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BIOCHEMICAL AND HISTOMORPHOLOGICAL EVALUATION OF *AGERATUM CONYZOIDES* ON ALUMINIUM CHLORIDE INDUCED HEPATOTOXICITY IN A RAT MODEL

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ABSTRACT

Aluminium is a hazardous heavy metal in the environment and accumulates intissues of humans and may cause health problems. *Ageratum conyzoides* is an herb used in traditional medicine in several countries of the world. This study evaluated the protective potential of *Ageratum convzoides* on oxidative stress mediated hepatoxicity following aluminium chloride administration. Twelve (n=12) male albino rats were assigned randomly into 3 groups of 4 animals each. Group 1 served as negative control and received pelleted growers feed and distilled water *ad libitum*. Group 2 served as positive control and was induced with 100mg/kg body wt. of aluminium chloride ($AlCl_3$). Group 3 received 100mg/kg body wt. of aluminium chloride and 100mg/kg body wt. of *Ageratum convzoide*s extract as treatment. All treatments were given orally and lasted for a period of fourteen (14) days. The results showed that MDA and ALT concentration increased significantly ($p<0.05$) in group 2 from 385.53±0.69 to 399.49±1.47 and 42.47±0.43 to 61.45±0.71 respectively. However, GST, SOD, body weight, liver weight and liver to body weightratio decreased from 4.80±0.26 to 2.57±0.14, from 5.87±0.14 to 7.78±0.28, 191.23±1.67 to 165.80±3.93, from 9.92±0.54 to 6.88±0.17 and from 0.05±0.32 to 0.04±0.04 respectively in group 2. In group 3, the elevated levels of MDA and ALT significantly reduced to normal whereas the decreased level of GST, SOD, body weight, liver weight and liver to body weight ratio was restored back to normal when compared to the control. The histomorphological studies revealed aluminium chloride induced hepato-degenerative changes which were ameliorated by*Ageratum conyzoides*. The results indicate that *Ageratum conyzoides* possesses hepatoprotective properties and attenuates the hepatotoxic effects of aluminium chloride through its antioxidant and membrane stabilizing effects.

KEYWORDS: Oxidative stress, hepatotoxicity, Aluminium chloride, *Ageratum conyzoides.*

1. INTRODUCTION

The use of herbs and plants in the treatment of diseases (traditional medicine) is gaining popularity in research and progressively changing the paradigm away from synthetic drugs towards natural products of herbs and plants with prophylactic and curative potentials.[1] In recent times, ethnomedical research is acknowledged as the most effective means of discovering novel therapeutic plants or refocusing on those that have already been reported for their therapeutic potentials based on their bioactive components. Traditional herbal remedies are used to heal illnesses/diseases and are made from naturally existing plant materials with little to no industrial processing. Traditional herbal therapy has recently received the most attention in global health.[2,3]

Scientific evidence has proven the anti-inflammatory, antioxidant, gastroprotective, anti-microbial, herbicidal, hepatoprotective, analgesic, antifungal, hypoglycemic, and antihyperglycemic properties of *Ageratum conyzoides*.[4] *Ageratum conyzoides*(AGC)is an annual herbaceous plant with many secondary metabolites and has been used traditionally for medicinal purposes in many different parts of the world. Phytochemical screening shows that flavonoids, alkaloids, chromene, terpenoids, coumarins, and sterols have all been discovered as metabolites in AGC.[5,6] Local farmers in East Africa use it as an astringent/haemostatic medication for healing wounds.[7] Also, AGC is used as a bacteriocide, antidysenteric, and antihelminthic by traditional communities in India, Asia, South America, and Africa. There is no literature on the potential

negative effects of AGC on the human body.[8] It is mostly widespread in tropical America, mainly Brazil, and all over Africa. In Nigeria, the Igedes (middle belt), Yorubas, and Igbos give it several tribal names, such as "ufuopioko,""imiesu," and "nriewu," respectively.[1]

The liver is the largest internal organ of the human body and plays a major role in detoxification of harmfulmaterials, regulation of numerous metabolic functions and maintenance of body homeostasis. The liver could be damaged by excessive exposure of an individual to toxic compounds like aluminium chloride.[9,10]

