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# PROPHYLACTIC EFFECT OF CUCURBITA PEPO SEED OIL ON BRAIN ACETYLCHOLINESTERASE AND ATPASES (NA+/K+ AND CA2+) ACTIVITIES OF TRAMADOL-INDUCED TOXICITY IN ALBINO RATS

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**Abstract**

Prophylactic effect of *Cucurbita pepo* seed oil (CPSO) on brain acetylcholinesterase and ATPases ($Na^+/K^+$ and $Ca^{2+}$) activities of tramadol-induced toxicity were investigated using a total of fifty six (56) Albino rats. The rats were assigned into seven (7) experimental groups (1-7) with eight rats in each group. They were acclimatized for one week, after which, rats in group 1 (Normal control) were allowed access to water and feed without restriction; rats in group 2 were administered 5 ml/Kg body weight (b.w) of normal saline. Toxicity was induced in groups 3-6 using 100 mg/Kg of tramadol (TM). Group 3 (TM control group) was left untreated; Groups 4, 5 and 6 (TM-CPSO treated groups) received 5, 2.5 and 1.5 ml/Kg of CPSO, respectively. Group 7 (CPSO group) received 5 ml/Kg of CPSO only. Group 2-7 were equally allowed free access to water and feed without restriction and the treatments were done by oral intubation once daily for 42 days. Acetylcholinesterase and ATPases activities were assayed using standard methods. TM toxicity was evidenced by significant ($p<0.05$) increase in the activity of acetylcholine esterase with significant ($p<0.05$) decreases in the activities of ATPases. However, treatment with CPSO significantly restored the activities of the altered biochemical parameters in a dose-dependent manner. The study suggests that TM-induced cerebral toxicity by increase in Ache and down regulations of ATPases activities. The inhibition of the alterations in the examined markers by CPSO could be relevant in the management of TM-induced toxicity.

Keywords: **Prophylactic, *Cucurbita pepo*, Acyetylcholinesterase, ATPases, Tramadol and Toxicity**

Tramadol is recommended for the management of acute and chronic pain of moderate to severe intensity (Lamont and Mathews, 2007). It is connected with a variety of diseases including osteoarthritis, fibromyalgia, diabetic neuropathy, neuropathic pain, low back pain, migraine, and even preoperative pain in human patients (Lamont and Mathews, 2007). Additionally, it could be powerful for alleviating symptoms of depression, anxiety and phobias (Rojas-Corrales *et al.*, 2004), and has a particular role in the treatment of premature ejaculation as well (Salem *et al.*, 2008).

Unfortunately, multiple cases of toxicity and abuse of tramadol have been reported. National Bureau of Statistics and Centre for Research and Information on Substance of Abuse revealed that prevalence of any drug use in Nigeria is estimated at 14.4 per cent or 14.3 million people aged between 15 and 64 years (UNODC, 2018). The extent of

drug use in Nigeria currently is comparatively high when compared with the 2016 global annual prevalence of any drug use of 5.6 per cent among the adult population (UNODC, 2016). UNODC (2018) reported that one in seven persons aged 15-64 years had used a drug (other than tobacco and alcohol) in the year 2017. In about 376,000 persons were estimated to be high risk drug users. The majority of high risk drug users were regular users of opioids such as tramadol. Around 1 in 5 high-risk drug users injects drugs, nearly 80,000 people (nearly 0.1 per cent of the adult population) were estimated to be people who inject drugs (UNODC, 2018). The majority (78 per cent) of those injecting drugs were men. The most common drugs injected are pharmaceutical opioids (such as tramadol, codeine, or morphine), followed by cocaine and heroin (WHO, UNODC, UNAIDS, 2012).Geographically, the highest prevalence of tramadol was found in the southern geopolitical zones (with a prevalence ranging between 13.8 per cent and 22.4 per cent) with south west being the highest followed by South-south and South-east compared to the northern geopolitical zones (with a prevalence ranging between 10 per cent and 13.6 per cent) (UNODC, 2018). The prevalence of the drug in the South-east zone was estimated at 13.8 per cent of the population or 1.5 million people aged 15-64 years. In overall, the prevalence in the south-eastern geopolitical zone is in the following order: Imo>Enugu>Ebonyi>Abia>Anambra (UNODC, 2018).

