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Hepatoprotective potential of *Tamarindus indica* following prenatal aluminum exposure in Wistar rat pups

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ABSTRACT

Over time, the use of plant-derived agents in the management of various human health conditions has gained a lot of attention. The study assessed the hepatoprotective potential of ethyl acetate fraction *Tamarindus indica* leaves (EFTI) during prenatal aluminum chloride exposure. Pregnant rats were divided into 5 groups (n = 4); Group I rats were administered 2 ml kg^{-1} of distilled water (negative control), Group II rats received only 200 mg kg^{-1} aluminum chloride (positive control), Group III rats were administered 200 mg kg^{-1} aluminum chloride and 400 mg kg^{-1} EFTI, Group IV rats were administered 200 mg kg^{-1} aluminum chloride and 800 mg kg^{-1} EFTI, Group V rats were administered 200 mg kg^{-1} aluminum chloride and 300 mg kg^{-1} Vit E (comparative control). On postnatal day 1, the pups were euthanized, and liver tissues were harvested for the biochemical study (tissue levels of malondialdehyde, caspase-3, tumor necrosis factor-alpha, aspartate aminotransferase, alkaline phosphatase, and alanine aminotransferases) and the liver histological examination. The administration of EFTI was marked with significant improvement in the tissue levels of malondialdehyde, caspase-3, tumor necrosis factor-alpha, aspartate aminotransferase, alkaline phosphatase, and alanine aminotransferases. There was a marked improvement in histopathological changes associated with prenatal aluminum chloride exposure. In conclusion, the administration of EFTI was protective during prenatal aluminum chloride exposure of the liver in Wistar rats, and is mediated by the anti-lipid peroxidative, antiapoptotic, and anti-inflammatory activity of EFTI.

1. Introduction

Aluminum is the most abundant metal in the earth's crust and occurs in a trivalent state [1]. Aluminum is released into the environment through anthropogenic activities and weathering, with consequent contamination of water bodies [2]. Humans are particularly exposed to aluminum in occupations related to mining, recycling of metals, and welding [3]. The use of aluminum-containing products in cooking utensils, aluminum foil, and plumbing in homes and aluminum compounds in the treatment of water, production of vaccines, and packaging for drugs increases the risk of possible human exposure [3–5]. The major route for aluminum entry into the body is via oral ingestion, with sequent accumulation in various tissues of the body including the liver [6], [7].

The liver is vulnerable to the effect of aluminum exposure considering its central role in detoxification [8]. Aluminum toxicity in the liver is mediated by possible disruption in the antioxidant defense system [9]. Aluminum toxicity is associated with an increase in lipid peroxidation, with consequent elevation in the levels of aldehydes generated, including malondialdehyde [10]. Elevated level of active caspase-3 and

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tumor necrosis factor-α (TNFα) is also implicated in Al toxicity [11]. Aluminum is associated with an elevated level of liver enzymes (aspartate aminotransferase (AST), alkaline phosphatase (ALP), and alanine aminotransferases (ALT)) [12]. A healthy liver is very important for human survival, hence the need for the present study [13].

Over time, the use of plant-derived agents in the management of various human health conditions has gained a lot of attention [14–20]. *Tamarindus indica* is rich in several phytochemical constituents, such as phenolic compounds, cardiac, malic acid, uronic acid, tartaric acid, pectin, xylose galactose, and glucose [21]. *Tamarindus indica* also contains essential elements like cadmium, arsenic, copper, calcium, iron, manganese, sodium, magnesium, phosphorus, potassium, zinc, and lead [22]. A preliminary phytochemical screening of ethyl acetate fraction of *Tamarindus indica* leaf (EFTI) revealed the presence of carbohydrates and flavonoids [20]. GCMS screening of EFTI revealed the presence of oleic acid, Phenol, 3,5-bis (1,1-dimethyl ethyl), and n-Hexadecanoic acid as major compounds present in the extract [20]. A previous study on the effect of EFTI during prenatal aluminum exposure revealed improvement in GFAP expression and oxidative stress parameters in the hippocampus [23]. Tablets formulation from *Tamarindus indica* leaves possessed anti-lipid peroxidative potential and maintained the redox balance in the experimental rats [24]. The tablets were also associated with an improvement in the levels of liver enzymes [24]. The administration of *Tamarindus indica* ameliorated cadmium-induced kidney and liver damage [25]. The administration of aqueous extract *Tamarindus indica* was hepatoprotective in anti-tuberculosis-induced oxidative liver damage [26]. However, there is no information on the hepatoprotective potential of EFTI during prenatal aluminum chloride exposure despite its rich phytochemical composition, hence the present study aimed to assess the hepatoprotective potential of EFTI following prenatal aluminum exposure in Wistar rat pups.

