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Lycopersicon esculetum drives apoptosis via downregulation of TNFα with positive immunomodulations of Caspase-3, Ki67, p53, MBP and GFAP in Ethidium Bromide-induced neurotoxicity in rats

AA Akinlolu[1], FA Sulaiman[2], S Tajudeen[3], SK Suleiman[3], O. Oloruntoba[3], AA Abdulsalam[3], and NT Asogwa[4]

Department of Anatomy[1], Olabisi Onabanjo University, Ago-Iwoye, Ogun State, Departments of Biochemistry[2] and Anatomy[3], University of Ilorin, Ilorin, Kwara State, Central Research Laboratory[4], Ilorin, Kwara State, Nigeria

Abstract

Background: Ethidium Bromide (EB) is an established mutagen, neurotoxin and Multiple Sclerosis (MS) model in rats. This study evaluated the immunomodulations of genes of immune response/ inflammation (TNFα), apoptosis (Caspase-3), proliferation (Ki67), tumour suppression (p53), demyelination (MBP) and gliosis (GFAP) in EB-induced neurotoxicity in rats treated with *Lycopersicon esculetum* (LE).

Materials and methods: Seventy adult male Wistar rats were divided into 23 groups (n = 5). 0.5 mls of EB solution (0.5 g/100 mls of ethanol) was applied to scraped ventral skin area of all rats. Groups 1 and 2 were treated with Normal Saline and 40 mg of Tamsulosin Hydrochloride respectively. Groups 3-14 were treated with 40 mg/kg bodyweight of aqueous, butanolic, ethanolic or n-hexane extracts of roots, stems or leaves of LE. Drugs/extracts were orally administered for 4 weeks. Subsequently, rats were anaesthetized by inhalation of diethyl ether. Homogenates of excised prefrontal cortices were obtained for ELISA analyses of TNFα, Caspase-3, Ki67, p53, MBP and GFAP concentrations. Computed data were statistically analysed using GraphPad Prism 2005 ($P \leq 0.05$).

Results: Results showed positive immunomodulations with statistically significant ($P \leq 0.05$) or non-significant ($P \geq 0.05$) lower or higher mean levels (ng/ml) of TNFα, Caspase-3, Ki67, p53, MBP and GFAP in rats of Groups 3-14 compared with Group 1. This implied that treatments with extracts of LE ameliorated EB-induced inflammation, hyperplasia, mutagenesis, anti-apoptosis, demyelination and gliosis via positive downregulations/upregulations of evaluated biomarker genes.

Conclusions: LE possesses neuro-regenerative, anti-inflammatory, anticancer and anti-MS potentials.

Correspondence: Dr. A.A. Akinlolu, Department of Anatomy, University of Medical Sciences Ondo, Ondo State, Nigeria. E-mail: aadelaja@unimed.edu.ng

Keywords: *Apoptosis, lycopersicon esculetum, ethidium bromide, mutagenesis, multiple sclerosis, neurotoxicity.*

Résumé

Contexte : Le bromure d'éthidium (BE) est un modèle établi de mutagène, de neurotoxine et de sclérose en plaques (SEP) dans les rats. Cette étude a évalué les immuno-modulations des gènes de la réponse immunitaire / inflammation (TNFα), de l'apoptose (Caspase-3), de la prolifération (Ki67), de la suppression tumorale (p53), de la démyélinisation (MBP) et de la gliose (GFAP) dans la neuro-toxicité induite par le BE dans les rats traité avec *Lycopersiconesculetum* (LE).

Matériels et méthodes : Soixante -dix rats Wistar mâles adultes ont été répartis en 23 groupes (n = 5). 0,5 ml de solution EB (0,5 g/100 ml d'éthanol) a été appliqué sur la zone de peau ventrale grattée de tous les rats. Les groupes 1 et 2 ont été traités respectivement avec une solution saline normale et 40 mg de chlorhydrate de tamsulosine. Les groupes 3 à 14 ont été traités avec 40 mg/kg de poids corporel de solution aqueuse, de butanol, d'éthanol ou d'extraits n-hexane de racines, de tiges ou de feuilles de LE. Les médicaments/extraits ont été administrés par voie orale pendant 4 semaines. Ensuite, les rats ont été anesthésiés par inhalation de diméthyle d'éther. Des homogénats de cortex préfrontal excisés ont été obtenus pour les analyses ELISA des concentrations de TNFα, Caspase-3, Ki67, p53, MBP et GFAP. Les données calculées ont été analysées statistiquement à l'aide de Prisme GraphPad 2005 ($p \leq 0,05$).

Résultats : Les résultats ont montré des immuno-modulations positives avec des niveaux moyens inférieurs ou supérieurs (ng/ml) statistiquement significatifs ($\leq 0,05$) ou non significatifs ($P \geq 0,05$) de TNFα, Caspase-3, Ki67, p53, MBP et GFAP chez des rats de Groupes 3 à 14 par rapport au groupe 1. Cela impliquait que les traitements avec des extraits de LE amélioraient l'inflammation induite par le BE, l'hyperplasie, la mutagenèse, l'anti-apoptose, la

démyélinisation et la gliose par des régulations négatives/positives positives des gènes bio-marqueurs évalués.*Conclusions* : LE possède des potentiels neuro-régénératifs, anti-inflammatoires, anticancéreux et anti-SEP.

Mots -clés : *Apoptose, Lycopersiconesculetum, bromure d'éthidium, mutagenèse, sclérose en plaques, neuro-toxicité.*

Introduction

Cancer is a group of diseases characterized by unrestricted growth/proliferation and metastasis of abnormal cells [1]. It was ranked the second leading cause of death after cardiovascular diseases since 2013 [1], and this imposes a huge burden on societies. Functionally, cancers comprise of cancer stem cells (CSCs), macrophages and vascular endothelial cells, with CSCs having tumourigenic capacity while others do not [2-4]. Cancer treatment regimens kill most cancer cells, but do not eliminate CSCs, which have protective and resistance mechanisms via up-regulation of biomarkers of proliferation (Ki67), drug resistance (Aldehyde dehydrogenase 1 and P-glycoprotein) and angiogenesis (VEGFR1); as well as de-regulation of proto-oncogenes (myc and src) and down-regulation of apoptotic and tumour suppressor genes (Caspase-3 and p53) [2-4]. Hence, the characteristic survival of CSCs provides explanations for failures of cancer treatments, as well as informed directions for the development of more potent anticancer drugs from plants or other sources.