Hazardous heavy metals including aluminum, cadmium, lead, and mercury build up in the tissues of humans, plants, and animals because they are natural components of the ecosystem.[11] Aluminum is the third most common element and is found everywhere. Aluminium compounds are used in the pharmaceutical industry to formulate medications such as antacids, vaccines, aspirin and phosphate binders. Additionally, it is used as a food additive as well as water purifier and found in toothpaste and antiperspirant.[12] A small amount of aluminum enters the human body through the skin, but the majority is ingested through food, water and drugs.[13,14] It has the ability to build up in all mammalian tissues, including the heart, liver, kidneys, blood, bones, and brain.[15] Hepatotoxicity, neurotoxicity (Alzheimers disease, sclerosis, encephalopathy), genotoxicity, blood disorder (microcytic hypochromic anemia), and male and female reproductive defects could occur due to aluminum intoxication.[16,14,12] According to studies, oxidative stress, which can cause tissue damage, production of reactive oxygen species (ROS), and DNA oxidative degradation, is a factor in aluminum toxicity.[17,18]

Although, humans are daily exposed to aluminium from drinking water, foods and drugs which is a potential danger, there has been no known remedy to mitigate the harmful effects of aluminium.[9] Based on some documented research evidence on the therapeutic properties of AGC, the current study aims to investigate the ameliorative effects of ethanolic extract of *Ageratum conyzoides*on aluminium chloride($AIC1_3$)induced hepatotoxicity in a rat model.

2. MATERIAL AND METHODS

2.1 Chemicals/Reagents

Ethanol (EmerekDartaatm W.46.07), Aluminum chloride ($AlCl_3$), (BDH chemical ltd. England), 30MM hydrogen peroxide, 6M hydrogen tetrasulphate (H_2SO_4) Phosphate buffer, Carbonate buffer $KMNO_4$, NaCl, NaOH, KH_2PO_4, Na_2HPO, HCl, Na_2CO_3, NaHCO3, EDTA (ethylenediaminetetraacetic acid), Adrenaline solution, TCA, DTNB, Chloroform and Formal Saline.

2.2 Preparation of *Ageratum conyzoides*LeafExtracts

Fresh leaves of *Ageratum conyzoides* wereobtained from a farm in Amassoma Town in Southern Ijaw Local Government Area of Bayelsa State and was identified and authenticated by the Pharmacognosy Department, Faculty of Pharmacy, Niger Delta University, and voucher specimen deposited in the herbarium.

The fresh leaves of *Ageratum conyzoides* were rinsed in clean water and shade-dried at room temperature. Afterwhich, the dried leaves were pulverized into fine powder with a mechanical grinder. 45g of the powdered leaf was dissolved with 350ml of 75% ethanol. The resulting mixture was filtered with the aid of a sterile cheese cloth and the filtrate was subjected to alow but complete solvent evaporation using a water bath at a temperature of 60^0C. The extract was stored in an air tight container, labeled and stored at room temperature.The preparation of *Ageratum conyzoides*leaf extract was according to the method of.[19]

2.3 Experimental Animals

Twelve (12) healthy adult male albino rats with an average weight of 124g to 186g were used for this study. They were procured from the animal house of the Department of Biochemistry, University of Port-Harcourt, Nigeria. The animals were moved to the animal house of the Department of Biochemistry, Niger Delta University, Wilberforce Island, Amassoma and were kept in cages under standard housing conditions (photoperiod: 12h natural light and 12h dark cycle). They were allowed to acclimatized for two (2) weeks and were fed pelletized growers feed and exposed to clean water throughout the period of the study.All procedures were carried out in compliance with the Institutional Animal Ethical Committee's (IAEC) guidelines for the Control and Supervision of Animal Experimentation (CPCSEA).

2.4 Experimental Design

The healthy male albino rats were randomly distributed to three (3) groups, with four (4) rats in each group.

- Group 1 (Control): Receivedpelleted grower's feed and distilled water *ad libitum* for fourteen days (14days).
- Group 11: (Positive Control): Received 100mg/kg body weight of aluminium chloride via orogastric intubation for fourteen days (14) days.
- Group 111: Received 100mg/kg body weight of ethanolic extract of *Ageratum conyzoides* followed by administration of 100mg/kg body weight of aluminium chloride one hour later via orogastric intubation for fourteen days (14) days.

