

# Cajanus Cajan and *Lycopersicon Esculentum* Ameliorated Ethidium Bromide-induced Dysregulations of Cytochrome C and Brain-derived Neurotrophic Factor Expressions in Rat Brain

Akinlolu A. A.<sup>1</sup>, Sulaiman F.<sup>2</sup>, Suleiman S.<sup>3</sup>, Abdulsalam A.<sup>3</sup>, Asogwa N.<sup>4</sup>

<sup>1</sup>Department of Anatomy, Faculty of Basic Medical Sciences, Federal University of Health Sciences Otuokpo, Benue State, Nigeria

<sup>2</sup>Department of Biochemistry, Faculty of Life Sciences, University of Ilorin, Ilorin, Kwara State, Nigeria

<sup>3</sup>Department of Anatomy, Faculty of Basic Medical Sciences, University of Ilorin, Ilorin, Kwara State, Nigeria

<sup>4</sup>Central Research Laboratory, Ilorin, Kwara State, Nigeria

**Disclose and conflicts of interest: none to be declared by all authors**

## ABSTRACT

**Introduction:** This study examined neuroprotection of *Cajanus cajan* (CC) and *Lycopersicon esculentum* (LE) on Cytochrome C (Cyt-c) and Brain-derived neurotrophic factor (BDNF) expressions in Ethidium Bromide (EB)-induced neurotoxicity.

**Materials and Methods:** One hundred and fifteen adult male Wistar rats were randomly divided into 23 Groups (n = 5). Rats were topically exposed to ½ ml of EB-solution (0.5 g EB/100 ml of ethanol) on Day 1. Groups 1 and 2 were post-treated with normal saline and 40 mg/kg body weight of tamsulosin hydrochloride respectively. Nine Groups (CC3-11) and another twelve Groups (LE3-14) were post-treated with CC and LE extracts respectively. Drugs and extracts were orally administered from Days 1-28. Histo-pathological and tissue-ELISA evaluations of Cyt-c and BDNF of prefrontal cortices were performed.

**Results:** Results showed normal histo-architectures of prefrontal cortices in all Groups. Furthermore, results showed significant downregulations of Cyt-c and BDNF in extract-treated groups compared with Group 1.

**Conclusion:** CC and LE could have antioxidant and neuro-regenerative potentials.

**Keywords:** Ethidium bromide; Cytochrome C; BDNF; *Cajanus cajan*; *Lycopersicon esculentum*.

## Introduction

Ethidium Bromide (3,8-diamino-5-ethyl-6-phenyl-phenanthridinium) (EB) is used for DNA visualization because of its efficient fluorescence property and cost-effectiveness<sup>1-5</sup>. However, EB is an effective intercalator and its toxicity is dependent on the exposed organism and the circumstances of the exposure<sup>1-6</sup>. EB can be absorbed via the skin and may irritate the eyes, mouth, and upper respiratory tract<sup>1-6</sup>. EB intercalates between adjacent base pairs in DNA of neuronal cells and deforms double-stranded DNA resulting in neurotoxicity, deranged biological processes and oxidative stress via increased generation of reactive oxygen species (ROS)<sup>1-5,7</sup>. EB accumulates in mitochondria, resulting in interference with replication and transcription, and mtDNA degradation<sup>7,8</sup>, which are underlying factors of neurological conditions<sup>7</sup>. In the brain of adults, aerobic glycolysis is restricted to specific regions, such as the dorsolateral prefrontal cortex, the superior and medial frontal gyrus, or the precuneus and posterior cingulate cortex<sup>9</sup>. Neurons and astrocytes depend on mitochondrial oxidative phosphorylation<sup>9,10</sup>. Therefore, brain mitochondria are required for proper functioning of the nervous system, while mitochondrial dysfunctions have been implicated in aetiology of neurodegenerative diseases<sup>9</sup>.

Cytochrome C (Cyt-c) is involved in electron transfer, redox-coupled protein import, cardiolipin oxidation,

radical scavenging and apoptosis<sup>11</sup>. Brain-derived neurotrophic factor (BDNF) is the main activity-dependent neurotrophin in the CNS, and it increases the respiratory control index of rat brain but not liver or heart mitochondria, resulting in a 64% increase in the efficiency of respiratory coupling<sup>10</sup>. Hence, BDNF plays impact roles in neurogenesis, neuroregeneration<sup>10</sup>, mitochondrial biogenesis, survival of nerve cells<sup>9</sup> and antidepressant treatments<sup>12</sup>.

*Cajanus cajan* (CC) (pigeon pea) contains potassium, calcium, vitamins (such as vitamin A, niacin, a small amount of thiamin, riboflavin, folate and pantothenic acid)<sup>3,5,13,14</sup>. *Lycopersicon esculentum* (LE) (tomato) contains carotenoids, ascorbic acid, phenolic compounds,  $\alpha$ -tocopherol and lycopenes<sup>4,5,15</sup>. Lycopene induces phase II enzymes that help eliminate carcinogens and toxins, thereby protecting lipids, proteins and DNA against cellular toxicity<sup>4,5,16</sup>. Lycopene equally inhibits cancer cells proliferation<sup>4,5,17</sup> and blocks cell transformation by reducing the loss of cancer cells inhibition contact<sup>4,5,18,19</sup>.

Our previous findings showed that EB-induced neurotoxicity resulted in increased levels of Glial Fibrillary Acidic Protein (GFAP), Ki67, tumor necrosis factor alpha (TNF- $\alpha$ ) and myelin basic protein (MBP), but decreased levels of pro-apoptotic Caspase-3 and p53 proteins in prefrontal cortices of rats<sup>3,4</sup>. However, post-treatments with different extracts of parts of CC<sup>3</sup>

and LE<sup>4</sup> ameliorated EB-induced neurotoxicity and resulted in decreased levels of GFAP, Ki67, TNF- $\alpha$  and MBP, but increased levels of pro-apoptotic Caspase-3 and p53 proteins in prefrontal cortices of rats. In addition, different extracts of parts of CC and LE ameliorated EB-induced increased drug resistance of the brain and resulted in significant downregulation of P-glycoprotein in cerebral cortices of rats<sup>5</sup>.