In addition, Multiple Sclerosis (MS) is an auto-immune and inflammatory disease characterized by neuronal demyelination, astrogliosis, and microglial activation [5-8]. MS results from T-cell induced reaction against myelin sheath of the white matter, though the grey matter is equally noted to be affected [5-8]. Cognitive and memory functions are impaired in MS due to adverse effects on the frontal cortex and hippocampus. Optic neuritis, an inflammatory condition may also result in the development of MS [5-8]. Developments of efficacious therapy for MS had been quite challenging because the etio-pathological mechanisms underlying MS is not yet clear [5-8]. Studies on drug candidates for the treatment of MS have been focused on in vivo animal models such as Ethidium Bromide (EB)-induced MS [9-10]. EB is an effective intercalator, a strong mutagen [11] and a possible carcinogen [12]. EB's toxicity is dependent on the exposed organism and the circumstances of exposure [13]. EB can be absorbed via the skin, and may cause irritation of the eyes, mouth, and upper respiratory tract. EB intercalates the adjacent base pairs of neuronal cells of the Central Nervous System (CNS) and deforms double-stranded DNA resulting in neurotoxicity [14], demyelination, inflammation and astrogliosis [9]. Hence, EB is used as an established model of mutagenesis, neurotoxicity and MS in rats.

Lycopersicon esculetum (LE) (tomatoes) contains carotenoids, ascorbic acid, phenolic compounds, α- tocopherol and lycopenes [15]. Lycopene induces phase II enzymes that help to eliminate carcinogens and toxins, thereby protecting lipids, proteins and DNA against cellular toxicity [16]. Lycopene equally inhibits cancer cells proliferation [17], and blocks cell transformation by reducing the loss of cancer cells inhibition contact [18,19].

In cancer studies, TNFα, Ki67, p53 and Caspase-3 are established biomarkers of immune response/inflammation, abnormal proliferation, tumour suppression and apoptosis respectively; while Tumour Necrosis Factor-alpha (TNFα), Myelin Basic Protein (MBP) and Glial Fibrillary Acidic Protein (GFAP) are biomarkers of immune response/inflammation, demyelination and gliosis respectively, and are evaluated in Multiple Sclerosis studies. Therefore, this study evaluated the immunomodulations of genes of TNFα, Caspase-3, Ki67, p53, MBP and GFAP in Ethidium Bromide-induced neurotoxicity in rats treated with of extracts of *Lycopersicon esculetum*, in-order to further determine which plant parts possess anti-inflammatory, anticancer and anti-Multiple Sclerosis potentials.

Materials and methods

Ethical approval

Ethical approval was sought and received from the University of Ilorin Ethical Review Committee with ethical approval number - UERC/ASN/2019/1820. This research study was conducted in accordance with the internationally accepted principles for laboratory animal use and care as provided in the European Community guidelines (EEC Directive of 1986; 86/609/EEC) and the US guidelines (NIH publication #85-23, revised in 1985).

Animal management

Seventy healthy 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 animals were housed in well ventilated plastics with saw dust 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.

Induction of Ethidium Bromide (EB) neurotoxicity

EB (Sigmal-Aldrich) (0.5 g) was dissolved in 100 mls of ethanol. 7 cm width of the skin of each rat was scraped anteriorly in the midline from the neck

to the pelvic region using a dissecting blade. 0.5 ml of EB solution was applied to the scraped skin area.

Equipment and reagents

Rotary evaporator (Heidolph 4011, Canada), Microtome (YD-315, Tokyo), Olympus binocular research Microscope (Tokyo) and Tamsulosin Hydrochloride. Tamsulosin Hydrochloride (Tamsulon-XL NRN: A4-0901) manufactured by Stallion Laboratories PVT. LTD, India was purchased from MOMROTA pharmacy, Ilorin, Kwara State, Nigeria.

Collection of plant materials and isolation of plant extracts

Freshly cut roots, stems and leaves of *Lycopersicon esculetum* (LE) were collected from the school forest of University of Ilorin, Ilorin, Kwara State, North Central region of Nigeria. Identification and authentication of plant materials were done at the Herbarium unit of the Department of Plant Biology, University of Ilorin, Ilorin, Kwara State, Nigeria. The collected plant materials were washed free of sand and debris, and then air dried under shade at room temperature (25-30 °C) for three weeks. Thereafter, the plant materials were pulverized and homogenized with an electric blender to increase the surface area for solvent extraction before usage in the experimental procedures.

Powdered sample of about 200 g of LE were weighed on an analytical balance (Sartorious BS 124S, China) with an accuracy of 0.1 mg and the aqueous extracts were prepared at 50 mg/L by infusions with distilled water for 5 - 10 minutes. The extracts were cooled at room temperature and centrifuged at 2000 revolutions per minute (rpm) for 10 minutes in a centrifuge (Centribio, MLW T24 D, Germany). The supernatant was filtered through a Whatman N0. 4 filter paper until the clarified aqueous extracts were obtained.

The butanolic extracts were prepared according to the modified procedures described by [20]. For ethanolic extracts, solutions of plant materials were prepared at a concentration of 0.1g/ml with 70% ethanol, which were macerated in the dark for 7 days and with periodic stirring at room temperature (25 ± 2 ^{0}C). These mixtures were concentrated to total dryness in a rotary evaporator (Heidolph 4011, Canada). With the resulting solid, infusions were prepared in distilled water until a concentration of 50 µg/ml was obtained. The extracts were centrifuged at 2000 rpm for 10 minutes. The supernatants were filtered with Whatman N0 4 filter paper until clarified.

Administrations of drugs and extracts

All 70 rats used in the present study were exposed to 0.5 ml of Ethidium Bromide (EB) solution (0.5 g of EB dissolved in 100 mls of ethanol). The rats were, thereafter, divided randomly into 11 Experimental Groups comprising of 5 rats per group. Rats of Group 1 (Negative Control) were treated with Normal Saline while rats of Experimental Group 2 (Positive Control) were treated with Tamsulosin Hydrochloride (used in the treatment of hyperplasia). Rats of Groups 3 - 14 were treated with 40 mg/kg bodyweight of Aqueous, Butanolic, Ethanolic or N-hexane extracts of roots, stems or leaves of *Lycopersicon esculetum* (LE) as detailed in Table 1. Normal Saline, Tamsulosin Hydrochloride and all fractions extracts of LE were orally administered to rats for 4 weeks.

Table 1: Groups of rats and doses of drugs/extracts administered.

