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Modulatory Role of Vitamins A and E on Memory and Motor Functions of Cyanide Induced Neurotoxicity in Adult Swiss Mice

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Summary: Cyanide is a potent neurotoxic substance that can initiate series of intracellular reactions leading to oxidative stress. To evaluate effect of sublethal administration of potassium cyanide (KCN) on sensorimotor functions and long term visuo-spatial learning and memory in adult Swiss mice and possible ameliorative role of vitamins A and E. These vitamins A and E (dietary) are antioxidants that have scavenging properties against free radicals and reactive oxygen species as a result of oxidative stress induced by cyanide. Thirty-five mice weighing between 18-22 g were used for the study. The animals were randomly divided into five groups (n = 7) and exposed to sublethal concentration of potassium cyanide (10% LD_{50}; 1.5 mg/kg). KCN was administered orally while vitamin A (25 mg/kg) and vitamin E (50 mg/kg) were administered intra-peritoneal (IP) once daily for 28 days. Potassium cyanide (KCN) was first administered and after 10 minutes intervals, followed by vitamin A and then E after 5 minutes, vitamin E were administered across the different treatment groups. Mice were examined for signs of toxicity. Vitamins pre-treatment ameliorated toxic signs. In the dynamics of wire grid, coat hanger and stationary beam test, the latency to fall in weeks 2 and 4 were statistically significant. In acquisition and retention, using elevated plus maze (EPM), KCN treated group recorded high transfer latencies in seconds (50.40±1.72 secs) and (57.60±0.93 secs) as compared to group IV (29.40±0.68 secs; 5.60±0.60 secs). Cyanide is a neurotoxin that affects motor functions with progressive decline in motor strength and coordination. KCN affects acquisition and retention memory while pre-treatment with antioxidant vitamins A and E ameliorated these deficits.

Keywords*:* Cyanide; Neurotoxicity; Memory; Motor function

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INTRODUCTION

Cyanide is a rapidly acting, potentially deadly chemical that can exist in various forms which is toxic to humans and animals; exposure can occur in various ways. Many substances are potential sources of cyanide exposure, including edible and non-edible plants (e.g., cassava), industrial operations (e.g., plastics processing), fires, and cigarette smoke (Ogundele and Olu, 2012). Although the primary natural source of cyanide poisoning is from plants, other natural sources include volcanoes, bacteria, and fungi. Its toxicity is well known but it is still used in the surgical dressing, metal-plating, mining, chemical and agricultural industries (Al-Ghanim and Mahboob, 2012). The toxic effects of cyanide (HCN) have traditionally been attributed to inhibition of cytochrome C oxidase, the terminal enzyme of respiratory chain, which compromises oxidative phosphorylation leading to cytotoxic hypoxia (Caxton *et al.*, 2010).

Neurological disturbances have been reported from parts of Africa with protein-deficient populations and attributed to cyanide (CN^-) exposure from prolonged dietary use of cassava (Mathangi *et al.*, 1999; Osuntokun, 1981). Acute cyanide intoxication causes confusion, agitation, disorientation and cognition deficits while, chronic cyanide exposure that results from consumption of improperly processed cassava leads to neurodegenerative disorders (Konzo and Tropical Ataxic Neuropathy) Cardosso *et al*., 2002. One form of cassava related disease is a slowly developing ataxic myeloneuropathy. It is a local Zairian term for a disease first described in 1938, in the democratic republic of Congo (formally Zaire), but has also been observed in Mozambique, Tanzania,

Central African Republic Cameroon and Nigeria (Ernesto *et al.,* 2000; Mathangi and Namasivayan, 2000).

The syndromes grouped as TAN can differ widely in clinical presentation, natural history and response to treatment. It is an upper-motor-neuron disease characterized by irreversible but non-progressive symmetric spastic paraparesis that has an abrupt onset (Oluwole *et al.,* 2003). The main clinical features of some of the syndromes have included sore-tongue, angular stomatitis, skin desquamations, optical atrophy, neurosensory deafness and sensory gait ataxia (Link *et al.,* 2000). The development of these syndromes is hypothesized to depend on 1) Amount and duration of exposure to dietary cyanide and 2) The ability of the body to detoxify cyanide, a function that may vary with nutritional status (Mathangi *et al.,* 1999).

