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Aqueous extract of *Aloe vera* ameliorates ethanol-ınduced liver damage and oxidative stress in Balb/c mice

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ABSTRACT: Exessive alcohol abuse is associated with physiological and pathological effects on the body. *Aloe vera* was selected because of its many beneficial qualities. 25 mice were given the following treatment protocol: distilled water (normal control), 20% ethanol at 10ml/kg (negative control), 25mg/kg Aqueous extract of *Aloe vera* (AA) plus 20% ethanol, 50mg/kg AA plus 20% ethanol and 100mg/kg silymarin plus 20% ethanol (positive control) respectively daily for 18 days. After sacrifice, blood was collected for biochemical analysis. The liver was analysed histologically, histochemically and part was macerated to determine oxidative stress biomarkers. AA significantly decreased (P< .05) AST activity but it did not significantly decrease ALT activity.It significantly increased (P<.05) albumin levels. Pre-treatment with AA at 25mg/kg significantly elevated (P< .05) catalase and SOD activities. However, GSH activity was not significantly changed. AA was found to significantly reduce (P< .05) lipid peroxidation. AA significantly increased the number of hepatocytes at telophase stage relative to the ethanol-treated mice. Histological and histochemical analysis revealed normal hepatocytes with few vacuoles and fewer fat droplets in the liver parenchyma of AA treated mice compared to the ethanol group. The results reveal that pretreatment with AA has hepatoprotective effects on ethanol-induced hepatic damage by reducing oxidative stress and preserving the histology of the hepatic tissue.

KEYWORDS: *Aloe vera*; biomarker; hepatic; histology; oil red o; oxidative stress

1. INTRODUCTION

Alcohol consumption is a widespread social practice among many populations worldwide because its moderate consumption is considered beneficial to cardiovascular health [1] and also for social interaction where alcoholic beverages are consumed without an intent to become intoxicated. However, alcohol can be consumed excessively and excessive alcohol abuse is also associated with several pathological conditions ranging from intoxication, to severe endangering states which are evolving causes of morbidity and mortality worldwide [1,2].

Ethanol which is contained in alcoholic cocktails is an interesting suject of nutritional consideration but no notable nutrient value, because, while being a source of energy (7 kcal/g), it has no functional and/or metabolic application, as described by [2,3]. It is a conceivably harmful substance for organisms and its misuse can cause damages both physiologically and psychologically. The physiological effects of high ethanol intake includes: an increased risk of cardio-cerebrovascular diseases, hepatic disease, gastrointestinal disorders and some forms of cancer. Alcohol is reported to induce oxidative stress either directly or indirectly [2,4]. Directly by increasing the production of reactive oxidizing species (ROS) indirectly action by reducing the antioxidant capacity of the cell [2,5].

A correlation between excessive alcohol intake, abuse and the manufacture of Reactive Oxygen Species (ROS), decrease in cellular antioxidant defenses, inbalance between oxidants and antioxidants and oxidative damages to proteins, lipids, carbohydrates and cell nucleic acids has been shown by several researchers [6,7]. Conversely, research has proven that several natural products including Aloe vera (AA)

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are able to reduce oxidative stress [2].The ongoing investigations in determining the therapeutic potentials of medicinal plants against alcohol induced toxicity has been an evolving and promising area of research [8-10]

Aloe barbadensis Miller (Family Liliaceae) was selected for the current study based on its phytomedical properties which have been studied by several researchers as several studies have been conducted to show the pharmacological evidence and the support of the traditional folklore medicinal usage of Aloe vera (AA) in the treatment of different toxicant induced tissue damages [11,12] The leaf gel extract and/or the extracted juice has been shown to possess: antibacterial, antioxidant, antidiabetic, anticarcinogenic, hypocholesterolemic, hepatoprotective, nephroprotective, laxative, antimicrobial, wound healing, anti-inflammatory properties [2, 13-19]. Most of medicinal properties of AA lies in the gel which is a clear gel or mucilaginous substances produced by parenchymal cells of the leaves and located in the central region of the leaf.

