

Original Research Article

Antioxidative Potential of *Telfairiaoccidentalis* Aqueous Leaves Extract in Mitigating Cyclophosphamide-Induced Neurotoxicity in Wistar Rats

ABSTRACT

Aim: This study sought to investigate the antioxidative potentials of *Telfairiaoccidentalis* aqueous leaves extract (TOAE) on cyclophosphamide (CP) induced neurotoxicity in rats.

Study design: This study sought to investigate the antioxidative potentials of *Telfairiaoccidentalis* aqueous leaf extract (TOAE) on cyclophosphamide induced neurotoxicity in rats.

Place and duration of the study: This study was carried out in the Department of Anatomy, Olabisi Onabanjo University, Sagamu, Ogun State, Nigeria between October, 2023 and September, 2024

Methods: 25 Adult wistar rats were assigned into five different groups I-V of (n=5) which were respectively exposed for 14 days as follow to Normal saline, TOAE only (400mg/kg b.w), 400mg/kg b.w TOAE with single dose of 100 mg/kg CP on 14th day, single dose 100 mg/kg CP only on day 1 and single dose of 100 mg/kg CP only on day 1 followed by 400mg/kg b.w of TOAE for 14days. CP was administered intraperitoneally and TOAE was administered orally. All the animals were sacrificed at the end of designated exposure and processed for biochemical evaluations using standard protocols.

Results: CP mediated inflammation in cerebellum of rats with a significant increase in MDA, CAT, IL-1 β and TNF- α significant decrease in SOD and Gpx levels compared with a control group. Lipid peroxidation was also induced following exposure of rats to CP. There is a marked decrease in antioxidant enzymes activities in CP group. However, treatment with TOAE markedly inhibited the inflammation by reversing the elevated levels of inflammatory markers. Furthermore, TOAE suppressed the lipid peroxidation chain reaction by reducing the level of malondialdehyde (MDA) and catalase CAT. TOAE attenuates CP-induced oxidative stress by increase the level of GPx and superoxide dismutase enhancing the activities of the cytoprotective enzymes (catalase and glutathione-S- transferase). Serum levels of Interleukin-2/1-beta and Tumor Necrosis Factor-alpha (TNF- α), were measured on the 15th day after

exposure. The effect of TOAE was deduced on anti-inflammatory and antioxidants properties. Taken together, TOAE inhibited inflammation, suppressed lipid peroxidation, attenuated oxidative stress, and enhanced the antioxidant enzymes activities.

Conclusion: These therapeutic effects of TOAE might be due to its phytochemicals. The findings of this study indicate that aqueous leaf extract of *T. occidentalis* could be a drug candidate for the treatment of the immunosuppressive effect of cyclophosphamide on neurotoxicity.

Keywords: *Telfairia occidentalis*, Cyclophosphamide, Cerebellum, Antioxidant, Oxidative stress, Neurotoxicity

INTRODUCTION

“Cyclophosphamide (CP) is a cytotoxic alkylating drug with a high therapeutic index and broad spectrum of activity against various cancers. It is known to cross the blood-brain barrier” (Zarei M and Shivanandappa T 2016). “Oxidative stress induced by CP exposure leads to functional changes in the brain associated with neuronal cell death” (Andersen JK 2004). “The brain is vulnerable to oxidative stress due to its high oxygen consumption, presence of unsaturated lipids, and limited antioxidant defenses” (Shulman RG 2004).

“Neuroprotective agents are of therapeutic interest due to their potential to ameliorate the effects of cytotoxic agents by protecting nervous tissue without compromising anti-tumor efficacy” (Zarei M and Shivanandappa T 2016). “Natural products, with their safety profiles and powerful antioxidant constituents such as polyphenols and flavonoids, have shown promise for developing safe therapeutics to alleviate the toxic side effects of anticancer drugs” (Zarei M and Shivanandappa T 2013).

“Oxidative stress is considered the cornerstone of cyclophosphamide-induced neurotoxicity pathophysiology. Brain tissues are critically vulnerable to oxidative damage due to their limited antioxidant capacity” (Garbarino VR et al, 2015). “Neurons and other brain cells are incapable of producing glutathione (GSH), relying on surrounding astrocytes for GSH precursors” (Mandal PK et al, 2015). “Various studies have demonstrated that CP-induced oxidative stress in the brain is mediated through overproduction of reactive oxygen species (ROS), GSH depletion, and

inhibition of antioxidant enzyme activities” (Singh S and Kumar A 2019; Zarei M and Shivanandappa T 2016).

