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Thymoquinone Mitigates Behavioural Deficit, Oxidative Stress, and Neurohistological Changes in the Brain of Wistar Rats Triggered by Exposure to Lead Acetate

Kabiru I. Adedokun[1], Kabirat B. Jimoh[1], Opeyemi S. Osuntokun[2], Olayemi O. Oladokun[1], Abdulquadri O. Abdulraheem[1], Tope G. Atere[3]

[1]Department of Physiology, Faculty of Basic Medical Sciences, Osun State University, Osogbo, Nigeria; [2]Department of Health and Sport Science, Regent College London, University of Bolton, England; [3]Department of Biochemistry, Faculty of Basic Medical Sciences, College of Health Sciences, Osun State University Osogbo, Nigeria

ABSTRACT

Lead is an environmental toxicant with adverse effects on the nervous system. This study evaluated the effect of thymoquinone (TQ) on learning, memory, motor coordination, oxidative stress, and neurohistology in lead acetate (PbA)-exposed male Wistar rats. Thirty rats (120–160 g) were randomly assigned to six groups (n = 5). The control group received distilled water (0.2 mL/day, p.o.), the PbA group received 15 mg/kg of PbA (i.p./week), and the TQ group received 5 mg/kg of TQ (p.o.), while the three remaining groups concurrently received PbA+TQ (5 mg/kg), PbA+TQ (3.75 mg/kg), and PbA+TQ (2.5 mg/kg), for 56 days. Rats were subjected to memory (novel object recognition and step-down latency) and motor coordination (rotarod and prehensile studies) tests. Twenty-four hours after the last administration, they were euthanised by cervical dislocation. The supernatants of the hippocampal and cerebellar tissue homogenates were spectrophotometrically analysed for indications of oxidative stress, while the haematoxylin and eosin staining technique was used to examine the histomorphology of the hippocampus, cerebellum, and prefrontal cortex. TQ protected memory and locomotor activities and maintained hippocampal and cerebellar malondialdehyde concentration while maintaining glutathione, catalase, and superoxide dismutase activities from PbA-induced deficits. The co-administration of TQ also preserved the cytoarchitecture of the hippocampus, prefrontal cortex, and cerebellar cortex. In conclusion, TQ at 5 mg/kg improved learning, memory, motor coordination, oxidative status, and neurohistology in male Wistar rats that had been exposed to PbA.

Keywords

Cerebellum, Prefrontal cortex, Lead acetate, Hippocampus, Oxidative stress, Thymoquinone

Correspondence: Kabiru I. Adedokun, PhD. Department of Physiology, Faculty of Basic Medical Sciences, Osun State University, Osogbo, Nigeria. E-mail: kabiru.adedokun@uniosun.edu.ng; Phone Number: + 2348036559099; ORCID: 0000-0003-1437-6079
Kabirat B. Jimoh – 0009-0007-3152-4623, kabiratbolajoko@gmail.com; Opeyemi S. Osuntokun - 0000-0002-1363-1843, OpeyemiS.Osuntokun@rcl.ac.uk; Olayemi O. Oladokun – 0000-0002-4685-3688, olayemi.oladokun@uniosun.edu.ng; Abdulquadri O. Abdulraheem - 0009-0000-7158-5614, abdulquadriabdulraheem92@gmail.com; Tope G. Atere - 0000-0001-9349-0990, tope.atere@uniosun.edu.ng

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INTRODUCTION

Heavy metal contamination has emerged as a significant global environmental concern, posing a substantial threat to human health and causing a multitude of detrimental health effects (Khan *et al*., 2015). Anthropogenic activities, ranging from coal-fired power plants to waste incineration, are accelerating the rate at which these metals enter the environment, consequently increasing human exposure (Jan *et al*., 2015). Among these contaminants, lead is the most prevalent (DiMaio, 2001). A report from the Institute for Health Metrics and Evaluation indicates that an alarming 815 million children worldwide have blood lead levels that exceed safe thresholds. Furthermore, data from the 2019 Global Burden of Disease study estimates that lead poisoning is responsible for approximately 900,000 adult deaths annually (Burki, 2020). Lead stands out as a prominent environmental toxicant due to its historical

significance and unique physico-chemical properties. Its abundance and widespread distribution pose a significant environmental challenge (Charkiewicz and Backstrand, 2020). Despite its historical importance and desirable properties such as softness, malleability, and resistance to corrosion, it remains a significant environmental challenge. The non-biodegradable nature of lead compounds coupled with their continued use leads to environmental accumulation, amplifying the associated hazards (Yang *et al*., 2020).

Exposure to lead and its compounds primarily occurs in occupational settings and through environmental sources, including historical use in leaded gasoline, industrial lead smelting and combustion processes, pottery glazes, boat building materials, lead-based paint, lead pipes in older plumbing systems, battery recycling, ammunition production, pigments in paints and dyes, and printing inks (WHO, 2019).

Despite the discontinuation of lead use in many nations globally, it persists in various industries, including car repair, battery production/recycling, and metal refining/smelting processes. Lead is a highly toxic metal that affects nearly every organ in the body, with the nervous system being the most vulnerable to lead toxicity in both children and adults. Due to their developing systems and softer tissues, both internally and externally, children experience a greater impact than adults. This vulnerability manifests as behavioural issues, learning deficits, and potentially lowered IQ scores. Even low-level exposure in infants and young children can contribute to such impairments. While adults exhibit some resilience, chronic lead exposure can lead to decreased cognitive performance in tests measuring nervous system function (Rubin *et al*., 2008). In severe cases, high lead levels can cause fatal brain damage in both children and adults. Blood disorders and nervous system damage are prevalent consequences of lead toxicity (Ara and Usmani, 2015).

