sap	227.4±10.71	231.4±9.75	4.0±0.96
E= Alcohol (1.34 mL/kg) + 264 mg/kg of TM stem-sap	219.2±27.16	222.2±26.57	3.0±0.59
	P= 0.8186	P= 0.8700	
	F= 0.3824	F= 0.3068	

Data is expressed as mean ± SEM at $P < 0.05$.

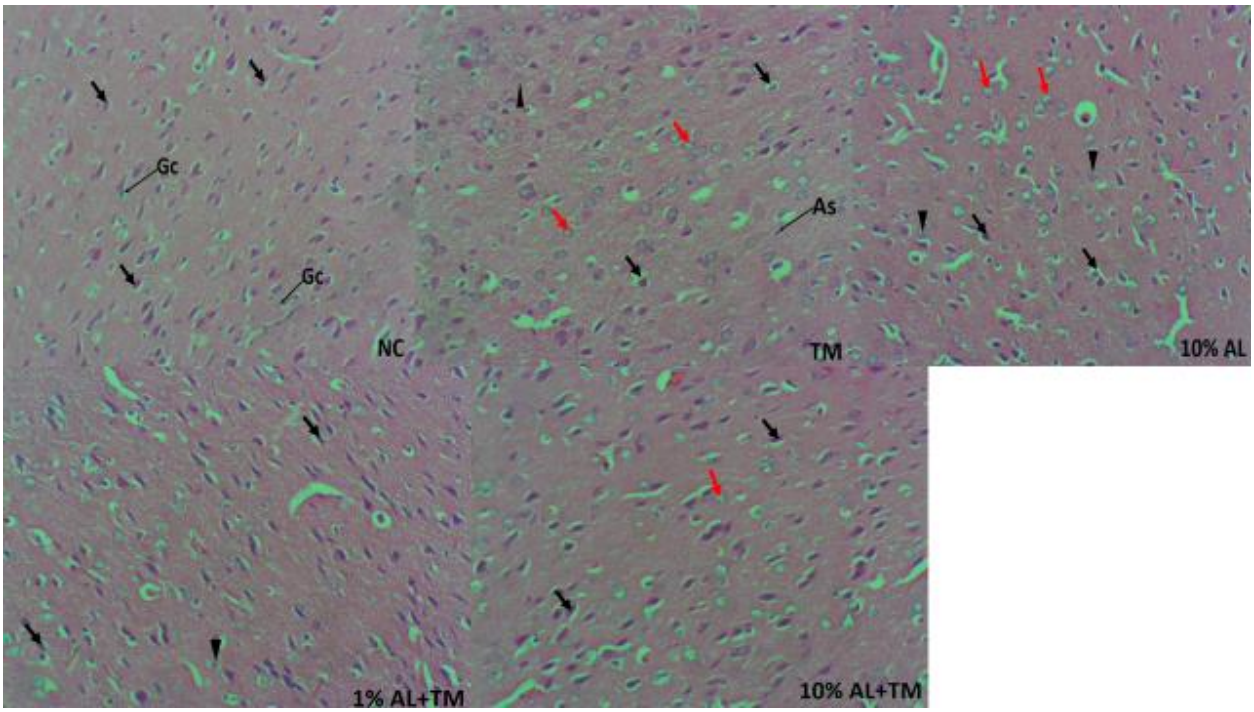

Figure 1 Photomicrograph of the sagittal section of Amygdala of normal control (Group A) that showed normal distribution of neurons (arrow) together with glial cells (Gc); Group B showed mildly affected cells; Group C showed hypertrophied cells; Group D showed mildly affected cells; and Group E showed moderately affected cells with vacuolations (black arrow), pyknotic (arrowhead), astrocytes (As) and karyorrhectic (red arrow) nuclei, H & E. Mag x 400. NC= Normal Control; TM = *T. macrogonus*; AL = Alcohol