The gel is comprises majorly of water (99%) and carbohydrate moieties which consists of 25% of the dry gel. In addition, AA contains 75 potentially active ingridients that comprise of minerals, sugars, vitamins, enzymes, lignin, saponins, salicylic acids and amino acids. Its medicinal properties are attributed to the presence of the polysaccharides found in the inner part of the parenchymatous tissue [8, 20].

In the present study, the authors sought to investigate the heptaoprotective potential of AA gel against ethanol-induced hepatotoxicity and to investigate its antioxidant properties in the animal model. The outcome of study will help to increase awareness for consumers of AA and its products, also, there will be an increase in the consumption of locally processed AA as a potential affordable and available remedy to remedy alcohol related disorders

2. RESULTS

2.1 The Effect of AA on Serum Liver Biomarkers

The serum AST and ALT activity of ethanol-treated mice was significantly high (P< .05) relative to the control. Pretreatment with AA significantly decreased (P< .05) the AST activity compared to the ethanol only treated mice. However, it did not significantly decrease the ALT activity relative to the ethanol only treated mice. No significant change (P> .05) was seen in the AST activity of 25mg/kg AA-pretreated mice compared to the control (Figure 1). Serum albumin level was reduced significantly (P< .05) in ethanol-treated mice compared to the control. Pre-treatment with AA and silymarin significantly increased (P< .05) the albumin level compared to the ethanol only treated mice (Figure 1).

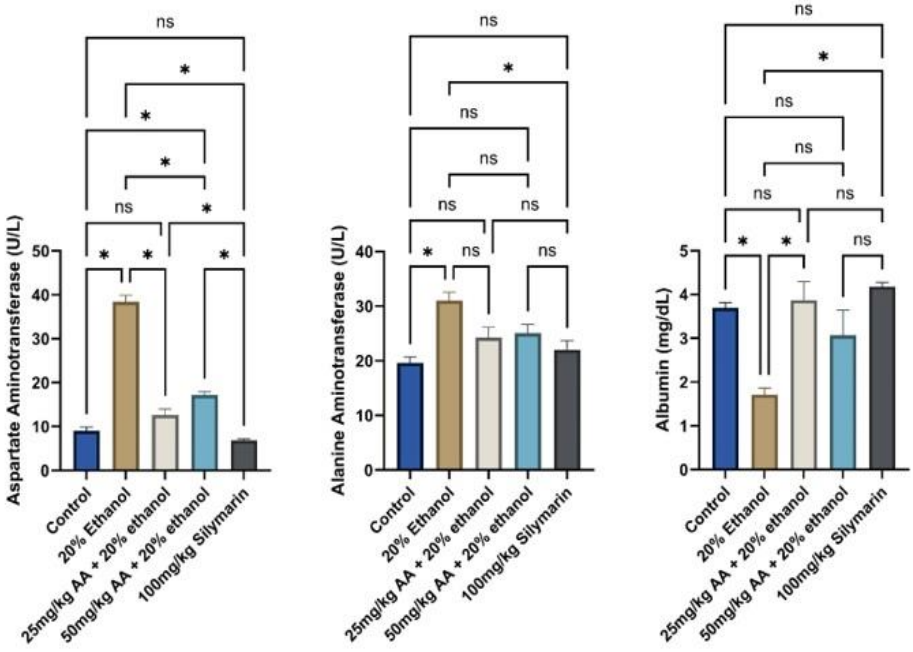

Figure 1. The liver function of ethanol-treated mice pretreated with aqueous extract of *Aloe vera*. The bars are presented as mean ±SEM. AA= Aqueous extract of *Aloe vera*, SEM= standard error of the mean, n=5.

2.2 The Effect of AA on Oxidative Stress Biomarkers

The catalase, GSH, and SOD activity significantly increased (P< .05) in the ethanol-treated mice relative to the control. Pre-treatment with AA at 25mg/kg significantly improve (P< .05) the catalase and SOD activity compared to the ethanol only treated mice. More so, Pre-treatment with AA and silymarin did not significantly change (P> .05) GSH activity compared to the ethanol-treated mice (Figure 2).

