Xylopia Aethiopica Suppresses Markers of Oxidative Stress, Inflammation and Cell Death in the Brain of Wistar Rats Exposed to Glyphosate

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Abstract

Background: The herbicide “Roundup” is used extensively in agriculture to control weeds. However, by translocation, it can be deposited in plants, their proceeds, and in the soil, thus provoking organ toxicities in exposed individuals. Neurotoxicity among others is one of the side effects of roundup which has led to an increasing global concern about the contamination of food by herbicides. *Xylopia aethiopica*is known to have medicinal properties due to its antioxidative and anti-inflammatory properties, it is hypothesized to neutralize roundup-induced neurotoxicity.

Methods: Thirty-six (36) Wistar rats were used for this study. The animals were shared equally into six groups with six rats each. Glyphosate administration to three of the six groups was done orally and for one week. Either *Xylopia aethiopica*or vitamin C was co-administered to two of the three groups and also administered to two other groups and the final group served as the control.

Results: Our studies demonstrated that glyphosate administration led to a significant decrease in antioxidants such as catalase, superoxide dismutase, glutathione, and glutathione peroxidase. We also observed a significant increase in inflammatory markers such as tumour necrosis factor- α, interleukin 6, C-reactive protein and immunohistochemical expression of caspase-3, cox-2 and p53 proteins ($p < 0.05$). However, *Xylopia aethiopica* co-administration with glyphosate was able to ameliorate the aforementioned changes when compared to the control ($p < 0.05$). Degenerative changes were also observed in the cerebellum, hippocampus, and cerebral cortex upon glyphosate administration. These changes were not observed in the groups treated with *Xylopia aethiopica* and vitamin C.

Conclusion: Taken together, *Xylopia aethiopica* could possess anti-oxidative and anti-inflammatory properties that could be used in combating glyphosate neurotoxicity.

Background

*P*hosphonomethyl-glycine known as glyphosate is a widely used broad-spectrum herbicide that partakes both in rural and non-rural activities (Cattani et al. 2017). It is a highly active herbicide that destroys both broadleaf plants and grasses (Valavanidis 2018). One major explanation for its widespread use is the enhancement of farming output that accounts for increased food-producing capacity in any population (Cattani et al. 2014a). Even though glyphosate is largely well-thought-out to be nontoxic and regarded as exerting negligible health hazards in many animal species including mammals (Williams et al. 2012), evidence exists relating this herbicide to neurological challenges (Samsel and Seneff 2015; Gallegos et al. 2016), resistance to the antibiotic, hepatic and renal toxicities (Bai and Ogbourne 2016) amid others, making it a very great public concern. The most widely studied health problems associated with glyphosate is neurological damage. Initiation of neuro-inflammation and oxidative stress by this chemical was announced by El-Shenawy, (2009) and Astiz et al., (2012). Besides, certain formulations of this herbicide were stated to express the ability in aggravating oxidative stress from definite brain sections including the hippocampus and cerebral cortex (Cattani et al. 2014a). Contact with glyphosate

has been proposed to be a key influence in neurodegenerative maladies (Gallegos et al. 2016) (Kirby et al., 2001; Patel et al., 2006; Samsel and Seneff 2015) with various mechanisms involving oxidative damage (El-Shenawy 2009), apoptotic and autophagic cell deaths in neuronal cells (Gui et al. 2012). Therefore, exploring promising therapeutic approaches to diminish neurotoxicity induced by herbicides will be highly beneficial for the public.

Materials of natural origin are considered profitable in lessening herbicide-induced neurotoxicity. Already, numerous organic constituents including ascorbic acid, and extracts from *Silybum marianum* and *camellia* species, were employed and they showed various protective activities against toxicities produced by the herbicide (Khan 2006; El-Hamid and Refaie 2009).

Xylopia aethiopica commonly known as Negro pepper is a member of the Annonaceae family. It is one of the kinds that survive in the perennial tropical African rain forests (Orwa et al. 2020). Negro pepper is employed in different continents as a spice in food processing or for various therapeutic measures (Fall et al. 2003; Ogunkunle and Ladejobi 2006). Additionally, bioactive ingredients in *X. aethiopica* have been known for their anticarcinogenic capacities (Ogunkunle and Ladejobi 2006) and ability to lessen radiation-provoked oxidative damage (Adaramoye et al. 2008)

X. aethiopica-derived Flavonoids were confessed to display anti-inflammatory and antioxidant assets (Biney et al. 2016). Of note is the neuroprotective effects displayed by this plant in various studies (Adaramoye et al. 2008);(Nneka Orish et al. 2021); (George et al. 2019); (Biney et al. 2016), it is therefore being hypothesized that *X. aethiopica* extract could be able to alleviate glyphosate-induced neurotoxicity. There is no information regarding this aspect, hence, we embark on this study to assess the prospective neuroprotective effects of ethanol *extract from X. aethiopica* stem bark.

Methods

Materials

The herbicide with the trade name “Roundup” containing 480 g active ingredient/L of glyphosate a product of Monsanto India Ltd (Mumbai, India) was used for the study. Rat interleukin 6 (IL-6) ELISA kit (CSB-E04640r), and rat TNF kit (CSB-E11987r) were procured from CUSABIO Biotechnology Company while rat- C-reactive protein (CRP) ELISA kit was gotten from e-Bioscience Inc. Antibodies such as anti-rat p53, anti-Rat-COX-2, and anti-Rat-CASPASE-3 were used to quantify protein expression in different sections of the brain.

Plant collection and authentication

A short branch with leaves of *Xylopia aethiopica* plant was obtained at around 11:05 am on the 18th of March, 2020 from Oba-Ile, Olorunda L.G.A, Osogbo, Osun State for authentication at the Herbarium in the Department of Plant Biology, University of Ibadan, Nigeria. Plant Authentication of the leaf was done by Mr. Donatus Esimehhuai, who is a Botanist and Assistant Chief Technologist at the University of Ibadan,

Nigeria. The voucher number assigned was UIH-22944. The stem bark was thereafter obtained from the same tree for the study.

