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Neuroprotective Effects of Annona Muricata Ethanolic Leaf Extract on Cyclophosphamide-Induced Neurotoxicity in the Cerebral Cortex Using Wistar Rats

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Abstract*—Alternative medicine has a lot of role to play in indigenous medicinal approach especially when it has to do with occupational hazards and natural products, especially those derived from plants, have been used to help mankind sustain its health since the dawn of medicine. Indigenous communities in Africa and South America extensively use annona muricata in their folk medicine due to its reported medicinal properties. This study evaluated the curative properties of ethanolic Annona muricata (soursop) leaves on cyclophosphamide induced cerebral cortex toxicity using adult male wistar rats. Twenty-four adult male wistar rats (180-200g) were assigned into six groups (n=4). Group 1 was the normal control group which received 1ml of normal saline and food. Group 2 received 40mg/kg of cyclophosphamide only, groups 3, 4 and 5 were administered with 100, 200 and 300mg/kg/day respectively of the Annona muricata extract respectively for 7 days and were induced with 40mg/kg/day of cyclophosphamide on day 8. Group 6 was administered with 50mg/kg of Vitamin E for seven (7) days and induced with 40mg/kg cyclophosphamide on day 8. At the end of the experiment, toxicity was measured histopathologically by light. Photomicrographs show severe tissue encephalopathy, cerebral infarction and selective neuronal necrosis in groups 2 and 3 while that of groups 4,5and 6 appeared normal suggesting protective properties of the leaf extracts. Hence, it was established that Annona muricata leaves had protective effects on cyclophosphamide induced toxicity*.

***Keywords*—** *Cyclophosphamide, Annona muricata, extract, cortex, toxicity*.

I. INTRODUCTION

Radiation therapy and severe chemotherapy are currently being used in the treatment and management of cancer; however, because chemotherapy is not tissue-specific, it damages both diseased and healthy tissues (Christina, 2008; Abdel-Hafez et al., 2017). Researchers frequently employ the well-known immunosuppressive and chemotherapeutic drug cyclophosphamide (CLP) to impair the immune systems of test animals (Elizab et al., 2021). However, despite having a wide range of therapeutic applications, CLP can have a wide range of negative side effects, such as neurotoxicity in both people and laboratory animals (Fraiser et al., 1991). The liver (Li et al., 2010), kidney (Sakr et al., 2017), and testis (Turner and Lysiak, 2008; Turk et al., 2010) have all been linked to cyclophosphamide toxicity. According to certain research, cyclophosphamide administered intraperitoneally to rats may cause enormous cellular damage (Hanaa et al., 2010), severe tissue oxidative stress (Bhatia et al., 2020; Olukole et al., 2006; Abdel-Hafez et al., 2017), and apoptosis (Napolitano and Singh, 2002), which ultimately results in the death of both normal and malignant cells (Stankiewicz et al., 2002; Abdel-Hafez et al., 2017). Oxidative stress is induced in CLP through the cytochrome P450 mixed functional oxidase system, which activates CLP metabolically and produces two metabolites: phosphoramide mustard and acrolein (Ludeman, 1999). According to Selvakumar et al. (2005), oxidative stress is the cause of CLP neurotoxicity. Accordingly, a large range of clinical conditions are treated with a variety of herbal medications made from plant extracts, even though relatively little is known about their mechanisms of action (Ratheesh & Helen, 2007). Herbal medicine has been demonstrated to be an invaluable aspect of modern healthcare in underdeveloped nations due to its economic and social benefits, making the advancement of research in this field imperative (Ighodaro et al., 2010).