Ethanol-treated mice showed a significant increase (P< .05) in MDA activity (lipid peroxidation) compared to the control. Pretreatment with AA and silymarin were found to significantly reduced (p< .05) lipid peroxidation relative to the ethanol-treated mice (Figure 3).

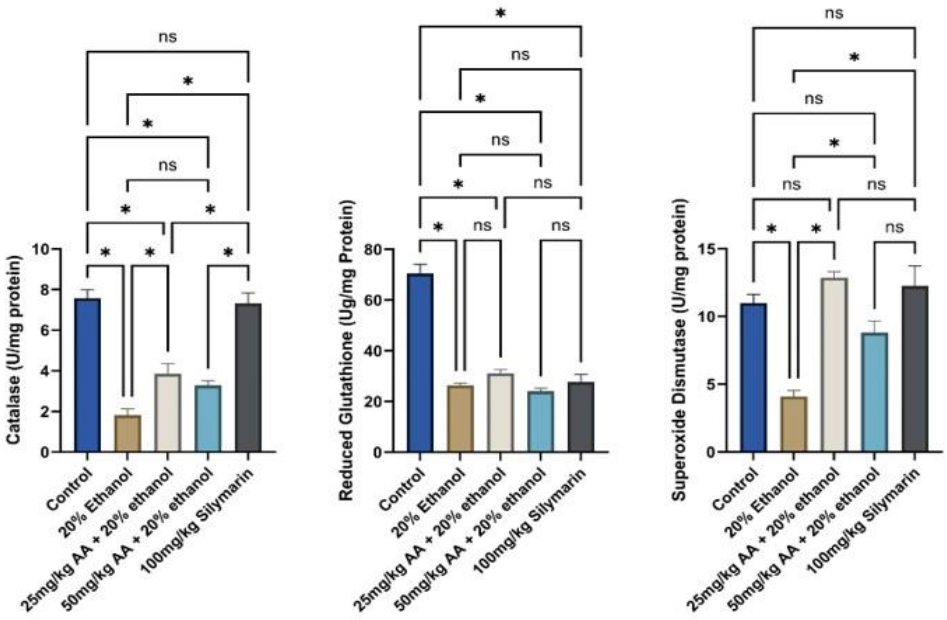

Figure 2. The antioxidant activity of ethanol-treated mice pretreated with aqueous extract of *Aloe vera*. The bars are presented as mean ±SEM. AA= Aqueous extract of *Aloe vera*, SEM= standard error of the mean, n=5.

2.3 AA Increases Number of Mitotic Figures in Treated Groups, Preserves the Histology of the Liver and Reduces Steatosis

Mitotic figures observed at telophase stage of cell division were significantly (P< .05) less in the ethanol-treated mice compared to the control. More so, pretreatment with AA and silymarin significantly increased the number of hepatocytes at telophase stage relative to the ethanol-treated mice (Figure 4).

The H&E-stained slides of the liver showed numerous hepatic vacuoles in ethanol-treated mice. Nevertheless, the liver of the control, AA-pretreated, and silymarin-pretreated mice revealed normal hepatocytes with few vacuoles. The histoachitecture of the hepatocytes remained intact as the cells were arranged in cords radiating away from the central vein. Sinusoidal spaces between the liver tissue was clear and there were several liver sinusoidal epithelial cells recognizable by their darkly stained nucleus in the perisinusoidal spaces. These cells were abundant in the control group but depleted in the negative control group. The cells were replenished again in AA treated groups (Figure 5).

The oil red O-stained slides of the liver showed large regions of positive fat staining (red) while those of the AA pretreated mice showed fewer fat staining. In the silymarin treated group, no lipid staining was viable in the hepatic tissue (Figure 6).