*Cucurbita pepo* (Pumpkin) as one of the natural products with antioxidant potentials is a leafy green vegetable belonging to the Cucurbitaceae family. Its fruits are variable in size, color, shape and weight. They have a moderately hard rind, with a thick, edible flesh below and a central seed cavity. There are numerous seeds in the fruit. Most seeds are plump and tan or soft white. They are all covered with a testa that serves as a protectant around the seeds (Robinson and Decker-Walters, 1997). Seeds belonging to Cucurbitaceous family are known to be as rich in oil as soybean, cotton and corn seeds (Esuoso *et al.*, 1998). Pumpkin seeds are excellent sources of both oil (37.8–45.4%) and protein (25.2–37%) (Lazos, 1986). This study was designed to investigate the prophylactic effect of *Cucurbita pepo* seed oil (CPSO) on brain acetylcholinesterase and ATPases ($Na^+/K^+$ and $Ca^{2+}$) activities of tramadol-induced toxicity in Albino rats.

## MATERIALS AND METHODS

### Biological Materials

Albino rats and *Cucurbita pepo* seed oil were the biological materials used in this study.

### Collection and Authentication of Biological Materials

Fresh seeds of *Cucurbita pepo* were collected from Aghara-oza village in Izzi Local Government Area of Ebonyi State and were identified by a Taxonomist in the Department of Applied Biology, Ebonyi State University, Abakaliki, Ebonyi State, Nigeria. Part of the identified plant was kept in Applied Biology Department, Ebonyi State University, Abakaliki, Ebonyi State, Nigeria, for reference purposes.

The albino rats used were purchased from the Animal Unit of Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Enugu, Nigeria. The rats undergone acclimatization for one week before the commencement of experiment in the Animal House of Department of Biochemistry, Ebonyi State University, Abakaliki, Ebonyi State, Nigeria.

### Methods

### Extraction of *Cucurbita pepo* Seed Oil

The seed oil of the *Cucurbita pepo* was extracted from the dried seed using mechanical pressing method according to Mathangi **(**2018).

**Experimental Design for the Study**

After acclimatization, total of 56 albino rats were randomly divided into seven experimental groups of 1-7 (*N = 8*). Group 1 (normal control): rats were fed on pellet and allowed free access to water; Group 2 (saline group): the animals received 5 ml/Kg body weight of normal saline. Group 3-6 received 100 mg/Kg body weight of tramadol (Abdel-Zaher *et al.*, 2011 and Ghoneim *et al.*, 2014); Group 3 (tramadol alone): the rats were left untreated; Group 4, 5 and 6 (Tramadol-CPSO treated groups): the animals received 5 ml/Kg, 2.5 ml/Kg and 1.5 ml/Kg body weight of *Cucurbita pepo* seed oil respectively; Group 7 (*Cucurbita pepo* seed oil group): the animals received 5 ml/Kg body weight of *Cucurbita pepo* seed oil only (Bandegi *et al.*, 2014; Tamaddonfard *et al.*, 2013). All the administrations were done by oral intubation once daily for six weeks. After the administrations, rats were anaesthetized to obtain the tissues for biochemical analyses.

**Biochemical Analyses**

Acetyl cholinesterase (AchE) activity was assayed according to the method of Ellman *et al.* (1961). $Na^+/K^+$ ATPase activity was assayed using the method of Fritz and Hamrick (1966) as reported by Desaiah and Ho (1979) while $Ca^{2+}$ ATPase activity was assayed according to the method of Lowry and Lopez (1946) as reported by Philips and Hayes (1977).

Statistical Analysis

Data were analyzed with one-way ANOVA using the GraphPad Prism 8.0.2 (263). Post hoc Tukey test was used to compare between the groups. The data are presented as the mean ± standard error of means (SEM). Differences were considered significant when the $p<0.05$.

**RESULTS**

Ache activity was significantly ($p<0.05$) elevated in tramadol intoxicated rats when compared with controls. However, CPSO treatment showed a significant ($p<0.05$) reversal in the trends of these markers to a level comparable to those observed in the control groups (NC, NSC and CPSO) when compared to the group that received tramadol without treatment. The effect of the CPSO was in a dose dependent manner The result equally showed that there was no significant ($p>0.05$) difference in the group administered CPSO only when compared with the normal controls.

