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The Mitigating Effects of Sour Sop (*Annona Muricata)* Extract and Aloe Vera *(Aloe Barbadenis Miller)* Gel on Aluminium-Induced Hepatotoxicity in Adult Wistar Rats.

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ABSTRACT
Annona muricata and *Aloe barbadenis miller* are multipurpose plants of great economic value, identified to contain different plant phytochemical and biological activities The aim is to study the mitigating effect of *Annona muricata* extract and *aloe barbadenis miller* gel on an aluminum induced hepatotoxicity on the Liver of an adult Wistar rat with the objectives on histology and Liver function enzymes. Twenty-five Wistar rats ranged (100-150g) were used, divided into five groups, with five rats in each group. In the course of this study twenty-five rats (100-150g) were divided into five groups and used, with five rats in each group. Group A: (control): received rats pellet and water. Group B: were given 100mg/kg/d of AlCl3. Group C: received 100mg/kg/d of AlCl3 and 3ml/kg/d of sour-sop. Group D: given 100mg/kg/d of AlCl3 and 3ml/kg/d of aloe vera. Group E: Received 100mg/kg/d of AlCl3 with 3ml/kg/d of sour-sop and 3ml/kg/d of aloe vera. The rats were weighed and sacrificed twenty-four hours after the last administration sedated with diethyl-ether and livers were autopsied. The histological observations revealed loss of normal liver architecture with hepatocyte that attained different shapes and dilated central vein in the aluminium group when compared with control group. However, treatment with *annona muricata* and *aloe barbadenis miller* attenuates the effect of $AlCl_3$. In liver function enzyme test $AlCl_3$ administration caused a significant increase in serum total protein (TP), serum level of serum glutamic pyruvic transaminase (SGPT) and Alkaline phosphatase (ALP). Results showed that $AlCl_3$ caused hepatotoxicity while significant decrease occurred in Serum glutamic pyruvic transaminase and Alkaline phosphatase level in *annona muricata* -treated rats ($p<0.05$). Animals treated with Aluminium along with *annona muricata* as well as *aloe barbadensis miller* showed recovery of liver tissue structure and improved hepatic morphology. Conclusively, the use of *annona muricata* as well as *aloe barbadensis miller* has shown to be effective in mitigating aluminium-induced hepatic toxicity, therefore *annona muricata* and *aloe barbadensis miller* mitigates hepatotoxic effect $AlCl_3$ administration.

Keywords: Liver, *annona muricata*, Hepotoxicity, *aloe barbadenis miller,* Aluminum Chloride.

INTRODUCTION
The liver as the main organ of metabolism is usually implicated by toxic activity mediated by medications, environmental toxicants and xenobiotics[1]. The prevalence of drug-induced hepatotoxicity is increasing world-wide, Although the rate of toxicity is difficult to ascertain due to low statistical data, detection or diagnostic problems and incomplete exposure observation. Previous research revealed that greater than 50 percent of cases has progression of liver diseases ranging from liver steatosis, liver cirrhosis and ultimately liver cancer[2]. Drug-induced liver injury can account for about 10% of All acute hepatitis, 5% of All hospital admissions, and 50% of All acute liver mistakes[3]. Over 75% of instances of idiosyncratic drugs responses lead to liver transplantation or death[3].

Aluminium is a slivery-whitish soft and malleable metal widely used as raw materials for producing several household utensils, window frames, beer kegs and drugs. This is due to its specific characteristics. It has low density, it is non-toxic, has elevated thermal conductivity, great resistance to corrosion, and can be casted, machined and shaped readily. It is non-magnetic as well as non-sparking. It is the second most malleable metal and sixth most ductile. Aluminum (Al) is the third most prevalent element and approximately 8% of total mineral components in the earth's crust[4]. It is widely distributed in the environment and extensively used in daily life, which causes its easy exposure to human beings[5]. It is present in medicines and is Also added to drinking water for purification purposes[6]. Al accumulates in all tissues of the mammals, including kidney, liver, heart, blood, bone and brain[7].

Herbal medicine originates from ancient cultures, including Egyptian, indian and Chinese cultures. It including using medicinal Plants to treat diseases and improves overall health and well-being. Indeed, many pharmaceutical drugs are discovered in crops based on the synthesized adaptations of naturally occurring compounds. Interest in herbal medicine has risen over

the years, Sour-sop *(Annona muricata)* has a long history of usage in herbal medicine in the tropical areas of South and North America.

Sour-sop and Aloe vera herbs have been recognise for its healing, medicinal and cosmetic benefits since the ages. These plants are fame for its curative and ameliorative effects on cancer, ulcer, radiation burns and arthritis. Aloe vera, *(Aloe barbadensis Miller)* a perennial succulent and drought-resistant plant, is well established for its medicinal capacity. Several positive impacts of Aloe vera have been recorded, including immunomodulatory, treatment of wounds and burns, hypoglycaemic, anticancer, gastro-protective, antifungal, and anti-inflammatory [8].