“The antioxidant effect is mainly due to components such as flavonoids. Antioxidants can interfere with the oxidation process by reacting with free radicals, chelating catalytic metals, acting as oxygen scavengers”(Nkereuwem AO et al, 2011; Shahidi, F and Wanasundara, PK 1992) and preventing lipid auto-oxidation (Brand-Williams W et al, 1995; Bondet V et al,1997). “Research has justified the use of *Telfairiaoccidentalis* leaves in Nigeria for treating certain diseases involving reactive oxygen species (ROS), attributing this to its antioxidant and free radical scavenging abilities” (Kayode AA and Kayode OT 2011).

“The cerebellum is responsible for coordinating skilled voluntary movements and is active in precision, motor learning, and timing of movements. It undergoes major development from the 3rd trimester to the infant period”(Ali AHA et al., 2021; Salem M et al, 2010) making it highly susceptible to injury, especially in young children (Wang SS et al, 2014). “The cerebellum's cells depend on food consumption and subsequent amino acid concentrations in the bloodstream”(Keshavarz M et al, 2013; Gouda SA et al, 2010). “It plays a crucial role in making postural adjustments to maintain balance and coordinating the timing and force of different muscle groups to produce fluid limb or body movements” (Drake RL et al, 2020).

“*Telfairiaoccidentalis* Hook. f. is an important staple vegetable grown in Nigeria, producing luxuriant edible green leaves rich in iron and vitamins. The leaves are known for analgesic and anti-inflammatory properties”(Osunkoya OA et al, 2016). “The plant is grown principally for its leaves and seeds, which are important soup condiments”(Oboh G 2005). Recent interest has focused on the antioxidant ability and benefits of phytochemicals in food and vegetables (Oboh G and Akindahunsi AA 2004) which have been used for various purposes, including nutrition, medicine, flavoring, and industrial applications.

“Many vegetables contain health-protective constituents essential for preventing diseases and maintaining well-being”(Oboh G 2005). “They contain phytochemicals with significant antioxidant capabilities that can lessen the toxic load (oxidative stress) on the liver by binding to various harmful substances, including lead, mercury, and chlorinated hydrocarbons. The use of these vegetables, along with fruits and other herbs, is increasing due to their numerous phytochemicals and antioxidants”(Oboh G 2004).

“Preliminary investigations revealed that *T. occidentalis* leaves are popularly consumed in many Nigerian homes due to various medicinal potentials ascribed to them. This could be attributed to the leaf's richness in phytochemicals with antioxidant activity, such as phenols and ascorbic acid”(Oboh G and Akindahunsi AA 2004). “Many phenolics, including flavonoids, have antioxidant capacities much stronger than vitamins C and E. Flavonols and flavones are particularly important because they possess antioxidant and free radical scavenging activity in foods, and some evidence shows that flavonoids can protect membrane lipids from oxidation”(Amic D et al, 2003).

“*T. occidentalis* is rich in flavonoids, phenolic compounds, and iron, possessing antioxidant, antihepatic, anticancer, antidiabetic, anti-inflammatory, and neuroprotective properties”(Aderibigbe AO et al, 1999; Oboh, G 2005; Kayode AA and Kayode OT 2011; Adejuwon AA and Olopade JO 2014). Given the health benefits of *T. occidentalis*, this study was designed to evaluate the protective effect of its aqueous extract on cyclophosphamide-induced oxidative stress in rat cerebellum.

MATERIALS AND METHODS

Experimental Animals

Twenty-five Wistar rats, weighing between 150-200g, were used for the experiment. The animals were obtained from Peter's Farm [Nig.] Enterprises in Sagamu, Nigeria. They were kept in wire mesh plastic cages in the animal holdings of the Department of Anatomy, Olabisi Onabanjo University, Sagamu campus, Nigeria, where they were allowed an acclimatization period of two weeks under standard laboratory conditions with free access to water and standard laboratory mouse chow as obtained from Boar feeds Ltd, Ikenne, Ogun state and were confirmed free from any pathological condition. Their daily body weights were monitored and documented.