Group of Rats\Doses of Drugs/Extracts Administered
1. 5g/100mls of EB + 0.5mls of NS
2. 5g/100mls of EB + 40mg/kg bw of TH
3. 5g/100mls of EB + 40mg/kg bw of LEER extract
4. 5g/100mls of EB + 40mg/kg bw of LEEL extract
5. 5g/100mls of EB + 40mg/kg bw of LEES extract
6. 5g/100mls of EB + 40mg/kg bw of LEAR extract
7. 5g/100mls of EB + 40mg/kg bw of LEAL extract
8. 5g/100mls of EB + 40mg/kg bw of LEAS extract
9. 5g/100mls of EB + 40mg/kg bw of LEBR extract
10. 5g/100mls of EB + 40mg/kg bw of LEBL extract
11. 5g/100mls of EB + 40mg/kg bw of LEBS extract
12. 5g/100mls of EB + 40mg/kg bw of LENR extract
13. 5g/100mls of EB + 40mg/kg bw of LENL extract
14. 5g/100mls of EB + 40mg/kg bw of LENS extract

EB = Ethidium Bromide, NS = Normal Saline,
bw = bodyweight,
TH = Tamsulosin Hydrochloride,
LE = *Lycopersicon esculetum,*
LEER = LE Ethanol root extract,
LEEL = Ethanol leaf extract, ES = LE Ethanol stem extract,
LEAR = Aqueous root extract,
LEAL = Aqueous leaf extract, LEAS = Aqueous stem extract,
LEBR = Butanol root extract,
LEBL = Butanol leaf extract, LEBS = Butanol stem extract,
LENR = N-hexane root extract,
LENL = N-hexane leaf extract, and LENS = N-hexane stem extract.

Enzyme Linked Immunosorbent Assay (ELISA) of concentrations of TNFα, Caspase-3, Ki67, p53, MBP and GFAP in cerebral cortices of rats of Groups 1 - 14

Each cerebral cortex was divided into two hemispheres. The right cerebral hemisphere was weighed and placed in 10 % formalin and processed for light microscopy using conventional histological

procedures, such as dehydration, clearing, impregnation (infiltration), embedding, sectioning, mounting and staining. The slices were stained with Hematoxyline and Eosin, and examined under the microscope for histopathological changes as earlier described [21]. Photomicrographs of the slides were prepared.

The left cerebral hemisphere was isolated and then subjected to thorough homogenization using 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 -4 <"C, and thereafter assayed for concentrations of TNFα, Caspase-3, Ki67, p53, MBP and GFAP in the prefrontal cortices of rats of Groups 1 – 14 using ELISA technique.

Statistical analyses

The statistical data acquired from the micro plate ELISA results were also analysed using one-way analysis of variance (ANOVA), and Tukey post hoc test was used for group comparison as appropriate on Graph pad prism 2005 version. The level of significance was set at $p<0.05$.

Results

Histopathological Evaluations

Histopathological evaluations showed normal histo-architecture of the prefrontal cortex in rats of Control and Experimental Groups 1 - 14. There was normal appearance of the cortical layers (molecular layer 1 to the multiform layer 6) with

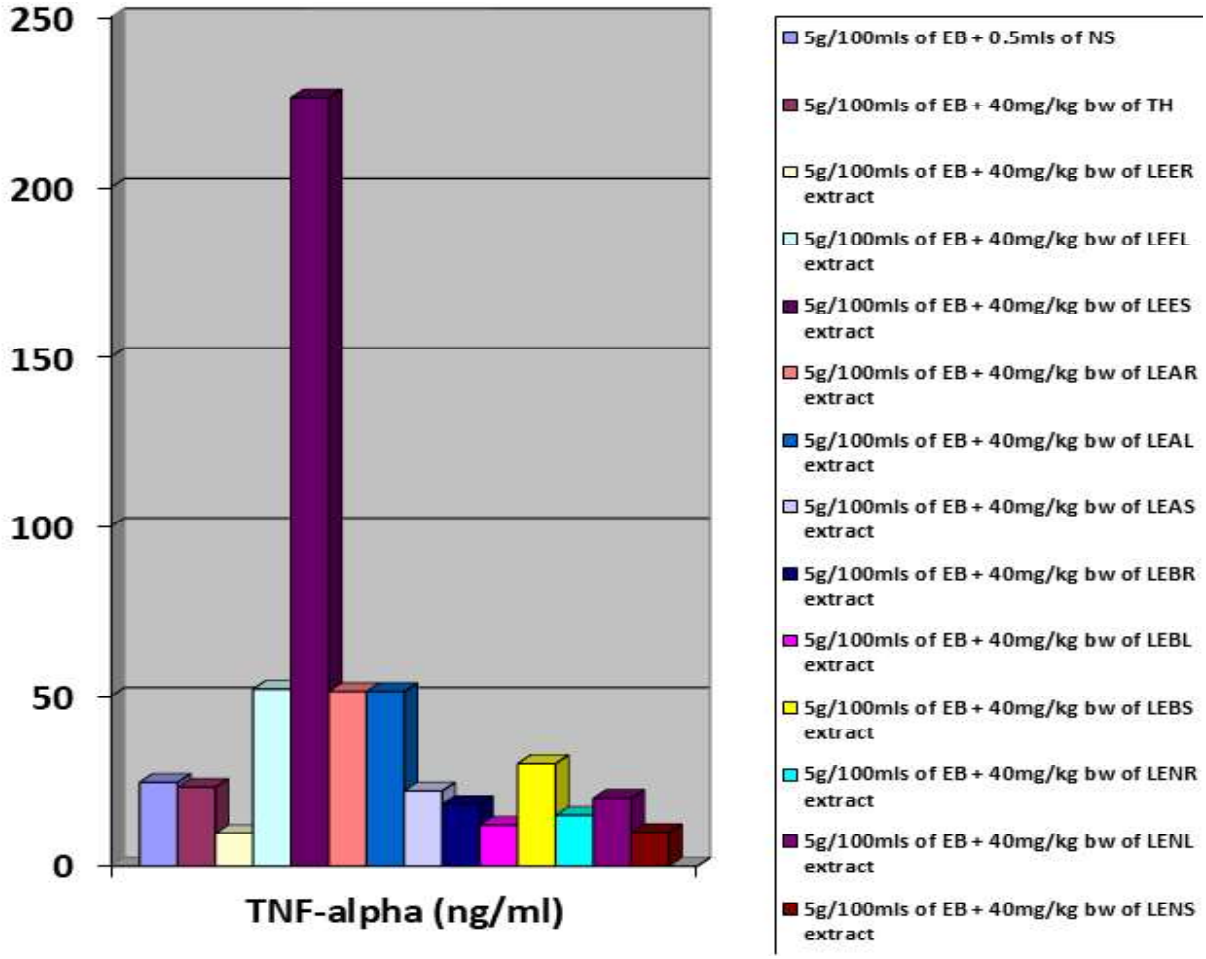

Fig. 1: *Concentrations of TNFα (Mean±SD) (ng/ml) in cerebral cortices of rats.*
EB = Ethidium Bromide, NS = Normal Saline, bw = bodyweight, TH = Tamsulosin Hydrochloride, LE = Lycopersicon esculetum, LEER = LE Ethanol root extract,
LEEL = Ethanol leaf extract, ES = LE Ethanol stem extract, LEAR = Aqueous root extract, LEAL = Aqueous leaf extract, LEAS = Aqueous stem extract, LEBR = Butanol root extract, LEBL = Butanol leaf extract, LEBS = Butanol stem extract, LENR = N-hexane root extract, LENL = N-hexane leaf extract, and LENS = N-hexane stem extract.

normal appearance of the pyramidal, granule and fusiform cell [22].