MATERIAL AND METHODS

Chemicals and Reagents: All chemicals used in the course of the study were of pure analytical grade. Aluminium Chloride was obtained from Pascal. The ELIZA kits for hormone profiles were bought from Nums Diagnostic Centre, Suleija. The histological staining was done in Anatomical-pathology Department, Obafemi Awolowo University, Ile-Ife, Osun state- Nigeria.

Preparation and Concentration of Sour Sop Fruit Extract: The extraction of Sour-sop pulp was achieved by sieving to separate the pulp from the seeds. In the production of Sour-sop concentrate,
(i) fruit selection; (ii) fresh water rinse; (iii) hand peeling; (iv)seed removal; (v) pulp scalding (1 min); (vi)cooling; (vii) soluble solids determination: (viii) addition of 0.1% sodium benzoate; (ix) blending (10 min); sieving; (x) sugar addition; (xi) air elimination and pulp concentration; (xii) packing of pulp in container; (xiii) covering; (xiv) cooling; (xv) labelling; and (xvi) Storage (in a refrigerator).

Plant Preparation and Extraction of the Aloe-Vera: Mature, healthy and fresh Aloe vera leaves were harvested from the botanical garden of the School of Agriculture and Agricultural Technology, Federal University of Technology, Akure. The leaves were washed with fresh water and the thick epidermis carefully removed. The mucilaginous gel was then homogenized with an electric blender. The homogenate was concentrated by filtration using Whatman paper 2. The thickened concentrated gel and the filtrate were kept at 4°C for use.

Preparation of Aluminium: Two gram (2g) of aluminium was weighed and dissolved in 5ml of distilled water. The aluminium chloride solution was administered 100mg/kg bw of rat.

Breeding of the Animals: Twenty-five adult female Wistar rats with an average weight of 100-200g g. The rats, after procurement, were housed in cages (made from wood, wire gauze and net) under natural light and dark cycles at room temperature in the animal house of the Department of Biochemistry animal house, Federal University of Technology Akure (FUTA). The floor of the cages were made with wood to make it comfortable for the rats and it was covered with sawdust to provide a soft floorfor the rats and to make cleaning of the cage convenient when littered. They were fed with rat pellets purchased from approved stores by Federal University of Technology Akure (FUTA) and water was given *ad libitum.* They were grouped and left to acclimatize for 2 weeks before the study commences.

Experimental Design and Procedure: The total numbers of animals were Twenty five (25). They were grouped into one (1) control, four (4) experimental groups with consideration towards size variation. Using a feeding tube (size-6), distilled water, Aloe Vera gel and Sour-sop extracts were administered to the treated animals respectively for a period of three (3) weeks or twenty and one (21) days.
Group 1 (control): (n-5): Received water and rat pellets only;
Group 2 (n-5): Given 100 mg/kg/d of $AlCl_3$ orally;
Group 3 (n-5): Given 3 ml/kg/d Sour-sop (*Annomia muricata*) fruit extract (SFE) simultaneously with 100 mg/kg/d $AlCl_3$ orally;
Group 4 (n-5): Given 3 ml/kg/d aloe Vera gel simultaneously with 100 mg/kg/d $AlCl_3$ orally;
Group 5 (n-5): Given 3 ml/kg/d of Sour-sop fruit extract and aloe vera gel in parallel with 100 mg/kg/d $AlCl_3$ orally.
All the groups were treated under the same housing conditions for a period of 1 month. The animals were weighed, then the animals were sacrificed under ethyl-ethers anaesthesia. The organs were removed, cleaned then weighed and the organ weight ratio was estimated, and the relative weight of organs was calculated as g/100 g BW.

Animal Sacrifice and Sample Collection: Approximately twenty-four hours after the administration of the last dose, All the overnight-fasted rats of the treatment and control groups were sacrificed under the use of ethyl-ethers. Blood samples was collected from each animal via cardiac puncture. The livers were dissected out, cleaned off from adherent fat and blood clot and was weighed on a digital electronic balance.

Histology Analyses: Tissue specimens were taken from the Ovary of female wistar rats from each of the five groups and were fixed in 10% Paraformaldehyde for 24 hours. Then each specimen was sliced into small slabs (3-5mm thick) and further fixed in a change of the same fixative for another 15 hours. The fixed tissue specimens were trimmed and washed in tap water for 12

hours. An alcohol series (methyl, ethyl, and absolute) was used to dehydrate the tissue specimens. The tissue specimens were cleared in xylene and embedded in paraffin. The paraffin blocks were sectioned in 5-micron slices on a rotary microtome. The obtained tissue sections were collected on glass slides and stained with Hematoxylin and Eosin.