Thymoquinone (TQ), the primary constituent of the volatile oil extracted from *Nigella sativa* seeds, exhibits a diverse range of pharmacological properties. These include anticonvulsant effects (Hosseinzadeh and Parvardeh, 2004), antitussive activity (Hosseinzadeh *et al*., 2008), anti-tumour properties (Attoub *et al*., 2013), as well as anti-inflammatory and antioxidant actions (Woo *et al*., 2012). TQ also demonstrates anti-cancer potential (El-Far, 2015), acts as an anti-hypertensive agent (Azzubaidi *et al*., 2015), and exhibits analgesic effects (Amin and Hosseinzadeh, 2016). Notably, TQ's potent antioxidant capacity is a significant aspect of its pharmacological profile (Darakhshan *et al*., 2015). Given its low systemic toxicity and high biological activity, TQ may be considered a promising therapeutic alternative (Darakhshan *et al*., 2015). TQ crosses the blood-brain barrier, influencing neuromodulatory activities. It has a neuroprotective effect and improves brain injuries resulting from Parkinson's disease (Ebrahimi *et al*., 2017) and status epilepticus (Shao *et al*., 2017). It is also useful in the treatment of glial tumours by inducing apoptosis of glial tumour cells (Elmaci and Altinoz, 2016). Several studies reported the protective role of TQ against neurotoxicity induced by heavy metals and radiation. It reduces the cerebral oxidative injuries induced by lead and ionising radiation (Elmaci and Altinoz, 2016). In addition, it has a nephroprotective role against lead (Mabrouk *et al*., 2016) and cadmium toxicity (Erboga *et al*., 2016). Based on this evidence, this study investigated the potential of TQ to ameliorate learning, memory, and motor coordination deficits in male Wistar rats exposed to lead acetate (PbA).

MATERIALS AND METHODS

Animal Care

Thirty male Wistar rats, weighing between 120 and 160 g, were obtained and housed in the Animal Holding Facilities of the College of Health Sciences, Osun State University, Osogbo, under standard laboratory conditions with a controlled temperature (20-24 ℃) and a natural light/dark cycle. Rat pellets and drinking water were accessible *ad libitum* to the rats.

This study followed the rules set out by the National Institutes of Health (NIH) in their "Guide to the Care and Use of Animals in Research and Teaching." Ethical approval for the study was obtained from the Health Research Ethics Committee (HREC) of the College of Health Sciences, Osun State University, Osogbo, Nigeria (Approval number: [UNIOSUNHREC 2024/006B]).

Drug Preparation and Experimental Design

TQ (Hebei Weibang Biotechnology Co. Ltd., CAS 490-91-5) was obtained from IBRA Hadad Nigeria Limited, Lagos, Nigeria, and was dissolved in 30% ethanol (Sigma Aldrich, CAS 64-17-5) in distilled water. Consequently, we obtained PbA (LOBA Chemie Laboratory Reagents and Fine Chemicals, CAS 6080-56-4) from Octopus Reagent, Nigeria, and dissolved it in distilled water.

Adult male Wistar rats were weighed and randomly divided into six groups of five animals each (n = 5) and assigned to receive the following treatments daily for 56 consecutive days between 8:00 and 9:00 am. The control group received distilled water (0.2 mL/day orally, p.o.), the PbA group received 15 mg/kg of PbA intraperitoneal (i.p.) per week (Moosavirad *et al*., 2016; Adedokun *et al*., 2023), and the TQ group received 5 mg/kg TQ p.o., while the three remaining groups concurrently received PbA+TQ (5 mg/kg), PbA+TQ (3.75 mg/kg), and PbA+TQ (2.5 mg/kg) p.o., respectively (Adedokun *et al*., 2023).

Behavioural Studies

Prior to the behavioural studies, rats were acclimated to the test room for 60 min to minimise environmental stress.

Novel Object Recognition Test

The novel object recognition (NOR) test was conducted using Ennaceur (2010) method. It was carried out in a simple wooden box with a transparent glass wall and measuring 50 cm in height and 40 × 60 cm in dimensions. The interior of the box was painted in a white colour. The

rats were first presented with two identical objects for a duration of 5 min, referred to as trial 1 (T1), which served as the sample phase. Upon completion of this training trial, the rats were removed from the box and returned to their home cage. After a retention delay of 3 h, the rats were reintroduced to two objects for a duration of 5 minutes during trial 2 (T2), one being the object from the initial exposure and the other being new. The time each rat spent investigating each of the objects during trial 2 was recorded and used to analyse NOR. The total time spent exploring each of the objects was recorded using two stopwatches. A rat was regarded to have spent time with an object when it pointed its nose at the object at a distance ≤2 cm or touched the object. To ensure that the test results are accurate, the apparatus was cleaned thoroughly with cotton wool and methylated spirit before and after every trial.

$$Percentage\ memory\ index/Discrimination\ index = \frac{Time\ with\ novel\ object - Time\ with\ familiar\ object}{Time\ with\ novel\ object + Time\ with\ familiar\ object} \times 100$$

Step-Down Latency Test

The step-down latency test is a long-term memory test that involved a rectangular box (50 x 50 cm) with an electrifiable grid floor and a 35 cm high platform. A shock device that administered scrambled foot shocks was connected to the grid floor. The test was in two steps:

Training: A rat was gently placed on the platform within the chamber (designated as the safe zone). When it stepped down from the platform onto the electrified grid on all four paws, a continuous electric shock was delivered until it returned to the platform to escape the aversive stimulus. Following this training trial, the rat was left in the cage for 5 min before returning it to its home cage. The apparatus was thoroughly cleaned before and after each trial.