*Cajanus cajan* (CC) and *Lycopersicon esculentum* (LE) were selected for this study because the two plants contain significant amount of antioxidants<sup>13,14</sup>, while LE contains anticancer compounds such as Lycopene<sup>15-19</sup>. Therefore, in order to further determine which plant parts possess antioxidant and neuro-regenerative potentials, this study examined the effects of the extracts of the roots, seeds, stems and leaves of *Cajanus cajan* and *Lycopersicon esculentum* on Cyt-c and BDNF expressions in Ethidium Bromide-induced toxicity of the cerebral cortex in rats. Furthermore, same doses of extracts of the different parts of CC and LE were used for the post-treatment of EB-induced neuro-toxicity in order to determine specifically which plant parts possess better antioxidant and neuro-regenerative potentials.

## Materials and Methods

### Ethics statement

Ethical approval for this study was sought and received from the University of Ilorin Ethical Review Committee (UERC) with ethical approval number-UERC/ASN/2019/1820.

### Collection of plant materials and isolation of plant extracts

Freshly cut seeds, stem and leaves of *Cajanus cajan* (CC); and roots, stem, and leaves of *Lycopersicon esculentum* (LE) were collected from the school forest of the University of Ilorin, Ilorin, Kwara State, North Central region of Nigeria. Identification and authentication of plant materials were done at the Herbarium unit of same university. The collected plant materials were washed free of sand and debris and then air-dried under shade for three weeks. Thereafter, the plant materials were pulverized, homogenized, fractionated and extracted with aqueous, butanolic, ethanolic and n-hexane solvents as previously modified and described<sup>3-5</sup>.

### Animal management

One hundred and fifteen (115) healthy adult male albino Wistar rats with an average weight of 150 g were obtained from the Animal House of the Department of Biochemistry of the University of Ilorin, Ilorin, Kwara State, Nigeria. The sample size was determined from previous studies<sup>3-5</sup> based on the UERC guidelines in accordance with the

internationally accepted principles for laboratory animal use and care. The animals were housed in well-ventilated plastics with sawdust or shavings as beddings, fed on standard rodent feed and allowed free access to tap water ad libitum. Proper aeration was maintained by using well-spaced and gauzed cages in a hygienic environment as previously described<sup>3-5</sup>.

### Induction of Ethidium Bromide (EB) neurotoxicity

EB (0.5 g) was dissolved in 100 ml of ethanol. With the aid of a dissecting blade, 7 cm width of the skin of each rat was scraped ventrally in the midline from the neck to the pelvic region after which 0.5 ml of EB solution was applied to the scraped skin area topically in-order to cause EB-induced toxicity<sup>3-5</sup>.

### Administration of drugs and extracts

All 115 rats used in the present study were exposed to single topical administration of 0.5ml of Ethidium Bromide (EB) solution (0.5 g of EB dissolved in 100 ml of ethanol) on Day 1. The rats were, thereafter, divided into 23 experimental Groups comprising of 5 rats per Group. Thereafter, rats of Group 1 (Toxic Control) and Group 2 (Positive Control) were treated daily with oral administrations of Normal Saline and Tamsulosin Hydrochloride (Tamsulon-XL NRN: A4-0901 from Stallion Laboratories PVT. LTD, India) respectively for 4 weeks (Days 1-28). In addition, following EB- exposure on Day 1 of experimental procedure, the rats of 9 Groups (CC3- 11) were treated daily with oral administrations of 40 mg/kg body weight of extracts of Ethanol leaf, Ethanol stem, Ethanol seed, Aqueous leaf, Aqueous stem, Aqueous seed, Butanol seed, Butanol leaf and Butanol stem of *Cajanus cajan* (CC) respectively for 4 weeks (Days 1-28) (Tables 1 and 2).

Similarly, following EB- exposure on Day 1 of experimental procedure, the rats of another 12 Groups (LE3- 14) were treated daily with oral administrations of 40 mg/kg body weight of extracts of Ethanol root, Ethanol leaf, Ethanol stem, Aqueous root, Aqueous leaf, Aqueous stem, Butanol root, Butanol leaf, Butanol stem, n- hexane root, n- hexane leaf and n- hexane stem of *Lycopersicon esculentum* (LE) respectively for 4 weeks (Days 1-28) (Tables 1 and 2). Tissue-exposure to toxins primarily elicits inflammation to cause toxicity<sup>3,4</sup>. Tamsulosin Hydrochloride was, therefore, used in this study as positive control because it is used in the treatment of benign prostatic hyperplasia as an anti-inflammatory agent as detailed in the information leaflet of Tamsulon-XL NRN: A4-0901. Doses of Ethidium Bromide, *Cajanus Cajan*, *Lycopersicon esculentum* and Tamsulosin Hydrochloride used in this study were determined from previous studies<sup>3-5</sup>. At the end of experimental procedure, all rats were sacrificed using cervical dislocation<sup>3-5</sup>.

**Table 1.** Concentrations of Cytochrome C (ng/ml) and BDNF (ng/ml) in cerebral cortices of *Cajanus cajan*-treated rats.