RESULTS

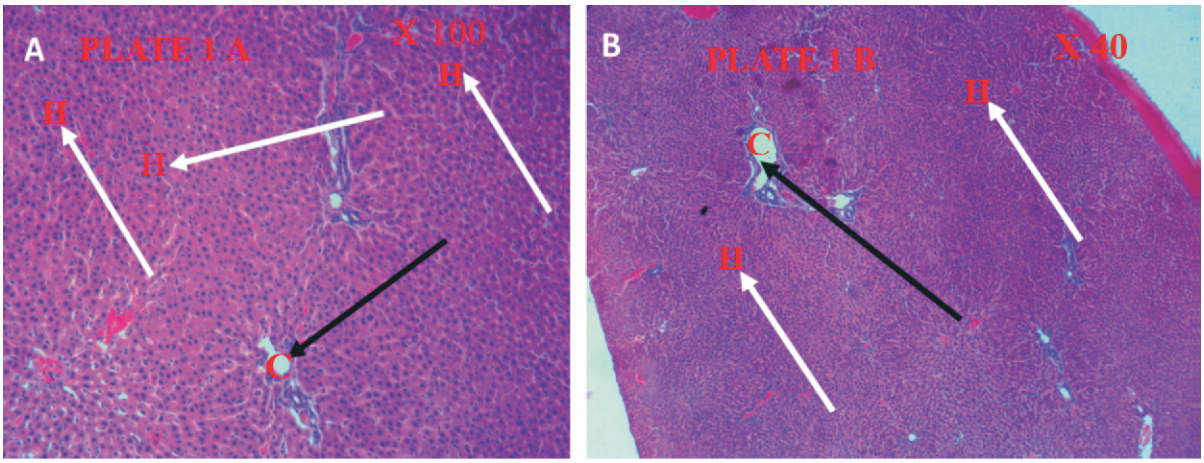

Plate 1A & B: A representative of Photomicrograph of wistar rat liver, group I (control) that receive feed and water showing normal architecture of the liver hepatocytes (BH) and Central vein (C). Stain is H&E and Magnification x100 & x40.

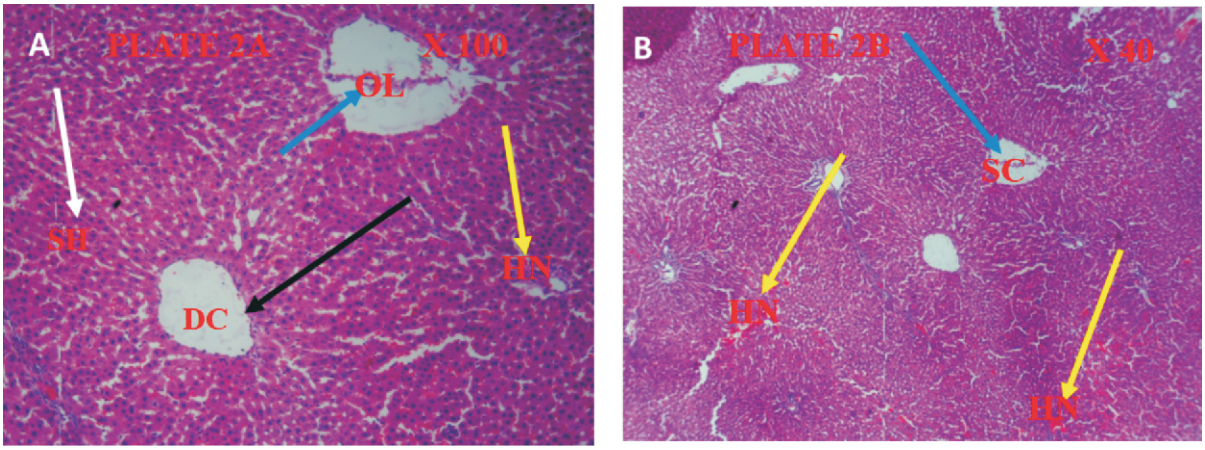

Plate 2A & 2B: A representative of Photomicrograph of wistar rat liver, group II (Aluminium-Chloride administered group 100 mg/kg BW) with abnormal liver morphology showing scattered hepatocytes (SH) (white arrow) and dilated Central vein (DC) (Black arrow) with accentuation of the sinusoidal spaces and portal spaces, (Blue arrow) (OL) shows the central vein indicating occlusion of the lumen and (yellow arrow) (HN) indicting where hepatocytes undergone necrosis. However, Plate 2B shows accentuation of the sinusoidal spaces and portal spaces, (Blue arrow) (SC) shows the central vein indicating stenosis and (yellow arrow) Stain is H&E and Magnification x100 & x40.