Tramadol administration to albino rats for six weeks caused a significant ($p<0.05$) decreased in the activities of $Na^+/K^+$ATPase and $Ca^+$ATPase when compared to controls. However, treatment with CPSO significantly ($p<0.05$) increased the activities of $Na^+/K^+$ATPase and $Ca^+$ATPase in a dose dependent manner when compared to groups that received tramadol alone. The result equally revealed that there was no significant ($p>0.05$) difference in the group administered CPSO only when compared with the normal controls.

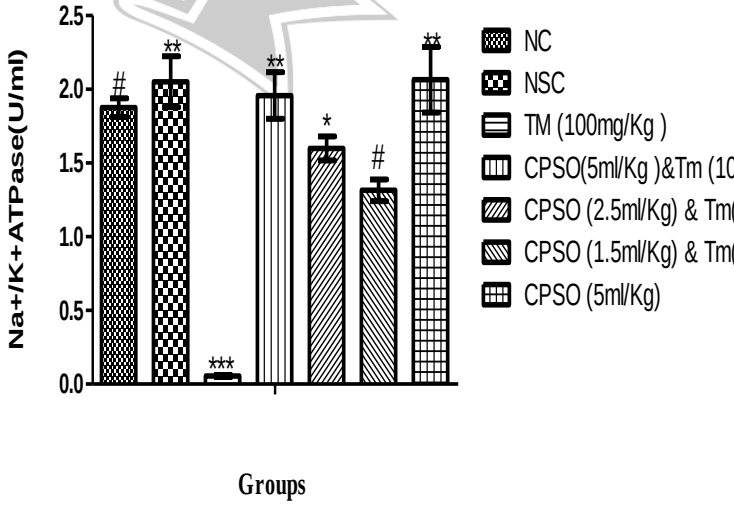

**Figure 1: $Na^+/K^+$-ATPase Activity of Tramadol Induced Toxicity in Albino Rats Treated with CPSO. Data are shown as mean ± S.D (n=8).**

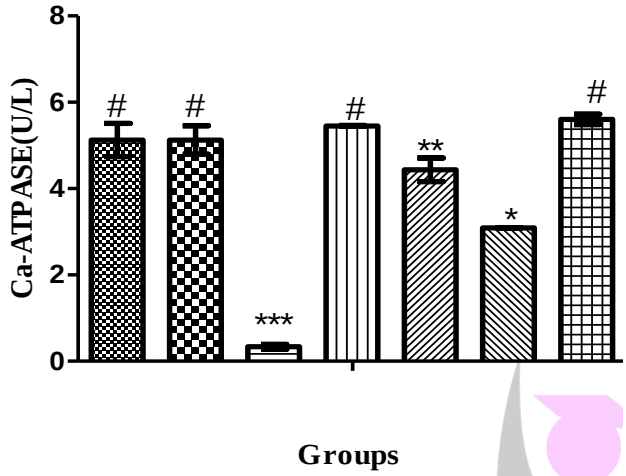


**Figure 2: $Ca^+$-ATPase Activity of Tramadol Induced Toxicity in Albino Rats Treated with CPSO. Data are shown as mean ± S.D (n=8).**

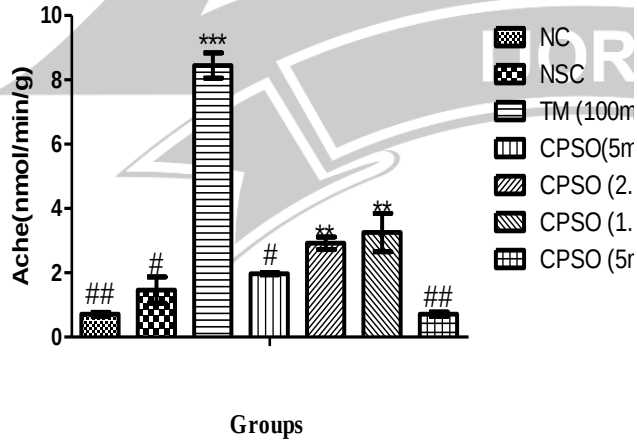


**Figure 3: Ache Activity of Tramadol Induced Toxicity in Albino Rats Treated with CPSO. Data are shown as mean ± S.D (n=8).**