Group of Rats	Drugs/Extracts Administered	Cytochrome C (Mean ± SD ng/ml)	p- value	BDNF (Mean ± SD ng/ml)	p- value
1	0.5 ml NS	99.12 ± 8.16		33.38 ± 0.40	
2	Tamsulosin Hydrochloride	22.58 ± 13.60	0.01*	9.17 ± 7.94	0.02*
CC3	Ethanol leaf	59.12 ± 23.39	0.02*	1.83 ± 0.71	0.01*
CC4	Ethanol stem	117.0 ± 4.08	0.05*	12.83 ± 1.96	0.04*
CC5	Ethanol seed	50.27 ± 25.02	0.01*	11.45 ± 0.01	0.03*
CC6	Aqueous leaf	201.6 ± 43.24	0.01*	18.56 ± 14.30	0.06
CC7	Aqueous stem	50.85 ± 5.17	0.01*	6.72 ± 0.71	0.01*
CC8	Aqueous seed	100.7 ± 25.02	0.94	7.28 ± 5.89	0.01*
CC9	Butanol seed	27.00 ± 1.90	0.01*	14.56 ± 0.47	0.04*
CC10	Butanol leaf	111.2 ± 6.26	0.24	4.84 ± 1.17	0.01*
CC11	Butanol stem	27.96 ± 3.81	0.01*	4.65 ± 3.43	0.01*

CC: *Cajanus cajan*. All groups were pretreated with topical exposure to 0.5 ml of Ethidium Bromide (EB)-solution (0.5 g EB/100 ml of ethanol). Group 2 rats were post-treated with 40 mg/kg body weight of Tamsulosin Hydrochloride. Groups CC3- 11 rats were post-treated with 40 mg/kg body weight of CC extracts. p<0.05: Groups 2 and CC3 - CC11 versus Group 1. \*: Statistical decrease at p<0.05.

**Table 2.** Concentrations of Cytochrome C (ng/ml) and BDNF (ng/ml) in cerebral cortices of *Lycopersicon esculentum*-treated rats.

Group of Rats	Drugs/Extracts Administered	Cytochrome C (Mean ± SD ng/ml)	p-value	BDNF (Mean ± SD ng/ml)	p- value
1	0.5 ml Normal Saline	99.12 ± 8.16		33.38 ± 0.40	
2	Tamsulosin Hydrochloride	22.58 ± 13.60	0.01*	9.17 ± 7.94	0.02*
LE3	Ethanol root	114.5 ± 20.13	0.42	5.67 ± 1.26	0.01*
LE4	Ethanol leaf	25.85 ± 5.71	0.01*	9.50 ± 0.08	0.01*
LE5	Ethanol stem	51.62 ± 7.89	0.02*	15.39 ± 0.39	0.04*
LE6	Aqueous root	59.69 ± 7.34	0.04*	3.06 ± 0.24	0.01*
LE7	Aqueous leaf	74.12 ± 8.66	0.05*	17.83 ± 6.68	0.05*
LE8	Aqueous stem	55.46 ± 32.36	0.02*	13.61 ± 1.34	0.03*
LE9	Butanol root	47.96 ± 27.74	0.01*	7.11 ± 3.93	0.01*
LE10	Butanol leaf	85.27 ± 9.79	0.04*	3.94 ± 1.96	0.01*
LE11	Butanol stem	67.96 ± 15.77	0.03*	7.83 ± 1.65	0.03*
LE12	n-Hexane root	196.6 ± 49.23	0.01**	5.11 ± 2.20	0.01*
LE13	n-Hexane leaf	106.6 ± 19.85	0.67	14.56 ± 1.89	0.05*
LE14	n-Hexane stem	28.35 ± 14.14	0.01*	-5.28 ± 6.99	0.001***

LE: *Lycopersicon esculentum*. All groups were pretreated with topical exposure to 0.5 ml of Ethidium Bromide (EB)-solution (0.5 g EB/100 ml of ethanol). Group 2 rats were post-treated with 40 mg/kg body weight of Tamsulosin Hydrochloride. Groups LE3- 14 rats were post-treated with 40 mg/kg body weight of LE extracts. p<0.05: Groups 2 and LE3 - CC14 versus Group 1. \*: Statistical decrease at p<0.05; \*\*: Statistical decrease at p<0.01; \*\*\*: Statistical increase at p<0.05.

### Histopathological evaluations of the cerebral cortices of rats

The skull was opened following animal sacrifice and the cerebrum excised. One cerebral hemisphere was fixed in 10% formalin and processed for light microscopy using conventional histological procedures, while the slices were stained with Hematoxyline and Eosin and examined under Olympus binocular research Microscope (Tokyo) for histopathological changes as

earlier described<sup>20</sup>. Photomicrographs of the slides were prepared.

### Tissue-Enzyme-Linked Immunosorbent Assay (ELISA) of concentrations of Cytochrome C and BDNF in cerebral cortices of rats

One cerebral hemisphere was isolated and subjected to thorough homogenization using a porcelain mortar and pestle in ice-cold 0.25 M sucrose, in the

proportion of 1 g to 4 ml of 0.25 M sucrose solution. The tissue homogenates were filled up to 5 ml with additional sucrose and collected in a 5 ml serum bottle. Homogenates were thereafter centrifuged at 3000 rpm for 15 minutes using a centrifuge (Model 90-1). The supernatant was collected with Pasteur pipettes and placed in a freezer at -20 °C, and thereafter assayed for concentrations of Cyt-c (Sigma-Aldrich C7150-1VL, United Kingdom) and BDNF (Sigma-Aldrich B3795, United Kingdom) in the prefrontal cortices of rats of Groups 1, 2, CC3- 11 and LE3- 14 using the ELISA technique as previously described<sup>3-5</sup>.

**Statistical analysis**

The statistical data acquired from the micro plate ELISA results were analysed. Comparisons between Groups 2 and CC3- 11 versus Group 1 were conducted for any significant difference using one-way analysis of variance (ANOVA), while Tukey post hoc test was used for Group comparison as appropriate. In addition, comparisons between Groups 2 and LE3- 14 versus Group 1 were conducted for any significant difference using one-way analysis of variance (ANOVA), while Tukey post hoc test was used for Group comparison as appropriate. The level of significance was set at p ≤ 0.05.