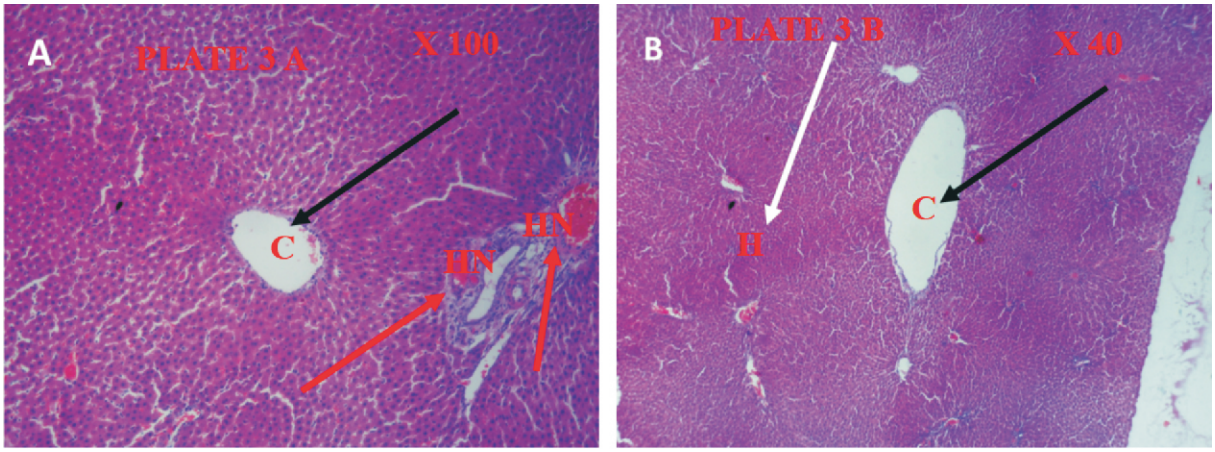

Plate 3A & B: A representative of Photomicrograph of AlCl3 wistar rat liver, group III treated with (*annona muricata)* (3ml/kg BW) showing nearly similar morphology with that of the control group. Hepatocytes (H), Central vein (black arrow) (C) and some Hepatocytes that has undergone necrosis (Red arrow) (HN) due to the induction of AlCl3. Stain is H&E and Magnification x100 & x40.

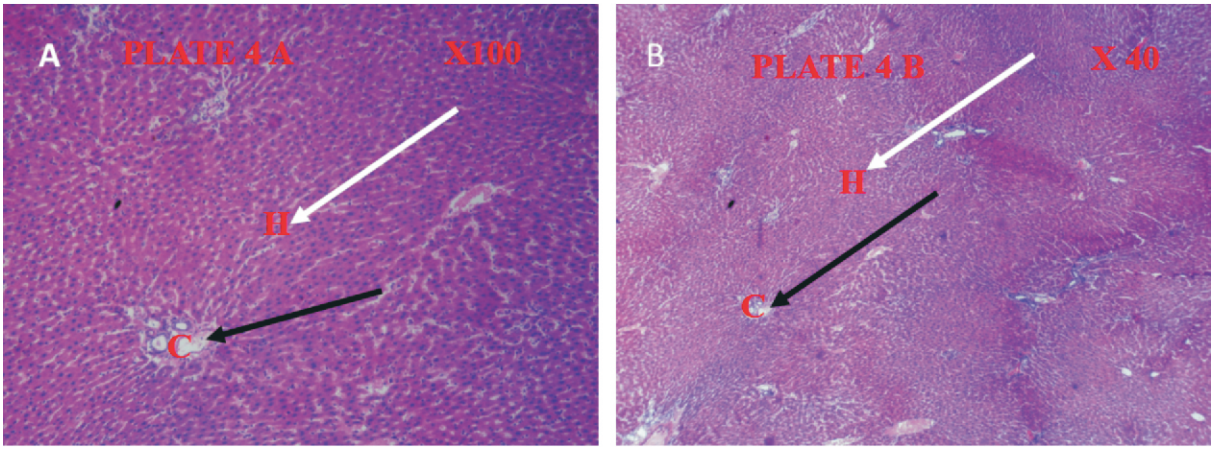

Plate 4A & B: A representative of Photomicrograph of AlCl3 wistar rat liver, group IV treated with (Aloevera *(aloe barbadenis miller)* (3ml/kg BW) showing nearly similar morphology with that of the control group. Hepatocytes (H) (White arrow) and Central vein (C) (Black arrow); Stain is H&E and Magnification x100 & X40.