Preparation of plant extract

The stem bark sample obtained was air-dried for 14 days. Cut into pieces and blend. The blended material was macerated with 50% ethanol for 72 hours with intermittent shaking in the morning and evening. The extract was filtered after 72 hours and concentrated using a rotary evaporator to obtain enough yield of the extract. The yield of the extract was freeze-dried using a lyophilizer and stored in the refrigerator for further use. The percentage yield was 35 %

Characterization of plant extract

Following the completion of the extraction process, the plant extract was characterized using the gas column mass spectrophotometry technique (GCMS).

Experimental animals

A total of thirty-six (36) healthy male albino Wistar rats (*Rattus norvegicus*) with weights ranging between 150-200 g were procured from the animal holding facility of the college of medicine, Osun State University, Osogbo. The animals were housed in clean cages and placed in a well-ventilated room at a temperature between 29 ± 4 ^{o}C. The animals were first acclimatized for one week before the experiment.

Experimental Design

Thirty-six (36) Wistar rats were shared equally into six groups and treated as shown in Table 1. The period of administration was for one week and the route of administration was oral. Twenty-four (24) hours after the last exposure to treatment, the animals were sacrificed under light ether anaesthesia.

Table 1: Experimental design

Group	Treatment
1	Negative control
2	150 mg/kg Glyphosate
3	126.49 mg/kg *Xylopia aethiopica* (1/10 of LD 50).
4	250 mg/kg Vitamin C
5	150 mg/kg Glyphosate + 126.49 mg/kg *Xylopia aethiopica*
6	150 mg/kg Glyphosate + 250 mg/kg Vitamin C

Preparation brain homogenate

The brain was homogenized in ice-cold 0.25 M sucrose buffer (pH 7.4), all homogenate was centrifuged at 10,000 rpm for 10 minutes at -4 ^{0}C and the supernatant were stored for biochemical assays

Estimation of Catalase activity.

Catalase activity in the brain supernatant was determined using the method of Claiborne, (1985)

Determination of Superoxide dismutase

Superoxide dismutase activity in the brain supernatant was estimated in tandem with the procedure as described by Misra and Fridovich, (1972).

Determination of Reduced Glutathione (GSH) level

The level of reduced glutathione (GSH) in the brain supernatant was determined using the method of Beutler *et al*., (1963).

Determination of Glutathione Peroxidase (GPx) activity

The activity of glutathione peroxidase in the brain supernatant was estimated following the method of Rotruck *et al*., (1973) using H_2O_2 as substrate in the presence of GSH.

Assessment of acetylcholine esterase (AChE) and butyrylcholinesterase (BChE)activity

The activities of AChE and BChE in the rat sera were assessed according to a modified method described by Thomsen et al. (1988)

Quantitative estimation of C-reactive protein

An enzyme-linked immunosorbent assay (ELISA) (rat C-reactive protein (CRP) ELISA Kit, e-Bioscience, Inc), was used to measure the concentration of high sensitive C reactive protein in the rat sera by following the instructional manual.

Determination of Tumour necrosis factor-alpha

An enzyme-linked immunosorbent assay (rat TNF-α CSB-E11987r from CUSABIO) ELISA kit was used to measure the concentration of tumour necrosis factor-α in the rat sera by following the instructional manual.

Estimation of Interleukin-6 level

An enzyme-linked immunosorbent assay (from CUSABIO) ELISA kit was used to measure the concentration of Interleukin-6 in the rat sera by following the instructional manual.

Histopathology

Different brain section samples (hippocampus, cerebral cortex and cerebellum) were fixed with formalin solution (10%), sectioned and stained with hematoxylin and eosin for microscopic examination.

Immunohistochemistry

The expression of three proteins caspase-3, cyclooxygenase-2 and p53 expression were accessed using anti-rat caspase 3, anti-rat p53 and anti-rat cyclooxygenase-2 antibodies. Image J software was used to access the densitometry of each protein after expression.

Statistical Analysis

The results of this study were expressed as mean ± standard deviation. One-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons was used to analyze differences between means. p-values less than 0.05 was considered statistically significant. Data analysis was carried out using GraphPad Prism 6 software.

Results

Xylopia aethiopica and Vitamin C modulate antioxidant capacity and brain function markers in glyphosate-induced brain toxicity

Results from our study show that there is a significant decrease in the levels of superoxide dismutase, catalase, reduced glutathione, glutathione peroxidase, acetylcholinesterase and butyrylcholinesterase upon glyphosate administration in the brain of Wistar rats ($p < 0.05$). However, Vitamin C and *Xylopia aethiopica* administration either alone or in combination with glyphosate showed significant modulation in these antioxidants and brain function markers levels when compared to the glyphosate-only administered group ($p < 0.05$) as shown in figure 1.

Xylopia aethiopica and Vitamin C modulate inflammatory markers in glyphosate-induced brain toxicity

We also accessed inflammatory markers including tumour necrosis factor-α, C-reactive protein and interleukin-6. Our findings reveal significant elevation in these proteins in the glyphosate-only treated group when compared to the negative control ($p < 0.05$). However, Vitamin C and *Xylopia aethiopica* administration either alone or in combination with glyphosate showed a significant modulation in these inflammatory markers levels when compared to the glyphosate-only administered group ($p < 0.05$).

Xylopia aethiopica and Vitamin C modulate cerebral cortex architecture in glyphosate-induced brain toxicity.