Cyclophosphamide powder [LobaCheme PVT Ltd, Mumbai, 40005, India] was purchased from MEX Pharmaceutical limited, Sagamu, Ogun State, Nigeria. All other chemicals and reagents were purchased from Jaybees Medical Limited Abeokuta, Ogun State. Phosphate Buffer Saline [PBS] at pH = 4.0 was prepared and stored in the refrigerator at 4°C. A fresh sample of *Telfairia occidentalis* leaves was purchased from Ikenne market, Ogun State, Nigeria. The plant was identified and deposited at the herbarium of the Department of Plant Science, Olabisi

Onabanjo University, Nigeria, in August 2023. This research was conducted between August 2023 and February 2024.



Picture 1: *Telfairia occidentalis* Plant (Airaodion, AI et al, 2019).

Preparation of Extract

Fresh leaves were thoroughly rinsed and air-dried at room temperature [24°C], then pulverized, crushed into fine powder using a manual blender, and weighed. Aqueous extracts of the plants were prepared by soaking 1000g of the dry powdered plant materials in 5 liters of double distilled water and then kept at room temperature for 48 hours [for thorough extraction]. At the end of the 48 hours, the extracts were filtered first through a Whatman filter paper No. 42 [125mm] and then through cotton wool. The filtrate was concentrated using a rotary evaporator with the water bath set at 40°C to one-tenth its original volume and then finally freeze-dried. The dried residue [crude extract] was then stored at 4°C. Aliquot portions of the crude plant extract residue were weighed and dissolved in distilled water for use on each day of the experiments.

Preparation, Dosage, and Administration of Extract

For each fresh preparation, one gram of the extract was dissolved in 10 mL of distilled water to make the stock solution termed *Telfairia occidentalis* aqueous extract [TOAE]. Previous reports of Ajao MY and Akindele AJ 2013; Owoeye O and Gabriel MO 2016) guided the experimental dosage.

Experimental Design

At the end of acclimatization period, all the rats were assigned into 5 groups (n=5) as Group 1 which served as control and were exposed to standard mouse chow and water ad libitum while Group 2 were exposed to daily 400mg/kg of TOAE via oral intubation for 14 days. The Group 3 animals were exposed to daily 400mg/kg of TOAE via oral intubation for 14 days with single dose of 100mg/kg of CP on the 14th day while Group 4 were exposed to single dose of 100mg/kg of CP only on the 1st day and given standard mouse chow and water ad libitum for 14 days. The

Group 5 animals were given single dose of 100mg/kg of CP only on the 1st day followed by daily 400mg/kg of TOAE via oral intubation and standard mouse chow with water ad libitum for 14 days. At the end of exposure all animals were sacrificed by cervical dislocation and processed for biochemical analyses of Superoxide dismutase (SOD), Glutathione peroxidase (GPx) and Malonaldehyde (MDA) as previously described by FakunlePB et al, 2013 as well as Catalase as previously described by OdubelaOO et al, 2024. The TNF- α and IL-1 β were processed as previously described by Dan-in J et al, 2021 and Gaffney EV et al, 1989 respectively.

RESULTS

The aim of this study was to determine the therapeutic and protective effects of *Telfairiaoccidentalis* against cyclophosphamide-induced neurotoxicity. We hypothesized that with the intervention of appropriate therapeutics, it should be possible to manage the CP damage and provide beneficial neuroprotection. Biochemical analysis results are outlined in Figures 1-4. The results of pro-inflammatory markers are shown in Figures 5 and 6.

Cerebellum lipid peroxidation, estimated as nmol malondialdehyde (MDA)/100 mg tissue, showed a significant ($P < 0.001$) increase in CP rats when compared to the control group. Treatment with 400 mg/kg TOAE significantly ameliorated the increased MDA and CAT levels (Fig. 1, 2). Similarly, the activities of cerebellum antioxidant enzymes, SOD and GPx, showed a significant decrease in CP rats (Figs. 3, 4). Along with concurrent administration of CP, TOAE significantly increased the activities of SOD ($P < 0.001$) and GPx ($P < 0.01$).

The effect of CP administration on serum TNF- α and IL-1 β expression in the cerebellum is represented in Figs. 5 and 6. The recorded data showed a significantly increased serum TNF- α concentration accompanied by a significant ($P < 0.01$) upregulation of both TNF- α and IL-1 β expression in CP rats when compared to the control group. TOAE significantly decreased the increased serum TNF- α and IL-1 β and the CP-induced expression of cerebellum TNF- α and IL-1 β .

The effect of *Telfairiaoccidentalis* and cyclophosphamide on the MDA and CAT levels

As shown in Fig. 1, in the CP group, the MDA levels were insignificantly increased compared to the control group. *Telfairiaoccidentalis* administration significantly reduced MDA concentration compared to the CP group ($P < 0.01$). Besides, CP administration decreased CAT levels in contrast to the control group. However, *Telfairiaoccidentalis* significantly elevated CAT levels in comparison with the CP group ($P < 0.05$).