2.5 Sample Collection

At the end of the experimental period, the animals in all groups were sacrificed under chloroform inhalation. 5ml of blood was collected from each animal by cardiac puncture using sterile needle and syringe. Part of the blood was dispensed into test tubes and allowed to clot for 30 minutes before centrifuging at 800g for 10 minutes, The supernatant was used for the biochemical analysis. Thereafter, the rats were dissected and the liver was excised and cleaned in normal saline, and part of it

was placed in a sample bottle containing 10% formalin for histological examination. After being weighed, 10 grams of liver tissue were homogenized in 0.1 M Tris buffer (pH 7.4) for 10 minutes at 3000 rpm. Malondialdehyde levels, catalase and superoxide dismutase activities were all measured using this homogenized liver solution.

2.6 Estimation of Biochemical Parameters

The homogenized liver and serum were used for biochemical estimations: superoxide dismutase (SOD) activity, malondialdehyde (MDA), and Alanine Transaminase (ALT/SGPT) were measured by standard spectrophotometric methods. Superoxide dismutase (SOD) and ALT levels in the blood were measured using test kits made by Randox Diagnostics in the UK. With the aid of analytical grade reagents, serum MDA was calculated.

2.7 Estimation of Superoxide Dismutase (SOD)

Misra and Fridovich's,[20] method was adopted for the measurement of superoxide dismutase (SOD) activity. Principle: It is based on the ability of the superoxide dismutase to inhibit the autoxidation of epinephrine at pH 10.2. Procedure: The spectrophotometer was zeroed using a blank made of 3.0 ml of distilled water after being set at 420 nm. 0.2ml of distilled water was dispensed to the reference tube, and 0.2ml of the proper enzyme extracts were introduced to the proper labeled test tube. 2.5ml of the phosphate buffer was dispensed into each of these tubes, and after equilibrating at room temperature, 0.3ml of the 0.3mM adrenaline solution was dispensed to the reference and each of the test solutions. This was then allowed to mix, and the absorbance at 420 nm was measured.

2.8 Determination of Malondialdehyde

Lipid peroxidation product was measured as an index of MDA formation by Shah and Walker's,[21] method. Principle: Malondialdehyde was determined as a conjugate with TBA. TCA was used to precipitate proteins, which were subsequently centrifuged to be eliminated. At 534 nm, the MDA-TBA complex was measured. Procedure: Two test tubes were labeled as test and blank, respectively. 1ml of the test solution was dispensed into the tube labeled test and 1ml of distilled water into the tube labeled blank. Reagents 1, 2, and 3 were dispensed in 1 ml volume to the test and the blank, respectively, and mixed. The tubes were incubated for 15 minutes in a boiling bath, followed by 20 minutes of cooling at room temperature. Then, the test tubes were centrifuged at 2000 rpm for 15 minutes and the supernatant layer was read at 534 nm. The concentration of MDA (nmol/ml) was calculated by using the following formula: Concentration of the test= Abs (test) –Abs (blank) / 1.56 x 1000000.

2.9 Alanine Transaminase (ALT/SGPT)

The enzyme Alanine Aminotransferase (ALT/GPT) reacts primarily with L-alanine and L-glutamate, but will also use aminobutyrate, ornithine or aspartate. Principle: In the presence of alanine, pyruvate is formed by the action of ALT. This in turn is converted to lactate by the enzyme lactate dehydrogenase (LDH) in the presence of NADH, which is simultaneously converted to NAD and this is monitored at 340nm. As the action of LDH is inhibited by increasing lactate concentration which actually favours the reverse reaction and formation of pyruvate, the assay is monitored by following the initial rate of NAD formation over 3 minutes.