Our studies revealed that the administration of glyphosate resulted in mild to severe degenerative changes in the cerebral cortex. These changes are characterized by the loss of nuclear and cytoplasmic materials and clustered pyknotic pyramidal neurons. Also, perineural spaces can be seen surrounding

degenerating neurons (black arrow), Axons and dendrites are scarcely appreciable around neurons in this group, and neuronal populations appear scarcely appreciable in this group. Lots of perineural spaces with empty content appear scattered across the micrographs in the groups with some presence of red inflammatory cells. Treatment with *Xylopia aethiopica* however was able to modulate these changes. As expected administration of *Xylopia aethiopica* or vitamin C only did not lead to changes in the architecture of the cerebral cortex as shown in figure 3.

Xylopia aethiopica and vitamin C modulate cerebellum architecture in glyphosate-induced brain toxicity.

Results from this study revealed that glyphosate administration induced mild to severe degenerative changes in the cerebellar cortex and was characterised by a fragmented Purkinje cell layer. Also, there appears to be a comparatively reduced cell density in the cortical granular layer of these groups. Treatment with *Xylopia aethiopica* however was able to modulate these changes in line with the morphologic appearance of the control. The cortical layers are better structured and delineated. Furthermore, glyphosate treatment caused depleted cytoplasmic and nuclear contents with loss of Purkinje cells alongside their cellular processes (black arrow). As expected administration of *Xylopia aethiopica* or vitamin C only did not lead to changes in the architecture of the cerebellum as shown in figure 4

Xylopia aethiopica modulates hippocampus architecture in glyphosate-induced brain toxicity

We also discovered that glyphosate administration induced mild to severe degenerative changes in the hippocampus and was characterised by fragmented pyramidal and granule cell layer loss of cellular processes and some loss in nuclear and cytoplasmic content. Treatment with *Xylopia aethiopica* however was able to modulate these changes as mild alterations were observed in the hippocampus but the granule cell layer, nuclear and cytoplasmic content appeared normal as compared to the control. As expected administration of *Xylopia aethiopica* or vitamin C only did not lead to changes in the architecture of the hippocampus as shown in figure 5.

Xylopia aethiopica and vitamin C modulate Caspase-3, p53 and COX-2 in glyphosate-induced brain toxicity

This study also investigated the effect of *Xylopia aethiopica* on apoptotic proteins and inflammatory proteins. Our studies reveal a significant upregulation in the activities of caspase-3, p53 and COX-2 upon glyphosate exposure in three different regions of the brain. This upregulation however was modulated by treatment with vitamin C and *Xylopia aethiopica* as shown in figure 6.

GC-MS analysis of *Xylopia aethiopica*

This study also documents the components of *Xylopia aethiopica* using Gas chromatography-mass spectrophotometry analysis to include scoparones, amphetamines and methenamines as shown in figure 7.

Discussion

Glyphosate is a major component of Roundup, it is an effective herbicide that kills both broadleaf plants and grasses (Valavanidis 2018). By absorption, some plants can take up a certain proportion of herbicides and metabolize them to prevent permanent damage to the plants (Duke et al. 2012). However, a considerable amount of these herbicides are translocated by the xylem and phloem tissues to different parts of the plants including fruits, roots and stems that serve as food for humans (Kanissery et al. 2019). There is an increasing concern about the rate of food contamination arising from herbicides and pesticides globally (Özkara et al. 2016). In this study, we attempted to underscore the potential beneficial effect of *Xylopia aethiopica* on glyphosate-induced toxicity in the brain of Wistar rats.

Phytomedicine has been acknowledged as an alternative form of medicine over the years (Ogbonnia et al. 2011). The phytochemical components of medicinal plants are primal to the pharmacological effects elicited by these plants against many diseases. *X. aethiopica* is one of the plants which has been reported to contain some phytochemicals which exhibit a wide range of biological effects. Among these are alkaloids, saponins, tannins, reducing sugar, anthraquinones, steroids, flavonoids, and glycosides (Aguoro *et al.*, 2016; Fleischer, 2003; Ehigiator and Ezeani, 2018). All of these account for its antioxidant, antimicrobial, anticancer, anti-inflammatory and anti-allergic potentials (Tatsadjieu et al. 2003; Fleischer et al. 2008; Aguoru and Olasan 2016). GCMS analysis of XASEE revealed the presence of Scoparones (Fig. 7) a member of the class of coumarins that have been shown to have antitumor activities (Thakur et al. 2015). Coumarins' impact on doxorubicin-induced oxidative stress has also been documented (Beillerot et al. 2008). The anti-oxidative potentials of XASEE may be due to the presence of scoparones as evident in this current study. GC-MS analysis also revealed the presence of amphetamines that act as a stimulant of the central nervous system. The beneficial effect of this class of compounds includes its cognition-enhancing properties and the treatment of attention deficit hyperactivity disorder (ADHD) and narcolepsy (Heal et al. 2013). At low doses, the levorotary form of amphetamine acts on norepinephrine while its dextrorotatory form acts on dopamine (Heal et al. 2013)

Cognitive function is generally coordinated by the brain (Kim and Won 2017). The brain coordinates major systemic activities by the activities of neurons distributed across the body (Schwartz 2016). The results of this study document a decrease in neurotransmitters such as acetylcholine esterase and butyrylcholinesterase upon glyphosate administration. Acetylcholine esterase is a cholinergic enzyme that is predominantly found in neuromuscular junctions such as nerves and muscles while butyrylcholinesterase supports the function of acetylcholine esterase by catalyzing its hydrolysis indicating its function in neurotransmission (Darvesh et al. 2003). The decrease in neurotransmitters upon glyphosate administration is in tandem with the studies of Glusczak et al., (2006) that documented a reduction in acetylcholine in fishes. Treatment with *Xylopia aethiopica* and vitamin C was able to significantly upregulate the activities of both neurotransmitters.