The effect of *Telfairiaoccidentalis* and cyclophosphamide on the SOD and GPx activity

The mean activity of GPx and SOD in the cerebellum of the CP group insignificantly decreased in comparison to the control group. The *Telfairiaoccidentalis* treated groups showed a considerable increase in SOD and GPx levels compared to the CP group (Figs.2 and 3).

The effect of *Telfairiaoccidentalis* and cyclophosphamide on the TNF- α and IL-1 β production

We assessed whether *Telfairiaoccidentalis* decreases the production of the pro-inflammatory cytokines TNF- α and IL-1 β induced by cyclophosphamide. The neurotoxic effects of CP resulted in significant elevation of TNF- α and IL-1 β concentrations in cerebellum tissues when compared to the control group ($P < 0.001$). However, the tissue levels of these cytokines were significantly reduced in the *Telfairiaoccidentalis* only ($P < 0.001$), TOAE plus CP, and CP plus *Telfairiaoccidentalis* ($P < 0.05$) groups in comparison to the CP group only (Figures 5 and 6). These results indicate that *Telfairiaoccidentalis* alone and co-administration of CP with *Telfairiaoccidentalis* decrease TNF- α and IL-1 β production.

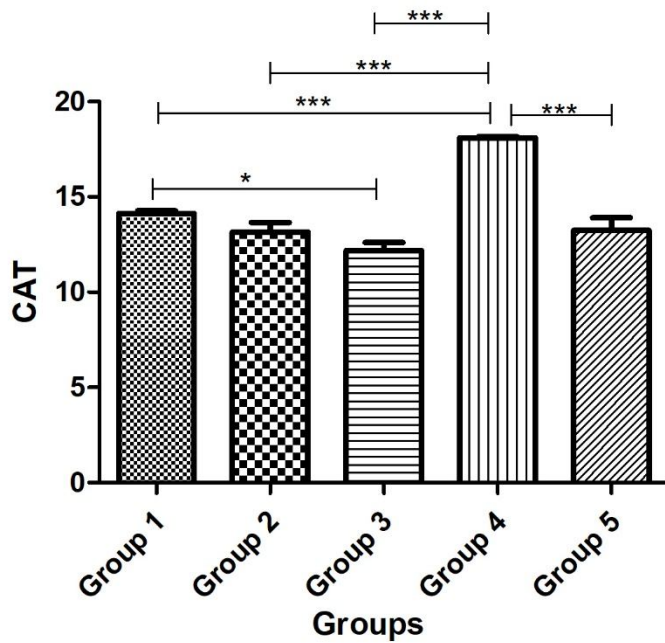


Fig. 1:The protective effect of TOAE cerebellar catalase [CAT] activity of Grp 1 (Control), Grp 2 (400 mg/kg TOAE only), Grp 3 (400 mg/kg of TOAE +100mg/kg CP), Grp 4 (100 mg/kg CPA only), Grp 5 (CP 100 mg/kg + TOAE 400mg/kg. Values are expressed as Mean ± Standard Error of Mean. Significant differences were denoted by ***($p < 0.001$), **($p < 0.01$) and and *($p < 0.05$).