The plant family Annonaceae, which includes *Annona muricata* L, is widely found in tropical and subtropical regions of the world and is usually referred to as soursop in English-speaking nations (Santos-Sánchez et al., 2018). Nonetheless, Annona muricata L has been traditionally utilized as a decoction or infusion for the treatment of a broad spectrum of maladies. These parts encompass the leaves, bark, fruit, and seed (Parveen et al., 2016; Arowora et al., 2016). Skin infections have been treated using water from cooked *Annona muricata* leaves (Longuefosse and Nossin, 1996; Boulogne et al., 2011) and the leaf's decoction has been used as an analgesic (Tene et al., 2007; Sreekeesoon and Mahomoodally, 2014). Additionally, the leaf has been explored for the treatment of cold, flu, asthma-related discomforts (Kossouoh et al., 2007; Joyeux, Mortier and Fleurentin, 1995) malarial, and hypertension in tropical countries, against headaches, insomnia, cystitis, liver problems, diabetes, anti-inflammatory, antispasm odic, and antidysenteric in folklore medicine (Badrie and Schauss, 2010; Ezuruike and Prieto, 2014).

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The fruits of the plant are eaten in Nigeria, and its infusions are used to treat or control breast cancer in its early stages as well as enlargement of the prostate. More than 200 bioactive chemicals have been found in A. muricata by earlier research, with acetogenins being the most abundant. Additionally, the plant's abundance in phenols, alkaloids, and other phytochemicals has been noted. The abundance of phytochemical components has been attributed to the antitumorigenic, hepatoprotective, anxiolytic, anti-nociceptive, anti-stress, hypotensive, wound healing, cytotoxic, antiprotozoal, insecticidal, antioxidant, antibacterial, antiviral, hypoglycemic, anticancer, and anti-tumorigenic properties of A. muricata (Coria-Téllez et al., 2018).

To the best of our knowledge, the possible role of *A. muricata* ethanolic leaf extract co-treated with cyclophosphamide in male rats remain enigmatic. The aim of the present study, therefore, is to evaluate the curative properties of ethanolic *Annona muricata* (soursop) leaf extract on cyclophosphamide induced cerebral cortex toxicity in adult male wistar rat.

II. Material and Methods

Cyclophosphamide used were of analytical grade purchased from Enugu state university teaching Hospital Pharmaceutical store, Parklane, Enugu State, Nigeria. Plant materials: A fresh sample of *A. muricata* leaves was obtained from local farms in Emene, Enugu East local government Area, Enugu State. The plant was identified by a taxonomist in the Plant Sciences and Biotechnology Department, in Enugu state university of science and technology, Nigeria.

Fresh unripe leaves of *A. muricata* were separated from the stalk and air-dried at room temperature (24 ◦C) and then pulverized into a fine powder using a manual blender. The methanol extract of the plant was prepared by soaking 342.1g of the dry powder plant material 1250 ml of 99% ethanol and shaken intermittently for 72 hours. At the end of the 72 hours, the extract was filtered with Whatman filter paper number 1. *A. muricata* methanol leaf extract was concentrated using a rotary evaporator at 40 ◦C.

Experimental Animal: Twenty-four (24) adult male wistar rats weighing 180-200g were purchased from breeding house in Animal House of the Department of Anatomy, Enugu State University college of Medicine Parklane. They were allowed free access to mouse pellets (Guinea Feed Nigeria Limited) and tap water ad libitum. The rats were kept in well-ventilated iron cages under the standard laboratory conditions (12-h light/dark cycle, 28 °C, humidity: 50%) for the two weeks of acclimatization and the period of the experiment.

Experimental Design:

The procedures employed in this study were all in line with the National Institutes of Health's protocol on handling of laboratory animals for biomedical research. The leaves were administered orally while cyclophosphamide was administered intraperitoneally.

At the end of the experiment, the animals were anaesthetized using ketamine chloride and perfusion methods was used in the preservation of the cerebral cortex. The cerebral cortex sample were collected for the histopathological examination and fixed in 10% formal saline.