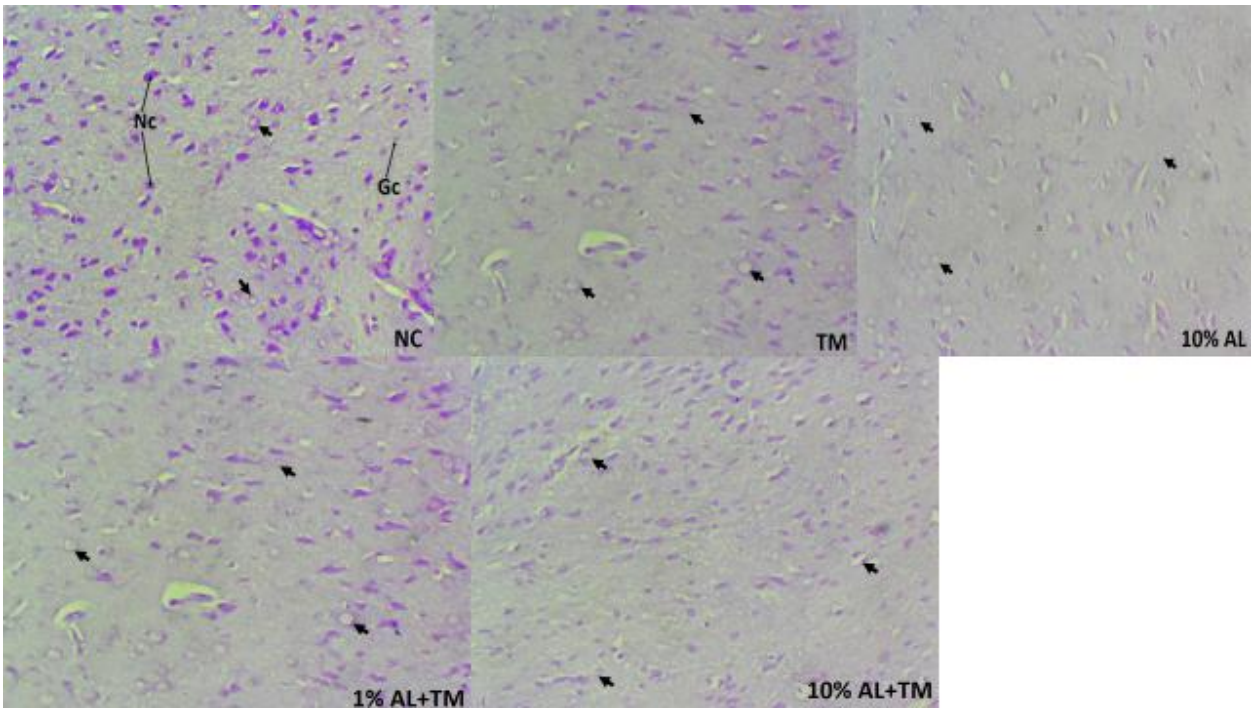

Figure 2 Photomicrograph of the sagittal section of Amygdala of normal control (Group A) that showed neural cells (Nc) together with glial cells (Gc) and strong expression of Nissl substance (arrow); Group B showed high expression of Nissl substance; Group C showed mild expression of Nissl substance; Group D showed strong expression of Nissl substance; and Group E showed moderate expression of Nissl substance (arrow), CFV. Mag x 400. NC= Normal Control; TM = *T. macrogonus*; AL = Alcohol

4. DISCUSSION

The adverse effects of neurotoxicity can vary depending on the type of chemical exposure and the duration of exposure. Alcohol is known to have neurotoxic effects on the brain, particularly on the amygdala. It is a pertinent brain structure for emotional processing, learning, memory, and social behavior, and is implicated in a range of mental health conditions, including anxiety disorders, depression, and post-traumatic stress disorder (PTSD). The study aims to assess the mitigating effect of *Trichocereus macrogonus* stem-sap following alcohol-induced toxicity in the amygdala of Wistar rats. There were no significant changes in the body weight in all groups. However, the group administered with 1.34 mL/kg of alcohol had an increase, which was not significant. This aligns with studies that show alcoholic drinks contain calories and slow down the body's fat-burning process, leading to weight gain (Griffith and France, 2018).

Histology showed mildly affected cells with vacuolations and pyknotic nuclei for the H & E stain, while Nissl bodies showed dense staining intensity for the CFV stain in the amygdala of group B and group D, administered with 88 mg/kg of *T. macrogonus* and 0.13 mL/kg body weight of alcohol + 88 mg/kg of *T. macrogonus,* respectively. Moderate expression with hypertrophied cells, vacuolations together with astrocytes for the H & E stain, and moderate expression of Nissl bodies in most of the cells for the CFV stain in group E, administered with 1.34 mL/kg of alcohol + 264 mg/kg of *T. macrogonus*, while adversely hypertrophied cells with pyknotic and karyorrhectic nuclei and astrocytes, and low expression of Nissl bodies indicating adverse effect of alcohol in the amygdala of group C, administered with 1.34 mL/kg of alcohol as compared to the control group that showed normal distribution of neurons together with glial cells. This indicates that alcohol affects the cells of the amygdala.

Vacuolations may be an identifiable indicator of neuronal degeneration in the amygdala due to neuronal deprivation of oxygen (Bzdok et al., 2013). Degeneration often precedes pyknosis and karyorrhexis, and they occur in the process of apoptosis or necrosis. Also, pyknosis may arise when a chemical agent traumatizes the brain (Reichard et al., 2005; Noureen et al., 2018). On the other hand, *T. macrogonus may have* had little or no damaging effect on the neuronal composition, rather a mitigating effect, as seen in group E, administered with 1.34 mL/kg of alcohol + 264 mg/kg of *T. macrogonus*. This confirms findings that *T. macrogonus* has potential

pharmacological significance, including antimicrobial, antioxidant, and anticancer properties (Rachael et al., 2020). Furthermore, the CFV staining specifically targeted the Nissl substance, providing a granular appearance to the cytoplasm of normal neurons (Piccinelli et al., 2019). In addition, it was used to evaluate brain lesions and neuronal plasticity following disease conditions and the effects of neuroprotective agents (Memudu et al., 2020).

5. CONCLUSION

This finding showed that group D with low doses of alcohol and *T. macrogonus* (0.13 mL/kg of alcohol + 88 mg/kg of *T. macrogonus*) caused mild histological alterations in the neuronal compositions of the amygdala, and group E with high doses of alcohol and *T. macrogonus* stem-sap (1.34 mL/kg of alcohol + 264 mg/kg of *T. macrogonus*), which had moderate histological alterations in the neuronal compositions of the amygdala when compared to group C, administered with only 1.34 mL/kg of alcohol that showed high alterations in the neuronal compositions of the amygdala. This suggests that *T. macrogonus,* as a neuroprotective agent, can mitigate the damaging effect of alcohol-induced neurotoxicity, however, dosage-dependent.

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Author Contributions

The design of the study, research and manuscript writing is by Ini-Obong G Essien. The analysis, interpretation of the result and discussion is by Christopher C Mbadugha. Proof reading is by Aquaisua N Aquaisua.

Ethical approval

The study was approved by the Faculty of Basic Medical Sciences Research and Ethical Committee (Ethical approval number: UU_FBMSREC_2024_008). As per the animal regulations in the Department of Human Anatomy, and the Faculty of Basic Medical Sciences' Animal House, University of Uyo, Nigeria, the Animal ethical guidelines are followed in the study for experimentation.

Informed consent

Not applicable

Conflicts of interests

The authors declare that there are no conflicts of interests.

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Data and materials availability

All data associated with this study are present in the paper.

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