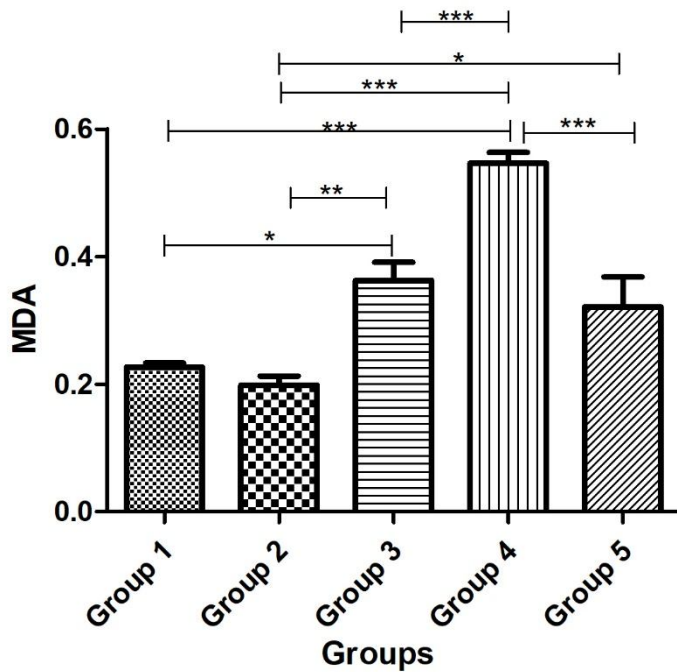


Fig. 2: The protective effect of *T. occidentalis* on malondialdehyde content of cerebellum of Grp 1 (Control), Grp 2 (400 mg/kg TOAE only), Grp 3 (400 mg/kg of TOAE +100mg/kg CP), Grp 4 (100 mg/kg CPA only), Grp 5 (CP 100 mg/kg + TOAE 400mg/kg). Values are expressed as Mean ± Standard Error of Mean. Significant differences were denoted by ***($p < 0.001$), **($p < 0.01$) and *($p < 0.05$).

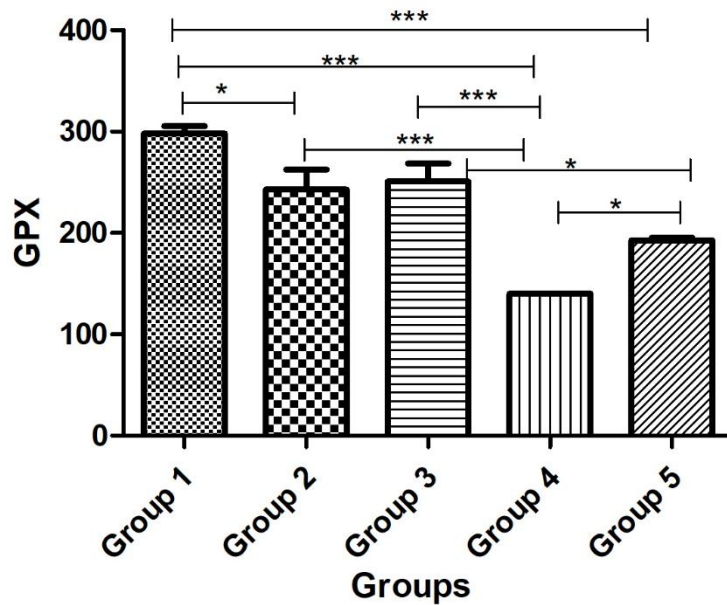


Fig. 3:The protective effect of TOAE Cerebellum of glutathione peroxidase activity of Grp 1 (Control), Grp 2 (400 mg/kg TOAE only), Grp 3 (400 mg/kg of TOAE +100mg/kg CP), Grp 4 (100 mg/kg CPA only), Grp 5 (CP 100 mg/kg + TOAE 400mg/kg). Values are expressed as Mean \pm Standard Error of Mean. Significant differences were denoted by ***($p < 0.001$), **($p < 0.01$) and and *($p < 0.05$).

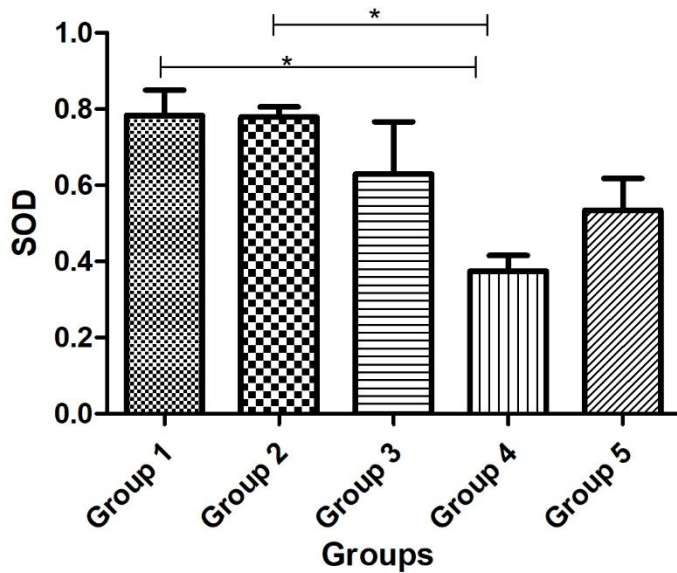


Fig. 4:The protective effect of TOAE Cerebellum superoxide dismutase activity of Grp 1 (Control), Grp 2 (400 mg/kg TOAE only), Grp 3 (400 mg/kg of TOAE +100mg/kg CP), Grp 4 (100 mg/kg CPA only), Grp 5 (CP 100 mg/kg + TOAE 400mg/kg). Values are expressed as Mean \pm Standard Error of Mean. Significant differences were denoted by ***($p < 0.001$), **($p < 0.01$) and and *($p < 0.05$).