**Results**

**Histopathological evaluations of the prefrontal cortices of rats**

Results were analysed by blinding using the expertise of a consultant Histo-pathologist. The results revealed normal histo-architectures of the prefrontal cortices in rats of toxic control Group 1 (Figures 1a and b), experimental Groups 2 (Figures 2a and b), CC3- 11 (Figures 1c- 1k) and LE3- 14 (Figures 2c- n). There

were normal appearances of the cerebral cortical cells. The pyramidal cells were well detailed. The staining intensity of nuclei, as well as the apical and basal projections of the inherent cells, are well delineated.

**Evaluations of concentrations of Cyt-c in cerebral cortices of rats post-treated with Cajanus cajan (CC)**

Results of post-treatments of EB-induced neurotoxicity with CC extracts showed statistically significant lower mean values of Cyt-c concentrations (ng/ml) in rats of Groups 2 and CC3, CC5, CC7, CC9 and CC11, when compared with Group 1 (99.12 ± 8.16 ng/ml) (Table 1). However, there were statistically significant higher mean values of Cyt-c concentrations (ng/ml) in rats of Groups CC4, CC6, CC8 and CC10, when compared with Group 1 (99.12 ± 8.16 ng/ml) (Table 1).

**Evaluations of concentrations of Cyt-c in cerebral cortices of rats post-treated with Lycopersicon esculentum (LE)**

Results of post-treatments of EB-induced neurotoxicity with LE extracts revealed statistically significant lower mean values of Cyt-c concentrations (ng/ml) in rats of Groups 2 and LE4- 11 and LE14, when compared with toxic control Group 1 (99.12 ± 8.16 ng/ml) (Table 2). However, there were statistically significant higher mean values of Cyt-c concentrations (ng/ml) in rats of Groups LE3, LE12 and LE13, when compared with Group 1 (99.12 ± 8.16 ng/ml) (Table 2).

**Evaluations of concentrations of BDNF in cerebral cortices of rats post-treated with Cajanus cajan (CC) and Lycopersicon esculentum (LE)**

Results of post-treatments of EB-induced neurotoxicity with CC extracts showed statistically significant lower mean values of BDNF concentrations

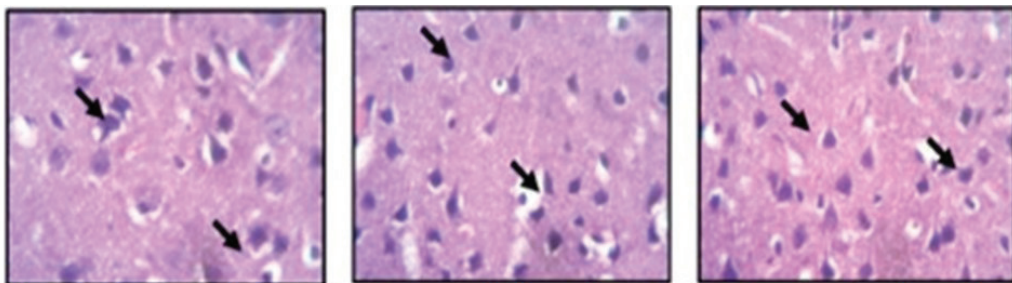


Fig. 1a. Normal Saline      Fig. 1b. Tamsulosin Hydrochloride      Fig. 1c. CC3: Ethanol leaf

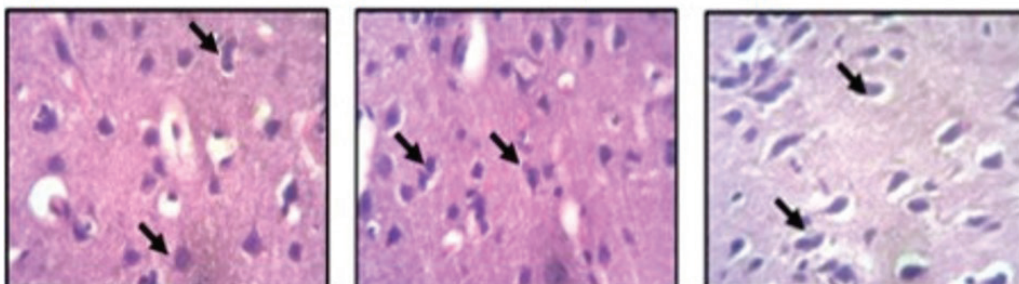


Fig. 1d. CC4: Ethanol stem      Fig. 1e. CC5: Ethanol seed      Fig. 1f. CC6: Aqueous leaf