TABLE 1. Showing the experimental design

GROUP	DAYS 1-7	DAY 8
1	Normal Saline	Normal Saline
2	Normal Saline	40mg/kg Cyclophosphamide only
3	100mg/kg *Annona muricata* extract daily	40mg/kg Cyclophospamide
4	200mg/kg *Annona muricata* extract daily	40mg/kg Cyclophospamide
5	300mg/kg *Annona muricata* extract daily	40mg/kg Cyclophospamide
6	Vitamin E 50mg/kg daily	40mg/kg Cyclophospamide

The formalin preserved cerebral cortex was embedded by a standard laboratory protocol. The tissue was sliced into 4 mm size and then fixed on the slides. After the slide was de-paraffinized with p-xylene and rehydrated in graded ethanol (100, 80, 70% and 50%) and then rinsed with water. Furthermore, the slide was stained with hematoxylin for 5 min and rinsed with water, and subsequently counter-stained in eosin, mounted in DPX, cover-slipped and viewed with digital light microscope. Photomicrography was carried out using Amscope 5.0 digital camera.

III. Results

Plate 1 shows a section of the cerebral cortex with normal histoarchitecture. It reveals several neuronal cells (N) within the neuropil.

Plate 2 shows a section of the cerebral cortex with severe cerebral infarction and selective neuronal necrosis (ARROW).

Plate 3 shows a section of the cerebral cortex revealing focal encephalopathic tissue (arrow) with mild glial cell activation.

Plate 4 shows a section of the cerebral cortex with mild general tissue traumatic encephalopathy. The result of this study shows that increase in the dosage of extract has potential to protect the cerebral cortex from encephalopathy.

Plate 5 shows a section of normal histoarchitecture of cerebral cortex with several neuronal cells (N) within the neuropil after receiving high dose of *Annona muricate* extract.

Plate 6 shows a section of the cerebral cortex with normal neuronal cell (N) distribution.

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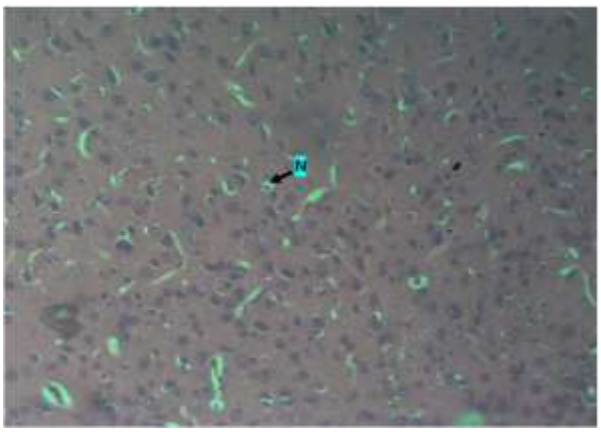
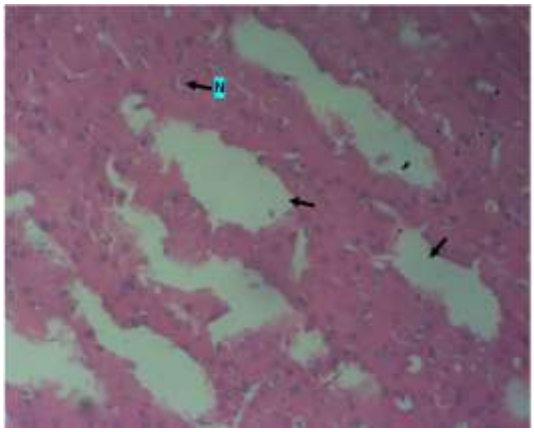


