

Tamarindus indica Ameliorates Neural Aluminum Chloride Toxicity in Neonatal Wistar rats

IBE MICHAEL USMAN (✉ gopama13@gmail.com)

Kampala International University - Western Campus <https://orcid.org/0000-0001-6624-1286>

Sunday Abraham Musa

Ahmadu Bello University

Ibrahim Abdullahi Iliya

Federal University Dutse

Eric Osamu Aigbogun Jr.

Kampala International University - Western Campus

Keneth Iceland Kasozi

Kabale University

Gaber El-Saber Batiha

Damanhour University

Samuel Sunday Adebisi

Ahmadu Bello University

Research note

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Abstract

Objective:

This was to determine levels of heavy metal expression following *Tamarindus indica* activity in neonatal rats. Pregnant timed rats were divided into 5 groups (n =4) and neonatal rats were used. Group 1 (negative control), Groups II-V were experimental groups treated with 100 mg/kg of AlCl₃ s/c. Group II (positive control), Groups III and IV receiving 400mg/kg and 800mg/kg of *Tamarindus indica* respectively, and Group 5 receiving 30mg/kg of Vitamin E (comparative control) for 3 weeks. Brain metal; copper, zinc, iron, and calcium (Cu, Zn, Fe, and Ca respectively) quantification was done using atomic absorption spectrophotometer.

Results:

Tamarindus indica contained 61.6% Oleic Acid, n-Hexadecanoic acid 11.03%, Phenol, 3,5-bis (1,1-dimethyl ethyl)- 8.48 %, and cis-9-Hexadecenal 7.79% as the main components in *Tamarindus indica*. The differential expression of brain metals in the treatment groups on post gestation day 7 and 21 revealed significantly high mean Zn, Fe, and Cu and lower Ca expression in the positive control compared to the negative control; but significantly lower mean Zn, Fe, and Cu and higher Ca expression was observed in the group treated with 400 and 800mg/kg bw of EATI and comparative control when compared to values observed in positive control.

Introduction

Pharmacological investigations on *Tamarindus indica* extracts revealed antibacterial and antifungal properties (1), however, there is limited information on the effect of the *Tamarindus indica* on the expression of trace elements in the brain following prenatal aluminum chloride exposure in Wistar rats, despite its rich phytochemical composition. Trace elements are often required in well-regulated and moderate quantity (2,3). Iron (Fe²⁺) acts as a coenzyme essential in phosphorylation in the body (4–6). Zinc (Zn²⁺) is associated with presynaptic vesicles of glutaminergic neurons and is often released into the synaptic cleft where they modulate the activities of various post-synaptic receptors (7,8), and regulation of oxidant generation in the neuronal cell (9). Copper (Cu²⁺) is an essential micronutrient essential for the maintenance of cellular integrity, production of energy, cell signaling, cellular proliferation, defense against oxidation, and effect of radiation (10) while Calcium (Ca²⁺) plays a role in gene transcription, neurotransmission, memory processing, synaptic plasticity, and apoptotic cell death (11,12). During pregnancy, the demand for energy and nutrients is usually increased especially for micronutrients such as Fe²⁺, Zn²⁺, Cu²⁺, and Ca²⁺ (13). One of the potential mechanisms of aluminum (Al³⁺) induced toxicity involves its ability to interfere with neural ionic homeostasis (14). The ability of aluminum chloride (AlCl₃) to cross the blood placental barrier, with subsequent ability to cross the fragile developing blood-brain barrier, gives it more access to the brain (15). The objective of the study was to

establish effects of *Tamarindus indica* on expression of heavy metals following prenatal exposure to AlCl_3 in neonatal Wistar rats.

Methods

Chemical and Drug Preparation

Aluminum chloride (CAS Number: 7446-70-0) was obtained from Sigma-Aldrich. Aluminum chloride stock solution was prepared by dissolving 1g of Aluminum chloride in 10mls of distilled water. Capsules of vitamin E (Gujarat liquid pharmacaps Pvt) were obtained from a reputable drug store in Zaria, Kaduna, Nigeria. The stock solution was prepared using Tween 80, containing 60 mg of vitamin E in 0.2 ml of the suspension. The stock solution containing vitamin E was then shielded from direct light to avoid photodegradation.