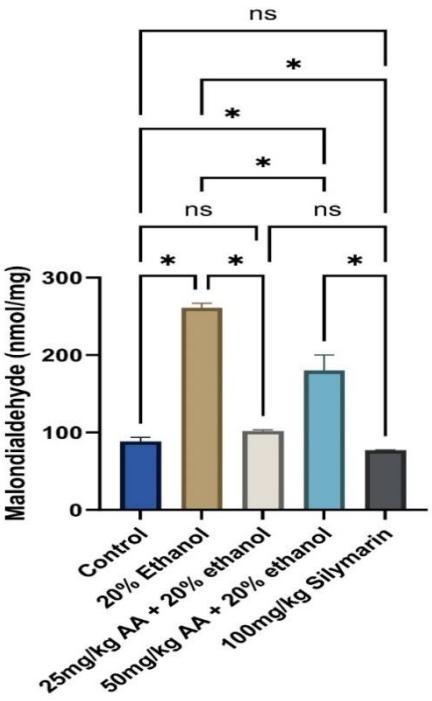

Figure 3. Malondialdehyde activity of ethanol-treated mice pretreated with aqueous extract of *Aloe vera*. The bars are presented as mean ±SEM. AA= Aqueous extract of *Aloe vera*, SEM= standard error of the mean, n=5.

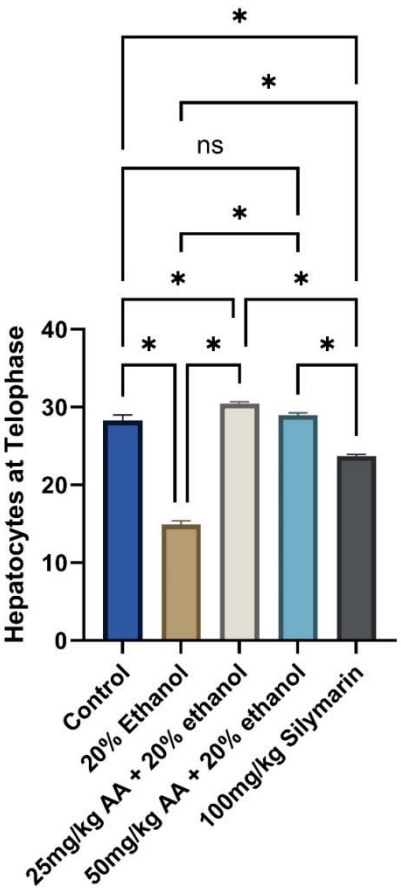

Figure 4. The number of hepatocytes at telophase stage of mitosis in ethanol-treated mice pretreated with aqueous extract of *Aloe vera*. The bars are presented as mean ±SEM. AA= Aqueous extract of *Aloe vera*, SEM= standard error of the mean, n=5.

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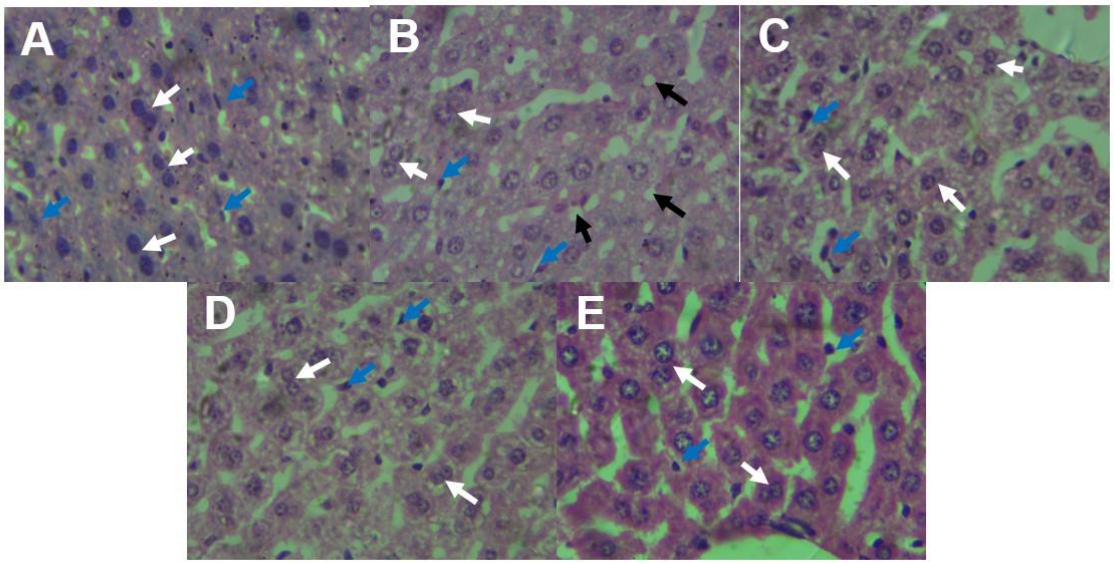