## DISCUSSION

Induction of toxicity to albino rats using tramadol caused significant ($p<0.05$) increase in the activity of acetylcholine esterase relative to the normal control. This result is in agreement with the study of Essam *et al.* (2014) who reported that oral administration of tramadol once daily for 28 days significantly ($p<0.05$) decreased the monoamine neurotransmitter (nor-epinephrine (NE), serotonin (5-HT) and dopamine (DA)) levels in streptozotocin-induced diabetes in sprague-dawley rats during painful diabetic neuropathy. It also correlate with the work of El-Baky and Hafez (2017) who reported a significant ($p<0.001$) increase in plasma monoamine oxidase (MAO) leading to serotonin (5-HT) and dopamine decrease in brain tissues proteins ($p<0.001$) in tramadol administered albino rats.

The significant ($p<0.05$) increase in brain acetyl cholinesterase (AChE) activity by this drug could explain in part, the cognitive and memory dysfunction in the users of tramadol or morphine. This comes in contact with Hosseini-Sharifabad *et al.* (2016), who reported memory impairing action for tramadol. Consequently, it may lead to many problems such as bad social adaptation and decreasing productivity of work or may even lead to many deaths. In addition, the increased dose levels of the drug did not induce more changes in most of the studied parameters as reported in the preliminary result, due to the fact that, the long-term use of opioids can, however, result in tolerance and dependence. Abd El-Hamid and Ghada (2017) reported that acetylcholine esterase (AChE) activity in the brain cerebral cortex significantly ($p<0.05$) increased following the administration of therapeutic repeated doses of either tramadol or morphine in different groups. Acetylcholine esterase (AChE) is the enzyme that catalyzes the hydrolysis of the neurotransmitter acetylcholine (ACh) into choline and acetic acid. Acetylcholine esterase hydrolyzes ACh faster than the other choline esters. Moreover, in brain normal activity of

acetylcholine esterase, it is essential for the healthy function of the brain, and changes in AChE activity are reported to be accompanied by clear signs of neurobehavioral toxicity. The central cholinergic neurotransmission in the brain is crucial for cognitive functions including memory and learning (Schliebs and Arendt, 2011). Consequently, changes in central cholinergic activity will be of an important factor on cognitive functions.

*Cucurbita pepo* seed oil (CPSO) treatment at the doses of 1.5, 2.5 and 5 ml/Kg body weight of the rats showed a significant ($p<0.05$) dose dependent reversal in the trends of these markers to a level similar to the level observed in both normal control and normal saline control when compared to group that received tramadol without treatment.

The effect of *Cucurbita pepo* seed oil could be attributed to the presence of pentadecanoic acid and methyl-thyl ester in the oil due to its ability to stabilize and protect the mitochondrial function by improving the mitochondrial membrane potential and reversing the decrease in ATP production as well as to its potent effect against oxidative stress. Vitamin E has been shown to increase neurotransmitters anabolism in the brain by activating the tyrosine hydroxylase, which is a rate limiting enzyme for the biosynthesis of the neurotransmitters in the brain. Adachi *et al.* (1999) demonstrated that the activity of tyrosine hydroxylase, which is a rate limiting enzyme for the biosynthesis of the neurotransmitters in the brain, was significantly lower in the vitamin E deficient rats than that of the controls. This means that monoamine anabolism in the vitamin E deficient rat brain could be impaired and the supplementation with vitamin E could reverse the effect. In addition, *Cucurbita pepo* seed oil may have the capability to preserve neuronal resistance and probably to recover the atrophying neurons (Roghani and Behzadi, 2001). The effect of *Cucurbita pepo* seed oil could equally be attributed to the antioxidant activity and scavenging capability of various reactive oxygen species generated by tramadol toxicity due to the presence of some antioxidant bioactive components.