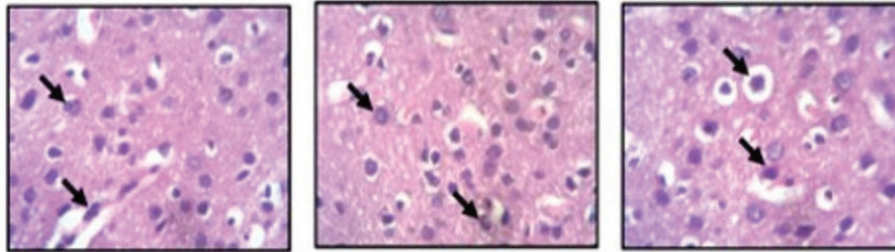


Fig. 1g. CC7: Aqueous stem Fig. 1h. CC8: Aqueous seed Fig. 1i. CC9: Butanol seed

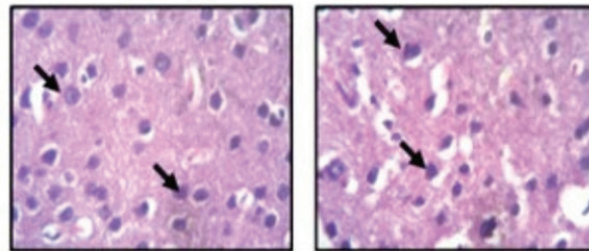


Fig. 1j. CC10: Butanol leaf Fig. 1k. CC11: Butanol stem

**Figures 1 a - k.** Representative photomicrographs of the external pyramidal layer of the prefrontal cortices of rats of groups 1, 2 and CC3- 11. Rats of groups 1, 2 and CC3- 11 were topically exposed to 0.5 ml of Ethidium Bromide (EB)-solution (0.5 g of EB dissolved in 100 ml of ethanol) on Day 1. Groups 1 (Figure 1a) and 2 (Figure 1b) were post-treated with Normal Saline and 40 mg/kg body weight of Tamsulosin Hydrochloride, respectively. Groups CC3- 11 were post-treated with 40 mg/kg body weight of the Aqueous, Butanolic and Ethanolic extracts of the seeds, stems or leaves of CC (Figure 1c- k). CC: *Cajanus cajan*. Magnifications: X 400 (Scale Bar: 100µm) Hematoxylin and Eosin. Histopathological evaluations showed normal histo-architectures of the prefrontal cortices. The pyramidal cells (short black arrows) are well detailed. The staining intensity of nuclei, as well as the apical and basal projections of the inherent cells, are well delineated.

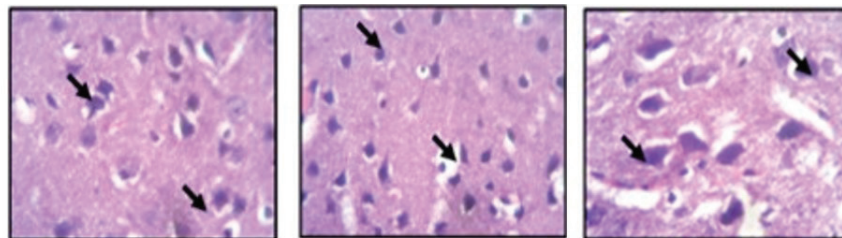


Fig. 2a. Normal Saline Fig. 2b. Tamsulosin Hydrochloride Fig. 2c. LE3: Ethanol root

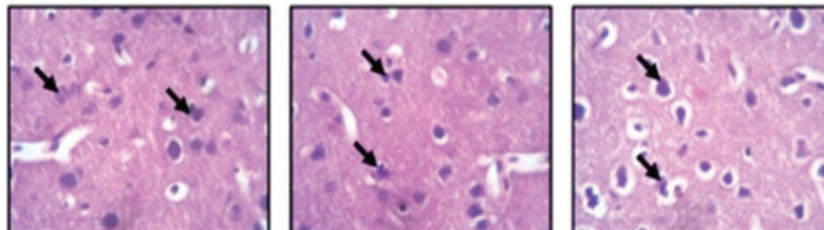


Fig. 2d. LE4: Ethanol leaf Fig. 2e. LE5: Ethanol stem Fig. 2f. LE6: Aqueous root

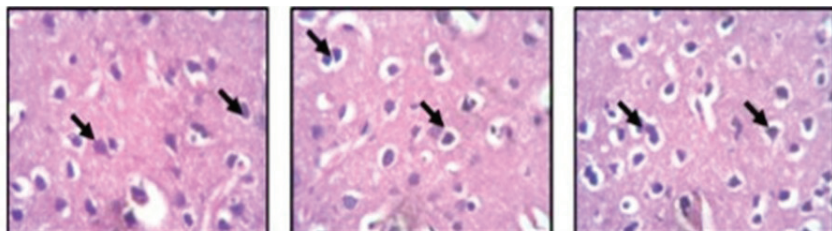


Fig. 2g. LE7: Aqueous leaf Fig. 2h. LE8: Aqueous stem Fig. 2i. LE9: Butanol root

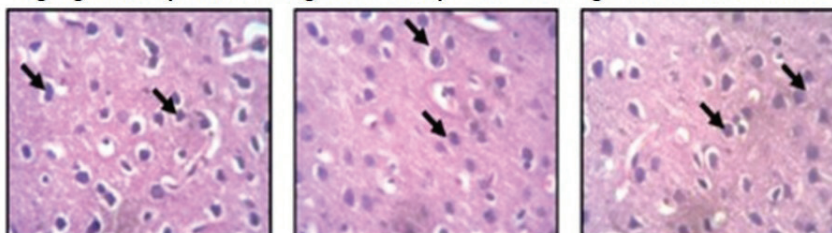
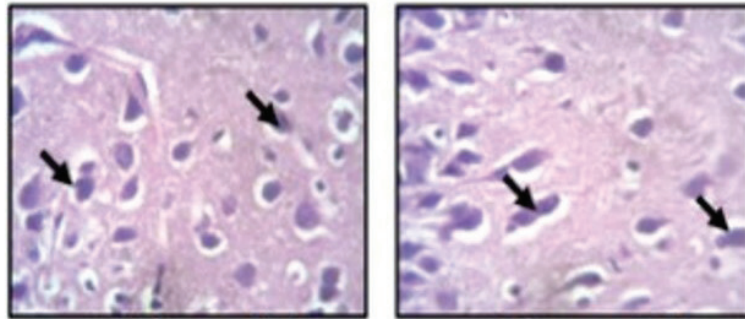


Fig. 2j. LE10: Butanol leaf Fig. 2k. LE11: Butanol stem Fig. 2l. LE12: n-Hexane root