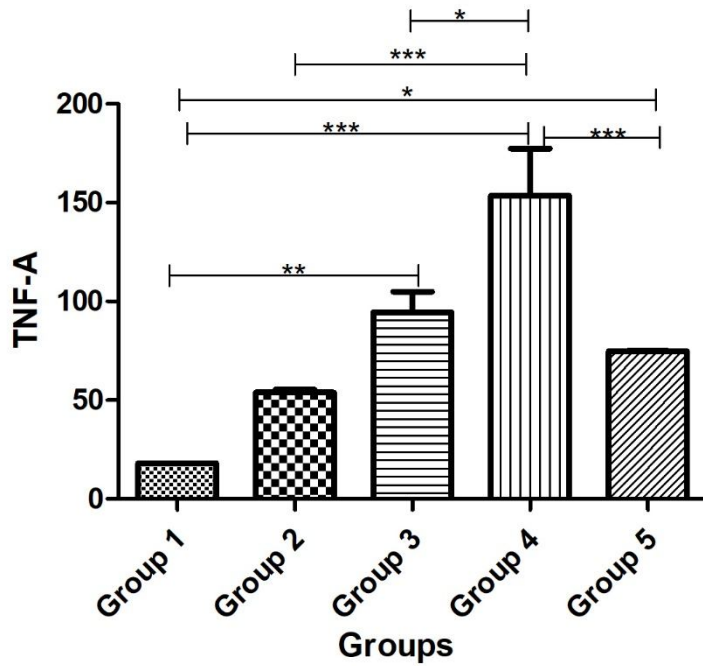


Fig. 5: The protective effect of TOAE cerebellum TNF- α expression of of Grp 1 (Control), Grp 2 (400 mg/kg TOAE only), Grp 3 (400 mg/kg of TOAE +100mg/kg CP), Grp 4 (100 mg/kg CPA only), Grp 5 (CP 100 mg/kg + TOAE 400mg/kg). Values are expressed as Mean \pm Standard Error of Mean. Significant differences were denoted by ***($p < 0.001$), **($p < 0.01$) and *($p < 0.05$).

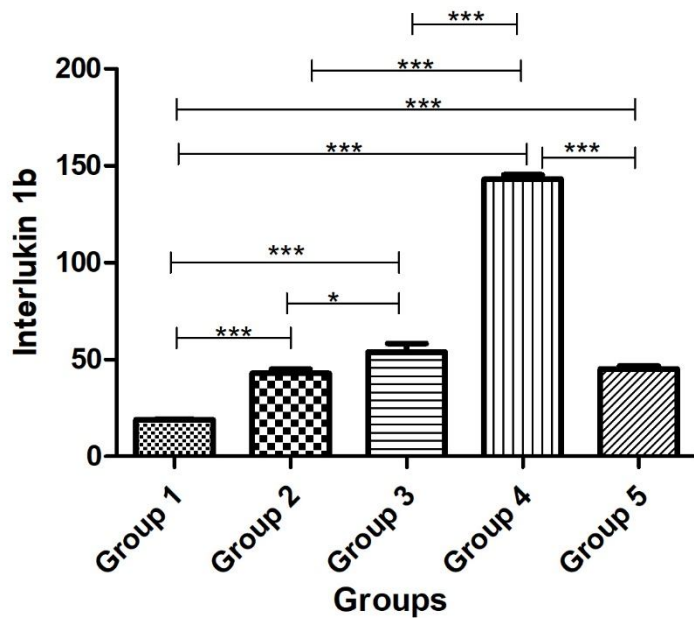


Fig. 6:The protective effect of TOAE cerebellum IL-1B expression of Grp 1 (Control), Grp 2 (400 mg/kg TOAE only), Grp 3 (400 mg/kg of TOAE +100mg/kg CP), Grp 4 (100 mg/kg CPA only), Grp 5 (CP 100 mg/kg + TOAE 400mg/kg). Values are expressed as Mean \pm Standard Error of Mean. Significant differences were denoted by ***($p < 0.001$), **($p < 0.01$) and *($p < 0.05$).