Evaluations of concentrations of TNFα in cerebral cortices of rats

Results showed significant lower mean values (P≤0.05) of TNFα concentrations (ng/ml) in rats of Group 3 (P=0.02), 9 (P-0.05) 10 (P=0.04), 12 (P = 0.04) and 14 (P = 0.03), and non-significant lower mean values (P≥ 0.05) in rats of Groups 2 (P = 0.77), 8 (P = 0.81) and 13 (P = 0.06) when compared with Control Group 1 (24.50 ± 5.05) (Figure 1). However, there were significant higher mean values (Pd") of TNFα concentrations (ng/ml) in rats of Groups 4, (P = 0.03), 5 (P = 0.01), 6 (P = 0.03), 7 (P = 0.03) and 11 (P = 0.05), when compared with Group 1 (24.50 ± 5.05) (Figure 1).

Evaluations of concentrations of Caspase-3 in cerebral cortices of rats

Results showed statistically significant higher mean values (P≤0.05) of Caspase 3 concentrations (ng/ml) in rats of Groups 4 (P = 0.04), 6 (P = 0.01), 7 (P = 0.04), 13 (P = 0.05) and 14 (P = 0.01), when compared with Control Group 1 (-9.42 ±

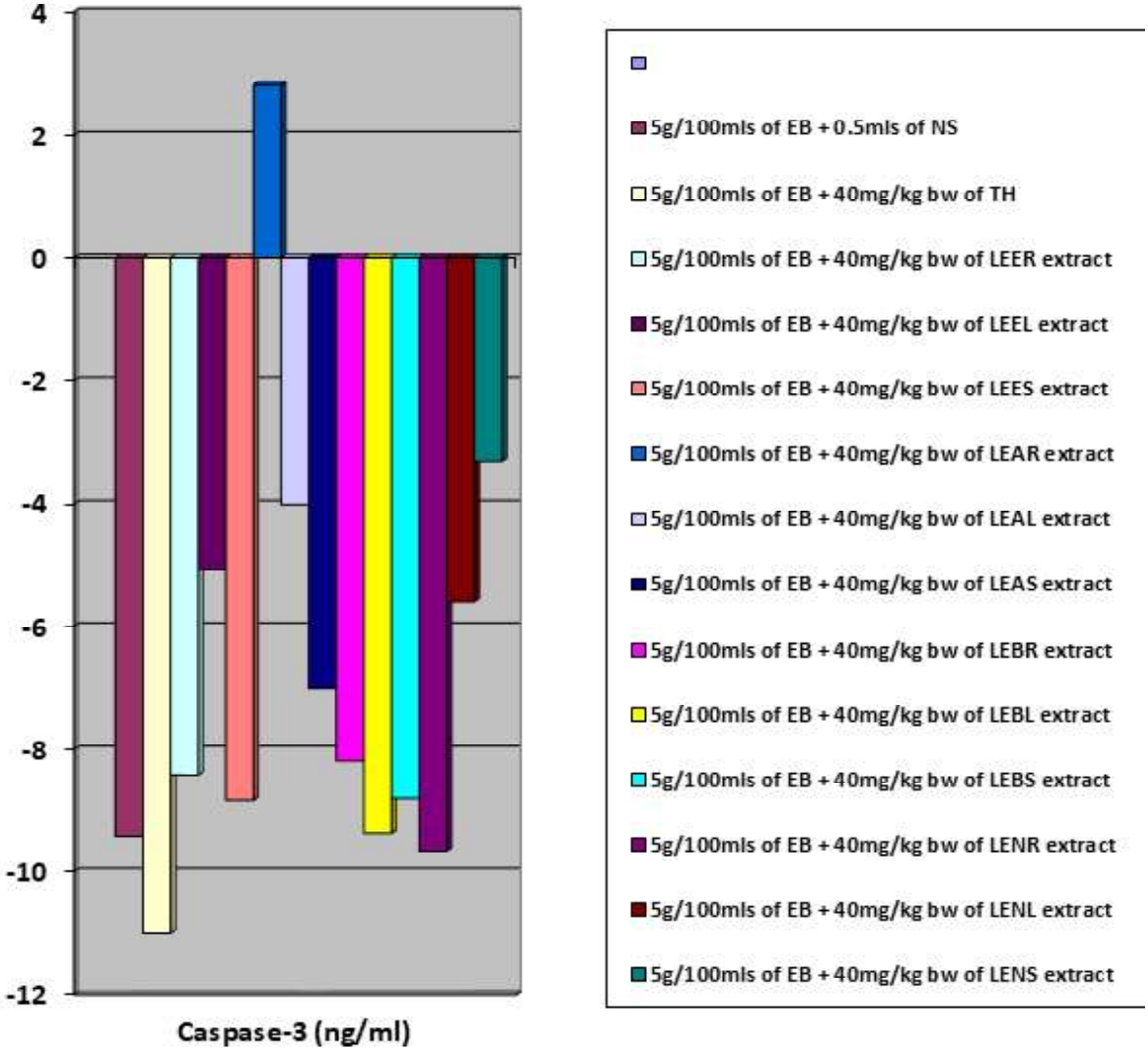

Fig. 2: Concentrations of Caspase-3 (Mean±SD) (ng/ml) in cerebral cortices of rats.
EB = Ethidium Bromide, NS = Normal Saline, bw = bodyweight, TH = Tamsulosin Hydrochloride,
LE = *Lycopersicon esculetum,* LEER = LE Ethanol root extract,
LEEL = Ethanol leaf extract, ES = LE Ethanol stem extract, LEAR = Aqueous root extract,
LEAL = Aqueous leaf extract, LEAS = Aqueous stem extract, LEBR = Butanol root extract,
LEBL = Butanol leaf extract, LEBS = Butanol stem extract, LENR = N-hexane root extract,
LENL = N-hexane leaf extract, and LENS = N-hexane stem extract.