**Fig. 2m. LE13: n-Hexane leaf Fig. 2n. LE14: n-Hexane stem**

**Figures 2 a - n.** Representative photomicrographs of the external pyramidal layer of the prefrontal cortices of rats of groups 1, 2 and LE3- 14. Rats of groups 1, 2 and LE3- 14 were topically exposed to 0.5 ml of Ethidium Bromide (EB)-solution (0.5 g of EB dissolved in 100 ml of ethanol) on Day 1. Groups 1 (Figure 2a) and 2 (Figure 2b) were post-treated with Normal Saline and 40 mg/kg body weight of Tamsulosin Hydrochloride, respectively. Groups LE3- 14 were post-treated with 40 mg/kg body weight of the Aqueous, Butanolic, Ethanolic and N-hexane extracts of the roots, stems and leaves of LE (Figures 2c- n). LE: *Lycopersicon esculentum*. Magnifications: X 400 (Scale Bar: 100 $\mu$ m) Hematoxylin and Eosin. Histopathological evaluations showed normal histo-architectures of the prefrontal cortices. The pyramidal cells (short black arrows) are well detailed. The staining intensity of nuclei, as well as the apical and basal projections of the inherent cells, are well delineated.

(ng/ml) in rats of Groups 2, CC3- 5 and CC7- 11, when compared with Group 1 ( $33.38 \pm 0.40$  ng/ml) (Table 1). In addition, there were statistically non-significant lower mean value of BDNF concentrations (ng/ml) in rats of Group CC6, when compared with Group 1 ( $33.38 \pm 0.40$  ng/ml) (Table 1). Furthermore, results of post-treatments of EB-induced neurotoxicity with LE extracts showed statistically significant lower mean values of BDNF concentrations (ng/ml) in rats of Groups 2 and LE3- 14 when compared with Group 1 ( $33.38 \pm 0.40$  ng/ml) (Table 2).

## Discussion

Histological analyses showed normal histo-architectures of the prefrontal cortices of rats of all groups (Figures 1a- k and 2a- n). The observed histo results of this study implied that the acute exposure of rats to topical administration of 0.5 ml of 0.5 g/100 ml EB-solution for 4 weeks did not result in evident histopathology of the prefrontal cortex of EB-treated rats post-treated with normal saline and rats post-treated with Tamsulosin Hydrochloride, *Cajanus cajan* (CC) and *Lycopersicon esculentum* (LE). This could have possibly resulted from the fact that cyto-toxicity of adverse chemical agents is dose and/or exposure-dependent. In addition, drug-induced toxicity is usually first elicited on molecular markers, while further exposure will result in evident histo-pathology at tissue level.

Mitochondrial dysgenesis and/or dysfunction results from increased oxidative stress, impaired functions of the Electron Transport Chain and upregulation of Cyt-c with consequent promotion of apoptosis<sup>11,21,22</sup>. Cyt-c therefore functions as an antioxidant<sup>11</sup>. Results of this study showed that the mean level of Cytochrome C in the prefrontal cortices of rats of Group 1 is  $99.12 \pm 8.16$  ng/ml (Tables 1 and 2). The observed elevated level of Cytochrome C in Group 1 is in obvious contrast with reported range of 0.01 ng/ml to 0.013 ng/ml of Cytochrome C in brain of rat fed with normal saline<sup>23</sup>. The observed elevated level

of Cytochrome C in Group 1 implied that EB-induced neurotoxicity resulted in increased generation of ROS, oxidative stress and upregulation of Cyt-c in rats of Group 1. Cyt-c is a cellular antioxidant<sup>11,21,22</sup>. Therefore, upregulation of Cyt-c in rats of Group 1 could have been due to its cellular response to ameliorating and mopping up of increased ROS levels as an antioxidant.

Post-treatments with CC extracts (Ethanol leaf, Ethanol seed, Aqueous stem, Butanol seed and Butanol stem) in rats of Groups CC3, CC5, CC7, CC9 and CC11 ameliorated EB-induced neurotoxicity and oxidative stress via down-regulation of Cyt-c (Table 1). In contrast, post-treatments with Ethanol stem, Aqueous leaf, Aqueous seed and Butanol leaf extracts of CC did not ameliorate EB-induced upregulation of Cyt-c (Table 1). These observations indicate that CC extracts (Ethanol leaf, Ethanol seed, Aqueous stem, Butanol seed and Butanol stem) could have antioxidant and neuroprotective potentials.

Post-treatments with LE extracts (Ethanol leaf, Ethanol stem, Aqueous root, Aqueous leaf, Aqueous stem, Butanol root, Butanol leaf, Butanol stem and n-Hexane stem) in rats of Groups LE4-11 and LE14 significantly ameliorated EB-induced neurotoxicity and oxidative stress via down-regulation of Cyt-c (Table 2). In contrast, post-treatments with ethanol root (LE3), n-hexane root (LE12) and n-hexane leaf (LE13) extracts of LE did not ameliorate EB-induced upregulation of Cyt-c (Table 2). These observations indicate that Ethanol leaf, Ethanol stem, Aqueous root, Aqueous leaf, Aqueous stem, Butanol root, Butanol leaf, Butanol stem and n-Hexane stem of LE could have antioxidant and neuroprotective potentials.

BDNF is the main activity-dependent neurotrophin in the CNS, and it increases the respiratory control index of rat brain resulting in a 64% increase in the efficiency of respiratory coupling<sup>10</sup>. This makes BDNF a biomarker of interest in neurogenesis and resolution of oxidative stress in the rat brain. BDNF levels ranged between 0.9 and 1.7 ng/g in rats that are one month's old<sup>24</sup>. Furthermore, BDNF level increases post-natally

but decreases with age in the cerebral cortex of rats<sup>25</sup>. In the present study, results showed elevated BDNF mean value of  $33.38 \pm 0.40$  ng/ml in prefrontal cortices of rats of Group 1 (Tables 1 and 2). This value is in sharp contrast with the normal low level of  $0.00024 \pm 0.005$  ng/ml in frontal cortices of adult male rats fed with normal saline<sup>26</sup>. The elevated BDNF level in rats of Group 1 agrees with previous observations of elevated BDNF levels in neurotoxin-treated neuro-degeneration models<sup>27</sup>. Hence, the elevated BDNF level in rats of toxic control Group 1 indicated cellular response of neuro-regeneration to resolve on-going neurodegeneration and oxidative stress in the brain.