2. Material and methods

The current submission emanated from the following studies [20,23,27,28], and part of a PhD thesis, hence the similarity in the experimental setting and methodology. The authors were compelled to look at the events in the liver during the study considering the important role the liver play in toxicological process [8].

2.1. Chemical and drug

Aluminum chloride (CAS Number: 7446–70–0) from Sigma-Aldrich (Mumbai, India) and Emzo vitamin E capsules (1000 mg) were purchased from reputable chemical and drug stores. The stock solution of aluminum chloride and vitamin E was prepared as used in previous studies [23,28]; 1 g of aluminum chloride was dissolved in 10 mls of distilled water to prepare aluminum chloride stock solution. On the other hand, vitamin E was mixed with tween 80 to ensure 0.4 ml of the suspension contained 120 mg of vitamin E, then protected against bright light to avoid photodegradation.

2.2. Plant material

Fresh leaves of *Tamarindus indica* were collected within Zaria town, Nigeria; and authenticated (verification number: 2417) in the Herbarium section of the Botany Department, Ahmadu Bello University, Nigeria.

The fresh leaves were rinsed in clean water, shade dried, then grinded using an electric blender for extraction by maceration method, followed by subsequent fractionation as outlined in previous studies [23,28,29]. The Stock solution of EFTI was prepared by dissolution in Tween 80, because EFTI was not soluble in water.

EFTI was chemically characterized and found to contain carbohydrates, polyphenols, and flavonoids. The GCMS screening of EFTI revealed it contained oleic acid, Phenol, 3,5-bis (1,1-dimethyl ethyl), and n-Hexadecanoic acid as major compounds present in the extract [20]. The LD50 of EFTI was determined to be above 3000 mg kg^{-1} using the up and down method (*Unpublished observation*).

2.3. Experimental animal

Twenty (20) adult nonpregnant females Wistar rats and 10 adult male Wistar rats were obtained and housed the departmental animal house. The rats were allowed unrestricted access to food and clean water. The experimental animals were cared for following standard guidelines for the care of experimental animals. Ethical approval was sort and obtained from The Ahmadu Bello University Committee on Animal Use and Care, and registered as ABUCAUC/2019/001.

Vaginal smears were collected from the female rats and studied under the light microscope as indicated in previous study for the determination of the estrous cycle [23,28,29]. Female rats in the proestrus phase were housed overnight with 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 [30–32].

2.4. Study design

Following the ARRIVE guidelines, the pregnant rats were randomly divided into five group, with each group containing 4 rats. Group I rats were administered 2 ml kg^{-1} of distilled water (normal control), Group II rats received only 200 mg kg^{-1} aluminum chloride, Group III rats were administered 200 mg kg^{-1} aluminum chloride and 400 mg kg^{-1} EFTI, Group IV rats were administered 200 mg kg^{-1} aluminum chloride and 800 mg kg^{-1} EFTI, Group V rats were administered 200 mg kg^{-1} aluminum chloride and 300 mg kg^{-1} Vitamin E. A dosage of 200 mg kg^{-1} was adopted for aluminum chloride based on previous studies in Wistar rat model [33], [34]. A dosage of 400 and 800 mg kg^{-1} was adopted for EFTI, while 300 mg kg^{-1} was adopted for vitamin E based on previous studies using the Wistar rat model [23,28].

The administrations of EFTI, aluminum chloride and Vitamin E were via gastric intubation for 14 days [prenatal day (PD) 7–21]. The animals were allowed to litter, on the post-natal day (PoND) 1, pups were euthanized using 5 mg kg^{-1} thiopental sodium intraperitoneally as described by Usman et al., [28]. This ensured the humane sacrifice of the experimental animal. The liver tissues were harvested following midline abdominal incision, then weighed. Liver tissues for the biochemical studies were stored at − 20 °C, while those for the histological studies were fixed in 10% neutral buffered formalin.