Plant Material Acquisition and Extraction

Tamarindus indica leaves were collected from the Botanical garden of Ahmadu Bello University, Zaria. The leaves were authenticated in the Herbarium unit of the Department of Botany and assigned a verification number of 2417. The extraction of the leaves was carried out by maceration, followed by subsequent fractionation (16).

Phytochemical Screening

Phytochemical screening of the crude extract and the fractions was carried out using standard methods (17), to reveal the presence of chemical constituents. Gas chromatography-mass spectrometry (GC-MS) analysis on the chemical composition of ethyl acetate leaf fraction of *Tamarindus indica* was done using GCMS-QP2010 PLUS SHIMADZU, Japan) (see additional file 1).

Experimental Animals

Apparently healthy (20) adult non-pregnant females and 10 adult male Wistar rats were acclimatized for two weeks in the Animal house of Human Anatomy Department, Ahmadu Bello University, after which, the vaginal smear was taken from all the female rats and examined under a light microscope for the staging of their estrous cycle. The female rats in the proestrus phase were caged overnight with the mature male in the ratio of 2:1 (female: male); the presence of vaginal plugs the following morning indicated mating and assumed to be day zero of pregnancy (18–20). The dams and their pups were allowed free access to feed and water before and during the experiment, under similar ventilated and spacious housing condition. A total of 40 male pups were used in the study.

Dosage Determination

A dosage of 200 mg/kg bw was adopted for AlCl_3 based on previous studies in Wistar rats (21,22). The adopted dosage for Ethyl acetate leaf fraction of *Tamarindus indica* (EATI) were 400 and 800 mg/kg bw

(low and high dose respectively). A dosage of 300 mg/kg bw was adopted for vitamin E based on previous studies using the Wistar rat model (23).

Experimental Design

Gestational rats on day 7 day (day 0 of the experiment) were administered the extracts for two weeks i.e. day 21 of gestation. To reduce biases, animals were assigned random numbers and independently assigned to groups (n=4) in line with the ARRIVE guidelines on experimental animals.

Group 1: 2 ml/kg bw of distilled water, p.o (negative control)

Group 2: 200mg/kg bw of AlCl₃, p.o (positive control).

Group 3: 200mg/kg bw of AlCl₃ and 400 mg/kg bw EATI, p.o

Group 4: 200mg/kg bw of AlCl₃ and 800 mg/kg bw EATI , p.o

Group 5: 200mg/kg bw of AlCl₃ and 300 mg/kg bw of Vitamin E, p.o (comparative control).

Neonatal rats on post gestation days 7 and 21 were euthanized using thiopental sodium since was ethically acceptable in experimental animals (24,25), by intraperitoneal injection of 5mg/kg thiopental sodium. The skull was dissected and the brain tissues were harvested from neonates for quantification of brain total Cu, Zn, Fe, and Ca.

Sample Preparation

Two grams of the harvested brain tissue were weighed and transferred to a beaker, then 10 ml of solvent (NH₃+HCl) was added. The mixture was then heated for 45 minutes to 1 hour at 100 °C, to allow complete digestion. The digested mixture was then allowed to cool for 15-20 minutes. The mixture was then filtered and readied for brain metal quantification using Atomic Absorption Spectrophotometer (AAS) (Perkin-Elmer Corp., Norwalk, Conn. 06856) and the concentrations were recorded in ppm.

Statistical analysis

Data obtained were analyzed using the GRAPH PAD prism. The results were expressed as mean ± SEM; differences in means were compared using one-way analysis of variance (ANOVA) and considered significantly different at $p \leq 0.05$.

Results

3.1 Phytochemical Screening of ethyl acetate of *Tamarindus indica*

This showed that the ethyl acetate fraction of *Tamarindus indica* had secondary metabolites (Additional file 1). There was a positive reaction for carbohydrates and flavonoids. The gas chromatography revealed

10 major peaks (Additional file 1), representing 10 chemical compounds in the ethyl acetate fraction of *Tamarindus indica* leaves. The mass spectrometry identified the following chemical components were Oleic Acid 61.63 %, n-Hexadecanoic acid 11.03%, Phenol, 3,5-bis (1,1-dimethyl ethyl)- 8.48 %, and cis-9-Hexadecenal 7.79% as the main components of our plant fraction (Table 1).