Oxidative stress arises from an imbalance between the production of free radicals and physiological antioxidant capability (Kapur *et al.,* 2004). However, during the oxidative stress as previously reported in cyanide poisoning, the body's antioxidant systems are overwhelmed by the oxidants. Under these circumstances, a provision of exogenous antioxidants becomes imperative to neutralize the damaging effect of reactive oxygen species. Vitamin A and its derivatives (retinoids) play important roles in many physiological processes. It is important for both development and maintenance of adult brain homeostasis (Bailey and Lane, 2005). Throughout adulthood, vitamin A remains important in other central nervous system (CNS) related functions, for instance learning and memory (Carta *et al.,* 2002). Furthermore, vitamin A and its related retinoids easily penetrate into blood-brain-barrier and mammalian CNS contains the molecular apparatus responsible for the production and maintenance of all-trans-retinoic acid in neurons, through retinal dehydrogenases and cellular retinoid proteins action (Deuster, 2000). Thus, the CNS is able to transport and metabolize retinoid molecules and may rapidly increase their concentrations. Vitamin E is an essential nutrient in humans and well known antioxidant substance that reduces free radicals and reactive oxygen species activity (Asonye and Okolie, 2004). Like other antioxidants, vitamin E slows or prevents memory impairments that accompany several conditions such as mental stress, diabetes, cerebral ischemic injury, Alzheimer's disease, stroke and aging (Mohammed *et al.,* 2 014). The aim of the present study is to investigate the effect of vitamins A and E on KCN-induced neurotoxicity.

MATERIALS AND METHODS

Drugs and Chemicals

Potassium cyanide was purchased from sigma Aldrich USA, It was dissolved in di-ionised water. Vitamin A (1000IU) and E (100 mg DL alpha tocopherol) were purchased from Patterson zoochonis Ltd, Nigeria) and were reconstituted in soy oil to 1% solution and 20 mg/ml respectively.

Animals

Thirty-five adult Swiss male mice (weighing between 18-22 g) were obtained from the animal house of Department of Physiology, Faculty of Medicine Ahmadu Bello University Zaria, Nigeria. All animals were housed in a steel cage, at room temperature and had free access to drinking water and their diets. The animals were acclimatized for 7 days to their environment and diet prior to commencement of the study.

Treatment of Animals

The animals were randomly grouped into five groups (n = 7) as follows: Group I (control, received deionised water), group II (1.5 mg/kg KCN only), group III (1.5 mg/kg KCN + 25 mg/kg vitamin A), group IV (1.5 mg/kg KCN + 50 mg/kg vitamin E), and group V (1.5 mg/kg KCN + 25 mg/kg vitamin A + 50 mg/kg vitamin E). KCN was administered orally while vitamins (A and E) were administered via intra-peritoneal (IP) route once daily for 28 days. Potassium cyanide (KCN) was first administered and after 10 minutes intervals, followed by vitamin A and then vitamin E after 5 minutes were administered across the different treatment groups. The animals were observed for toxic signs and possible deaths throughout the study period. The experiment was performed according to the guidelines on animal research of the Animal Research Ethic Committee of the Ahmadu Bello University, Zaria.

NEUROBEHAVIORAL ASSESSMENTS

All behavioural parameters were assessed by two trained observers blind to the animals' treatment status in order to eliminate bias.

Wire Grid Test

Motor strength was evaluated using wire grid test. Strength suspension (four paws) was assessed on a grid bordered with masking tape to prevent the mouse from walking off the edge (Delatour *et al.,* 2008). The mouse was placed on the centre of the grid that was slowly turned upside down at a 20 cm height above the floor. Latency to fall was recorded (cut off: 1 min).

Coat Hanger Test

The assessment of motor coordination was evaluated using coat hanger test as described by Delatour *et al*., (2008). An iron horizontal bar (1.3 mm diameter) was placed at a height of 30 cm from the floor; a cardboard wall was inserted at each end of the bar in order to prevent mice from escaping sideways. Mice were placed in the middle part of the horizontal bar. Each trial began only when the forepaws gripped the bar at the moment of release. Three trials were recorded

(inter-trial interval: 5 min), and after each trial, mice were re-isolated. The latencies of the traction reflex (i.e. time taken by the animal to catch the bar by one of its hind paws) and alternatively the latencies before falling (cut off: 30 s) were measured.

Stationary Beam Test

Beam walk was conducted in order to detect any neurological deficits in sensory, balance, or motor performance. The materials used were Methylated spirit, cotton wool, and the Beam walk apparatus which is made up of 75 cm strips of two smooth woods. One is flat like a ruler of 25 mm width by 10 mm thickness. The other is round and a diameter of 20 mm. Narrow supports stand to hold up the start section of the raised beam (15 mm cross-section, 40 cm high) was also used, together with the Goal box (20 cm x 15 cm, with a 4 × 5 cm entrance hole) secured on top of a support stand.