2-9 Histopathological Analysis

Routine tissue processing was carried out using automatic tissue processor; Histokinette (LEICA TP 1020). The liver tissues were embedded in paraffin wax in tissue embedder (LE1CA EG 1160) and trimmed in a rotary microtome (LEICA P.M 2125 RTS) at 20 microns and sectioned at 5 microns thickness. The sectioned tissues were attached to slides and subsequently dewaxed in xylene and stained in haematoxylin and eosin using the method of,[22] for general tissue architecture. The stained slides were then examined using compound light microscope at X400 magnification and photomicrographs were produced.

2.10Statistical Analysis

The mean and standard deviation for every piece of data were shown. The SPSS Software, version 23.0, was used to analyze the data that had been collected. The findings between the control group and the test group were compared using one-way ANOVA, and the group means were compared using Bonferroni multiple comparison. To determine the level of significance, a probability level of $p< 0.05$ was utilized. A probability level of $p< 0.05$ was used to assess the degree of significance.

RESULTS

Results presented in Table 1 reveals that rats treated with 100mg/Kg b.w of $AICI_3$ significantly ($P<0.05$) decreased in body weight (165.80±3.93), liver weight (6.88±0.17) and liver to body weight (4.01) ratio in comparison with the control (191.23±1.67;9.92±0.54 and 5.21) respectively. In contrast, the body weight (190.79±1.19), liver weight (9.90±0.56) liver to body weight (5.20)of rats administered $AIC1_3$and treated with 100mg/kg b.w leaf extract of *Ageratum conyzoides* had a significant ($p<0.05$) increase in weight close to normal when compared with the control group (191.23±1.67; 9.92±0.54 and 5.21).Results presented in Table 2 reveals that there was a significant ($p<0.05$) increase in serum ALT level (61.45±0.7) and a significant ($p < 0.05$) decrease in SOD level (5.87±0.14)compared with control (7.77±0.28) following administration of 100mg/kg b.w of aluminium chloride. In contrast, the group that was administered with $AICl_3$and *Ageratum conyzoides*leaf extract had a significant ($p<0.05$) increase in SODlevel (7.78±0.28) and a significant ($P<0.05$) decrease in ALT level (43.47±1.35). The resultshown in table 3 reveal that in the $AICl_3$ treated group, GST level was significantly ($P<0.05$) decreased (2.57±0.14) compared to the control

(4.80±0.26). In contrast, the group treated with $AIC1_3$and *Ageratum conyzoides* leaf extract had a significant ($p<0.05$) increase (4.7±0.26) close to normal. On the other hand, MDA level was significantly ($P<0.05$) increased (399.49±1.47) on $AICl_3$ treatedgroup compared to the control (385.53±0.69). In contrast, the group treated with $AIC1_3$and *Ageratum convzoides*leaf extract had a significant ($p<0.05$) decrease (386.53±0.69) close to normal ((385.53±0.69). Histology of animals induced with aluminium chloride shows degeneration of the central vein (CV)with areas of necrosis, occluded sinusoidal space (S) and ballooning of hepatocytes (Figure 2) While the group that received aluminium and *Ageratum convzoides*showsa normal central vein (CV), polygonal shape hepatocytes(H) and sinusoids(S) consistent with normal histology of the liver (Figure 3)

Table 1: Effect of $AICI_3$; and *Ageratum conyzoides* on Body weight, Liver weight and Liver to Body Weight ratio.

Treatment	Body Weight(g)	Liver Weight(g)	Liver Weight/Body weight x 100 (%)
Control (distilled water)	191 .23±1 .67[a]	9.92±0.54a	5.21[a]
$AIC1_3$ 100mg/kg b.wt.	165.80±3.93[b]	6.88±0.17[b]	4.01[b]
Ageratum conyzoides (100m/kg)+$AlC1_3$	190.79±1.19[a]	9.90±0.56[a]	5.20[a]

Values are presented as mean±SD (n = 4). Values with the same superscript are not statistically significant ($p<0.05$) at 95% confidence level.

Table 2: Effect of Ageratum conyzoides on liver marker enzymes.

Treatment	ALT	SOD (unit/mgprotein)
Control distilled water)	42.47±0.431	7.77±0.28[a]
$AICl_3$ 100mg/kg	61.45±0.7	5.87±0.14[b]
Ageratum conyzoides (100mg/kg) + $AlCl_3$	43.47±1.35[a]	7.78±0.28[a]

Values are presented as mean±SD (n = 4). Values with the same superscript are not statistically significant ($p<0.05$) at 95% confidence level.