In the present investigation, $Na^+/K^+$ATPase and $Ca^+$ATPase activities were significantly ($p<0.05$) decreased in tramadol administered rats when compared to controls. This agrees with the following findings; Abd El-Hamid and Ghada (2017) who reported a significant decrease in the activities of $Na^+/K^+$-ATPase in the brain cerebral cortex of repeated therapeutic doses of tramadol or morphine for 21 consecutive days. Hanaa *et al.* (2009) who revealed that rotenone administration for 50 days produced significant decrease in brain $Na^+/K^+$-ATPase activity. Visweswari *et al.* (2010) who reported that activities of three ATPases ($Na^+/K^+$, $Mg^{2+}$ and $Ca^{2+}$) were decreased in brain during pentylenetetrazol (PTZ)-induced epilepsy. Sushma *et al.* (2007) who reported that with sublethal dose (3.5 mg/Kg body weight) of aluminium acetate, total ATPase activity was decreased in brain, liver, kidney, heart, muscle and testis of albino mice with decrement enhanced with the increase of aluminium acetate.

Tramadol as a well known neurotoxin reduced $Na^+/K^+$-ATPase activity due to inhibition of complex I of the mitochondrial electron transport chain, which in turn reduces the ATP-producing capacity of cells. Since ATPases play a pivotal role in maintenance of cellular ionic gradient, their inhibition probably enhance neuronal excitability and facilitates the appearance of excitatory activity and convulsions (Vasilets and Schwartz, 1993). Accordingly, ATPase inhibition leads to uncontrolled dendrite discharge in purkinje cells of rat cerebellum and causes electrographically recorded seizures in mice (Jamme *et al.*, 1995). Since the $Na^+$, $K^+$ and $Ca^{2+}$ ions are important in the development and conduction of action

potential, the decrease in the activities of respective ATPases may alter the rate of influx and efflux of cations correlating with altered membrane permeability properties. Further, the decrement in the activities of ATPases reflects the decreased turnover of ATP, presumably due to inhibition of the oxidoreductase system and uncoupling of oxidative phosphorylation. In addition, the significant decreases in the activities of brain ATPases recorded in this study may lead to cell injury and death of neurons, consequently, brain disorders (Balzan *et al.*, 2000; de Souza Wyse *et al.*, 2000) due to alterations in the gradients of sodium and potassium across the cell membrane. Tramadol may also exert its inhibiting effects by direct action on the brain enzyme protein itself. Since $Na^+$ and $K^+$ play a role in body functions including transmission of nerve signals, fluid balance and various chemical reactions, abnormal activity of brain $Na^+/K^+$-ATPase could be the cause of many different types of neurological disorders.

Treatment with CPSO significantly ($p<0.05$) increased the activities of this ATPases towards normal control values in a dose dependent manner when compared with untreated group. This is in line with the work of Visweswari *et al.* (2010) who reported that activities of three ATPases ($Na^+/K^+$, $Mg^{2+}$ and $Ca^{2+}$) were increased in brain regions of PTZ-induced epileptic rats pretreated with different extracts of *Centella asiatica*. This also agrees with the work of Hanaa *et al.* (2009) who reported that co-administration of CPSO produced significant increase in brain $Na^+/K^+$-ATPase activity of albino rats under rotenone administration. This equally concur with the studies on Bacoside A isolated from *Bacopa monniera* which showed that administration of Bacoside A inhibited lipid peroxidation, thus, improving the activities of $Na^+/K^+$-ATPase, $Ca^{2+}$-ATPase, and $Mg^{2+}$-ATPase, hence maintaining the ionic equilibrium (Anbarasi *et al.*, 2000). The effect of the oil observed in this study could be due to the antioxidant properties (Bastianetto and Quirion, 2002) of the seed oil, since Nicolson demonstrated that administration of antioxidants can prevent excess oxidative membrane damage, restore mitochondrial and other cellular membrane functions (Abdel-Rahman, 2006; Nicolson, 2007). This property also suggests that the bioactive factors such as octadecadienoic acid, methyl ester and octadecenoic acid present in this oil could offer neuroprotection by directly or indirectly modulating the activities of ATPases and thus may be helpful in the management of neurological associated diseases. This study suggests that TM-induced cerebral toxicity by increase in Ache and down regulations of ATPases activities. However, the inhibition of the alterations in the examined markers by CPSO could be relevant in the management of TM-induced toxicity.

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