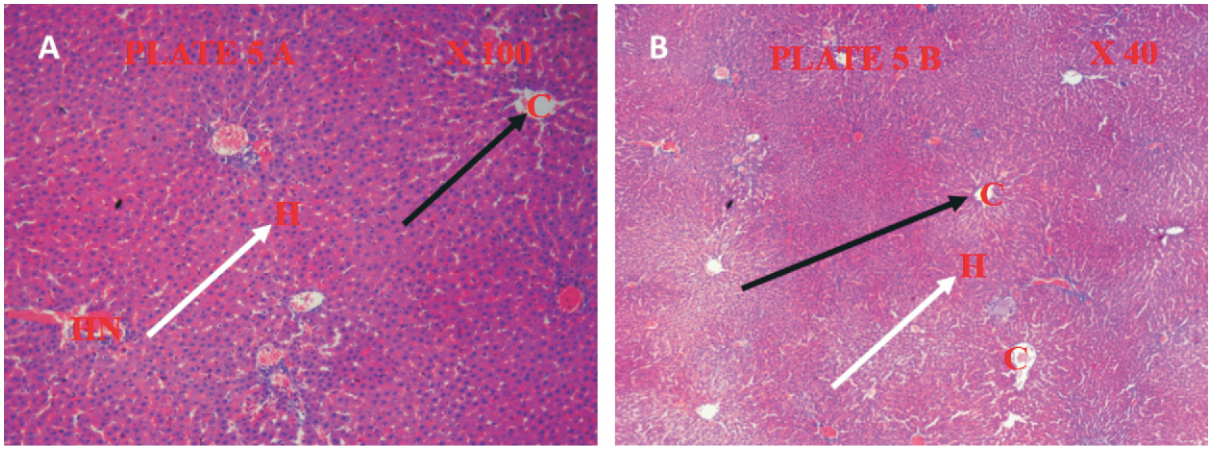

Plate 5A & B: A representative of Photomicrograph of AlCl3 wistar rat liver, group V treated with (Aloevera *(aloe barbadenis miller)* 3ml/kg BW and Sour sop *(annona muricata)* 3ml/kg BW) showing nearly similar morphology with that of the control group. Hepatocytes (H) (white Arrow), (HN) some hepatocyte that has undergone necrosis and Central vein (C) (Black arrow); Stain is H&E and Magnification x100 & x40.

Liver Function Enzyme Tests: Alkaline Phosphatase [ALP] (Male)

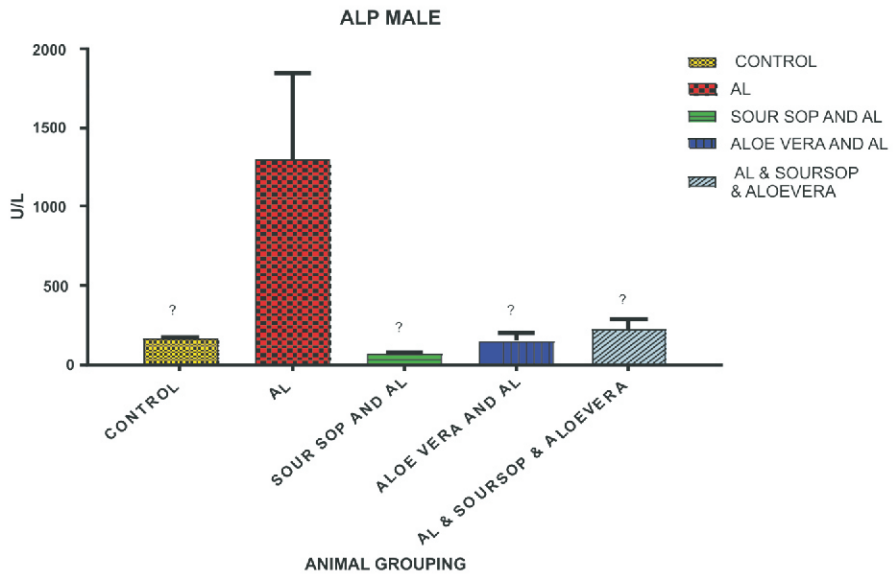

Figure 1A: A Chart showing Alkaline Phosphatase level in experimental male animals. * *AL group was significantly higher than all the other groups at P <0.05. ALP - Alkaline Phosphatase, AL – Aluminum Chloride, Sour Sop - Annona muricata, Aloe Vera - Aloe Barbadenis Miller.*
Data on Alkaline Phosphatase in experimental male Animals expressed in Fig 1A. Alkaline Phosphatase was significantly high at (P<0.05) in ($AlCl_3$) Al group (1296.624 ± 548.789) when compared with other groups.