2.5. Tissue collection and preparation

Tissue preparation for histological studies was carried out as described in a previous study [29,35]. Sections of the liver tissues were stained using Hematoxylin & Eosin (H and E) [36]. The photomicrographs were taken using a light microscope (Olympus BH2) mounted with a Nikon Digital Sight DS-L1 (Nikon Corporation, Japan). The histological slides were qualitatively interpreted by a blinded independent pathologist since this was a globally accepted histopathological approach to reduce bias.

2.6. Caspase-3, malondialdehyde, and tumor necrosis factor- α assay

Tissue levels of caspase-3, malondialdehyde (MDA), and tumor necrosis factor-alpha (TNF-α) were determined using ELISA kit methods (MyBio Source, San Diego, CA, United States).

2.7. Liver enzyme assay

ALT, AST, and ALP were estimated with the kits obtained from Randox Laboratories Ltd., Ardmore, Crumlin Co., UK; using a semi autoanalyzer (Biosystem, BTS-330, Barcelona, Spain).

2.8. Statistical analysis

The GraphPad Prism® version 5.01 (San Diego, CA) was used for the data analysis. Quantitative variables were analyzed using one-way analysis of variance (ANOVA), followed by a Tukey post hoc test. Differences among the groups were considered significant at $p < 0.05$. The result for the caspase-3, malondialdehyde, tumor necrosis factor - α, and the liver enzyme were presented as bar charts.

3. Result

3.1. Malondialdehyde

The administration of aluminum chloride was marked with significant ($p < 0.05$) elevation in the levels of MDA when compared with the normal control. The administration of 800 mg kg^{-1} EFTI was associated with marked improvements in the mean levels of MDA ($p < 0.05$) when compared to the 200 mg kg^{-1} aluminum chloride treated group (Fig. 1).

3.2. Caspase-3

The administration of aluminum chloride was marked with significant ($p < 0.05$) elevation in the levels of caspase-3 when compared to the normal control group. The administration of 800 mg kg^{-1} EFTI and 300 mg kg^{-1} of Vitamin E were associated with marked improvements in the mean levels of caspase-3 ($p < 0.05$) when compared to the 200 mg kg^{-1} aluminum chloride treated group (Fig. 2).

3.3. Tumor necrosis factor- α

The administration of aluminum chloride was marked with significant elevation in the levels of TNF-α when compared to the normal control group. The administration of 800 mg kg^{-1} EFTI and 300 mg kg^{-1} of Vitamin E were associated with marked improvement in mean levels of TNF-α ($p < 0.05$) when compared to the 200 mg kg^{-1} aluminum chloride treated group (Fig. 3).