Retention Test: It was performed 24 h after the training session; the retention test involved placing the rat back on the platform (safe zone). The duration in which it took the rat to step down from the platform (step-down latency) was recorded. The test continued until the cutoff time (5 min), at which point it concluded when the animal either stepped down or remained on the platform (Izquierdo and Dias, 1983).

Open Field Test

The open field test is a widely used experimental method in scientific research to assess general locomotor activity, anxiety, and exploratory behaviour in animals, typically rodents (Crusio, 2013). In this study, locomotor activity was performed using the method of Küçük *et al*. (2008). Briefly, each rat was allowed a maximum of 300 s in a square open field, which was an enclosed area marked with a grid to prevent escape. The frequency with which the Wistar rat crossed grid lines with all four paws served as a measure of locomotor activity.

Accelerated Rotarod Test

To test how the TQ-rich fraction affected rats' motor coordination in an accelerated rotarod apparatus, a modified method of Haryash *et al*. (2017) was used. The retention time, defined as the duration between placing the animal on the rotating drum and its subsequent fall (detected by hind limb release), was recorded as the primary outcome measure. To ensure optimal performance, rats underwent a training session to familiarise themselves with the test apparatus and maintain equilibrium. On the test day, each rat was allowed a maximum of 300 s to navigate the rod, which accelerated linearly from 20 to 50 rpm, with intermediate increments of 40 rpm.

Prehensile Reflex Test

The prehensile reflex test is a behavioural assay that evaluates an animal's ability to grasp and suspend itself from a horizontal wire using its forepaw, thereby assessing muscle strength (Shukkitt Hale *et al*., 1998). In this test, rats were gently lifted and placed on a taut horizontal wire (12-gauge, 55 cm above the tabletop), and the time (in seconds, up to a maximum of 60 s) during which the animal can maintain its grip with both forepaws is recorded. This test provides a quantitative measure of forelimb muscle strength and coordination.

Animal Sacrifice and Sample Collection

Twenty-four hours following the final drug administrations, the rats were euthanised through cervical dislocation. Their brains were excised and placed on a pre-cooled metal platform so that the hippocampus, prefrontal cortex, and cerebellum could be studied separately. An electronic balance was then used to measure their weight. The hippocampi and cerebella from the rats (n = 5 per group) were respectively divided into two halves. The right halves were used for biochemical assays, while the left halves and the prefrontal cortex were used for histological protocols. Tissues for biochemical assays were homogenised, and diluted in four times the volume of phosphate buffer. These homogenates were placed in sample bottles and spun in a centrifuge at 3,000 revolutions per minute for 10 min. The supernatants were collected and subsequently stored at -20°C for biochemical analysis. Tissues for histological evaluation were immediately fixed in 10% formalin and further processed.

Assessment of Oxidative Stress Markers

The spectrophotometry technique was applied to analyse aliquots of the supernatants of the hippocampal and cerebellar tissues for oxidative stress. Four key markers of oxidative stress, including malondialdehyde (MDA) as described by Beheshti *et al*. (2017), superoxide dismutase (SOD) as described by Madesh and Balasubramanian (1997), catalase (CAT) as described by Aebi (1984), and glutathione peroxidase (GSH) activities according to the procedure described by Hu (1994).

Histological Studies

The fixed prefrontal cortex, hippocampus, and cerebellum were dehydrated in descending grades of alcohol, cleared in chloroform, and impregnated in paraffin. Then 5-6 µm sections were placed on clean slides, deparaffinized in xylene, and stained with haematoxylin and eosin. Morphological changes were then observed under a light

microscope at ×400 magnification. This was carried out according to Adedokun *et al*. (2022).

Statistical Analysis

A Gaussian distribution test was done using the Anderson-Darling and Shapiro-Wilk normality tests. This was to ensure that data were normally distributed. Data were then analysed using one-way analysis of variance (ANOVA and a post-hoc test (Tukey test) using GraphPad Prism 5. A significance level of $P < 0.05$ was considered statistically significant.

RESULTS

Effects of PbA-TQ Combination on Short-Term/ Working Memory of Male Wistar Rats using the NOR Test

The percentage memory index in the NOR test significantly decreased ($p < 0.0001$) in the PbA group, as well as in the PbA + TQ (3.75 mg/kg) and PbA + TQ (2.5 mg/kg) groups compared to the control. However, no significant decrease in memory index was observed in the PbA + TQ (5 mg/kg) group, which was significantly higher ($p = 0.0006$) than the PbA group (Fig. 1).