Elevated plus-maze test

An elevated plus maze was conducted as described by Komada *et al.,* (2008). Briefly, the elevated plus maze consisted of two open arms (25 x 5 cm) and two closed arms of the same size with 15 cm high wooden walls. The arms and central square were made of wood, elevated 55 cm above the floor. Each mouse was placed at the distal part of the open arm maze (5 x 5 cm), facing away from the closed arm. On the training day (first day), each animal was placed at the end of one open arm, facing away from the central platform. The latency of the mouse to move from the open arm to the enclosed arms was recorded within 90 seconds. Following entry into the arm, the animals were allowed to explore the apparatus for 20 seconds. Twenty-four hours later, the second trial (retention test) was performed and the animals were observed for 90 seconds. Reduction in latencies between day one (acquisition) and day two (retention) indicates memory of the learned task. After each trial, the maze was wiped with a cloth dipped in 70% ethanol, and allowed to dry to remove any olfactory cue. An overhead camera video camera recorded movement of the mice for later quantification.

Statistical analysis

Results were expressed as the mean ± standard error of the mean (SEM). Data for multiple variable comparisons were analyzed by one-way analysis of variance (ANOVA). For the comparison of significance between groups, Duncan's test was used as a *post hoc* test according to the statistical package program (SPSS version 17.0). All values $p < 0.05$ was considered as significant for all statistical analysis in this study.

RESULTS

Signs of toxicity

Toxic signs observed in the potassium cyanide treated group included huddling, asphyxiation, dyspnoea, mild tremors, piloerection and soft faecal bolus (diarrhoea). With progressive weeks, one death was recorded in group II. The vitamin pre-treated groups (III, IV and V) showed milder toxic signs compared to the KCN-treated group and these include piloerection, soft faecal bolus (diarrhoea) and dyspnoea.

Effect of treatments on wire grid test

As shown in figure 1, analysis of neurological performance in the WGT showed a significant decrease ($p < 0.05$) of motor strength in the KCN-treated group as compared with control and vitamins pre-treated group. A global decline of performance was noted with progressive weeks 2 and 4 respectively (12.60±0.40 secs) and (9.20±0.58 secs) in group II as compared to group V (27.60±0.93 secs) and (38.00±1.05 sec) $p < 0.05$.

Effect of treatments on coat hanger test

The latency to fall from the coat hanger was also measured as an alternative index for motor strength. Analysis of variance of this variable indicated a global decline of performance in KCN-treated group with progressive increase in weeks 2 and 4 respectively (11.00±0.45 secs; 8.20±0.58 secs) when compared to control (24.40±0.68 secs; 26.60±0.51 secs). Pretreatment with vitamins showed increase in motor performance as seen in group IV in contrast to group II (8.20±0.58 secs and 24.60±0.68 secs) $p < 0.05$.

Effect of treatments on stationary beam test

Motor coordination was evaluated with a stationary beam test. Analysis of the number of crossed segments in group II showed decreased activity in KCN treated group (19.60±0.51 secs; 15.80±0.58 secs) when compared with group IV (6.40±0.51 secs; 4.60±0.68 secs), $p < 0.05$. A decline in motor performance as the week progresses, as it was recorded in their transfer latencies.

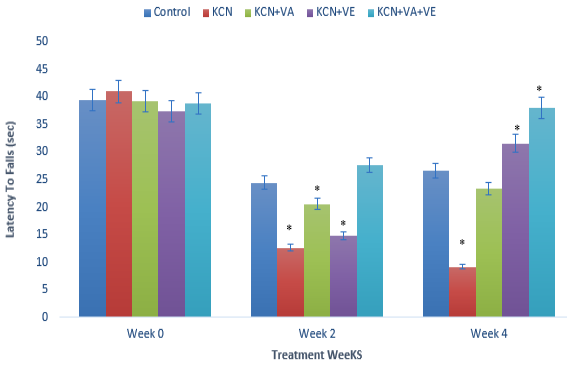

Fig. 1: Effect of Sublethal Administration of Potassium Cyanide and Administration of Vitamins A and E on wire grid Test in Swiss Mice. *$p < 0.05$.