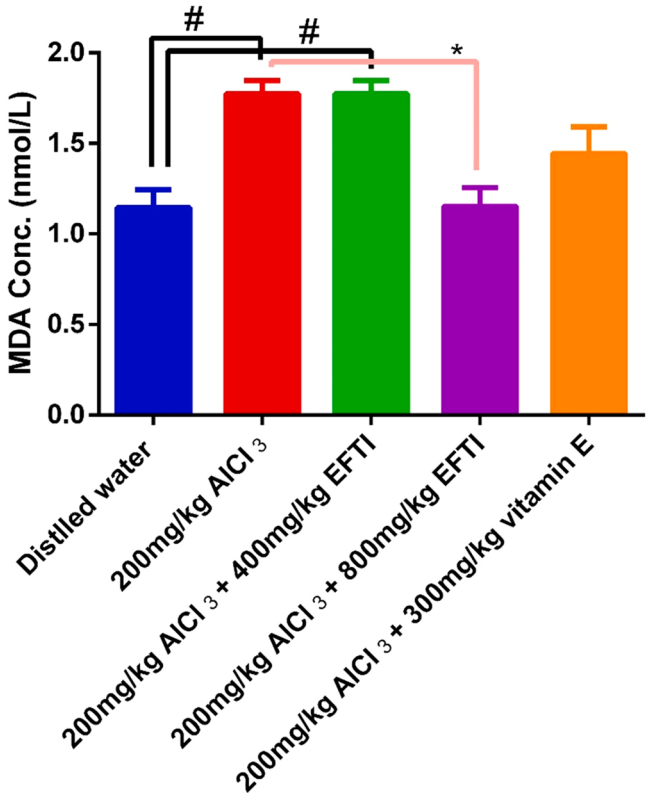

Fig. 1. Result of MDA analysis following treatment with EFTI during prenatal $AlCl_3$ exposure (n = 5). Values are presented as mean ± SEM, # represents significant difference ($p < 0.05$) compared to normal control, * represents significant difference ($p < 0.05$) compared to the 200 mg kg^{-1} treated group.

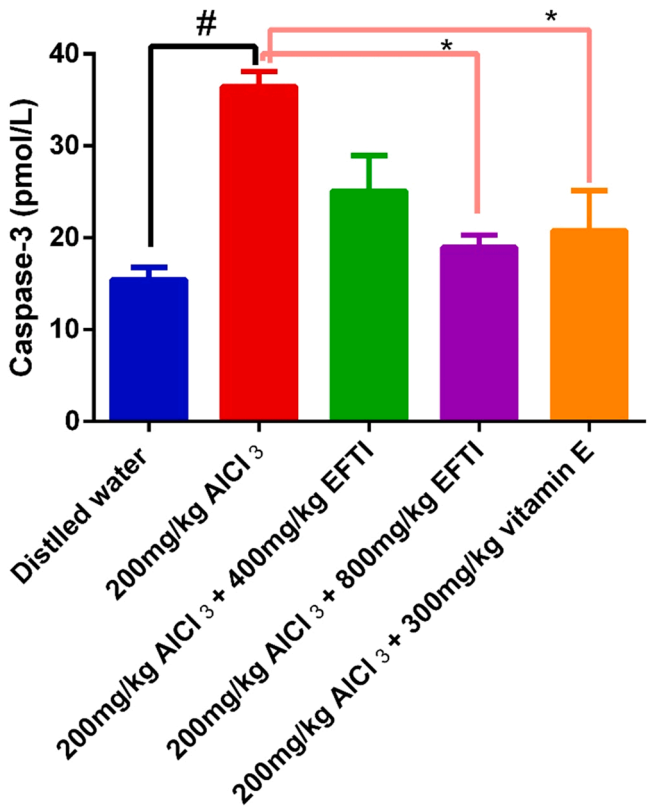

Fig. 2. Result of caspase-3 analysis following treatment with EFTI during prenatal $AlCl_3$ exposure (n = 5). Values are presented as mean ± SEM, # represents significant difference ($p < 0.05$) compared to normal control, * represents significant difference ($p < 0.05$) compared to the 200 mg kg^{-1} treated group.

3.4. Liver Enzyme

The administration of aluminum chloride was m with significant elevation in the levels of AST, ALT, and ALP ($p < 0.05$) when compared to the normal control group. The administration of 800 mg kg^{-1} EFTI and 300 mg kg^{-1} of Vitamin E were associated with marked improvement in the mean levels of AST, ALT, and ALP when compared to the 200 mg kg^{-1} aluminum chloride treated group (Fig. 4A–C).

3.5. Histological and histochemical studies

The histological examination of the liver from Wistar rat neonates on PoND 1 showed relatively normal histo-architecture in Fig. 5A, evidence of pathological changes in Fig. 5B & C, with improvement in the observed histological changes in Fig. 5D & E when compared to the normal control.