3.2 Brain metal expression in brain tissue of neonatal rats

On days 7 and 21, there were significant differences in ionic concentrations between the negative and positive controls (Table 2) in which the positive control was associated with high ionic levels compared to other experimental groups. EATI lowered Zn (Fig 1A) concentrations comparable to the comparative control ($P > 0.05$). EATI lowered Cu levels and there were significant differences in the dosages at which 800 mg/kg bw was most effective on day 7, although this wasn't reproduced on day 21 (Fig 1B). Furthermore, Fe levels in the positive control were the highest, however, EATI at 400 mg/kg bw and 800 mg/kg bw reduced Fe concentrations in the brain tissue on both days 7 and 21 (Fig 1C). Significant decreases in Fe levels were significant on day 7 at 400 mg/kg. Furthermore, the positive control Ca levels were lowest, however, total Ca levels were increased by EATI at 800 mg/kg to the comparative control levels ($P > 0.05$) (Fig 1D).

Discussion

The current study showed that *Tamarindus indica* contains flavonoids known for their unprecedented antioxidant property. Flavonoids also possess anti-inflammatory and anti-mutagenic properties (26). Flavonoids-rich plant materials are shown to significantly improve cognition (27), These effects range from memory and learning enhancement to the said general cognition improvements (28). Ethyl acetate leave fraction of *Tamarindus indica* was reported to contain oleic acid. Oleic acid is involved in some very important developmental processes in the nervous system (29). Although, the nervous system can produce enough quantities it needs for these processes; however, the importance of dietary sourced oleic acid to other tissues of the body cannot be overruled. The reported n-Hexadecanoic acid present in ethyl acetate leave fraction of *Tamarindus indica* is said to possess hypo-cholesterolemic, anti-inflammatory, antioxidant, and antitumor activities (30).

During pregnancy, demand for energy and nutrients is usually increased especially for micronutrients such as Cu, Zn, Fe, and Ca (13). In the present study prenatal exposure to $AlCl_3$ adversely antagonized neural homeostasis of essential elements and this was in agreement with previous findings (31). The study showed that administration of ethyl acetate leaves fraction of *Tamarindus indica* (EATI) at both 400 and 800mg/kg bw improved neural ionic homeostasis. Al exerts its toxicity by displacing Zn from the tissues demonstrating the importance of EATI in protecting neural tissue. Since Zn plays a crucial role in DNA and RNA enzyme activity, neural transmission, and antioxidant balance (7–9), findings in the study would help in alternative and complementary medicine. Also, the study showed that EAT lowered Cu levels especially at 800 mg/kg bw in the first week demonstrating its effects to be associated with chronic effects. Returning Cu levels to baseline levels is essential in mammals for the maintenance of

cellular integrity, cell signaling, and antioxidation, (10,32). Furthermore, EATI lowered Fe levels to comparable levels to the negative and comparative controls. Since Fe acts as a coenzyme essential in phosphorylation in the body (4–6) and the body lacks mechanisms to metabolize excessive levels, administration of EATI offers protective effects. The study also showed that Al toxicity was associated with a suppression of Ca^{2+} levels. Ca^{2+} is essential for neural transmission, cellular signaling, and apoptosis (11,12), showing that EATI administration helps to restore tissue physiological function. Since trace elements are needed in moderate quantities (2,3), administration of EATI showed that this helped to modulate and promote physiological function following AlCl_3 toxicity. The current study, though basic offers a preview of the roles of EATI in preventing heavy metal toxicity. Furthermore, Al toxicity was associated with elevation of trace elements in the tissues except Ca^{2+} demonstrating a need to investigate cellular mechanisms responsible for ionic efflux/influx.

Limitations

The study fails to investigate in-vitro trace element concentrations following challenges in infrastructure. Also, limited funding meant a thorough phytochemical analysis on the plant was impossible thus creating room for further research on EATI. Cellular mechanisms interrupted and gene expression studies were not investigated, and these would help offer a pluralistic interpretation of our study findings.

Abbreviations

Ca Calcium

Cu Copper

DNA Deoxyribonucleic acid

EATI Ethanoic aqueous leaf *Tamarindus indica*

Fe Iron

RNA Ribonucleic acid

Zn Zinc

Declarations

Ethics approval and consent to participate

Expediated ethical approval from Ahmadu Bello University Committee on Animal Use and Care was acquired and registered as ABUCAUC/2019/001. Consent to participate was not applicable.