Figure 5. Liver photomicrograph of ethanol-treated mice pretreated with aqueous extract of *Aloe vera* showing numerous hepatic vacuoles in B and hepatocytes at the telophase indicated by black and white arrows respectively. Sinusoidal epithelial cells are represented by blue arrow. A= control, B= 20% ethanol, C= 25mg/kg aqueous extract of *Aloe vera*, D= 50mg/kg aqueous extract of *Aloe vera*, and E= 100mg/kg silymarin. H&E stain x400.

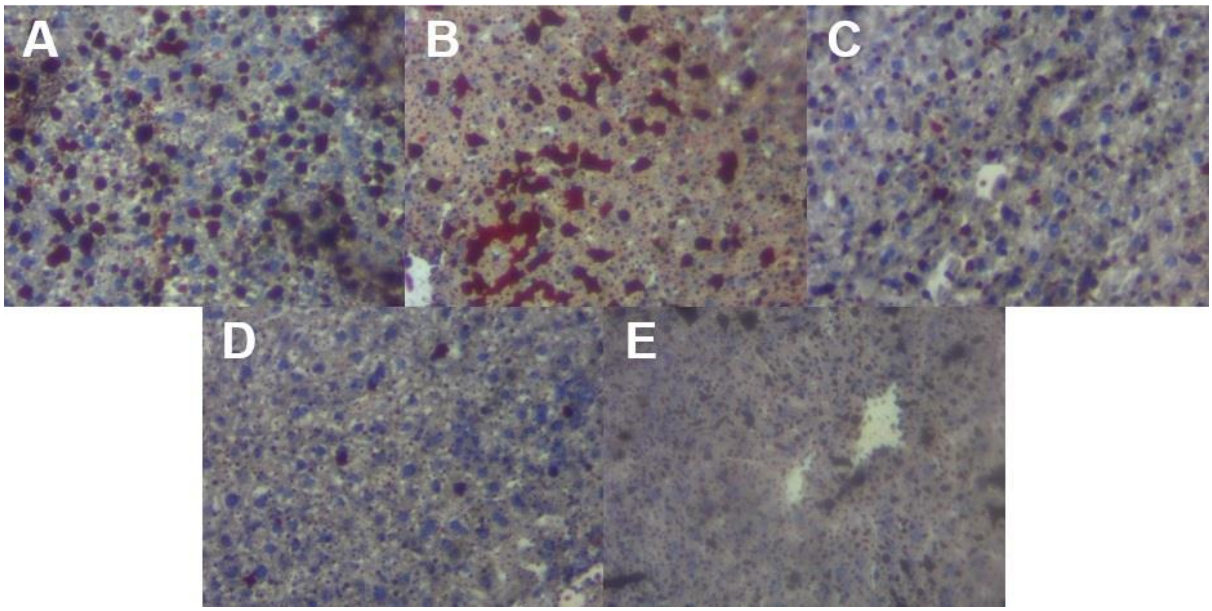

Figure 6. The oil red o-stained liver of ethanol-treated mice pretreated with aqueous extract of *Aloe vera* showing positive fat staining in B with negative fat staining in A, C, & D. A= control, B= 20% ethanol, C= 25mg/kg aqueous extract of *Aloe vera*, D= 50mg/kg aqueous extract of *Aloe vera*, and E= 100mg/kg silymarin. Oil red O stain x200.