Table 3. Effect of*Ageratum convzoides* on liver antioxidants.

Treatment	GST (unit/mg protein)	MDA (unit/mg protein)
Control (distilled water)	4.80±0.26[a]	385.53±0.69[a]
$AICl_3$ 100mg/kg	2.57±0.14[b]	399.49±1.47[b]
Ageratumconyzoides 100m 1k +AlC13	4.7±0.26[a]	386.53±0.69[a]

Values are presented as mean+SD (n = 4). Values with the same superscript are not statistically significant ($p<0.05$) at 95% confidence level.

Histology Photomicrograph

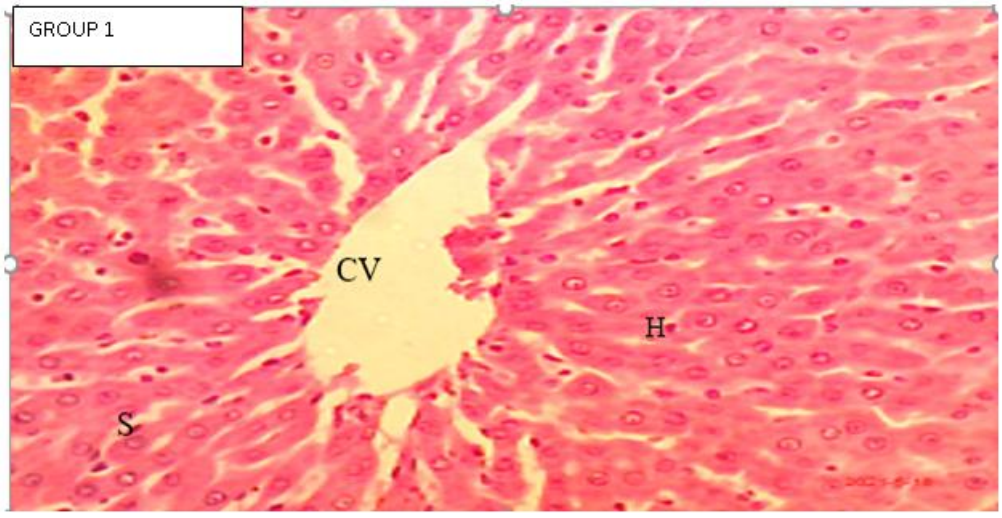

Fig 1: Photomicrograph of liver of rat (negative control) showing normal central vein (CV),polygonal shape hepatocytes (H) and normal sinusoids (S). The section is consistent with normal histology of the liver. (X400; H&E).