Alkaline Phosphatase [ALP] (Female)

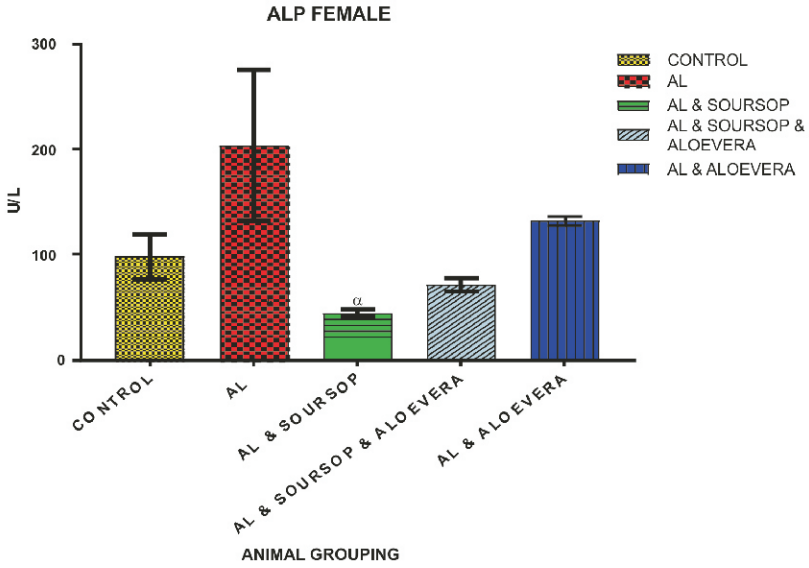

Figure 1B: Chart showing Alkaline Phosphatase level in experimental female animals: *α Al + Sour sop was significantly P <0.05 lower when compared with AL group.*
Data on ALP test in experimental female Animals expressed in Fig 1B. ALP level shows no significantly difference in all groups at (P<0.05) when compared with control group, but AL + Sour sop group (44.00 ± 3.502), was significant lower when compared with AL ($AlCl_3$) (203.500 ± 72.242) group.

Serum Glutamic Pyruvic Transaminase [SGPT] (Male)

Figure 2A: Chart showing SGPT level in experimental male animals*: α* Control group was significantly lower at P <0.05 when compared AL, AL + Soursop and AL + Aloe Vera groups. β AL group was higher when compared with AL + Soursop and AL +Aloe Vera groups. μ AL + Soursop was lower when compared with AL + Soursop and AL + Aloe Vera groups.
Data on SGPT test in experimental male Animals expressed in Fig 2A. SGPT was significantly high at (P<0.05) in ($AlCl_3$) AL group (24.430 ± 0.140), Sour sop + AL ($AlCl_3$) group (20.940 ± 0.173) and Aloe vera + Al group (20.70 ± 0.829) when compared with Control group (13.960 ± 0.149). Also, there was significant difference in all groups when compared with Al group. While Aloe vera + AL group (20.70 ± 0.829) was significantly (P<0.05) higher when compared with AL + Sour sop + Aloe vera group (16.58 ± 0.853)

Serum Glutamic Pyruvic Transaminase [SGPT] (Female)

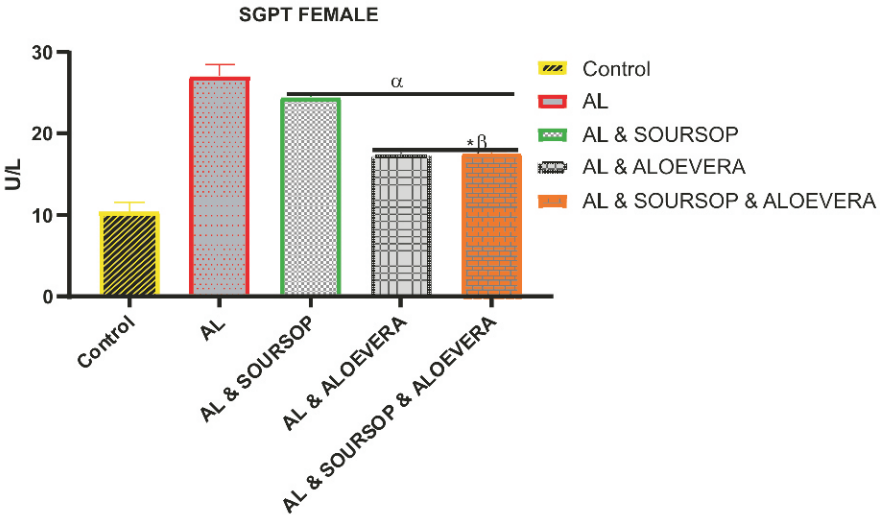

Figure 2B: Chart showing Serum Glutamic Pyruvic Transaminase level in experimental female animals. *α* Control was significantly lower at P <0.05 when compared AL, β AL was higher when compared with AL+ Soursop, AL & Soursop and aloe Vera groups., *AL + Soursop was higher when compared with AL + Aloe Vera and AL + Soursop + Aloe Vera groups.
Data on SGPT test in experimental female Animals expressed in Fig. 2B. SGPT level was significantly high (P<0.05) in all groups when compared with control group (10.47 ± 1.130). Also, there was significant difference in AL + Soursop (24.43 ± 0.266) and AL + Aloe vera group (17.470 ± 0.380) when compared AL group (27.050 ±1.388). AL + Soursop group (24.43 ± 0.266) was significantly high when compared AL + Aloe vera (17.470 ± 0.380) and AL + Soursop + Aloe vera group (17.450 ± 0.396).