4. Discussion

The present study revealed the administration of aluminum chloride was associated with an elevation in the tissue levels of caspase-3, MDA, TNF-α, AST, ALP & ALT, with disruption in the general histoarchitecture of the liver. The administration of 800 mg kg^{-1} EFTI were associated with lowered tissue levels of caspase-3, MDA, TNF-α, AST, ALP, ALT,

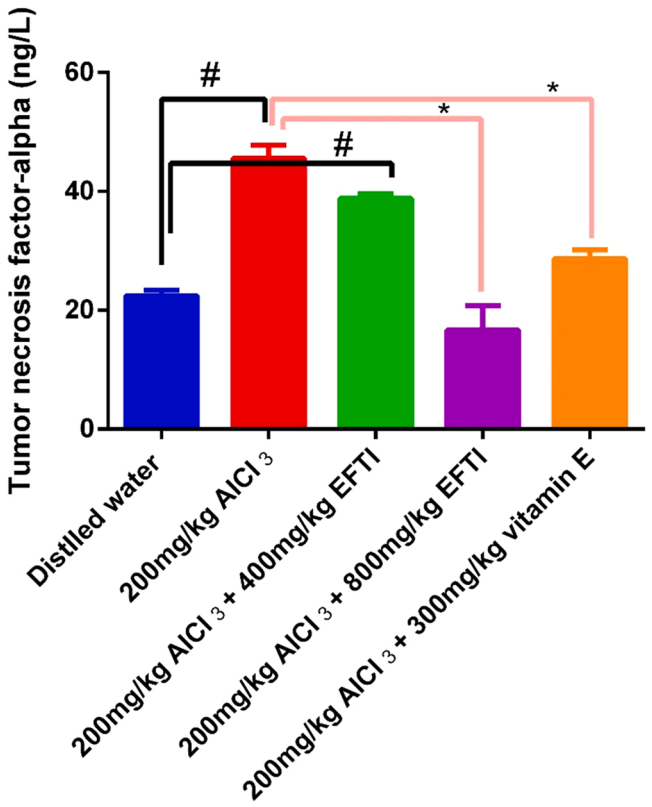

Fig. 3. Result of the analysis of TNF-α level (n = 5) following treatment with EFTI during prenatal $AlCl_3$ exposure (n = 5). Values are presented as mean ± SEM, # represents significant difference ($p < 0.05$) compared to normal control, * represents significant difference ($p < 0.05$) compared to the 200 mg kg^{-1} treated group.

and the general histoarchitecture of the liver.

The observed deleterious effect of aluminum exposure on the liver is in line with the finding of Anane [10] who reported that aluminum exposure is associated with an increase in lipid peroxidation, with consequent elevation in the level generated aldehydes, including MDA. Generation of aldehyde including MDA has some implication in cellular protein synthesis, as they form an adduct with the DNA, which possess cellular programming consequence and cell death since MDA is known to be mutagenic [37].

Generated free radicals sensitize hepatocytes to TNF-α toxicity; hence, the correlation between oxidative stress and inflammation during aluminum exposure [38]. Numerous inflammatory cytokines are often upregulated, such as TNF-α in liver injuries [39]. Following liver injury, TNF-α rapidly moves into the injured tissue to suppress the death of hepatic cells, and promote cellular proliferation [11,40]. The involvement of TNF-α in liver injury results in subsequent fibrosis [11]. Generally, TNF-α mediates cellular apoptosis, metabolism, insulin sensitivity, and vascular functions [41,42]. The observed elevation in the levels of TNF-α following prenatal aluminum exposure in the liver confirms that the negative health impact of aluminum exposure elicits possible inflammatory responses [11].

Caspases play a very important role in apoptotic cell death, among which caspase-3 is the most frequently activated [43,44]. Caspase-3 catalyzes the cleavage of many key cellular proteins [45]. Caspase-3 activation is indispensable in programmed cell death associated with chromatin condensation and DNA fragmentation, therefore very important in the disintegration of cells and consequent formation of apoptotic bodies [46]. Therefore, explaining the observed alteration in the liver histo-architecture in the present study. Free radicals contribute to disturbances in permeability of the mitochondrial membrane and its transition potential [38], resulting in the release of proapoptotic factors including caspase-3 [38].