DISCUSSION

“Brain cells are more at risk from free radical damage because the brain contains more free form of iron that is responsible for the formation of ROS [reactive oxygen species]”(Ali, AHA, et al,2021; Leelavinothan P and Muniappan L 2004). “The vulnerability of the brain to oxidative stress produced by ROS is due to its utilization of about one-fifth of the total oxygen demand of the body and its relatively poor antioxidant enzyme content”(Vincent AM et al, 2024; Seo EJ et al, 2018). “Neurotoxicity caused by cyclophosphamide can manifest in numerous ways, including anxiety, depression, motor dysfunction, and cognitive deficits”(Ibrahim S et al, 2023).

“Several investigations have demonstrated that oxidative stress is a significant component of cyclophosphamide-induced neurotoxicity”(Singh S and Kumar A 2019). Additionally, “different

reports estimated the endogenous antioxidant proteins and several oxidative stress markers to assess the dimension of oxidative stress in cyclophosphamide-exposed animals, which leads to excessive oxidative stress and causes cell toxicity”(Singh S and Kumar A 2019; Gonzalez H et al, 2014; Sahinturk V et al, 2018).

“Considering oxidative stress as a fundamental component of cyclophosphamide-induced neurotoxicity”(Singh S and Kumar A 2019) and given the high antioxidant property of *Telfairiaoccidentalis* that has been established (Eseyin O et al, 2014), it is likely that the cancer chemopreventive activity of the seed could be attributed to the antioxidant components and activity of the plant (Eseyin O et al, 2014). “Numerous research works on the leaves of *T. occidentalis* have affirmed its antioxidant effect”(Eseyin O et al, 2017; Obog G et al, 2006; Nkereuwem AO et al, 2011).

“The current medical treatments for this disease, such as anti-inflammatory and immunosuppressant drugs, are associated with adverse effects, thus necessitating the need for an alternative, safe, affordable, and effective drug candidate. *T. occidentalis* is an herb with much therapeutic and biological significance, such as anti-inflammatory, antioxidant, and cytoprotective properties. This study investigated the curative ability of the aqueous leaf extract of *T. occidentalis* in cyclophosphamide-induced neurotoxicity in rats” (Eseyin O et al, 2017).

This study demonstrated that *T. occidentalis* inhibited experimental oxidative stress, lipid peroxidation, and inflammation. The 100 mg/kg B.W of cyclophosphamide induced neurotoxicity in rats for this experiment. “Rats that were intraperitoneally exposed to 100 mg/kg B.W of cyclophosphamide alone exhibited different kinds of clinical symptoms such as weight loss, hair loss, loss of appetite, and change in body color. The preclinical manifestations were markedly expressed toward the end of the experiment. These findings are in tandem with previous reports documented in other DSS-induced colitis model studies”(Hasnat MA et al, 2015; Li Y et al, 2016) . However, rats treated with 400 mg/kg bw of *T. occidentalis* markedly improved the clinical score, indicating the curative ability of *T. occidentalis* in this model of neurotoxicity.

One of the major players in the pathogenesis of cyclophosphamide is oxidative stress. Cellular macromolecules including DNA, lipids, and proteins are prone to oxidative cellular damage via attack by free radicals and reactive oxygen/nitrogen species (ROS/RNS). “In this study, rats

received 100 mg/kg cyclophosphamide intraperitoneally on the first day followed by 14 days of *Telfairiaoccidentalis aqueous extract* administration at 400 mg/kg orally. *Telfairiaoccidentalis aqueous extract* could effectively guard against cyclophosphamide-induced oxidative brain damage by upgrading activities of the antioxidant enzymes CAT and SOD, together with reducing brain MDA levels and glutathione peroxidase (GPx). The antioxidant property of *Telfairiaoccidentalis* is attributable to the high content of polyphenols, especially flavonoids” (Eseyin O et al, 2014; Potterat O (1997). “These findings are in tandem with previous reports documented in other cyclophosphamide-induced neurotoxicity model studies”(Ali AHA et al, 2021; Singh S and Kumar A 2019).