PLATE 1 H & E. X4 00



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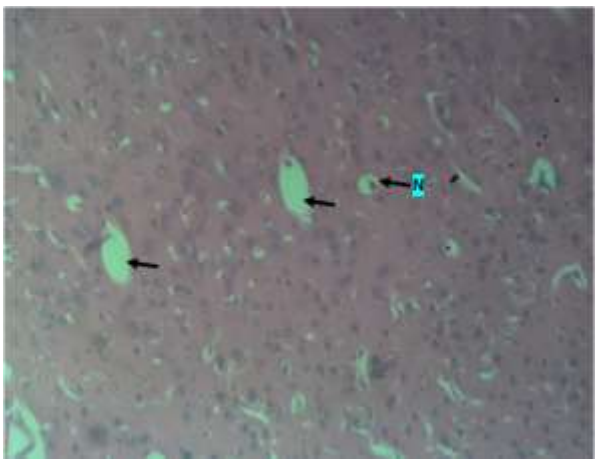

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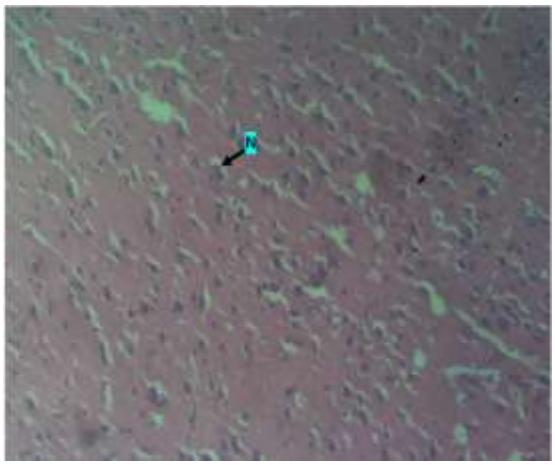

PLATE 4 H & E. X4 00

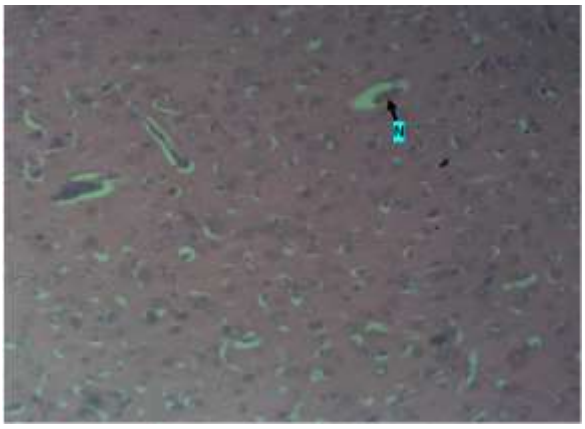

PLATE 5 H & E. X4 00

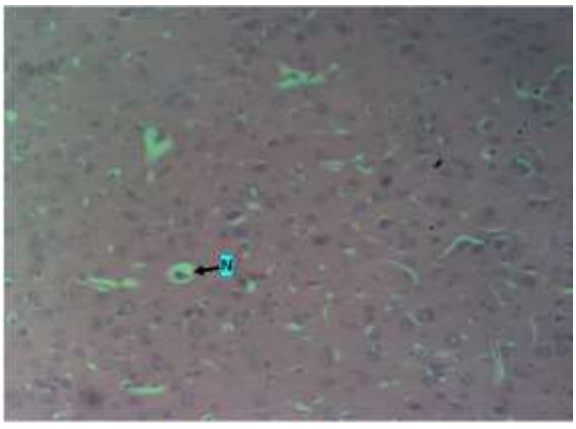

PLATE 6 H & E. X4 00

IV Discussion

Cyclophosphamide is an anti-cancer chemotherapeutic and immunosuppressive agent for the treatment of a wide range of neoplastic as well as some autoimmune diseases (Patti, Lo Fermo, 2011; Altayli et al., 2012). Several studies revealed that cyclophosphamide induce multi organs injuries including liver, lung, spleen and kidneys (Ozkok et al., 2012; Shokrzadeh et al., 2015). Where, the mechanism of cyclophosphamide induced toxicity was due to an oxidative stress and the generation of toxic reactive oxygen species (ROS) (Motawi, Sadik & Refaat, 2010). It has been reported that oxidative DNA damage is caused by a hydroperoxide derivative of cyclophosphamide through generation of H_2O_2 (Murata et al., 2004). This study was carried out to evaluate the curative properties of ethanolic *Annona muricata* (soursop) leaf extract on cyclophosphamide induced cerebral toxicity in adult male wistar rat.