Ighodaro et al. [12] reported that aluminum exposure is associated with the elevation of liver enzymes (AST, ALP, and ALT). An elevated level of tissue AST, ALP, and ALT are implicated in both acute or chronic injury to the liver [47]. Elevation in tissue levels of AST, ALP, and ALT is usually a result leakage of these enzymes into the bloodstream [47]. AST, ALP, and ALT are very important liver enzymes, and catalyze the transfer of α-amino groups from alanine and aspartate to the α-keto group of ketoglutaric acid, with the resultant generation of pyruvic and oxalacetic acids, which are important contributors to the citric acid cycle [47]. An elevated level of the liver enzyme is often associated with an elevated level of TNF-α with consequent suppression of hepatic cell

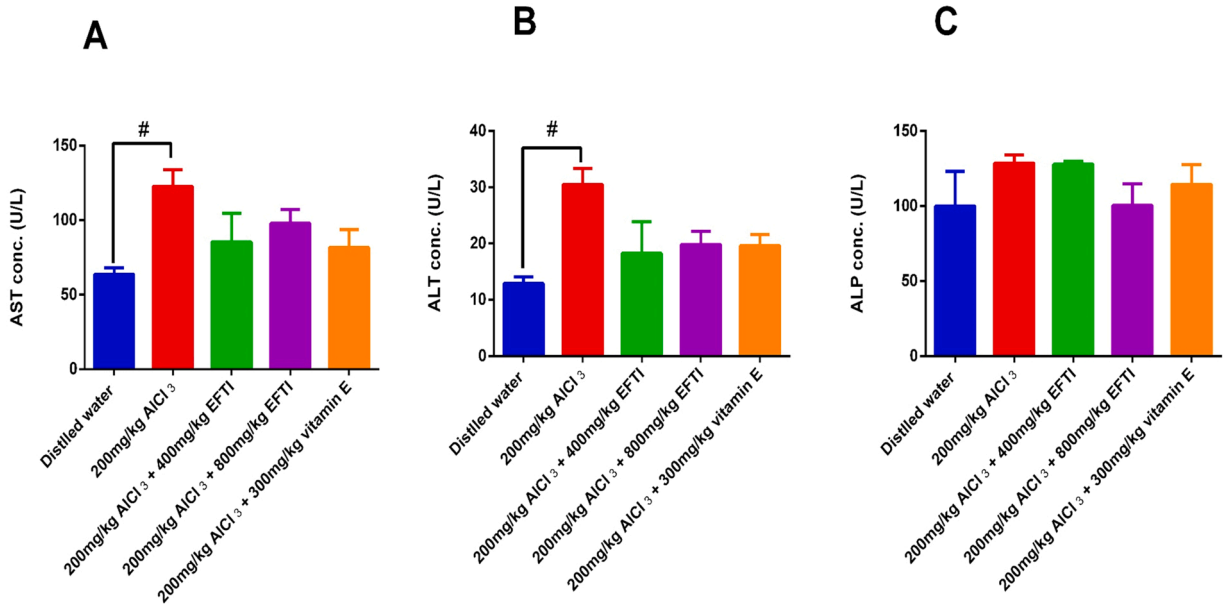

Fig. 4. Result of the analysis of liver enzymes; mean concentration of Alanine amino transaminase (A), Aspartate amino transaminase (B), and Alkaline phosphatase (C) on PoND 1 following administration of EFTI during prenatal $AlCl_3$ exposure. ALT: Alanine amino transaminase, ALP: Alkaline phosphatase, AST: Aspartate amino transaminase; Values are presented as mean ± SEM, # represents significant difference ($p < 0.05$) compared to normal control.