Anatomically, the brain can be said to be composed of three major structural divisions that include the cerebrum, the brainstem and the cerebellum (Schwartz 2016). Exposure of organisms to toxic

components can have a great effect on brain homeostasis and in turn the entire system (Jaishankar et al. 2014). Results from this study show that the architecture of the brain is affected by glyphosate administration. Degenerative changes are seen in the cerebral cortex, cerebellum and hippocampus. These alterations are characterized by changes in Purkinje cells, pyramidal cells, nuclear materials, neurons etc. These results are in tandem with the studies of Cattani *et al.*, (2014) that reported similar degenerative changes in the brain upon glyphosate administration although in chickens.

This effect termed oxidative stress is combated by the body's ability to effectively utilize antioxidants in abating exposure to free radicals (Lobo et al. 2010). These antioxidants are oxidizable species produced in little quantities and can mitigate cellular damage (Kurutas 2016). Enzymatic antioxidants include catalase, superoxide dismutase, glutathione-s-transferase, glutathione peroxidase, xanthine oxidase etc (Soto et al. 2014).

Oxidative stress has been implicated in the aetiology of brain associated disorders including Parkinson's Alzheimer's, and amyotrophic lateral sclerosis (Kim et al. 2015). Superoxide radical (O_2^-) is one of the most potent free radicals that cause damage to the brain cells due to its high reactivity (Phaniendra et al. 2015). Superoxide dismutase can catalyze the dismutation of superoxide radicals in the presence of water thereby generating hydrogen peroxide and oxygen molecules (Wang et al. 2018). Hydrogen peroxide (H_2O_2) is equally a reactive oxygen species and thus capable of causing cellular damage. Another enzymatic antioxidant catalase can split hydrogen peroxide into water and oxygen thereby mitigating its toxicity (Sharma et al. 2012). Administration of vitamin C and *Xylopia aethiopica* either alone or in combination with glyphosate resulted in significantly elevated levels of superoxide dismutase when compared to the glyphosate-only treated group.

Ascorbic acid is a water-soluble vitamin, its anti-oxidative properties have been documented extensively in the literature (Pehlivan 2017). It can scavenge free radicals and also act as a cofactor for other antioxidant enzymes thereby playing a central role in oxidative and nitrosative stress mitigation. Similarly, *Xylopia aethiopica*'s ability to restore the activities of superoxide dismutase may be due to the presence of bioactive compounds that includes flavonoids and tannins (Gbadamosi and Kalejaye 2017). These bioactive compounds facilitate the synthesis and production of antioxidants either directly or by ensuring the supply of cofactors that ensure their generation (Yu et al. 2021). Our previous studies document that the protective role of *Xylopia aethiopica* may be due to the presence of scoparones a class of coumarins that was identified through GCMS analysis.

Glutathione is a tripeptide that consists of cysteine, glycine and glutamic acid. Its central role in mitigating oxidative stress stems from its ability to participate in suicide inhibition upon cellular exposure to oxidative stress (Sreekumar et al. 2021). Glutathione is also an efficient hydrogen peroxide quencher. By utilizing glutathione peroxidase, hydrogen peroxide is converted to water while glutathione is converted from its reduced form (GSH) to its oxidized form (GSSG) (Sarıkaya and Doğan 2020). Our results show that glyphosate administration led to a significant decrease in both glutathione and glutathione peroxidase. Administration of vitamin C and *Xylopia aethiopica* either alone or in

combination with glyphosate resulted in significantly elevated levels of glutathione and glutathione peroxidase when compared to the glyphosate-only treated group. The ability of vitamin C and *Xylopia aethiopica* to ensure the amelioration of reduced glutathione capacity is similar to as described previously by Adikwu and Ehigiator, (2020).

Inflammation is one of the body immune's responses to harmful stimuli that include toxins, xenobiotics and harmful contaminants (Sajid 2016). Upon the invasion of the immune system, certain proteins known as cytokines are synthesized and recruited to the site of injury to combat the effect at various stages of inflammation (Chen et al. 2018). These proteins could either be pro-inflammatory such as tumour necrosis factor, interleukin-6, interleukin-1β etc or an anti-inflammatory such as interleukin-4, interleukin-11, interleukin-13 etc. Results from this study revealed that glyphosate administration increased the level of interleukin-6, TNF-α and C - reactive protein. However, treatment with vitamin C and *Xylopia aethiopica* either alone or in combination with glyphosate resulted in a decline in the levels of these proteins.

This study also attempted to understand the effect of glyphosate on the expression of some apoptotic and inflammatory proteins in different sections of the brain as well as the protective effect of *Xylopia aethiopica*. Apoptosis is one of the several forms of cell death that has been identified to regulate cell proliferation and an increase in apoptosis in the brain has been correlated with neurodegenerative diseases such as Parkinson's, and Alzheimer amongst others. This form of cell death is programmed and is usually characterized by the activation of certain caspases (cysteine-rich aspartic proteases) that could be classified as either initiators or executioners (Green and Llambi 2015). One of the executioner caspases is Caspase-3 which converges both the intrinsic and extrinsic apoptotic pathways. Our studies reveal that glyphosate administration increases the expression of Caspase 3 in the three regions of the brain accessed. These results are similar to the other studies that document the activation of caspase-3 by glyphosate although *in vitro* (Kwiatkowska et al. 2020) and the placental cells (Benachour and Séralini 2009). *Xylopia aethiopica* and vitamin C administration were able to significantly modulate the expression of caspase-3 in the brain. The ability of ascorbic acid to protect against apoptotic changes induced by glyphosate was previously documented by Abu Zeid *et al*., (2018)

Another important apoptotic regulator is p53. Studies have shown that p53 can regulate the activities of caspase-3 by first activating Apaf-1 which in turn activates caspase-9 and caspase-9 can, in turn, activate caspase-3 in the intrinsic apoptosis pathway (Cavalcante et al. 2019). Similarly, p53 can activate Bax protein a pro-apoptotic protein that can inactivate the Bcl-2 protein and further allow apoptosis to progress. This study revealed that administration of glyphosate significantly increases the expression of p53 in different regions of the brain accessed. This increase in p53 is indicative of increased apoptosis in different regions of the brain (Mendrysa et al. 2011). Treatment with *Xylopia aethiopica* and vitamin C was able to modulate the expression of p53.