Histological observations gotten from this study showed that 40mg/kg of cyclophosphamide caused severe cerebral

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infarction and selective neuronal necrosis in granular and molecular layer of cerebral cortex this is in accordance with the works of Jnaneshwari *et al., (*2014) and Mansour *et al., (*2017) who reported that cyclophosphamide induces proinflammatory cytokines in their studies. Also in accord with our research are the works by Diaz Montero *et al.,* (2009) who reported that loss of blood supply caused by cyclophosphamide resulted to cerebral infarction and neuronal necrosis. Cyclophosphamide treatment induces oxidative stress by the generation of the free radicals and reactive oxygen species according to Motawi, Sadik & Refaat (2010) which aligns with the present study. Because of this excessive production of free radicals, cyclophosphamide treatment reduces the levels of glutathione and glutathione peroxidase, catalase, and superoxide dismutase activities in cerebral cortex resulting to neuronal necrosis (Liu, Chen & Clair, 2008) and is in accord with our present research.

Animals that were given 100mg/kg, 200mg/kg and 300mg/kg of flavonoid extract of *A. muricata* revealed focal pyramidal nerve encephalopathy, mild general tissue traumatic encephalopathy and normal histoarchitecture respectively. It can be that flavonoid has curative properties in the histoarchitecture of cerebral cortex when compared with vitamin E. *Annona muricata* administration normalized the oxidant status of the neuronal cells, due to *Annona muricata* antioxidant activity (George, Kumar & kumar, 2014). *Annona muricata* has a protective role against free radicals (OH) and H_2O_2 (Baskar, Rajeswari & Kumar, 2007) thus it stopped the elevation of lipid peroxide (Spitz *et al.,* 2004) and converted the ROS to nontoxic or dangerous goods (Ekaluo *et al.,* 2016). *Annona muricata* possesses potent antioxidant properties due to the presence of acetogenins, which can play an essential and significant role in free radical scavenging (Baskar, Rajeswari & Kumar, 2007). The present result showed that the *Annona muricata* extract normalized the level of proinflammatory cytokines due to the presence of anti-inflammatory agents in *Annona muricata* extracts, such as alkaloids, saponins, flavonoids, and tannins, which inhibit inflammation this is in accordance with the research of Serafini, Peluso & Raguzzini, (2010); Foong and Hamid (2012) but it's inconsistent with the research of Laksmitawati *et al.,* (2016).

This study shows that the administration of cyclophosphamide led to significant increase in P53, caspase-3, and apoptotic proteins and a substantial reduction in antiapoptotic proteins. *Annona muricata* was found to decrease the higher levels of apoptosis, caspase 3, and P53 and significantly increase glial cell cyclophosphamide-treated rats. These results indicate that cyclophosphamide injured the surface structure of cerebrum but after administration of different concentrations of extracts, the surface structure of the cerebral cortex obviously proliferated and several neuronal cells could be observed and glial cell activation among rat group that received medium dose (200mg/kg) of *Annona muricata* extract indicating that the cerebral injury could be cured by *Annona muricata* extracts on dose dependent.

The extract of *Annona muricata* exhibited curative properties when compared with vitamin E and the normal control group, therefore revealing that the cyclophosphamide-caused cerebral injury could be cured by the extracts from *Annona muricata.*

IV. Conclusion

This study evaluated the medicinal value annona muricata leaves in the cerebral cortex using adult wistar rats. Many sufferers of cancer who undergo specific chemotherapies end up having other complications in other body areas. Thus, this will proffer a natural front into the field of pharmacocognosy.

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