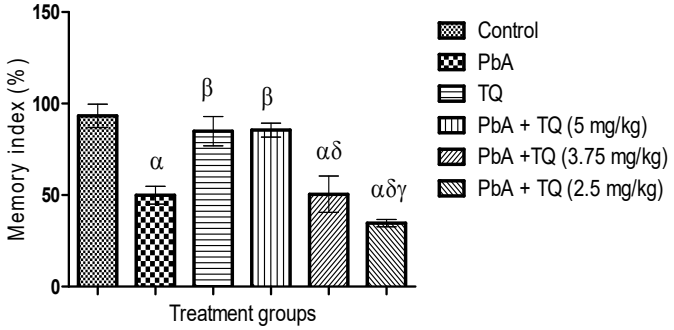

Fig. 1: Effects of PbA-TQ combination on short term/working memory of male Wistar rats using the NOR test. α: decrease compared to the control ($p = 0.0001$); β: increase compared to PbA ($p = 0.0006$); δ: decrease compared to TQ ($p = 0.0011$); Ɣ: decrease compared to PbA + TQ (5 mg/kg) ($p = 0.0024$)

Effects of PbA-TQ Combination on Long-Term Memory of Male Wistar Rats using the Step-Down Latency Test

There was a significant reduction ($p = 0.0001$) in step-down latency in the PbA group, as well as in the PbA + TQ (3.75 mg/kg) and PbA + TQ (2.5 mg/kg) groups compared to the control. However, no significant decrease in long-term memory was observed in the PbA + TQ (5 mg/kg) group, which was significantly higher ($p = 0.0001$) than the PbA group (Fig. 2).

Effects of PBA-TQ Combination on Locomotor Activities of Male Wistar rats using the Open Field Test

There was significant decrease ($p = 0.0001$) in the locomotor activities in the PbA group, as well as in the PbA + TQ (5 mg/kg), PbA + TQ (3.75 mg/kg), and PbA + TQ (2.5 mg/kg) groups compared to the control. However, the locomotor actvity of the PbA + TQ (5 mg/kg) group was signifiacantly higher ($p = 0.0007$) than the PbA group (Fig. 3).

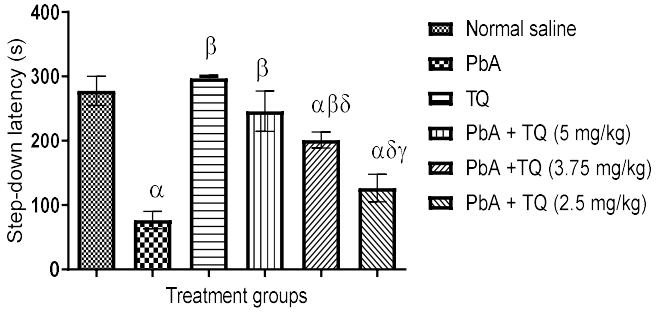

Fig. 2: Effects of PbA-TQ combination on long-term memory using the step-down latency test on male Wistar rats. α: decrease compared to the control ($p = 0.0001$); β: increase compared to PbA ($p = 0.0001$); δ: decrease compared to TQ ($p = 0.0042$); Ɣ: decrease compared to PbA + TQ (5 mg/kg) ($p = 0.0362$)

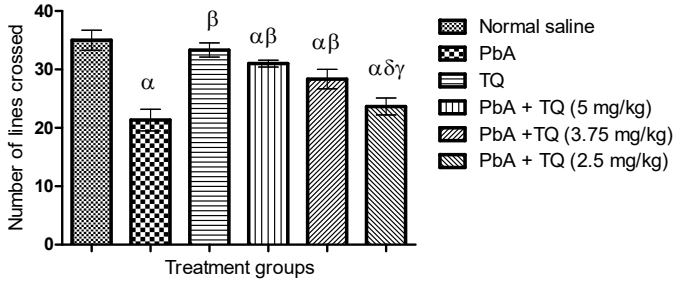

Fig 3. Effects of PbA-TQ combination on the locomotor behaviour of male Wistar rats. α: decrease compared to the control ($p = 0.0001$); β: increase compared to PbA ($p = 0.0007$); δ: decrease compared to TQ ($p = 0.0040$); Ɣ: decrease compared to Pb + TQ (5 mg/kg) ($p = 0.0208$)

Effects of PbA-TQ Combination on the Traction Score of Male Wistar Rats in Prehensile Test

The traction score, a measure of forelimb grasping ability, was significantly reduced ($p = 0.0001$) in the PbA group compared to the control group. However, there was no significant difference in the traction score in the PbA + TQ (5 mg/kg), PbA + TQ (3.75 mg/kg), and PbA + TQ (2.5 mg/kg) groups compared to the control, but these were significantly higher than the PbA group (Fig. 4).

Effects of PbA-TQ Combination on Motor Coordination of Male Wistar Rats in the Rotarod Test

There was a significant reduction ($p = 0.0001$)in latency to fall from the accelerating rotarod in the PbA group, as well as in the PbA + TQ (5 mg/kg), PbA + TQ (3.75 mg/kg), and PbA + TQ (2.5 mg/kg) groups compared to the control. However, the latency to fall from the accelerating rotarod of the PbA + TQ (5 mg/kg) group was signifiacantly higher ($p = 0.0001$) than the PbA group (Fig. 5).

The Effect of PbA-TQ combination in Brain Tissue Oxidative Stress Biomarkers in Male Wistar Rats

The hippocampal and cerebellar levels of MDA were significantly higher in the PbA group. However, a significant decrease in MDA was observed in hippocampal and cerebellar tissues in the PbA + 5 mg/kg TQ and PbA + 3.75 mg/kg TQ treatment groups compared to the PbA group. In both the hippocampi and the cerebellum, the PbA + 2.5 mg/kg TQ group had significantly higher levels of MDA than the control group (Table 1).