Furthermore, cyclooxygenase-2 (COX-2) is the enzyme that is mostly responsible for generating inflammation, which is a typical disease mechanism (Attiq et al. 2018). Upon administration of

glyphosate, there was a significant upregulation in COX-2 expression in different sections of the brain indicating inflammation. This increase in COX-2 expression was significantly modulated by treatment with vitamin C and *Xylopia aethiopica.* This modulation is indicative of the potential of *Xylopia aethiopica* to modulate inflammation in different sections of the brain.

Conclusion

Taken together, *Xylopia aethiopica* could possess anti-oxidative and anti-inflammatory properties that could be used in combating glyphosate neurotoxicity

Abbreviations

CAT, catalase; GPx, glutathione peroxidase; AcHE, acetylcholinesterase; GSH, glutathione; SOD, superoxide dismutase; BcHE, Butyrylcholinesterase; IHC, immunohistochemistry; GSH, reduced glutathione; CRP- c-reactive protein, GLY- glyphosate TNF-α- tumour necrosis factor-α; IL-6; interleukin-6; VIT. C-vitamin C; COX-2; cyclooxygenase-2; Caspase-3; cysteine-rich aspartic protease 3.

Declarations

Ethics approval and consent to participate

Ethical approval was obtained from our Institution's ethics committee before the commencement of this study (UNIOSUNHREC/2021/003C) and authentication of the plant material was carried out at the University of Ibadan, Ibadan Nigeria (UIH-22944). C**onsent to participate is not applicable in this research**

Consent for publication

Not applicable

Availability of data and materials

All data generated or analyzed during this study are included in this published article [and its supplementary information files.

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

OOA; Supervision, conceptualization, methodology, initial draft, review and editing; OAA; initial draft, review, editing and data analysis; TAO; Methodology, data analysis and review and editing; DAA; Co-supervision, methodology and review; IOB; Study design, performed the experiments, acquisition and analysis of data; EOA; Study design, performed the experiments, acquisition and analysis of data.

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Figures

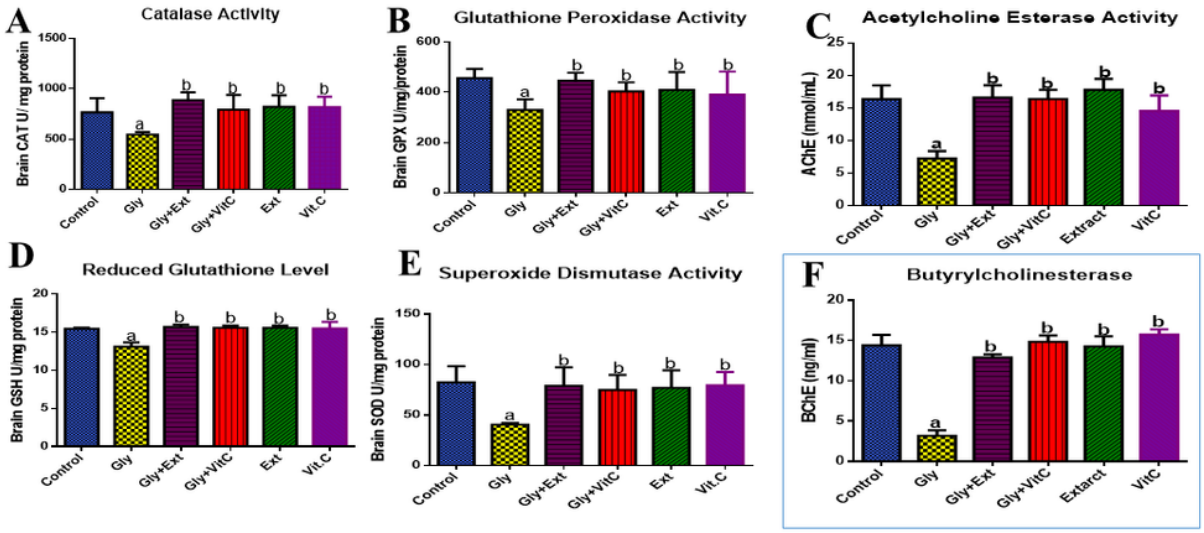

Figure 1

Effect of *Xylopia aethiopica* on oxidative stress and brain function markers in glyphosate-induced brain toxicity.

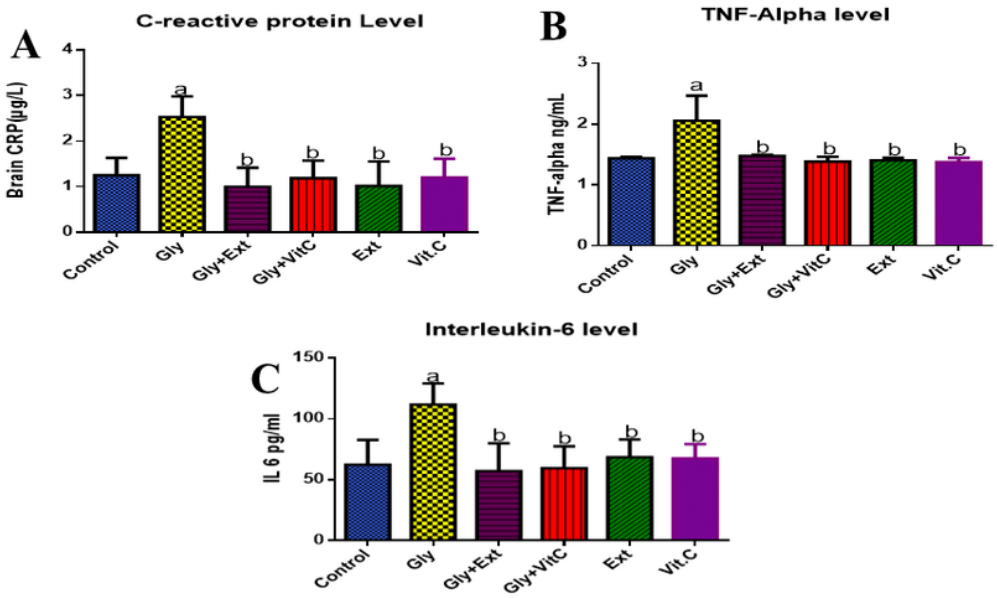

Figure 2

Effect of *Xylopia aethiopica* on selected inflammatory markers in glyphosate-induced brain toxicity.