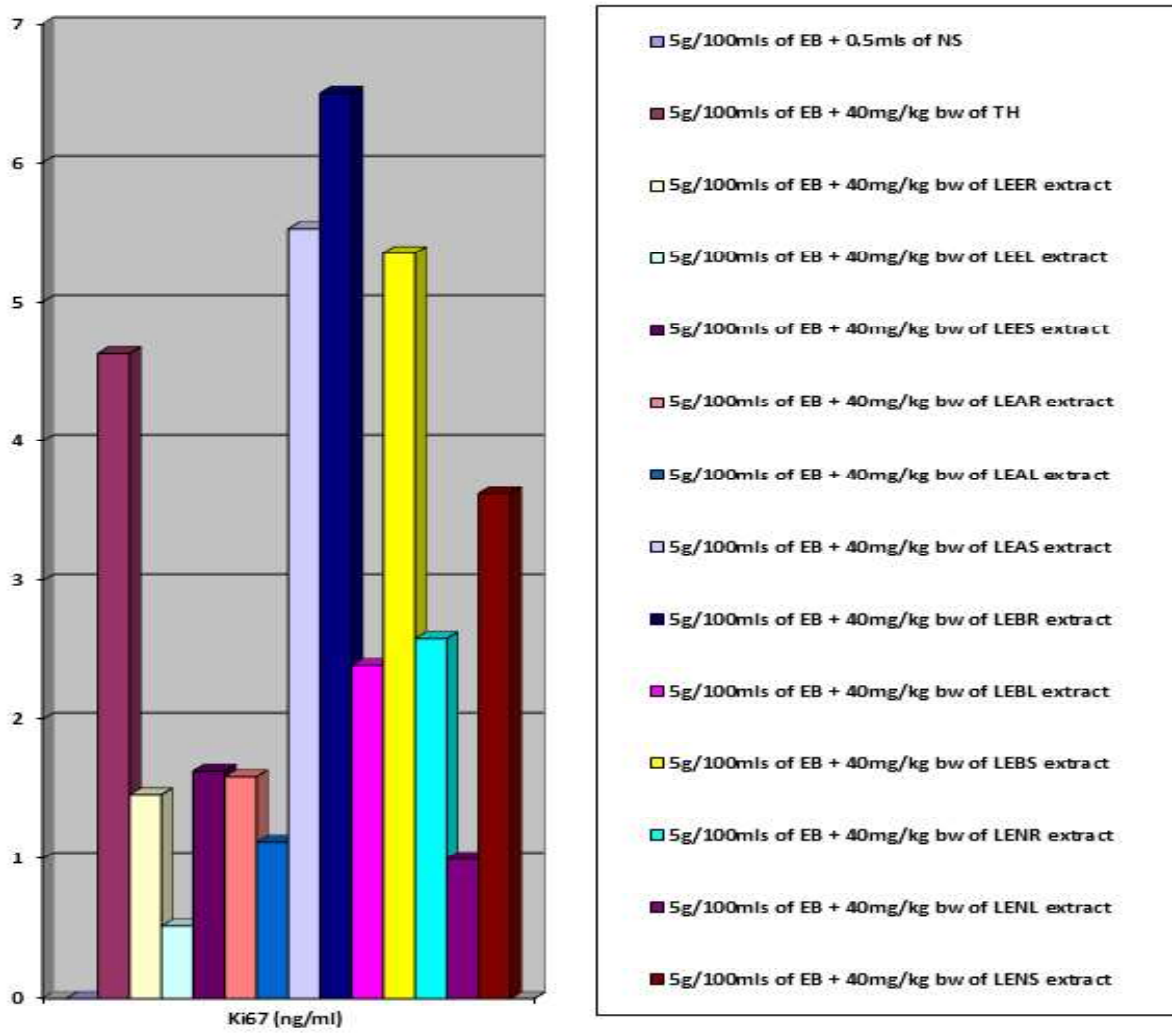

Fig. 3: *Concentrations of Ki67 (Mean±SD) (ng/ml) in cerebral cortices of rats.*
EB = Ethidium Bromide, NS = Normal Saline, bw = bodyweight, TH = Tamsulosin Hydrochloride,
LE = Lycopersicon esculetum, LEER = LE Ethanol root extract, LEEL = Ethanol leaf extract,
ES = LE Ethanol stem extract, LEAR = Aqueous root extract, LEAL = Aqueous leaf extract,
LEAS = Aqueous stem extract, LEBR = Butanol root extract, LEBL = Butanol leaf extract,
LEBS = Butanol stem extract, LENR = N-hexane root extract, LENL = N-hexane leaf extract, and
LENS = N-hexane stem extract.

3.83). (Figure 2). There were statistically non-significant higher mean values ($P \geq 0.05$) of Caspase 3 concentrations (ng/ml) in rats of Groups 3 (P = 0.82), 5 (P = 0.63), 8 (P = 0.60), 9 (P = 0.74), 10 (P = 0.99) and 11 (P = 0.67) when compared with Control Group 1 (-9.42 ± 3.83) (Figure 1). There were statistically non-significant lower mean values ($P \geq 0.05$) of Caspase 3 concentrations (ng/ml) in rats of Groups 2 (P = 0.06) and 12 (P = 0.95) when compared with Control Group 1 (-9.42 ± 3.83) (Figure 2).

Evaluations of concentrations of Ki67 in cerebral cortices of rats

Results showed statistically significant lower mean values ($P \leq 0.05$) of Ki67 concentrations (ng/ml) in rats of Groups 2 (P = 0.05), 3 (P = 0.01), 4 (P = <0.01), 5 (P = <0.01), 6 (P = 0.02), 7 (P = <0.01), 10 (P = 0.03), 12 (P = 0.03), 13 (P = <0.01) and 14 (P = 0.04), when compared with Control Group 1 (10.16±4.13). (Figure 3). There were statistically non-significant lower mean values ($P \geq 0.05$) of Ki67 concentrations (ng/ml) in rats of Groups 8 (P = 0.06), 9 (P = 0.07) and 11 (P = 0.06), when compared with Control Group 1 (10.16±4.13). (Figure 3).