Post-treatments of EB-induced neurotoxicity with all tested extracts of CC (Groups CC3- 11) and LE (Groups LE3- 14) resulted in down-regulations of BDNF (Tables 1 and 2). These findings implied that the regulatory roles of BDNF in neuroprotection, neurogenesis and neural plasticity<sup>10</sup> as well as in resolution of oxidative stress in the brain were promoted in rats treated with extracts of CC and LE. Our observations are in agreement with those of previous studies<sup>28</sup>, which noted that BDNF was involved in immune neuroprotective interaction in neurodegenerative disorders such as Multiple Sclerosis. In addition, these findings implied that CC and LE possibly possess antioxidant, neurotrophic and neuro-regenerative potentials.

Furthermore, post-treatments of EB-induced neurotoxicity with Tamsulosin Hydrochloride resulted in similar significant downregulation of Cytochrome C when compared with CC extracts (Ethanol leaf, Ethanol seed, Aqueous stem, Butanol seed and Butanol

stem) and LE extracts (Ethanol leaf, Ethanol stem, Aqueous root, Aqueous leaf, Aqueous stem, Butanol root, Butanol leaf, Butanol stem and n-Hexane stem) (Tables 1 and 2). In addition, post-treatments of EB-induced neurotoxicity with Tamsulosin Hydrochloride resulted in similar significant downregulations of BDNF when compared with CC extracts (CC3- 11) and LE extracts (LE3- 14) (Tables 1 and 2). However, post-treatment with n-Hexane stem extract of LE achieved higher significant downregulation of BDNF against EB-induced neurotoxicity (Table 2), and deserves further evaluation for the discovery of neuro-protective and neuro-regenerative compounds.

## Conclusion

Overall, the findings of this study revealed that post-treatments of EB-induced neurotoxicity with extracts of CC and LE resulted in down-regulations of Cyt-c and BDNF concentrations. These findings indicate that CC and LE conferred some degree of neuro-protection against EB-induced neurotoxicity in rats. Hence, CC and LE could have antioxidant and neuro-regenerative potentials. Furthermore, the usage of CC and LE as edible supplements or as sources of possible candidate compounds in the treatments of oxidative stress and neurodegeneration deserve further investigations.

## Acknowledgements

The technical supports of laboratory scientists of the Departments of Anatomy and Biochemistry of University of Ilorin, Nigeria, are acknowledged.

## References

1. James WA, Caroline JC, Caroline J, Anjanapriya S. Toxic and mutagenic impact of Ethidium Bromide traces on the growth of *Escherichia Coli* KL226. *Int J Curr Sci* 2013;8:28-32.
2. Saeidnia S, Abdollahi A. Are other fluorescent tags used instead of ethidium bromide safer? *DARU J Pharmaceut Sci* 2013;21:71. <https://www.darujps.com/content/21/1/71>.
3. Akinlolu AA, Sulaiman FA, Tajudeen S, Suleiman SK, Abdulsalam AA, Asogwa NT. *Cajanus cajan* *esculentum* drives apoptosis via activation of caspase3/p53 pathway and possesses remyelination and anti-gliosis potentials in Ethidium Bromide-induced neurotoxicity in rats. *Nigerian J Sci Res* 2020;19(4):286-293.
4. Akinlolu AA, Sulaiman FA, Tajudeen S, Abdulsalam A, Suleiman SK, Asogwa NT. *Lycopersicon esculentum* drives apoptosis via activation downregulation of TNF $\alpha$  with positive immunomodulations of Caspase-3, Ki67, p53, MBP and GFAP in Ethidium Bromide-induced neurotoxicity in rats. *Afr J Medicine Med Sci* 2022a;51:41-51.
5. Akinlolu AA, Sulaiman FA, Olawuyi O, Suleiman SK, Abdulsalam A, Asogwa NT. Drug delivery resistance of Blood-Brain-Barrier: *Cajanus cajan* and *Lycopersicon esculentum* down-regulated Multi-drug resistance1 expression in cerebrum of rats. *East Cent Afr J Pharm Sci* 2022b;25(1):9-16.
6. Akorlha EE, Niemogha MT, Edozor O. Mutagenic and genotoxic screening of eight commonly used skin whitening creams in Nigeria. *Bayero J Pure Appl Sci* 2011;5(1):5-10.
7. Warren EB, Aicher AE, Fessel JP, Konradi C. Mitochondrial DNA depletion by ethidium bromide decreases neuronal mitochondrial creatine kinase: Implications for striatal energy metabolism. *PLoS ONE* 2017;12:12. <https://doi.org/10.1371/journal.pone.0190456>.
8. Luo Y, Hu Y, Zhang M, Xiao Y, Song Z, Xu Y. EtBr-induced selective degradation of mitochondria occurs via autophagy. *Oncol Rep* 2013;30:1201-1208.
9. Magistretti PJ, Allaman I. A cellular perspective on brain energy metabolism and functional imaging. *Neuron* 2015;86(4):883-901.
10. Markham A, Bains R, Franklin P, Spedding M. Changes in mitochondrial function are pivotal in neurodegenerative and psychiatric disorders: how important is BDNF? *British J Pharmacol* 2014;171:2206-2229.
11. Hüttemann M, Pecina P, Rainbolt M, *et al.* The multiple functions of cytochrome c and their regulation in life and death decisions of the mammalian cell: from respiration to apoptosis. *Mitochondrion* 2011;3:369-381. doi:10.1016/j.mito.2011.01.010.
12. Perera TD, Coplan JD, Lisanby SH, *et al.* Antidepressant-induced neurogenesis in the hippocampus of adult nonhuman primates. *J Neurosci* 2007;27:4894-4901.
13. Akande KE, Abubakar MM, Adegbola TA, Bogoro SE, Doma UD. Chemical evaluation of the nutritive quality of pigeon pea [*Cajanus cajan* (L.) Millsp.]. *Int J Poultry Sci* 2010;9(1):63-65.
14. Lawal OU. The mineral and phytochemical analysis of leaves of *Senna alata* and *Cajanus cajan* and their medicinal value. *Int J Biol Pharmacy Allied Sci* 2012;1:1-11.
15. Balestrieri ML, Prisco RDe, Nicolaus B, *et al.* Lycopene in