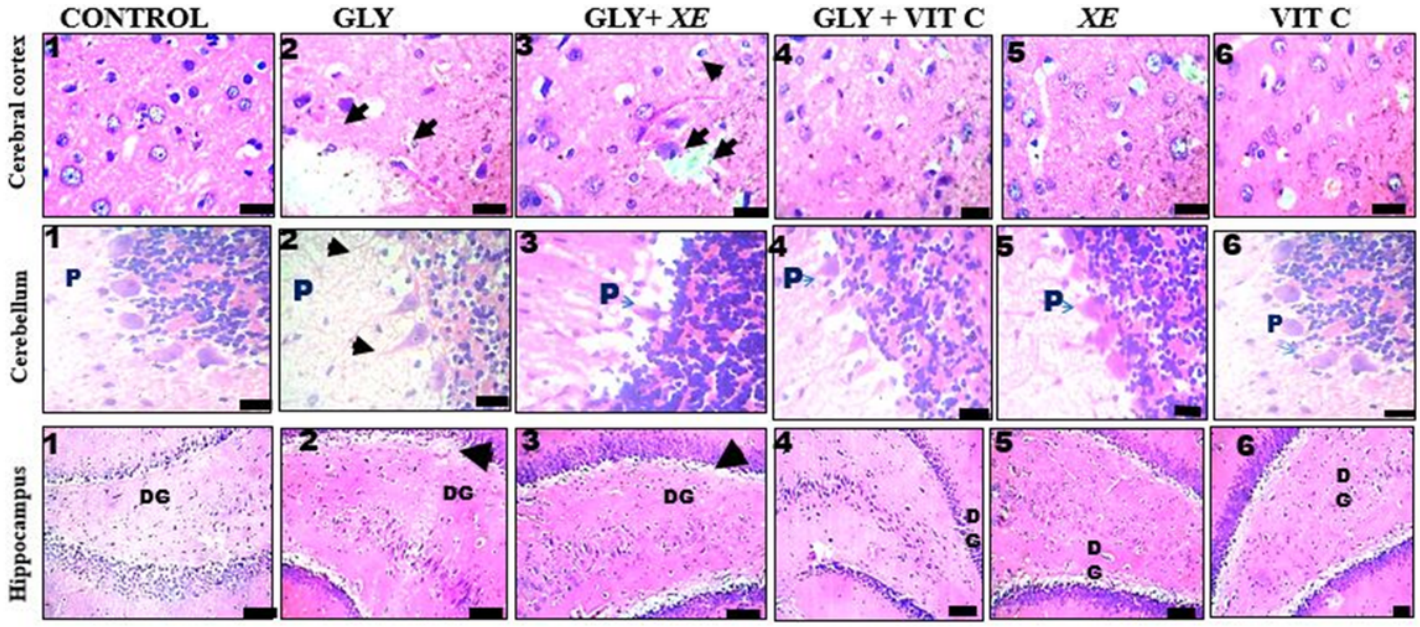

Figure 3

Photomicrographs detailing the effect of *Xylopia aethiopica* and vitamin C on glyphosate-induced toxicity in the cerebral cortex, cerebellum and hippocampus of Wistar rats.