Evaluations of concentrations of p53 in cerebral cortices of rats

Results showed statistically significant higher mean values ($P \leq 0.05$) of p53 concentrations (ng/ml) in rats of Groups 3 (P = 0.05), 5 (P = 0.01), 6 (P = 0.02), 7

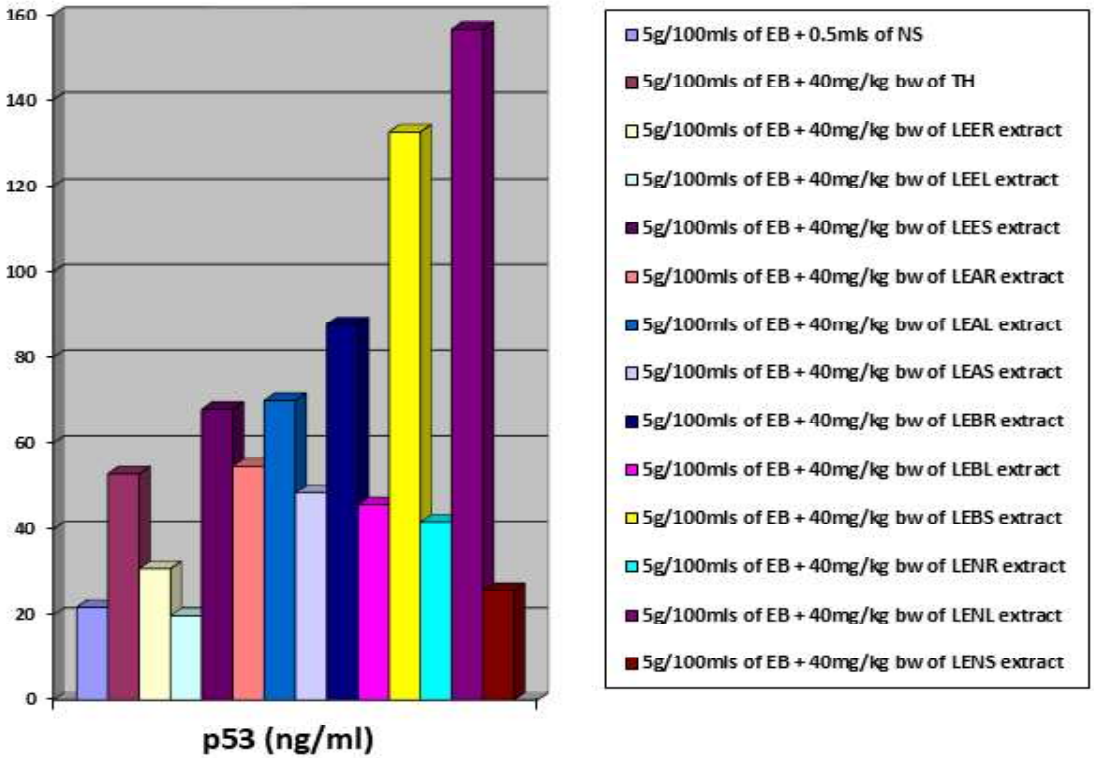

Fig. 4: *Concentrations of p53 (Mean±SD) (ng/ml) in cerebral cortices of rats.*
EB = Ethidium Bromide, NS = Normal Saline, bw = bodyweight, TH = Tamsulosin Hydrochloride, LE = Lycopersicon esculetum, LEER = LE Ethanol root extract, LEEL = Ethanol leaf extract, ES = LE Ethanol stem extract, LEAR = Aqueous root extract, LEAL = Aqueous leaf extract, LEAS = Aqueous stem extract, LEBR = Butanol root extract, LEBL = Butanol leaf extract, LEBS = Butanol stem extract, LENR = N-hexane root extract, LENL = N-hexane leaf extract, and LENS = N-hexane stem extract.

(P = 0.01), 8 (P = 0.03), 9 (P = <0.01), 10 (P = 0.03), 11 (P = <0.01), 12 (P = 0.04) and 13 (P = <0.01), but statistically non-significant higher mean values (P≥0.05) of p53 concentrations (ng/ml) in rats of Group 14 (P = 0.71), when compared with Control Group 1 (21.79±2.88). (Figure 4). However, there were statistically non-significant lower mean values (P≥0.05) of p53 concentrations (ng/ml) in rats of Group 4 (P = 0.84), when compared with Control Group 1 (19.48±2.88) (Figure 4).

Evaluations of concentrations of MBP in cerebral cortices of rats

Results showed statistically significant lower mean values (P≤ 0.05) of Myelin Basic Protein concentrations (ng/ml) in rats of Groups 2 (P = 0.04), 3 (P = <0.01), 4 (P = <0.01), 5 (P = 0.03), 6 (P = 0.05), 7 (P = 0.01), 8 (P = 0.04), 9 (P = <0.01), 10 (P = 0.02), 11 (P = 0.03), 12 (P = 0.01), 13 (P = 0.04) and 14 (P= <0.01), when compared with Control Group 1 (7.38±0.22)

Evaluations of concentrations of GFAP in cerebral cortices of rats

Results showed statistically significant lower mean values (P≤ 0.05) of GFAP concentrations (ng/ml) in rats of Groups 3 (P = 0.05), 4 (P = 0.05), 5 (P = 0.05), 6 (P = 0.04), 7 (P = 0.05), 8 (P =0.05) and 9 (P =0.05), but non-significant lower mean values (P≥0.05) in rats of Groups 13 (P = 0.92) and 14 (P = 0.89), when compared with Control Group 1 (0.59±0.02) (Figure 6). In addition, there were statistically non-significant higher mean values (P≥0.05) of GFAP concentrations (ng/ml) in rats of Groups 2 (P = 0.99), 10 (P = 0.95), 11 (P = 0.65) and

12 (P = 0.99), when compared with Control Group 1 (0.59±0.02).

Discussion

Exposures to chemical or physical injury, anoxia or starvation results in inflammation via cell damage characterized by leakage of cell contents into the adjacent tissues and capillary transmigration of granulocytes to the injured tissue [23]. In neuroinflammation, there is activation of microglial cells resulting in the release of pro-inflammatory factors and cytokines such as tumour necrosis factor alpha (TNFα). In addition, TNFα-induction of apoptosis and necrosis of oligodendrocytes in experimental animal models has been well reported [23]. Hence, in chemicals-induced mutagenesis and neurotoxicity, elevated TNFα level is a characteristic immune response mechanism of induction of apoptosis.

TNFα stimulates acute phase reactions in inflammation [23], hence, the observed up-regulation of TNFα in rats of Control Group 1 exposed to topical administration of 5 g/100 mls of EB implied EB-induction of neuro-inflammation (Figure 1). However, post-treatments of EB-induced neurotoxicity with Ethanol root, Aqueous stem, Butanol root, Butanol leaf, N-hexane root, N-hexane leaf and N-hexane stem extracts of *Lycopersicon esculetum* (LE) resulted in decreased and downregulations of TNFα concentrations in rats of Groups 2 - 3, 8 - 10 and 12 - 14, when compared with Control Group 1 (Figure 1). In contrast, post-treatments of EB-induced neuro-inflammation with Ethanol leaf, Ethanol stem, Aqueous root, Aqueous leaf and Butanol stem extracts of LE resulted in significant upregulation of TNFα concentrations in rats of Groups 4 – 7 and 11, when compared with Group 1 (Figure 1). This implied that these extracts did not ameliorate EB-induced neuro-inflammation.