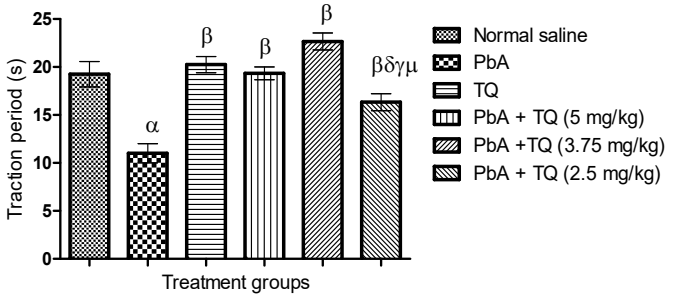

Fig 4: Effects of PbA-TQ combination treatment on the traction score of male Wistar rats in prehensile test. α: decrease compared to control (p = 0.0001); β: increase compared to PbA (p = 0.0001); δ: decrease compared to TQ treated (p = 0.0046); Ɣ: decrease compared to PbA + TQ (5 mg/kg) treated (p = 0.0046); μ: Ɣ: decrease compared to PbA + TQ (3.75 mg/kg) treated (p = 0.0046)

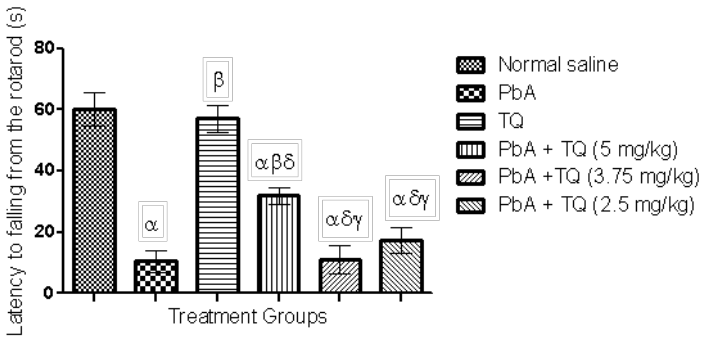

Fig. 5: Effects of PbA-TQ combination on the motor coordination of male Wistar rats in the rotarod test. α: decrease compared to control (p = 0.0001); β: increase compared to PbA (p = 0.0001); δ: decrease compared to TQ (p = 0.0001); Ɣ: decrease compared to PbA + TQ (5 mg/kg) (p = 0.0132)

The hippocampal and cerebellar activities of SOD significantly decreased following treatments with PbA compared to the control. The PbA + 5 mg/kg TQ, PbA + 3.75 mg/kg TQ, and PbA + 2.5 mg/kg TQ groups, on the other hand, had significantly higher levels of SOD in their hippocampal and cerebellar tissues compared to the PbA group. A significant decrease in cerebellar SOD was equally observed in PbA + 5 mg/kg TQ, PbA + 3.75 mg/kg TQ, and PbA + 2.5 mg/kg TQ treated groups compared to the control group (Table 1).

The PbA group had a significantly lower level of CAT in the hippocampi, but there was no difference in the level of CAT in the cerebellum compared to the control group. Furthermore, only hippocampal CAT significantly increased in PbA + 5 mg/kg TQ, PbA + 3.75 mg/kg TQ, and PbA + 2.5 mg/kg TQ when compared to the PbA group (Table 1).

The hippocampal level of GSH showed a significant decrease in the PbA group, while the cerebellar level of GSH showed no significant difference compared with the control group. Furthermore, hippocampal levels of GSH significantly decreased in PbA + 5 mg/kg TQ, PbA + 3.75 mg/kg TQ, and PbA + 2.5 mg/kg TQ compared to the control. However, only PbA + 2.5 mg/kg TQ showed a significant decrease in cerebellar GSH compared to the control. No significant changes in hippocampal and cerebellar GSH following treatments with PbA + 5 mg/kg TQ, PbA + 3.75 mg/kg TQ, and PbA + 2.5 mg/kg TQ when compared to the PbA group (Table 1).

Effects of PbA-TQ Combination on the Prefrontal Cortex Histomorphology of Male Wistar Rats

The external pyramidal and internal granular layers of the prefrontal cortex were the focus in this study across all groups. There were normal histological features of the prefrontal cortex in the control and TQ groups (PbA + 5 mg/kg TQ, PbA + 3.75 mg/kg TQ, and PbA + 2.5 mg/kg TQ), characterised by large pyramidal neurons with long axons that extended from the soma to adjacent neurons within the neuropil. Apical and basal dendrites extended from the well-delineated soma of the pyramidal neurons in both groups. Rats exposed to PbA only showed degenerative changes in the prefrontal cortex that were

Table 1: Effect of TQ on lead acetate-induced toxicity on hippocampal and cerebellar oxidative stress biomarkers in male Wistar rats