association with  $\alpha$ -tocopherol or tomato lipophilic extracts enhances acyl-platelet-activating factor biosynthesis in endothelial cells during oxidative stress. *Free Radical Biol Med* 2004;36(8):1058-1067.

16. Aust OAA, Ale-Agha N, Zhang L, Wollersen H, Sies H, Stahl W. Lycopene oxidation product enhances gap junctional communication. *Food Chem Toxicol* 2003;41(10):1399-1407.

17. Nahum A, Hirsch K, Danilenko M, *et al.* Lycopene inhibition of cell cycle progression in breast and endometrial cancer cells is associated with reduction in cyclin D levels and retention of p27 Kip1 in the cyclin E-cdk2 complexes. *Oncogene* 2001;20(26):3428-3436.

18. Wertz K, Siler U, Goralczyk R. Lycopene: modes of action to promote prostate health. *Arch Biochem Biophys* 2004;430(1):127-134.

19. Karppi J, Kurl S, Nurmi T, Rissanen TH, Pukkala E, Nyssönen K. Serum lycopene and the risk of cancer: The Kuopio Ischaemic Heart Disease Risk Factor (KIHD) study. *Ann Epidemiol* 2009;19(7):512-518.

20. Omotoso GO, Kadir ER, Lewu SF, *et al.* Moringa oleifera ameliorates cuprizone-induced cerebellar damage in adult female rats. *Res J Health Sci* 2018;6(1):13-25.

21. Xuan P, Sarah JS, Yimin Z, *et al.* Caspase-3 and caspase-8 expression in breast cancer: caspase-3 is associated with survival. *Apoptosis* 2017;22:357-368.

22. Arnold S. Cytochrome c Oxidase and its role in

neurodegeneration and neuroprotection. *Adv Exp Med Biol* 2012;748:305-339.

23. Davoudzadeh F, Babaei P, Jafari A. Mespilus germanica leaves flavonoids improve passive avoidance memory and apoptosis in a rat model of amyloid- $\beta$  neurotoxicity. *Physiol Pharmacol* 2018;22:219-227.

24. Katoh-Semba R, Takeuchi IK, Semba R, Kato K. Distribution of brain-derived neurotrophic factor in rats and its changes with development in the brain. *J Neurochem* 1997;69(1):34-42.

25. Katoh-Semba R, Semba R, Takeuchi IK, Kato K. Age-related changes in levels of brain-derived neurotrophic factor in selected brain regions of rats, normal mice and senescence-accelerated mice: a comparison to those of nerve growth factor and neurotrophin-3. *Neurosci Res* 1998;31(3):227-234.

26. Elfving B, Plougmann PH, Wegener G. Detection of brain-derived neurotrophic factor (BDNF) in rat blood and brain preparations using ELISA: Pitfalls and solutions. *J Neurosci Methods* 2010;187:73-77.

27. Kim J, Lee S, Kang S, Kim SH, Kim JC, Moon C. Brain-derived neurotrophic factor and GABAergic transmission in neurodegeneration and neuroregeneration. *Neural Regener Res* 2017;12(10):1733-1741.

28. Stadelmann C, Kerschensteiner M, Misgeld T, Brück W, Hohlfeld R, Lassmann H. BDNF and gp145trkB in multiple sclerosis brain lesions: neuroprotective interactions between immune and neuronal cells? *Brain* 2002;125(1):75-85.

## Mini Curriculum and Author's Contribution

1. Akinlolu A.A. Qualification: BSc; MSc.; PhD. Contribution: Main contributor in writing the manuscript. Conceptualized, designed and supervised the experimental procedures, performed the experimental procedures, analyzed and interpreted the data, read and approved the final manuscript. Rank: Professor - ORCID: 0000-0002-2374-8754.

2. Sulaiman F. Qualification: BSc.; MSc.; PhD. Contribution: Conceptualized, designed and supervised the experimental procedures, performed the experimental procedures, analyzed and interpreted the data, read and approved the final manuscript. Rank: Associate Professor - ORCID: 0000-0002-2374-8754.

3. Suleiman S. Qualification: BSc; MSc. Contribution: Conceptualized, designed, performed the experimental procedures, analyzed and interpreted the data, read and approved the final manuscript. Rank: Former postgraduate student - ORCID: 0000-0002-2374-8754.

4. Abdulsalam A. Qualification: BSc; MSc. Contribution: Conceptualized, designed, performed the experimental procedures, analyzed and interpreted the data, read and approved the final manuscript. Rank: Former postgraduate student - ORCID: 0000-0002-2374-8754.

5. Asogwa N. Qualification: BSc.; MSc.; PhD. Contribution: Designed and performed the experimental procedures, analyzed and interpreted the data, read and approved the final manuscript. Rank: Research Scientist - ORCID: 0000-0002-2374-8754.

Received: November 26, 2023

Accepted: December 12, 2023

Corresponding author

Adelaja Akinlolu

E-mail: adelaja.akinlolu@fuhso.edu.ng