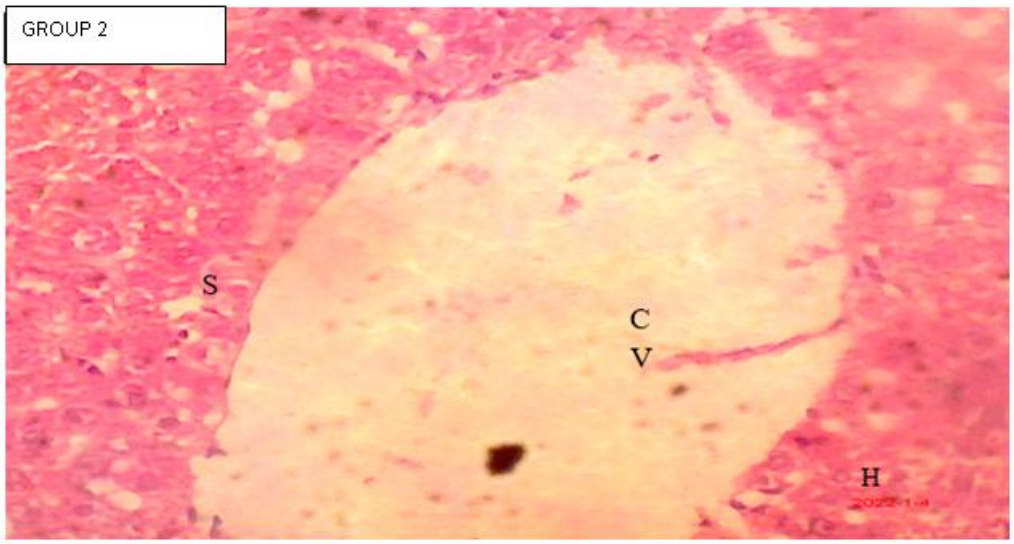

Fig. 2: Photomicrograph of liver of rat administered with100mg/kg per body weight (b.w.) of aluminium chloride showing degeneration of thecentral vein (CV)with areas of necrosis, occluded sinusoidal space (S) and ballooning of hepatocytes(H)(X400; H&E).

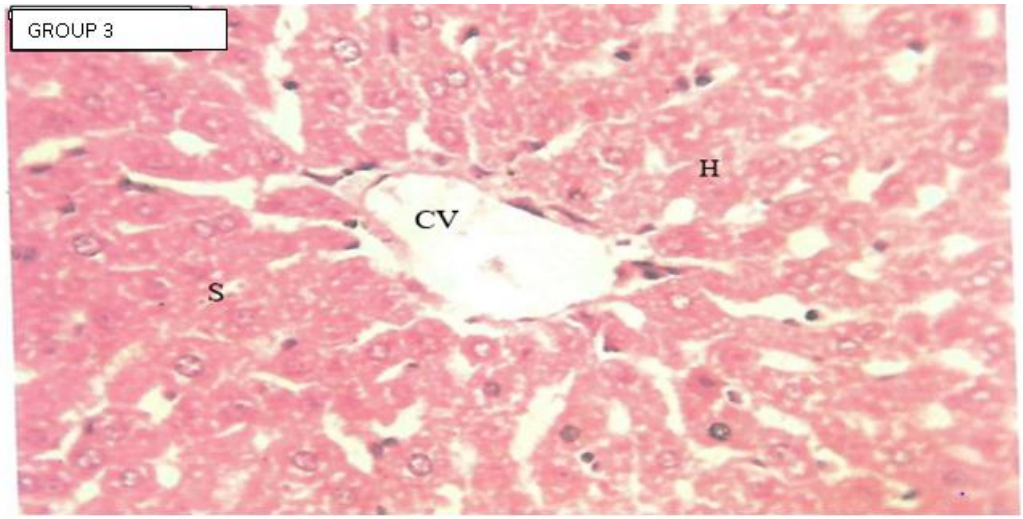

Figure 3: Photomicrograph of liver of rats treated with 100mg/kg b.w of $AICl_3$ + *Ageratum conyzoides*showing normal section of the liver with a central vein(CV), polygonal shaped hepatocytes(H)and normal sinusoids. (S) The section is consistent with normal histology of the liver(X400; H&E).

DISCUSSION

Aluminium toxicity in tissues of humans and other biotic life is a public health challenge because of its bioavailability in the environment.[12,13] As a result, the need to identify a potent pharmacological formulation to mitigate the life-threatening effects of $AlCl_3$ via oxidative stress has become imperative. This study is aimed at evaluating the effect of *Ageratum conyzoides* on aluminium chloride induced hepatotoxicity in albino rats.

Studies have associated aluminium chloride intoxication with lysosomal damage, hepatopathy, periportal inflammation and cholestasis of the liver.[17] One mechanisms of aluminium chloride induced hepatotoxicity is via reduced hepatic antioxidant function. Hepatocytes cell membranes are damaged by free radicals with a resultant increase in liver enzymes activities. As a result, liver enzymes which are normally found inside cell cytosols migrates into the blood circulation. Thus, the degree of liver damage is directly proportional to the increase in the activity of these enzymes.[33,23,12]

This study observed a significant increase in serum ALT level which is a marker of liver damage or injury and a significant decrease in SOD level following the administration of aluminium chloride. This observation is in agreement with previous studies by.[33,24,25,26] Superoxide Dismutase (SOD) is a key defense against the toxicity of the superoxide radical because it catalyzes the dismutation of the superoxide radical into hydrogen peroxide (H_20_2) and elemental oxygen. Superoxide Dismutase (SOD) overexpression prevents apoptosis and

enhances cell development in murine fibrosarcoma cells.[27]