	MDA (umol/g)		SOD (U/mg)		CATALASE (U/mg)		GSH (U/mg)	
	Hip	Cer	Hip	Cer	Hip	Cer	Hip	Cer
Control	0.46 ±0.09	0.53±0.02	4.23±0.43	9.43±0.30	0.45±0.04	0.67±0.19	0.03±0.00	0.02±0.00
PbA	1.35±0.10 $^{\alpha}$	1.10±0.10 $^{\alpha}$	1.83±0.11$^{\mu}$	1.67±0.17$^{\mu}$	0.13±0.05$^{\mu}$	0.13±0.04	0.02±0.00$^{\mu}$	0.02±0.00
5 mg/kg	0.48±0.05$^{\Upsilon}$	0.51±0.03$^{\Upsilon}$	4.50±0.50$^{\lambda}$	10.33±1.49$^{\alpha\lambda}$	0.44±0.02$^{\lambda}$	0.74±0.23	0.03±0.00$^{\lambda}$	0.02±0.00
PbA + 5 mg/kg TQ	0.58±0.04$^{\Upsilon}$	0.67±0.01$^{\alpha\Upsilon}$	3.67±0.48$^{\lambda}$	8.10±0.67 $^{\mu\lambda}$	0.32±0.01$^{\lambda}$	0.41±0.08	0.02±0.00$^{\mu}$	0.02± 0.00
PbA + 3.75 mg/kg TQ	0.65±0.07$^{\Upsilon}$	0.68±0.02$^{\alpha\Upsilon}$	2.93±0.18$^{\lambda}$	5.67±0.88$^{\mu\lambda}$	0.25±0.04$^{\mu}$	0.31±0.06	0.02±0.00$^{\mu}$	0.02±0.00
PbA + 2.5 mg/kg TQ	0.83±0.06$^{\alpha\Upsilon}$	0.86±0.04$^{\alpha}$	2.67±0.33$^{\lambda}$	3.67±0.33$^{\mu\lambda}$	0.24±0.03$^{\mu}$	0.25±0.45	0.02±0.00$^{\mu}$	0.02±0.00$^{\beta}$

α = significant increase compared to the control group; μ = significant decrease compared to the control group; Ɣ = significant decrease compared to the PbA group; λ = significant increase compared to the PbA group; PbA = Lead acetate; MDA = malondialdehyde; SOD = superoxide dismutase; GSH = glutathione peroxidise; Hip = hippocampus; Cer = cerebellum

characterised by clustered pyknotic pyramidal neurons that appeared with fragmented cytoplasm and condensed nuclei within their soma. Axons and dendrites were not prominent around neurons. Rats that received different doses of TQ in combination with PbA showed much more similar morphology with the control and thymoquinone-only groups. These groups were characterised by neurons with prominent axons and dendrites observable within the neuropil (Fig. 6).

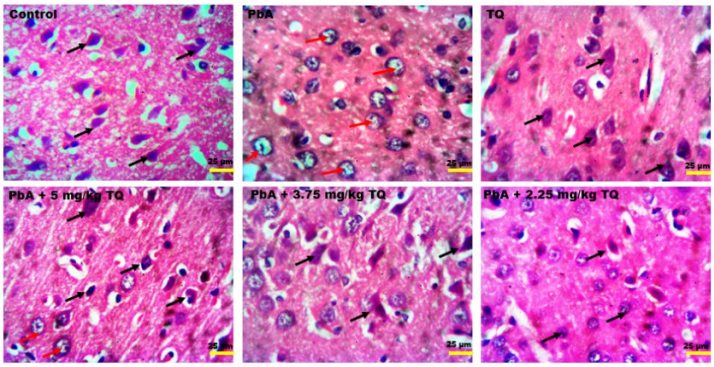

Fig. 6: Photomicrographs of the prefrontal cortex (external pyramidal and internal granular layers) shows the effects of PbA-TQ combination. Black arrows: normal pyramidal neurons; red arrows: pyknotic and fragmented pyramidal neurons. Control: PbA: Lead acetate only; TQ: Thymoquinone only; PbA + 5 mg/kg TQ: concomitant exposure to lead acetate and 5 mg/kg of thymoquinone; PbA + 3.75 mg/kg TQ: concomitant exposure to lead acetate and 3.75 mg/kg of thymoquinone; PbA + 2.25 mg/kg TQ: concomitant exposure to lead acetate and 2.25 mg/kg of thymoquinone. Scale bars: 25 µm, H&E

Effects of PbA-TQ Combination on the Hippocampus (Dentate Gyrus) Histomorphology in Male Wistar Rats

The dentate gyrus revealed notable histopathological changes across the experimental groups. Rats exposed to PbA exhibited distortion in the cytoarchitecture of the dentate gyrus when compared to the control group, characterised by disorganisation of the granule cell layer. In contrast, treatment with TQ (5, 3.75, and 2.25 mg/kg) preserved the structural integrity of the dentate gyrus, as evidenced by an organised granule cell layer and normal neuronal distribution (Fig. 7).

Effects of PbA-TQ Combination on the Cerebellum Histomorphology in Male Wistar Rats

The layers of the cerebellar cortex (molecular, Purkinje, and granule layers) were the focus of the study, which was to observe neuronal arrangements and morphology. There were normal morphological features of the cerebellar cortex in the control and thymoquinone groups. Cellular morphology in both groups was characterised by Purkinje cells having conspicuous cell bodies and dendrites that projected into the molecular layers. In contrast, degenerating Purkinje cells with pyknotic cell bodies and dendritic processes were observed around the indistinctly demarcated cerebellar layers of the PbA-treated rats. Furthermore, their neuropils appeared fragmented with irregularly shaped and sized neuronal cells. However, the neuronal morphology and cerebellar layers in groups that received both TQ and PbA were generally characterised by neurons with appreciable axons and dendrites. The transitional regions between the cerebellar layers were also generally better delineated (Fig. 8).