3. DISCUSSION

The liver is an important organ which plays many important roles including production of bile for fat metabolism, filtration of blood, immune functions, supporting the formation of blood clots, absorption and metabolism of bilirubin, storage of vitamins and minerals, and production of many hormones [23, 24], in physiological processes including bile secretion, metabolism of proteins, carbohydrates and lipids, balancing metabolism, detoxification sna synthesis of blood clotting factors, proteins and biochemicals [11, 25]. Excessive alcohol consumption damages these organs, and may lead to alterations in their metabolic

functions hence, hepatoprotective substances are important [9,10]. Aloe vera gel extract in the current study has been demonstrated to show a potential protective effect against acute alcohol-induced hepatotoxicity.

The results of this study demonstrated that chronic alcohol ingestion resulted in an increase in serum AST and ALT activity, reduced serum albumin concentration, reduced liver catalse, reduced glutathione, superoxide dismutase and increased lipid peroxidation evidenced by an elevated malondialdehyde levels. Histologically, there were numerous vacuoles found in the hepatic cells and also numerous lipid accumulation (steatosis). This is in agreement with earlier studies carried out on the effect of alcohol damage on the liver [1,26],

Serum transaminases and ALP have been reported to be sensitive indicators of liver injury; ALP and AST are responsive biomarkers implicated directly in liver tissue damage and toxicity [1, 10, 27]. In the current study, AA significantly decreased AST levels in treatment groups, the decrease in ALT levels were non-signifcant when compared to the ethanol treated mice. Serum albumin is produced in the liver and it is the most abundant blood plasma protein which plays a key role in the transport of hormones, anaesthetics, endogenous ligands and free fatty acid [1,28]. In the current study, AA also significantly increased serum albumin concentration in the treated groups.The results of this investigation is in line with the finding of Cui et al. [12], Akintola et al. [1], Ahmad et al. [9], who reported that administration of Aloe vera juice found to be effective in reducing liver enzymes and elevating serum albumin concentration in toxicity induced groups. These restorative effects of AA on the specific activities of these tissue damage marker enzymes might be due to its hepato-renoprotective activities as reported by Nahal [10], Akinloye [1], Cui et al. [12], Hussein et al. [16], Al-Shinnawy [26], Saito [11] which enable it to protect the liver tissue in alcohol induced damage.

Oxidative stress plays a key function in the development of hepatotoxicity and alcohol liver disease. Several studies have demonstrated that alcoholic liver disease is connected with increased lipid peroxidation, destruction to the mitochondria, generation of free radicals and a decrease in liver antioxidant defense, providing a stong proof for the pathogenic role of oxidative stress [12, 25]. In the current study, AA was found to attenuate alcohol induced oxidative stress by significantly increasing liver catalase, superoxide dismutase and non-significantly increased liver reduced glutathione concentrations.It also significantly reduced lipid peroxidation in the treatment groups and this is in concurrence with the results obtained by several researchers [2, 6, 10, 12-14]. It has been postulated that the attenuation of chronic alcohol-induced oxidative stress by AA is partly due to its ability to alleviate lipid peroxidation and scavenge free radicals. These antioxidant capacities could be partly attributed to the acetyl groups and the reductive nature of the monosaccharides in AA polysaccharides [25].

The regeneration of the liver tissue is a crucial process that is necessary for the hepatic tissue to recover from alcohol-induced [23]. The process of regeneration of healthy liver depends mostly on hepatocyte proliferation, growth, and apoptosis [24]. The liver is frequently exposed to stresses and injury throughout life as a result of lifestyle choices. In the adult human liver parenchyma, hepatocytes are slowly replaced, with each cell having a normal mean lifespan of approximately 200–300 days, but they retain the ability to proliferate rapidly in response to aggression [29]. This replication is evidenced by mitotic figures found in the hepatic parenchyma which indicates that hepatocytes are being replaced to replenish damaged cells. There was a significant increase in the mitotic figures found in the micrographs in the groups treated with AA when compared with the ethanol group, indicating that AA plays a role in hepatic cell regeneration following ethanol-induced damage. Similar studies have also been carried out by [30, 31] indicating that administration of Aloe vera gel extract significantly modulated serum electrolytes imbalances with concomitant lowering of ALT, AST, ALP, GGT, LDH and GST rates when compared to the control group suggesting the restorative ability of alcohol induced injury using Aloe vera gel.