Consent for publication

Not applicable

Availability of data and materials

Data files used can be accessed at <https://figshare.com/s/327de6b61acf74ed5e09>

Competing interests

The authors declare no conflicts of interest.

Funding

Not applicable

Authors' contributions

IMU conceptualized the study; IMU, SSA, SAM, IAI designed the study; IMU collected the data; IMU, GEB, KIK conducted data analysis while IMU; KIK, GEB interpreted the data. IMU drafted the initial version, SAM, IAI, EOA, KIK, GEB, SSA revised it critically for intellectual content. All authors approved final version for published and remain in agreement to be accountable for all aspects of the work.

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Not applicable.

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Tables

Table 1. Chemical Composition of the sample analyzed (% of peak areas) determined by GC-MS

No.	R.t.*	Compound Name	Area%	Molecular formula	Molecular weight	RetIndex	CAS
1	7.958	Cyclopropane, nonyl-	1.36	C ₁₂ H ₂₄	168	1216	74663-85-7
2	10.717	1-Tridecene	2.02	C ₁₃ H ₂₆	182	1304	2437-56-1
3	12.708	Phenol, 3,5-bis(1,1-dimethylethyl)-	8.48	C ₁₄ H ₂₂ O	206	1555	1138-52-9
4	13.267	1-Pentadecene	2.55	C ₁₅ H ₃₀	210	1502	13360-61-7
5	15.725	9-Eicosene, (E)-	2.74	C ₂₀ H ₄₀	280	2017	74685-29-3
6	19.033	n-Hexadecanoic acid	11.03	C ₁₆ H ₃₂ O ₂	256	1968	57-10-3
7	20.642	9-Octadecenoic acid, methyl ester, (E)-	1.13	C ₁₉ H ₃₆ O ₂	296	2085	1937-62-8
8	20.975	Octadecanoic acid, methyl ester	1.27	C ₁₉ H ₃₈ O ₂	298	2077	112-61-8
9	21.467	Oleic Acid	61.63	C ₁₈ H ₃₄ O ₂	282	2175	112-80-1
10	24.017	cis-9-Hexadecenal	7.79	C ₁₆ H ₃₀ O	238	1808	56219-04-6

*R.t. retention time

Table 2. Multiple comparisons tests amongst experimental groups on Zn, Cu, Fe and Ca content in brain tissue following exposure to EATI.

Tukey's multiple comparisons test	N	Adjusted P values			
		Zn	Cu	Fe	Ca
Post Gestation Day 7					
Negative control vs. Positive control	8	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Negative control vs. 400 mg/kg bw EATI	8	0.5308	< 0.0001	< 0.0001	0.0407
Negative control vs. 800 mg/kg bw EATI	8	0.0554	0.0002	0.8437	0.1916
Negative control vs. Comparative control	8	0.1288	0.9931	0.0264	> 0.9999
Positive control vs. 400 mg/kg bw EATI	8	< 0.0001	0.0003	< 0.0001	< 0.0001
Positive control vs. 800 mg/kg bw EATI	8	< 0.0001	< 0.0001	< 0.0001	< 0.0001
Positive control vs. Comparative control	8	< 0.0001	< 0.0001	< 0.0001	< 0.0001
400 mg/kg bw EATI vs. 800 mg/kg bw EATI	8	0.6989	0.0003	< 0.0001	0.9434
400 mg/kg bw EATI vs. Comparative control	8	0.8985	< 0.0001	0.0015	0.0306
800 mg/kg bw EATI vs. Comparative control	8	0.9943	0.0006	0.0019	0.1527
Post Gestation Day 21					
Negative control vs. Positive control	8	0.0071	< 0.0001	0.1423	> 0.9999
Negative control vs. 400 mg/kg bw EATI	8	> 0.9999	0.031	0.3728	0.9972
Negative control vs. 800 mg/kg bw EATI	8	> 0.9999	0.9609	0.9743	< 0.0001
Negative control vs. Comparative control	8	0.9998	0.9971	0.9986	0.4261
Positive control vs. 400 mg/kg bw EATI	8	0.0069	0.003	0.9788	0.9923
Positive control vs. 800 mg/kg bw EATI	8	0.0067	< 0.0001	0.3897	< 0.0001
Positive control vs. Comparative control	8	0.0046	< 0.0001	0.2364	0.3706
400 mg/kg bw EATI vs. 800 mg/kg bw EATI	8	> 0.9999	0.1333	0.7299	0.0001
400 mg/kg bw EATI vs. Comparative control	8	0.9999	0.0664	0.5351	0.6278
800 mg/kg bw EATI vs. Comparative control	8	0.9999	0.9971	0.9976	0.0062