Cells exposed to cytotoxic injuries resulting in inflammation are usually destroyed via necrosis, which involves leakage of cellular components into surrounding extracellular spaces. The resolution of inflammatory reactions by adjacent cells involves apoptosis, thereby increasing tissue damage due to inflammation [23-25]. The intrinsic or mitochondrial and the extrinsic or death receptor pathways of apoptosis involve activation of effector caspases such as Caspase-3 [24,25], hence its increased levels implied promotion of apoptosis.

The exposure of rats of Control Group 1 to topical administration of 5 g/100 mls of EB resulted in decreased and downregulation of Caspase 3 concentrations (Figure 2), indicating EB-induction of anti-apoptosis. However, post-treatments of EB-induced anti-apoptosis with all tested extracts of LE resulted in increased and upregulations of Caspase 3 concentrations in rats of Groups 3 - 11 and 13 - 14, when compared with Control Group 1 (Figure 2). These observations implied that EB-induced anti-apoptosis was ameliorated via promotion of apoptosis and up-regulation of Caspase-3 by treatments with all the tested leaf, stem and root extracts of LE except the N-hexane root extract. In other words, post-treatments of EB-induced anti-apoptosis with Tamsulosin Hydrochloride and N-hexane root extract of LE did not ameliorate EB-induced anti-apoptosis.

Ki-67 protein is detected during all the active phases of the cell cycle and it is usually used as a complement to grading systems that include mitotic counting as a sign of proliferation [26,27]. Ki-67 is not expressed by quiescent or resting cells in the G0 phase, and it is one of the five genes (out of 16 cancer-associated genes) of proliferation that is of important weight to the Oncotype score implying that it is an excellent operational marker for evaluation of the proliferation of a given cell population and the aggressiveness of malignancies [26-28].

The exposure of rats of Control Group 1 to topical administration of 5 g/100 mls of EB without further treatment resulted in increased and upregulation of Ki-67 concentrations (Figure 3), implying EB-induction of increased and abnormal proliferation. This observation is in agreement with the review work by [29], which noted that all tissues tested showed Ki-67 staining in proliferating cell populations and no convincing evidence has been presented that the Ki-67 protein may not be expressed by any proliferating human or animal cell type. However, post-treatments with all tested extracts of LE ameliorated EB-induced increased proliferation and resulted in decreased and downregulations of Ki-67 concentrations in rats of Groups 3 – 14 (Figure 3).

Uncontrolled proliferation in cancer or other anomalies is associated with a high level of apoptosis. The tumour suppressor gene p53, which is regarded as the guardian of the genome is central to tissue protection against abnormal proliferation and the development of cancer. p53 promotes cell cycle arrest or apoptosis in response to DNA damage, hypoxia, oncogene expression, nutrient deprivation and ribosome dysfunction [28]. p53 induces apoptosis via both the intrinsic and extrinsic pathways involving activation of effector Caspase 3. Hence, increased p53 levels correlates with increased Caspase 3 levels

and promotion of apoptosis in anti-proliferation and tumour suppression.

Post-treatments of EB-induced neurotoxicity and anti-apoptosis with all tested extracts of LE resulted in increased and upregulation of p53 in rats of Groups 3 and 5 – 14, when compared with Control Group 1 (Figure 4). This implied that all tested leaf, stem and root extracts of LE (except LE Ethanol leaf extract) ameliorated EB-induced anti-apoptosis via the up-regulation of p53 in rats.

EB-induced demyelination was associated with inflammation and depletion of neuroglia (astrocytes) in the early hours of intoxication in Wistar rats exposed to 1 µl of 0.1 % EB [9]. The depletion of astrocytes results in a breach in the glial-limiting membrane allowing invasion by Schwann cells for the repair of lost myelin sheaths [9]. The destruction of the intact myelin sheath further results in the dissociation of Myelin Basic Protein (MBP) from the plasma membrane allowing it to act in a free, membrane-unbound manner in the extracellular matrix thereby evoking an immune response in the nervous system [30]. Hence, demyelination is associated with increased MBP levels.

Post-treatments of EB-induced neurotoxicity with all tested extracts of LE resulted in decreased and downregulation of MBP in rats of Groups 3 – 14, when compared with Control Group 1 (Figure 5). This implied that all tested leaf, stem and root extracts of LE ameliorated EB-induced demyelination via the downregulation of MBP in rats.

Gliosis which is characterized by transformation of microglia and astrocytes into their activated (possibly phagocytic) phenotypes is the dominant and universal response to drug or chemical-induced nervous system damage [31]. Glial fibrillary acidic protein (GFAP) is the oldest and most well documented of glial genes expressed in gliosis. In gliosis, enhanced expression of GFAP is very rapid, linked to the time course and location of damage, and occurs at toxicant doses below those associated with evident cytopathology/histology and/or behavioural changes [31].

Post-treatments of EB-induced neurotoxicity with LE extracts resulted in decreased and down regulation of GFAP in rats of Groups 3 – 9 and 13 – 14, when compared with Control Group 1 (Figure 6). This implied that all tested leaf, stem and root extracts of LE (except Butanol leaf, Butanol stem and N-hexane root extracts) ameliorated EB-induced astrocytes damage, disruption of brain homeostasis, neurodegeneration and gliosis. This observation is in agreement with [32], which reported that GFAP aggregates are toxic to astrocytes resulting in astroglial degeneration, Rosenthal fiber formation and the subsequent white matter degeneration pathology observed in Neurodegenerative Diseases.

Conclusion

In conclusion, elevated TNFα levels has been previously noted to result in induction of apoptosis in neural cells, especially oligodendrocytes. Post-treatments of EB-induced neurotoxicity with extracts doses of *Lycopersicon esculetum* (LE) resulted in reduced levels of TNFα, but elevated levels of Caspase 3 and p53 in rats. This implied that LE drives apoptosis via downregulation of TNFα with consequent activation of the Caspase-3/p53 apoptotic pathway. In addition, post-treatments of EB-induced neurotoxicity with extracts of LE resulted in positive immunomodulations of the concentrations of biomarkers of carcinogenesis (TNFα, Ki67, p53 and Caspase-3) and biomarkers of Multiple Sclerosis (TNFα, MBP and GFAP) in rats. This further implied that the roots, stems and leaves extracts of LE conferred a degree of neuro-regenerative, anti-inflammatory, anticancer and anti-Multiple Sclerosis potentials against EB-induced neurotoxicity.