“Oxidative stress, which is an imbalance between antioxidant and pro-oxidant factors, has been suggested to play a significant role in the induction of mucosal tissue injury and intestinal inflammation in colitis (Sengul N et al, 2011. SOD performs a beneficial function by converting superoxide radical [O⁻] into hydrogen peroxide [H₂O₂], thereby shielding cells against oxidative damage. The generated H₂O₂ is then bio-transformed into water [H₂O] by catalase and glutathione peroxidase to protect against the deleterious effects of H₂O₂. Previous studies have demonstrated that these antioxidant enzymes' activity levels were reduced in cyclophosphamide-induced neurotoxicity”(Ramchandani S et al, 2016). The results from this study agreed with this, as activity levels of SOD and GPx decreased in the cerebellum of cyclophosphamide-treated rats. However, these antioxidant enzymes' activity levels were markedly increased in rats treated with 400 mg/kg bw of *Telfairiaoccidentalis*. These results indicated that treatment with *Telfairiaoccidentalis* in neurotoxicity might inhibit the degree of cerebellar injury via its free radical scavenging and antioxidant activities.

“Superoxide dismutase [SOD] plays a key role in the detoxification of superoxide radical, thereby protecting cells from damage induced by free radicals”(Adejuwon AA et al, 2014). “The observed decrease in SOD activity after cyclophosphamide exposure suggests increased production of superoxide radicals”. Farombiet al. (Farombi EO 2012) suggested that superoxide radicals by themselves, or after their formation of H₂O₂, caused oxidation of GPx enzymes and thus decreased SOD activity. In this study, the reduction in GPx activity may indicate the inability of the brain cells to get rid of H₂O₂ generated due to exposure to cyclophosphamide as previously reported by(Adejuwon AA et al, 2014). It may also imply enzyme inactivation caused

by excess ROS formation in the brain. The brain contains less CAT levels, and hence GPx has a major role in quenching H₂O₂ and other peroxides, which otherwise would lead to the production of hydroxyl and peroxy radicals in the presence of insult (Adejuwon AA et al, 2014; Halliwell B and Gutteridge, JM 2015; Dringen R et al, 2005).

“Unsaturated lipids are predominantly prone to lipid peroxidation and oxidative alterations, which are remarkable indicators of oxidative stress. Lipid peroxidation is a deteriorating pathway of membrane components [unsaturated fatty acid and arachidonic acid] which is induced by the presence of radicals to produce highly reactive lipid peroxy radicals that facilitate chain reactions which further attack other unsaturated fatty acids”(Ramchandani S et al, 2016; Ayala A et al, 2014). “An increase in the production of free radicals stimulates lipid peroxidation, which leads to increased levels of malondialdehyde”(Niki E 2009). In this study, cyclophosphamide induction marked elevation in the level of MDA as an indicator of lipid peroxidation. However, treatment with *Telfairia occidentalis* results in a significant reversal in the levels of MDA. The inhibitory effect of the extract on lipid peroxidation may be due to the presence of flavonoids and other phytochemicals that have been documented to inhibit the lipid peroxidation chain reactions.

“Cyclophosphamide produced inflammatory responses”(Ahlmann M and Hempel G 2016). “The coordinated activation of signaling pathways that regulate the levels of inflammatory mediators in tissue-resident cells and blood-derived inflammatory cells is called inflammation. Inflammation is the root cause of many chronic diseases, including cardiovascular disease, bowel disease, diabetes, arthritis, and cancer. Inflammatory response mechanisms are similar regardless of the initial stimulus. When cell surface pattern receptors recognize harmful stimuli, inflammatory pathways are activated, inflammatory markers are secreted, and inflammatory cells are recruited. Inflammation is caused by primary inflammatory stimuli such as cytokines like IL-1 β and TNF- α ”(Chen L et al, 2018). “TNF- α is an inflammatory cytokine that is involved in a variety of pain models”(Zhang JM and & An J 2007).

In this study, the increased blood levels of the essential cytokines IL-1 β and TNF- α in CP-induced neurotoxicity rats were markedly decreased following treatment with 400 mg/kg bw of TOAE as shown in Fig. 1, TNF- α , and IL-1 β [Figs. 5 and 6]. This finding indicates the beneficial and anti-inflammatory effects of TOAE against neurotoxicity via downregulating IL-1 β and

TNF- α . This may be due to the presence of phytochemicals with anti-inflammatory properties in the extract.

CONCLUSION

The results of this study indicated that CP induced neurotoxicity as shown in the increased of the oxidative stress biomarkers. *T. occidentalis* leaves was able to remedy this effect by regulating the oxidative stress biomarkers, thus possesses therapeutic effect against CP induced neurotoxicity and can protect the body against free radicals arising from oxidative stress. Consequently, these study suggests that co-administration of TOAE supplement with cyclophosphamide may be used as a protective effective method to overcome cyclophosphamide induced neurotoxicity.

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- 3.

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