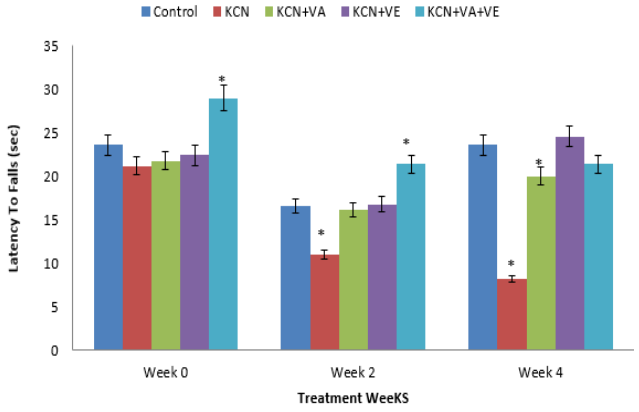

Fig. 2: Effect of Sublethal Administration of Potassium Cyanide and Administration of Vitamins A and E on Dynamics of Coat Hanger Test in Swiss Mice. *p < 0.05.

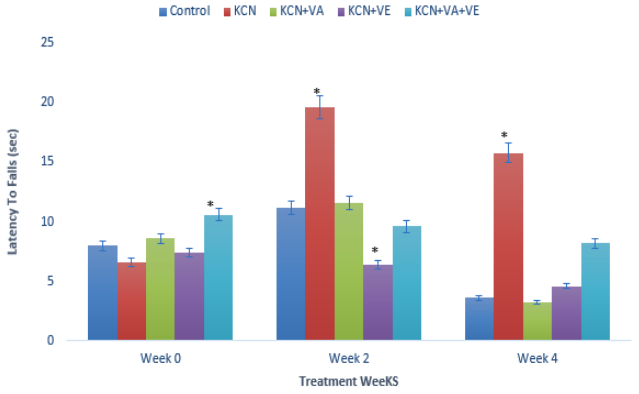

Fig. 3: Effect of Sublethal Administration of Potassium Cyanide and Administration of Vitamins A and E on Dynamics of Beam Walk Performance Test in Swiss Mice. *p < 0.05.

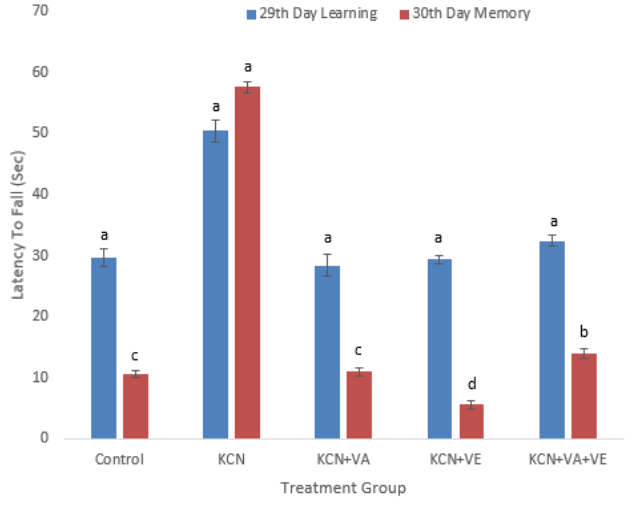

Fig. 4: Effect of Sublethal Administration of Potassium Cyanide and Administration of Vitamins A and E on Learning and Memory in Swiss Mice. *p < 0.05.

Effect of treatments on elevated plus-maze test

In the EPM test, this was assayed for long-term visuo-spatial memory acquisition and retention latencies. Acquisition latencies (seconds) for KCN treated group and control (50.40±1.72 secs and 29.60±1.43 secs) while retention transfer latencies were (57.60±0.93 secs and 10.60±0.51 secs) for both groups. In vitamins pre-treated groups III and V acquisition latencies (28.40±1.81 secs and 32.40±0.93 secs) while retention transfer latencies were (11.00±0.71 secs and 14.00±0.70 secs), figure 4. The KCN-treated group mice spent more time in open arms than in the closed arms in contrast to the vitamin groups underlining avoidance of the anxiogenic area of the apparatus.

DISCUSSION

The toxic signs observed in the KCN-treated group which included tremors, convulsion and salivation in the KCN-treated group were consistent with symptoms observed in cyanide poisoning. The mitigation of toxic signs and mortality in mice pretreated with vitamins A and E demonstrated the protective role of vitamins (A and E) on cyanide induced toxic signs and deaths. This shows the role of oxidative stress in toxic signs evoked by cyanide.