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References

1. Serguei V and Xin W. Cancer stem cells and drug resistance: the potential of nanomedicine. Nanomedicine (London) 2002; **7**(4): 597-615.
2. Alfarouk KO, Stock C-M, Taylor S, *et al*. Resistance to cancer chemotherapy: failure in drug response from ADME to P-gp. Cancer Cell Int 2015; 15: 71. DOI:101186/s12935-015-0221-1.
3. Chen K, Huang Yh and Chen JI. Understanding and targeting cancer stem cells: therapeutic implications and challenges. Acta Pharmacologia Sinica 2013; 34: 732-740.
4. Plaks V, Kong N and Werb Z. The Cancer Stem Cell Niche: How Essential Is the Niche in Regulating Stemness of Tumor Cells? Cell Stem Cell, 2015; 16(3): 225-238.
5. Omotoso GO, Gbadamosi IT, Afolabi TT, Abdulwahab AB and Akinlolu AA. Ameliorative effects of Moringa on cuprizone-induced memory

decline in rat model of multiple sclerosis. J Anatomy Cell Biol 2018; 51: 119-127.
6. Masvekar R, WU T, Kosa P, *et al*. Cerebrospinal fluid biomarkers link toxic astrogliosis and microglial activation to multiple sclerosis severity. Multiple Sclerosis Related Disorder 2019; 28: 34-43.
7. Nabil KEA and Samia JK. Blood Biomarkers as Outcome Measures in Inflammatory Neurologic Diseases. Neurotherapeutics 2017; 14: 135-147.
8. Oh SJ, Hemmi S and Hatanaka Y. Diagnostic markers for axonal degeneration and demyelination in sensory nerve conduction. Muscle Nerve 2016; 6: 866-71.
9. Riet-Correa G, Fernandes CG, Pereira LAV and Graça DL. Ethidium bromide-induced demyelination of the sciatic nerve of adult Wistar rats. Brazilian J Med Biol Res 2002; 35: 99-104.
10. Baukje JVDS, Daphne YSV, Markus K, *et al*. In Vitro and In Vivo Models of Multiple Sclerosis. CNS Neurolog Disorders - Drug Targets 2012; 11: 570-588.
11. James WA Caroline JC Caroline J and Anjanapriya S. Toxic and Mutagenic Impact of Ethidium Bromide Traces on the Growth of *Escherichia Coli* KL226. Int J Current Sci 2013; 8: 28-32.
12. Saeidnia S and Abdollahi M. Are other fluorescent tags used instead of ethidium bromide safer? DARU J Pharmaceutical Sciences 2013; 21(1): 71. doi: 101186/2008-2231-21-71.
13. Akortha EE, Niemogha MT and Edobor O. Mutagenic and Genotoxic Screening of Eight Commonly Used Skin Whitening Creams in Nigeria. Bayero J Pure Appl Sci 2011; 5(1): 5-10.
14. Warren EB, Aicher AE, Fessel JP and Konradi C. Mitochondrial DNA depletion by ethidium bromide decreases neuronal mitochondrial creatine kinase: Implications for striatal energy metabolism. PLOS ONE 2017; 12(12): e0190456 https://doiorg/101371/journalpone0190456.
15. Balestrieri ML, Prisco RDe, Nicolaus B, *et al*. Lycopene in association with α-tocopherol or tomato lipophilic extracts enhances acyl-platelet-activating factor biosynthesis in endothelial cells during oxidative stress. 2004; Free Radical Biol Medicine 36(8): 1058-1067.
16. Aust OAAN, Ale-Agha N, Zhang L, *et al*. 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 Biophy, 2004; 430(1): 127-134
19. Karppi J, Kurl S, Nurmi T, *et al*. Serum lycopene and the risk of cancer: The Kuopio Ischaemic Heart Disease Risk Factor (KIHD) study. Ann Epidemiol 2009; 19(7): 512-518.
20. Tan SP, Parks SE, Stathopoulos CE and Roach PD. Extraction of Flavonoids from Bitter Melon. Food Nutrition Sci 2014; 5: 458-465.
21. Zahr NM, Luong R, Sullivan EV and Pfefferbaum A. Measurement of Serum Liver and Brain Cytokine Induction Thiamine Levels and Hepatopathology in Rats Exposed to a 4-Day Alcohol Binge Protocol. Alcohol Clinical Exp Res 2010; 34(11): 1858-1870.
22. Akinlolu AA, Ameen M, Quadri T, *et al*. Extraction, isolation and evaluation of anti-toxic principles from *Moringa oleifera* (MOF6) and *Myristica fragrans* (*Trimyristin*) upregulated Acetylcholinesterase concentrations in Sodium arsenite-induced neurotoxicity in rats. J Phytomed Therap 2020; 19(2): 503-519.
23. Akinlolu AA, Sulaiman FA, Suleiman SK, *et al*. Cajanus cajan and Lycopersicon esculetum ameliorated Ethidium Bromide-induced mitochondrial dysfunction and neuro-degeneration in rats. Nigerian J Basic and Appl Sci 2020 (Manuscript Under Review).
24. 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.
25. Liu P-F, Hu Y-C, Kang B-H, *et al*. Expression levels of cleaved caspase-3 and caspase-3 in tumorigenesis and prognosis of oral tongue squamous cell carcinoma. PLOS ONE 2017; 12(7): e0180620. https://doiorg/101371/journal pone0180620.
26. Guo M and Hay BA. Cell proliferation and apoptosis. Current Opinion Cell Biol 1999; 11: 745–752.
27. Luporsi E, Andre F, Spyratos F, *et al*. Ki-67: level of evidence and methodological considerations for its role in the clinical management of breast cancer: analytical and critical review. Breast Cancer Resistant Treatment 2012; 132: 895–915.
28. Xiao LI-J, Shuang Z, En-Hong, *et al*. Clinicopathological and prognostic significance of Ki-67 caspase-3 and p53 expression in gastric carcinomas. Oncology Letters 2013; 6: 1277-1284.

29. Scholzen T and Gerdes J. The Ki-67 protein: from the known and the unknown. J Cell Physiol 2000; 182: 311–322.
30. Zhang J, Sun X, Zheng S, *et al*. Myelin Basic Protein Induces Neuron-Specific Toxicity by Directly Damaging the Neuronal Plasma Membrane. PLOS ONE 2014; (9): e108646. doi:101371/journalpone0108646.
31. James POC and Krishnan S. Glial fibrillary acidic protein and related glial proteins as biomarkers of neurotoxicity. Expert Opinion Drug Safety 2005; 4(3): 433-442.
32. Yang Z and Wang KK. Glial fibrillary acidic protein: from intermediate filament assembly and gliosis to neurobiomarker. Trends Neurosci 2015; 38(6): 364-374.

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