Total Protein [TP] (Male)

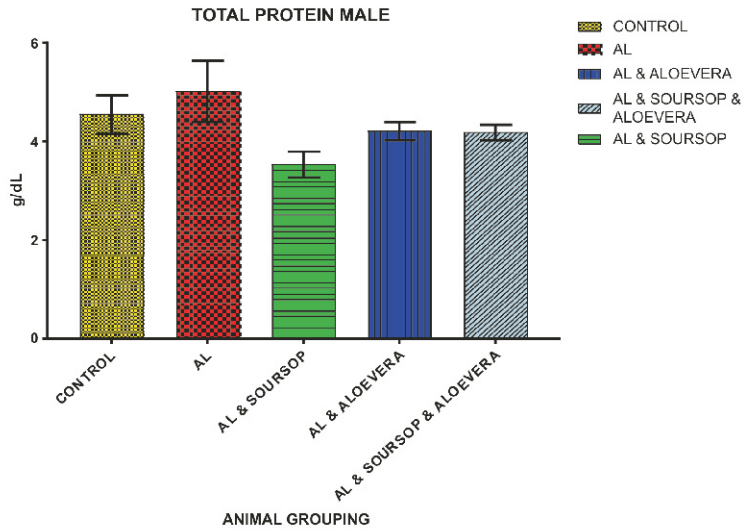

Figure 3A: Chart showing Total Protein level in experimental male animals. *AL was higher than the other groups but not statistically significant.*

Data on TP test in experimental male Animals expressed in Fig 3A. In Total protein test there is no significant level at ($P<0.05$) in AL group (5.01 ± 0.268), in control group (4.550 ± 0.391), in AL & Soursop (3.53 ± 0.627), AL+ Aloe vera (4.210 ± 0.184) and AL + Soursop + Aloe vera group (4.185 ± 0.262). The AL group shows an increased TP level compared to the control group but the difference is not statistically significant, AL + Soursop + Aloe vera group shows a slightly decreased difference TP level compared to the control group but the difference is not statistically significant, AL + Sour sop shows no significant difference of Total protein level compared to the control group same Also AL + Aloe vera compared to control group but not statistically significant.

Total Protein [TP] (Female)

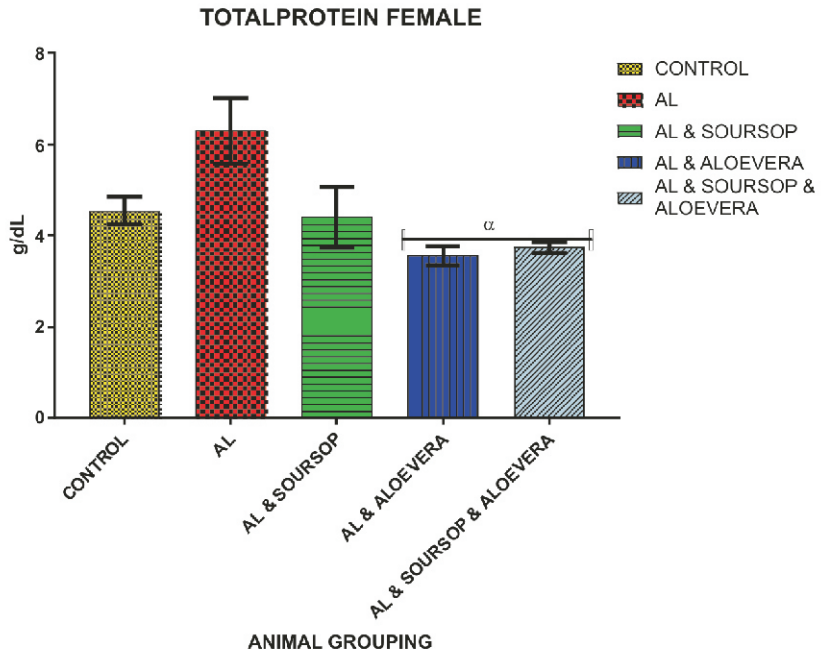

Figure 3B: *Chart showing Total Protein level in experimental female animals: α AL group was significantly (at $P<0.05$) higher when compared with AL + aloe Vera group and AL + Soursop + aloe Vera group.*

Data on TP test in experimental female Animals expressed in Fig 3B. In Total protein test AL group (6.300 ± 0.711) was significantly (at $P<0.05$) higher when compared with *AL + aloe Vera group* (3.560 ± 0.215) and *AL + Soursop + aloe Vera group* (3.745 ± 0.121). AL group (6.300 ± 0.711) was higher than control group (4.550 ± 0.313) and AL + Soursop group (4.400 ± 0.675) but not statistically significant.