Analysis of neurological performance in the wire grid test showed a significant reduction of all paws grip, reflecting deficit in all paws motor strength following sublethal exposure to cyanide treated group in Swiss mice. The impairment can be as a result of lesion in cerebellar cortex, motor area or maybe explained by the triggering of region specific toxic pathways in which oxidative stress maybe a common activator (Borowitz *et al.*, 1999; Hahm *et al.*, 2010). This may be due to partly reduced brain and perhaps muscle oxidative damage. The antioxidant pre-treated groups motor deficits were less compared to potassium cyanide treated groups. The group that received vitamin A and E had better grip and longer latency in comparison to group that received KCN + vitamin A, and KCN + vitamin E. The vitamins group (A and E) was able to mitigate the effects of cyanide induced oxidative damage via their antioxidant properties.

The latency to fall from the coat hanger was also measured as an index of motor-strength. Analysis of this variable indicates a decrease of performance as week progresses, reflecting deficits in forepaw motor strength following sublethal exposure to KCN in Swiss mice. The impairment of motor strength by potassium cyanide may have been due to oxidative damage to muscles resulting in muscular rigidity (Calabressi *et al.*, 2008). Similarly, reports have also shown that reduced hand strength and loss of muscle strength following acute cyanide poisoning (Messing, 1991). Antioxidant vitamins treated groups ameliorated the effects of cyanide induced neurotoxicity on the muscle mass.

Motor co-ordination was evaluated using the stationary beam walk. It assayed for muscular coordination and balance. It is an integrated form of behaviour requiring pertinent level of consciousness, memory, sensorimotor and cortical functions mediated by the cortical areas (De-Latour *et al.*, 2008). The loss

of equilibrium followed by erratic movements was observed together with imbalanced body activity and muscular incoordination was seen in group pretreated with KCN. This correlates with the work of Ogundele *et al.,* 2014. The progressive increase in which the mice slipped off the beam indicates impairment of motor co-ordination. This impairment can be as a result of either due to cortical damage (Ayuba, 2014), cerebellar damage (Ogundele *et al*., 2014) and or pyramidal motor system damage, which controls all of the voluntary movements (Hamakawa *et al*., 2011). Furthermore, cyanide has been shown to induce cell death in a varying mode of different brain areas (Hahm et al., 2010). This might have been responsible for the deficit in beam walk performance in KCN treated group. The impairment of motor strength by cyanide may have also been due to the reduced motor-strength which has been observed in humans following prolonged cyanide exposure as seen in endemic exposed areas (Osuntokun, 1981). It is well documented in the literature that the brain is a critical site for anoxia, which may be due to changes in electrical activity which cause damage to the region of the brain associated with the maintenance of equilibrium (De-Latour *et al*., 2008, 2012) as was observed in the present study. However, groups pre-treated with antioxidant vitamins (A and E) had improved motor performance and decreased number of slipping. When these groups were compared with control, the transfer latency was increased, which shows that antioxidant vitamins may not be the only mechanism involved in motor coordination deficits induced by cyanide exposure.

Deficits in behaviour and performance in visuo-spatial memory and learning tasks was observed in KCN treated mice. The underlying thread is that cyanide impairs performance in memory and learning. Mice spent more time in the open arms, less explorative to find the enclosed arms, showing less aversion to open space and exhibiting avoidance behavior. The present study also corroborates with the work of Bukachi *et al.,* 2014, which showed memory deficits is associated with sublethal cyanide poisoning relative to cyanate toxicity in rodents. The impairment can be as a result of different hippocampal neurotransmitters (Ayuba, 2014) or cholinergic deficiency; leading to reduced acetylcholine synthesis, or combination of both parameters.

On the other hand, antioxidant vitamin administered was able to ameliorate these acquisition and retention decline through their antioxidant properties. Vitamin E administration from the results showed better performance as compared to vitamin A, or co-administered vitamins. This is in agreement with the work of Abe *et al.,* 2005 that oxidative stress induced by hyperoxia significantly impaired cognitive performance of 3 month old mice, effects that were partially attenuated by dietary vitamin E supplementation. Furthermore, the brain is rich in polyunsaturated fatty acids and relies heavily on the antioxidant properties of the lipid-soluble vitamin E (α-tocopherol) (Rashid *et al*., 2011).

The present study revealed that (i) potassium cyanide significantly decreased motor performance (motor strength and coordination) in Swiss mice and also a decline in cognitive functions; (ii) the antioxidant vitamins (A and E) significantly ameliorated the deficits in motor performance and also improved learning and memory functions of the brain in Swiss mice.

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