The study also observed that body weights, liver weight and liver/body weights of the rats significantly (P<0.05) decreased following the administration of aluminium chloride. This is consistent with the findings of.[28,29,30] who also noted a decrease in the body and organ weights of rats attributable to the ability of aluminium to inhibit digestive activities leading to loss of appetite due to altered biosynthesis of serotonin and dopamine. However, the rats treated with aluminum chloride and *Ageratum conyzoides* demonstrated significant (p<0.05) increase in body and organ weight close to normal compared with the control (Table 1). This may be due to the antioxidant properties of *Ageratum conyzoides*; because reduced hepatic antioxidant function has also been suggested as one of the mechanism of aluminium chloride induced hepatotoxicity.Aluminum toxicity might be related to oxidative stress.[14]

Hepatocellular damages are identified by increase in serum ALT and MDA levels, and a significant (P<0.05) decrease in SOD and GST levels; in that these enzymes and antioxidants are in the cytoplasm and after cellular damage; they enter the blood circulation.[31,33] This study also observed a significant decrease in serum GST level and an increase in MDA level after the administration of aluminium chloride. Increase in the MDA and decrease in Glutathione- S transferase (GST) level may suggest decrease in the synthetic function of the liver cell following administration of aluminium chloride. However, the damage caused by aluminium chloride was ameliorated with the administration of *Ageratum conyzoides* as the serum levels of GST and MDA were restored to their normal values.

Histomorphological studies reveals that histology of animals induced with aluminium chloride manifested with degeneration of the central vein with areas of necrosis, occluded sinusoidal spaceand ballooning of hepatocytes. While the group that received aluminium and *Ageratum convzoides*showsa normal centralvein, polygonal shaped hepatocytes and sinusoidsconsistent with normal histology of the liver. Exposure to aluminium chloride may cause an accumulation of this metal in the liver and cause alterations of the hepatic function. Increased permeability of cell membranes can result from hepatocyte damage-induced degeneration, inflammation, and necrosis.[23] This study is in agreement with other research which observed that aluminum chloride causes a significant increase in alkaline phosphatase, AST, ALT and lactate dehydrogenase, and causes sinusoidal dilatation, congestion of central vein, lipid accumulation and lymphocyte infiltration in the liver.[25]

Ageratum conyzoides have been reported to have various phytochemicals responsible for its medicinal potentials. The observed antioxidant property exhibited in vitro by *Ageratum conyzoides*is due to the presence of some of the afore-mentioned compounds as also demonstrated in other studies.[32] Another study observed that the use of antioxidants ameliorated aluminum-induced hepato-renal toxicity and restored antioxidant system.[35,34,36] Antioxidants combat the process of oxidation by neutralizing free radicals and have the potential to inhibit the oxidant chain reactions and ultimately reconstitute the damaged membrane. Therefore, the hepatoprotective effect of *Ageratum convzoides* extract against aluminium chloride induced hepatotoxicity as observed in this study could be as a result of the antioxidant activity against the free radicals produced by aluminium chloride. The ability of *Ageratum convzoides* to ameliorate the aluminium chloride induced hepatic damage in this study justifies the use of the plant in traditional medicine for liver problems.

CONCLUSION

Aluminium is a known toxicant and is ingested by humans through various means. The result from this study revealed that rats exposed to aluminum chloride resulted in marked alterations in biochemical and histologicalparameters such as malondialdehyde (MDA), superoxide dismustase (SOD) and Glutathione S-transferase (GST).*Ageratum conyzoides* have been reported to have various phytochemicalsresponsible for its medicinal properties. This study revealed that *Ageratum conyzoides* exhibited potential hepatoprotective activity and attenuated the hepatotoxic effects of aluminium chloride; most likely through its antioxidant and membrane stabilizing effects. It restored the liver enzymes/functions and weights of the albino rats.Thus, *Ageratum conyzoides* may be a promising candidate for amelioration of some chemical induced liver pathologies.

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Disclosure of Conflict of Interest

The authors declare that there is no conflict of interest.

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