Figures

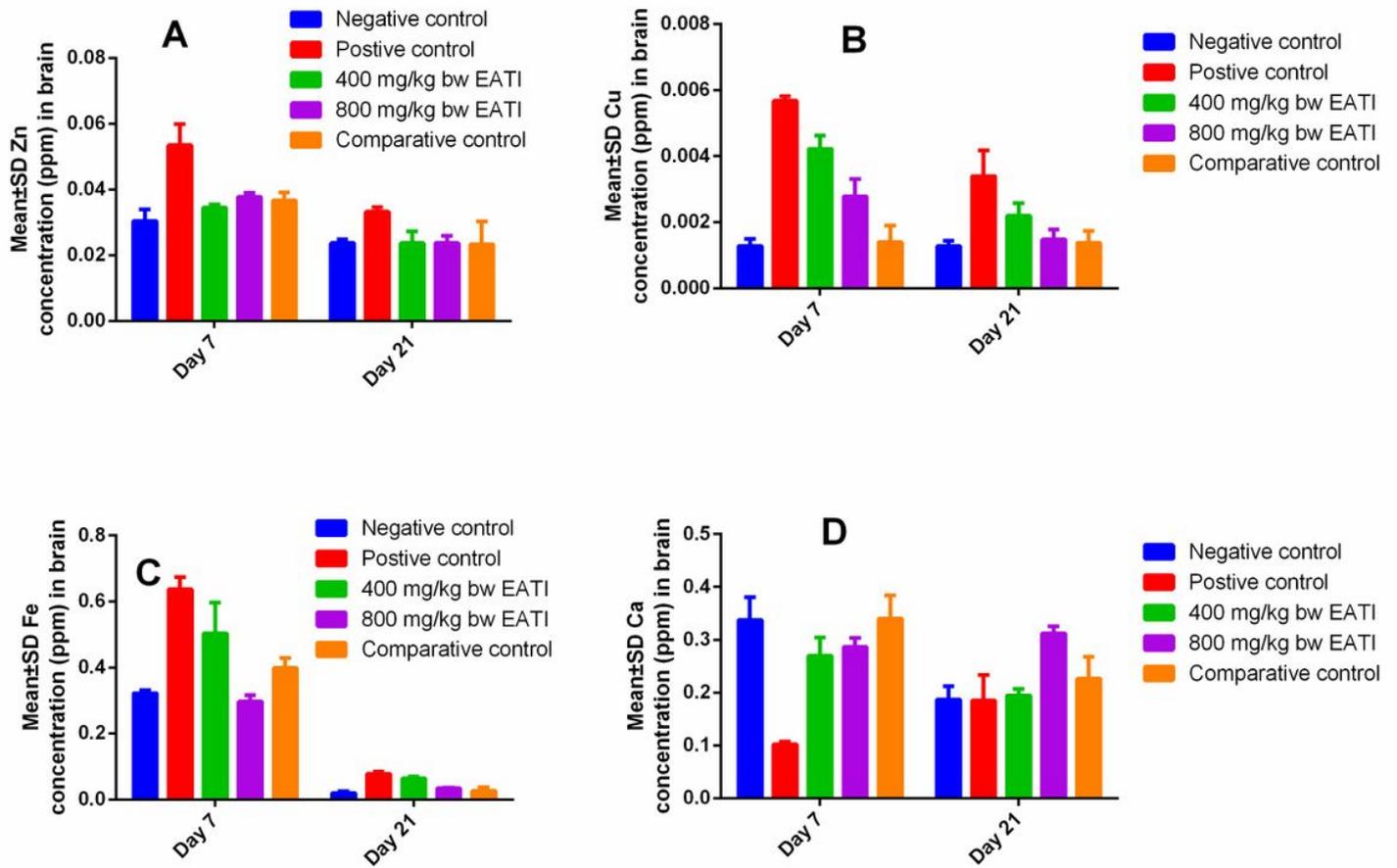


Figure 1

Mean concentration of brain metals following EAT1 administration following aluminum chloride exposure.

Supplementary Files

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