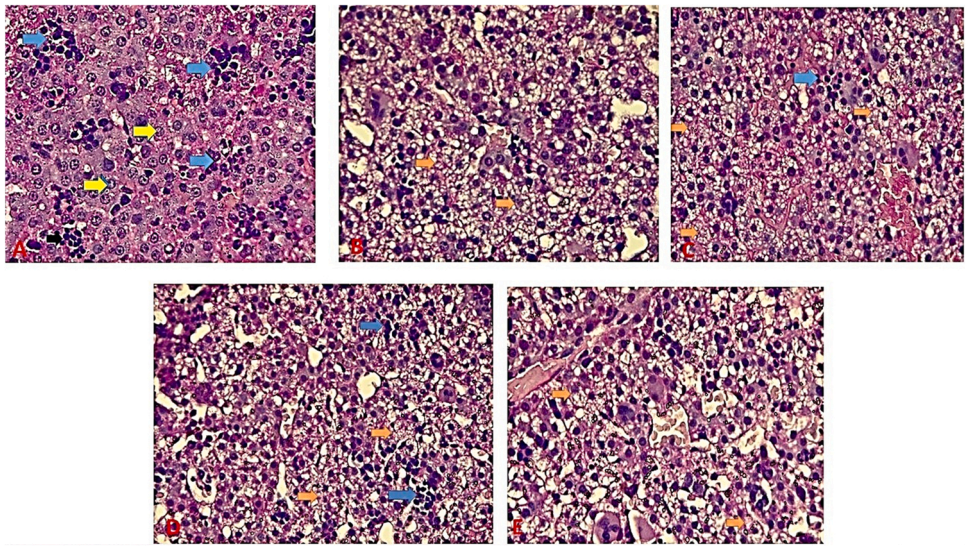

Fig. 5. Liver sections from the different treatment groups on PoND 1, hepatocytes (yellow arrow), aggregation hemopoietic cells (blue arrow), vacuolation (orange arrow) (H and E × 250). Legend: Negative control (distilled water) (3 A), positive control (200 mg kg^{-1} bw of $AlCl_3$) (3B), 200 mg kg^{-1} bw of $AlCl_3$ + 400 mg kg^{-1} bw EFTI (3 C), 200 mg kg^{-1} bw of $AlCl_3$ + 800 mg kg^{-1} bw EFTI (3D), and 200 mg kg^{-1} bw of $AlCl_3$ + 300 mg kg^{-1} bw of Vit E (3E).

proliferation [48].

The observed improvement following treatment with EFTI is in line with the finding of Usman et al., [23] who reported that EFTI was protective following prenatal Aluminum chloride exposure, an effect hinged on the presence of biological active phytochemicals such as flavonoids and polyphenols in EFTI. Other study on EFTI showed it possesses antioxidant potentials [23]. Flavonoids and polyphenols possess extracellular and intracellular antioxidant properties [49]. Flavonoids enhance the activities of endogenous antioxidant enzymes [50], in addition to direct potentiation of free radical scavenging [51]. This process involves the inhibitory initiation of a chain reaction altering/halting the propagation of free radical reaction, through the donation of a hydrogen atom to stabilize free radical substance [52–54]. Consequently, improving the antioxidant defense system, membrane integrity, tissue levels of caspase-3, MDA, and TNF-α even in prenatal aluminum chloride exposure. Lowered lipid peroxidation is associated with a decreased generation of aldehydes including MDA. The decrease in the tissue levels of caspase-3, MDA, and TNF-α suggest possible anti-lipid peroxidative, antiapoptotic, and anti-inflammatory activity of EFTI. An observation that could explain reported decrease in the mean tissue level of AST, ALP, ALT, and improvement in the general histoarchitecture of the liver.

This study though basic offers significant insight into the fact that treatment with EFTI was hepatoprotective, as observed in the reported anti-lipid peroxidative, antiapoptotic, and anti-inflammatory activity of EFTI. However, the author recommends the conduct of studies detailing gene expression, ultra-microscopy, and sterology in order to elucidate the mechanism via which EFTI acts. The authors faced significant resource constrain, therefore limiting the scope of the study which we intend to expand in the next phase of the work.

5. Conclusion

The administration of EFTI and Vitamin E were protective during prenatal aluminum exposure of the liver in Wistar rats. The antiapoptotic, anti-lipid peroxidative and anti-inflammatory activity of the EFTI may have mediated the observed improvement.

Ethical statement

The animal study was reviewed and approved by The Ahmadu Bello University Committee on Animal Use and Care, and registered as ABU-CAUC/2019/001.

CRediT authorship contribution statement

HRY, SAM, ANA, and IMU: conception or design of the work; HRY, SAM, ANA, EDE, AAO, IO, TP, VA, MEFD, JJO, NK, BYS, and IMU: the acquisition, analysis, or interpretation of data for the work; HRY, SAM, IMU: Drafting the work; HRY, SAM, ANA, EDE, AAO, IO, TP, VA, MEFD, JJO, NK, BYS, and IMU: revising it critically for important intellectual content; HRY, SAM, ANA, EDE, AAO, IO, TP, VA, MEFD, JJO, NK, BYS, and IMU: Final approval of the version to be published; All the authors agreed to be accountable for all aspects of the work.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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