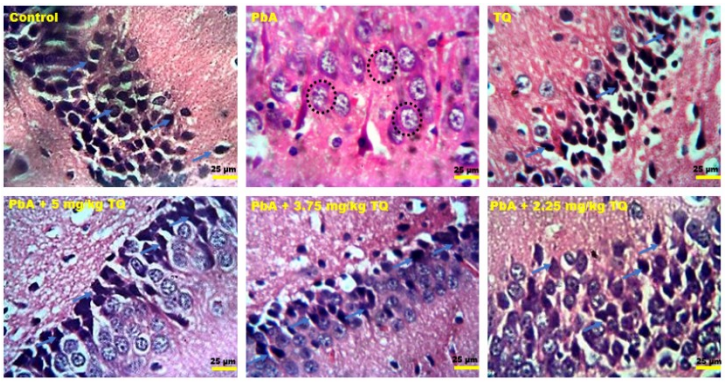

Fig. 7: Photomicrographs showing hippocampal (dentate gyrus) general morphological presentations in Wistar rats across the various study groups. Blue arrows: normal neurons; black dotted circle: shrunken nuclei. Control: PbA: Lead acetate only; TQ: Thymoquinone only; PbA + 5 mg/kg TQ: concomitant exposure to lead acetate and 5 mg/kg of thymoquinone; PbA + 3.75 mg/kg TQ: concomitant exposure to lead acetate and 3.75 mg/kg of thymoquinone; PbA + 2.25 mg/kg TQ: concomitant exposure to lead acetate and 2.25 mg/kg of thymoquinone. Scale bars: 25 µm, H&E

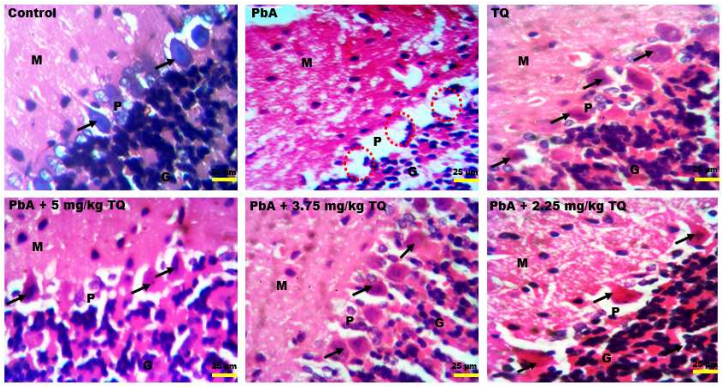

Fig. 8: Photomicrographs of the cerebellar cortex of Wistar rats across various study groups. Black arrows: normal Purkinje cells; dotted red circles: degenerating Purkinje cells. Control: PbA: Lead acetate only; TQ: Thymoquinone only; PbA + 5 mg/kg TQ: concomitant exposure to lead acetate and 5 mg/kg of thymoquinone; PbA + 3.75 mg/kg TQ: concomitant exposure to lead acetate and 3.75 mg/kg of thymoquinone; PbA + 2.25 mg/kg TQ: concomitant exposure to lead acetate and 2.25 mg/kg of thymoquinone. Scale bars: 25 µm, H&E

DISCUSSION

The brain is particularly sensitive to lead exposure (Cleveland *et al*., 2008), which disrupts the development of neurochemicals, including neurotransmitters, and affects the organisation of ion channels (Casarett, 2008). This study investigated the protective effects of TQ on learning, memory, and motor coordination in male Wistar rats exposed to PbA. The NOR test assesses cognition, specifically recognition memory, in rodent models. There

was a significant decrease in the NOR following PbA administration, indicating cognitive impairment, and consistent with a previous finding (Ramírez Ortega *et al*., 2021). TQ administration prevented the harmful behavioural effects of PbA, indicating that TQ may be effective against lead-related neuron damage. This aligns with Khan *et al*. (2022), who reported TQ's neuroprotective properties against cognitive impairment and related dementias.

The step-down avoidance task is a simple method for assessing aversive memory and learning, with memory inferred from behavioural responses to prior experiences. In this study, a significant decrease in the step-down latency following PbA administration indicates a memory retention impairment. However, the administration of TQ mitigated this toxic effect. The present result aligns with Radad *et al*. (2014), who reported that TQ co-treatment reduced the incidence of lead-induced brain lesions in rats. The protective effect of TQ on memory retention may be attributed to its antioxidant properties, which is consistent with previous studies (Padhye *et al*., 2008; Ahmad *et al*., 2013; Tavakkoli *et al*., 2017).

The open-field test is commonly used to measure locomotor activity, anxiety, and exploration in rodents. The test showed a significant decrease in exploration after PbA administration. This decrease is indicative of PbA's interference with the development of neurochemicals, including neurotransmitters, and the organisation of ion channels, as previously reported by Casarett (2008). However, the administration of TQ alleviated the toxic effects of PbA in a dose-dependent manner. The actions of TQ could be due to its diverse potentials, including antioxidant, anti-inflammatory, and neuroprotective effects, as previously reported (Su *et al*., 2016; Samarghandian *et al*., 2014; Velagapudi *et al*., 2017; Barkat *et al*., 2018).

The accelerated rotarod test serves as a method for assessing the impact of TQ on rat motor coordination. The present result showed a notable decline in rat performance on the accelerated rotarod test after PbA administration, suggesting a capacity to impair motor coordination. Nonetheless, the adverse effects of PbA were mitigated when it was co-administered with TQ, consistent with the findings of Houghton *et al*. (1995), who reported TQ's broad and diverse pharmacological effects, including robust antioxidant activity against agents that generate free radicals. The amelioration of PbA's toxic effects on motor coordination may also be attributed to its antioxidant properties. This finding was further supported by the results of a prehensile experiment, which showed a significant decrease in latency arising from PbA exposure and a subsequent alleviation of this toxic effect following TQ co-administration.