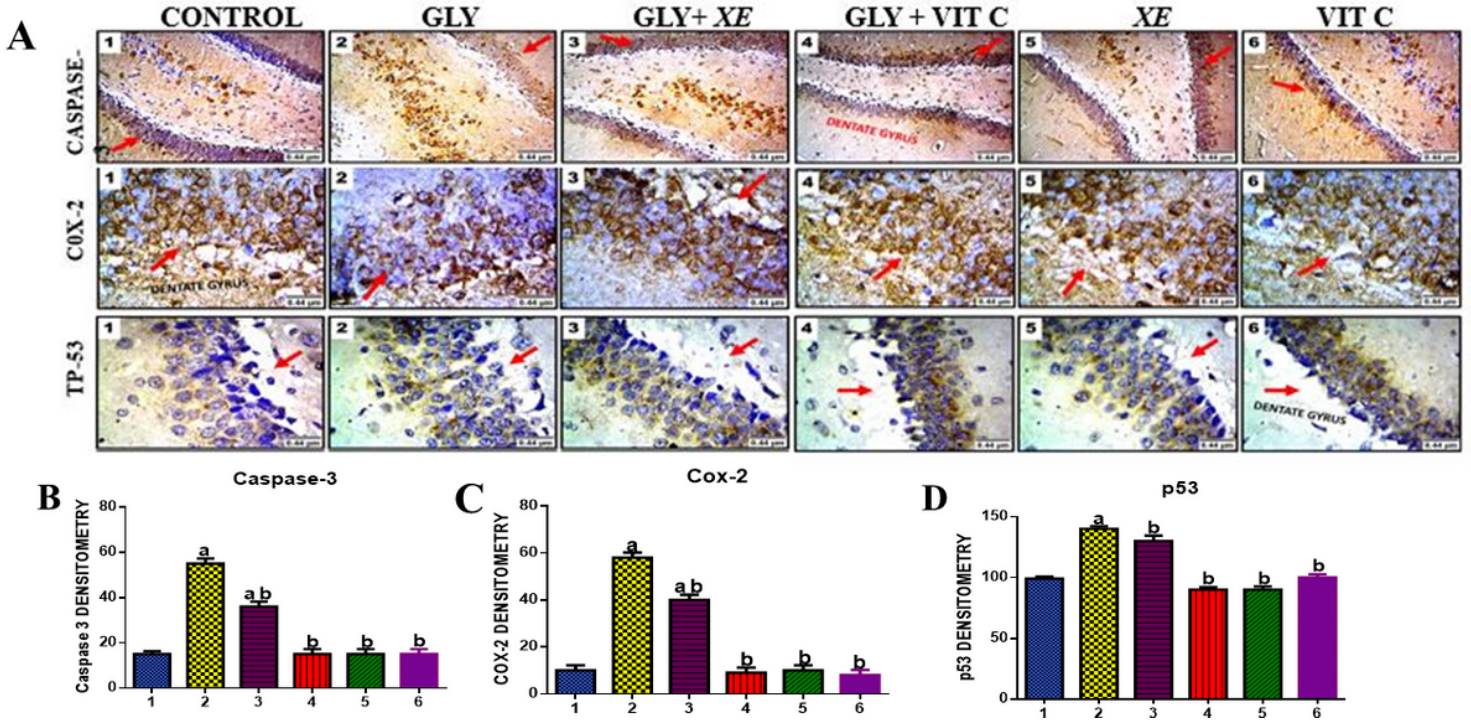

Figure 4

Micrographs showing Caspase-3, COX-2 and p53 immunohistochemical expression in the hippocampus of the treatment groups 1-6.

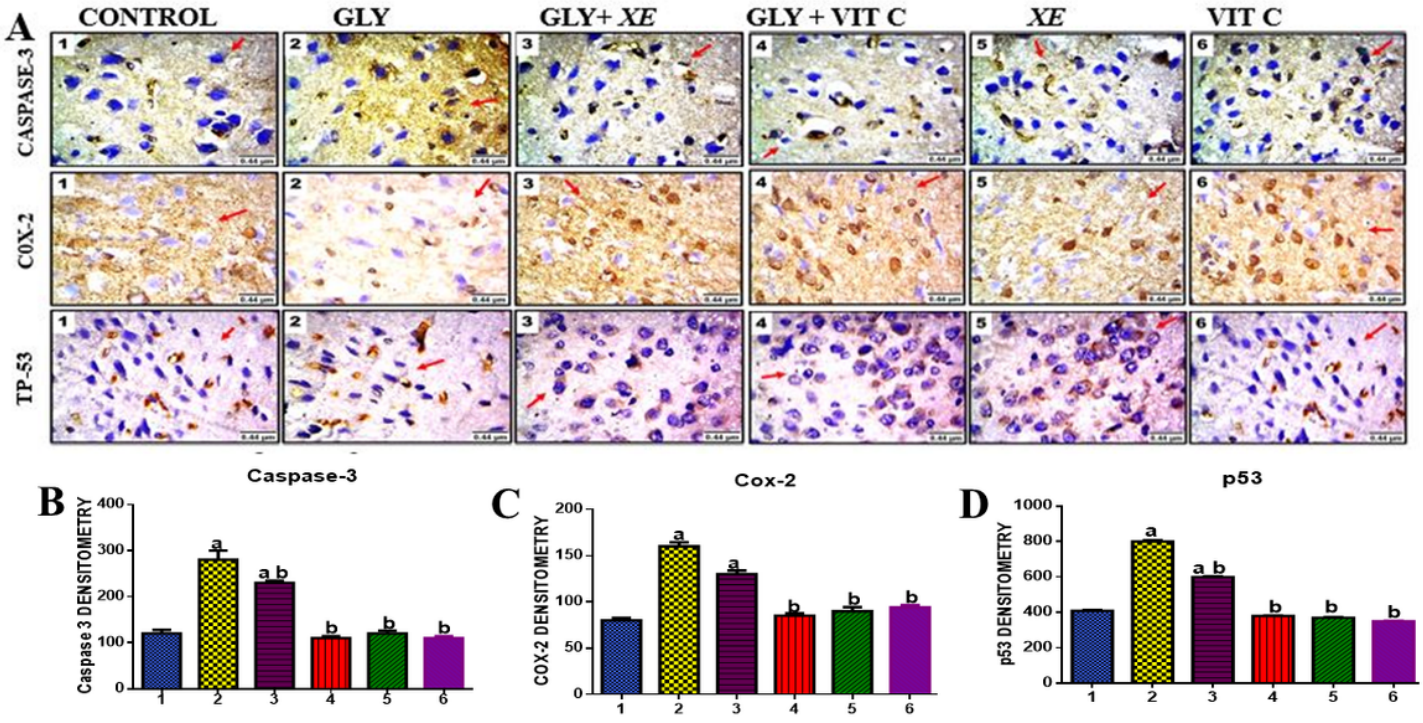

Figure 5

Micrographs showing Caspase-3, COX-2 and p53 immunohistochemical expression in the cerebral cortex of the treatment groups 1-6.