DISCUSSION

The present study was carried out to investigate the mitigating effect soursop *(annona muricata)* and Aloe vera *(aloe barbadenis miller)* on Aluminium induced hepatotoxicity in adult wistar rats. Aluminum exposure usually result in the build-up of aluminium compound within the liver tissue thereby causing hepatic toxicity due to its high concentration[9]. The liver, which is the primary site for the biotransformation of foreign compounds, is especially vulnerable to chemical assaults. It performs high activity in metabolism and has a chief role in the detoxification process and withdrawal of many toxic substances which enter the body[10]. Various enzymes are usually susceptible to several environmental toxicants, insecticide and drug toxicity thereby affecting the metabolic function of the liver[11].

In most cases these enzymes leak out from the necrotic hepatocytes into the blood stream in abnormal amounts thereby causing malfunction of the liver and damage to the liver tissue [11]. Changes in serum glutamic pyruvic transaminase, alkaline phosphatase activities could be expected to occur in association with a pathology involving the toxicity of the liver [12]. In this study *Annona muricata* and *Aloe barbadenis miller* inhibited the leakage of liver maker enzymes into circulation and, therefore limits the damages caused by aluminium toxicity.

Alkaline phosphatase concentration level (ALP) in male wistar rat was significantly high in aluminium group when compared with control group ditto with the rats treated with *Annona muricata*, *Aloe barbadenis miller* and rats treated with both *Annona muricata* and *Aloe barbadenis miller* this is related to Newsome *et al*[12].

Alkaline phosphatase (ALP) in female wistar rats shew no significant difference in all groups when compared with control group, but extract of aluminium with *Annona muricata* was significant lower when compared with aluminium group.

In male wistar rats Serum glutamic pyruvic transaminase concentration level was high in rats treated with aluminium when compared with the control group and the rat treated with treated with *Annona muricata* and *Aloe barbadenis miller* rat treated there were decrease.

In female wistar rats Serum glutamic pyruvic transaminase level was significantly increased in rats treated with aluminium compound when compared with the control group. The groups treated with aluminium with *Annona muricata* and *Aloe barbadenis miller* in different combination this reaveled visible decreased in the level of Serum glutamic pyruvic transaminase.

In both male and female wistar rats Total protein level was high in rats treated with aluminium chloride when compared with control group and the groups treated with aluminium with *Annona muricata* and *Aloe barbadenis miller* in different combination this shew that there were derailment in liver function enzymes which have been improved by treatment with extracts. This study shows that elevated level of SGPT, ALP and serum Total protein causes damages to the liver. The effect of aluminium was observed from this study, the high concentration of aluminium in liver caused hepatotoxicity[13].

A variety of medicinal properties have been found in *annona muricata* extract and *Aloe barbadenis miller* gel, this study was carried out to determine the curative impact of *annona muricata* and *Aloe barbadenis miller* gel on hepatotoxicity caused by aluminium. Proof from results and studies also show that both *Annona muricata* and *Aloe barbadenis miller* gel have the ability to protect the liver and enzymes from aluminium detrimental operations, both extract increased body weight. But there was a decrease in body weight of the animal treated with aluminium compound this is related to Newsome *et al*[12].

The photomicrographs from this study showed that the control livers were of normal morphology and normal of histoarchitecture each showed the Central vein and hepatocytes which were distorted by induction with aluminium chloride which resulted in several alterations indicating abnormal morphology of the liver, with scattered hepatocytes and enlarged central vein, group treated with *Annona muricata* and *Aloe barbadenis miller* gel showed that they were similar to the control plate. Histology of the group treated with *Annona muricata* was closer and more similar to the control slide showing normal morphology and histoarchitecture as that of the control plate.

CONCLUSION

This study showed that aluminium caused hepatotoxicity on adult wistar rats, which was observed after administration of aluminum chloride through elevated level of serum marker enzymes ALP and SGPT and serum Total Protein which was due to the severe hepatic damage. However, the co-administration of *Annona muricata* and *Aloe barbadenis miller* gel extracts treated rats showed that liver enzymes nearly reach that of the control group thereby indicating the protective role of these extracts against aluminium hepatotoxicity.

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Ethics approval
The experimental procedures were in conformity with national and international standards on the use of laboratory animals. Also, the study was approved by institutional committee on the care and use of animal for experiments.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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