An elevated hippocampal MDA level suggests that PbA exerts its toxic effects through the generation of lipid peroxidation. Previous research also reported that rats exposed to PbA led to a significant rise in lipid peroxidation and high MDA levels in their brains (Kalender *et al*., 2014; Abdulmajeed *et al*., 2016). Notably, the administration of TQ mitigated the PbA-induced increase in MDA levels. These results are in line with earlier studies where TQ countered free radical-generating agents and highlight its antioxidant properties (Abdel-Zaher *et al*., 2013; Sedaghat *et al*., 2014; Gülşen *et al*., 2016).

The levels of brain SOD, CAT, and GSH were markedly reduced in rats exposed to PbA compared to those in the control group, consistent with the findings of Ayman *et al*. (2021). This suggests that alterations in both enzymatic and non-enzymatic antioxidants in the brain may result from the inactivation of their functional sulphydryl groups through irreversible binding to PbA or oxidation induced by reactive oxygen species (ROS) overproduction due to PbA (Valko *et al*., 2005; Matović *et al*., 2015). However, the administration of TQ, particularly at a dose of 5 mg/kg, prevented the depletion of antioxidants.

TQ mitigates oxidative stress through a potent scavenging of free radicals (Kruk *et al*., 2000; Mansour *et al*., 2002; Badary *et al*., 2003; Khalife and Lupidi, 2007; Khattab and Nagi, 2007) and the upregulation of antioxidant enzyme genes (Ismail *et al*., 2008; Sayed-Ahmed *et al*., 2010; El-Sayed, 2011). The significant capacity of TQ to neutralise ROS can be attributed to the inherent redox properties of its quinone structure, as well as its ability to readily traverse morphophysiological barriers and access subcellular compartments (Badary *et al*., 2003).

The increased level of MDA in the cerebellum of this study suggests PbA's adverse effect on the brain through the stimulation of lipid peroxidation. This finding aligns with Patrick (2006), who hypothesised that free radicals generated by PbA can steal electrons from lipids within cell membranes, causing cellular damage. However, the administration of TQ reversed the elevated MDA levels. These results support an earlier study that reported TQ's action on various medicinal qualities, including strong antioxidant effects against free radical-generating substances (Houghton *et al*., 1995).

PbA significantly decreased the brain SOD levels, which is in line with Flora *et al*. (2006), who reported that a reduction in SOD level impairs superoxide radicals disposal, while a decrease in CAT impairs the scavenging of superoxide radicals. However, the co-administration of TQ mitigated these effects. This conclusion is consistent with the established antioxidant and anti-inflammatory properties of TQ, as reported by Chehl *et al*. (2009). These findings suggest that TQ's protective effects against PbA neurotoxicity may involve enhancing the cellular antioxidant defence system.

Although the CAT and GSH levels in the cerebellar tissue decreased, this reduction was not statistically significant. However, co-administration of TQ in graded doses protected against the observed decrease. This finding is consistent with the report by Flora *et al*. (2006). Kassab and El-Hennamy (2017) reported that using TQ after arsenate exposure lowered high levels of 5-HT and MDA, while it raised the levels of norepinephrine, dopamine, and glutathione. They also found that the antioxidant system, which includes GPx, GR, SOD, and CAT, was improved in the cerebral cortex, cerebellum, and brain stem.

Finally, PbA distorted the structures of the hippocampus and the prefrontal cortex and exhibited severe chromatolysis within the Purkinje cell layer. This finding

aligns with Ahmed *et al*. (2013), who reported that lead is a potent neurotoxin that damages the nervous system and causes brain disorders. Co-administration of TQ with PbA reduced damage in the hippocampus, prefrontal cortex, and cerebellum. The findings further demonstrated that TQ can help protect these brain structures from damage caused by PbA. This observation is consistent with already reported broad pharmacological properties of TQ (Houghton *et al*., 1995). The antioxidant properties of TQ may help protect against cerebellar damage caused by PbA.

Conclusion

PbA significantly impaired learning, memory, and motor coordination in male Wistar rats, likely due to oxidative stress and disruption of the hippocampus and prefrontal cortex, as well as the cerebellum. This impairment was evidenced by decreased performance in exploratory, rotarod, and prehensile tests, along with increased lipid peroxidation. However, TQ showed promise in protecting against these impairments, potentially through its antioxidant properties that neutralised lipid peroxidation and improved tissue antioxidants, leading to better behavioural outcomes and enhanced hippocampal, prefrontal, and cerebellar histomorphology.

DECLARATION

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Conflict of Interest

None declared.

Ethical Approval

Ethical approval for the study was obtained from the Health Research Ethics Committee (HREC) of the College of Health Sciences, Osun State University, Osogbo, Nigeria (Approval number: [UNIOSUNHREC2024/006B]).

Consent to Participate and Publish Data

Not Applicable.

Authors' Contribution

KIA: Conceptualisation, methodology and draft preparation; KBJ: Methodology, collection of data, draft preparation; OSO: Conceptualisation, reviewing, and editing; OOO: Visualisation and investigation; AOA: Collection of data, software analysis; TGA: Software analysis, reviewing, and editing.

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