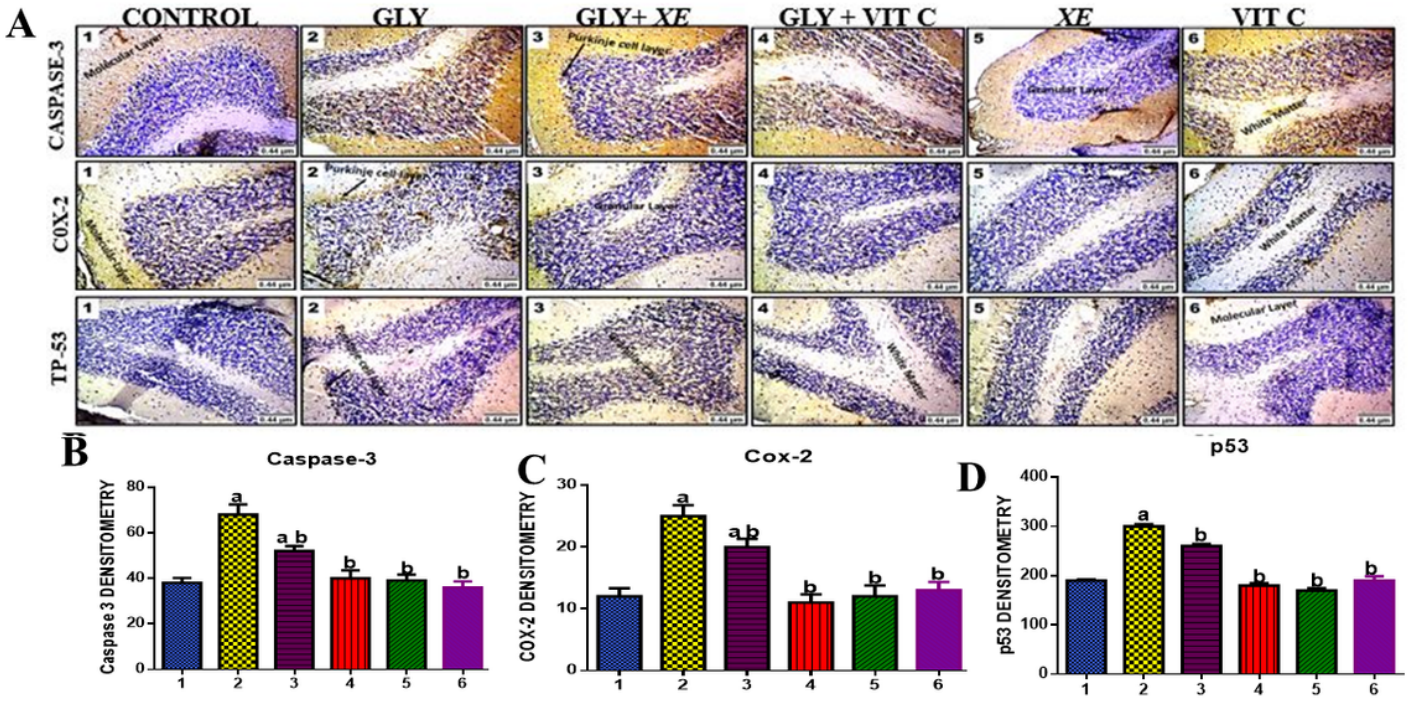

Figure 6

Micrographs showing Caspase-3, COX-2 and p53 immunohistochemical expression in the cerebellum of the treatment groups 1-6.

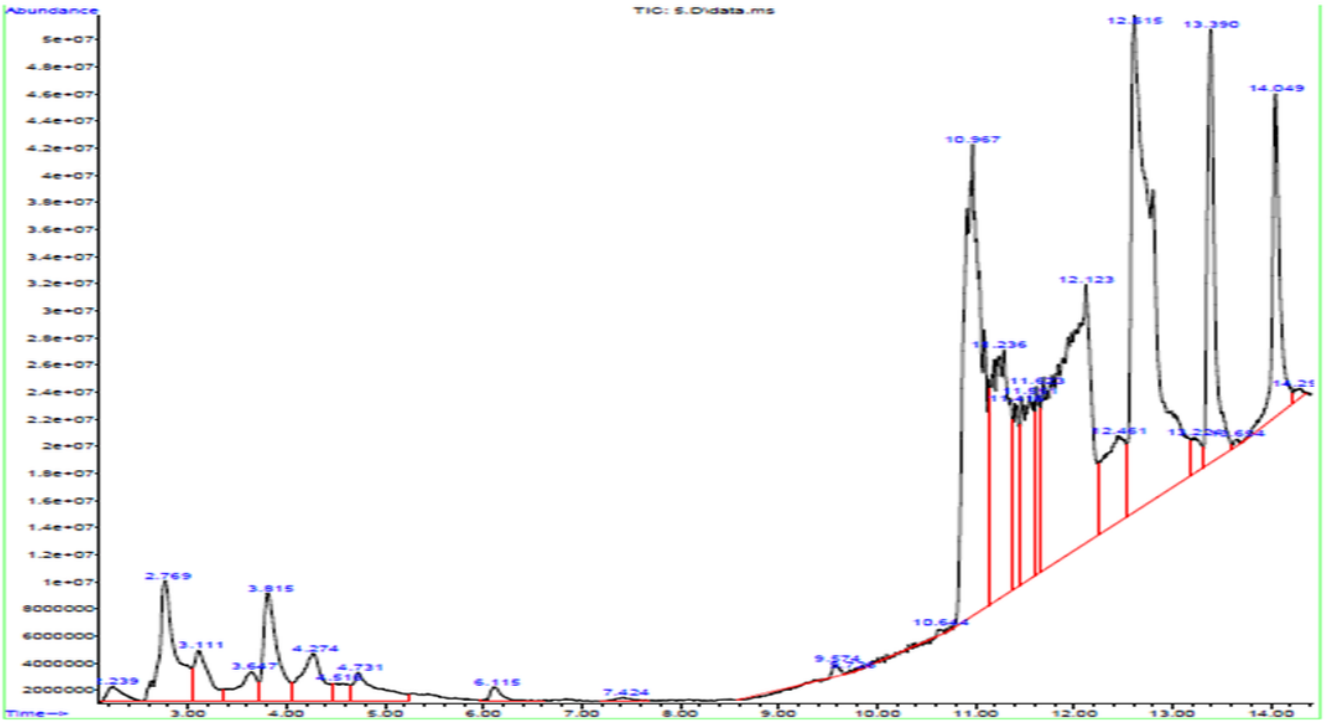

Figure 7

GCMS analysis of *Xylopia aethiopica* stem bark ethanol extract