Oil red O (also known as Oil Red 4B) is a lysochrome used in histochemistry for the staining of neutral triglycerides [32]. Lipids in frozen tissue stained with Oil Red O appears as red drops, making them easy to distinguish from other tissue components, which will appear blue because of the nucleus taking up dye. Oil Red O staining is applied for quantifying liver steatosis [33] and alcoholic ketoacidosis [32]. Alcohol abuse and alcoholism leads to alcohol liver disease such as alcoholic fatty liver [34]. In the present study, lipid accumulation in the primary hepatic cells were investigated by staining the liver tissue with Oil red O. The AA treated groups did not show any signs of lipid steatosis as red droplets were fewer and the tissue remained blue in contrast with the ethanol group, thus indicating its hepatoprotective properties [11].

The histological appearance of the groups treated with AA also showed preserved hepatic architecture and no observed damage to the microscopic structure of the hepatocytes as observed in studies carried out by [9,10,12,14].

4. CONCLUSION

Aloe vera gel has been used for many medicinal purposes and in the current research, its protective effect against ethanol-induced liver damage was investigated. The aqueous extract of Aloe vera was found to effectively ameliorate the effects by decreasing serum biomarkers of hepatic damage, elevating serum albumin, catalase, reduced glutathione, superoxide dismutase and reducing lipid peroxidation in ethanol induced liver injury. Histological examination of the liver tissue revealed normal hepatic tissue with no lipid deposit in the hepatic parenchyma. The results of the research reveals that Aloe vera gel has the potential to alleviate the effects of ethanol-induced liver damage.

5. MATERIALS AND METHODS

5.1 Chemicals

All chemicals used are of analytical grade and are products of Poole, England of Signa Aldrich, USA. Ketamine injection was used as an anesthetic, it was purchased from a local pharmacy in Maiduguri, Borno State.

5.2 Plant Material

Aloe vera was harvested from a garden in the University of Maiduguri and authenticated at the Faculty of Pharmacy Herbarium (UMM/FPH/ASH/002). The leaves were cleaned, homogenized and macerated with cold distilled water for 24 hours. The concoction was filtered and evaporated in an oven at 45 ^{o} C to obtain the aqueous extract.

5.3 Animals and Ethics

Twenty-five (25) mice, 6-8 weeks old (19-24) g were housed at the Animal House, Department of Biochemistry, University of Maiduguri. They had free access to hybrid feed (Chikun Feed, Nigeria) and water. The study was approved by the Postgraduate Board of Studies, University of Maiduguri, and conducted conferring to the National Institute of Health Guide for the Care and Use of Laboratory Animals and ARRIVE Guidelines.

5.4 Experiment Design

The mice were randomly allotted to five groups, each consisting of five mice. The groups received the following treatment protocol: distilled water (normal control), 20% ethanol at 10ml/kg (negative control), 25mg/kg Aqueous extract of Aloe vera (AA) plus 20% ethanol, 50mg/kg AA plus 20% ethanol and 100mg/kg silymarin plus 20% ethanol (positive control) respectively daily for 18 days (this duration corresponds to two years regular alcohol drinking in the human beings). The mice in the treatment groups were administered 25mg/kg Aqueous extract of Aloe vera (AA) and 50mg/kg Aqueous extract of Aloe vera (AA) prior to administration of the ethanol.

Ethanol was used to induce liver damage and this was given as a single dose administered on day 1 of the experimental study. Administration of the extract was via the oral route and this continued for a period of 18 days. The mice were euthanized by ketamin injection on day 19 and the blood was collected in plain bottles for biochemical analysis. The liver tissue was dissected and processed for histological and oxidative stress study to determine the effect of the extract.

5.5 Biochemical Analysis

The blood that was drawn was centrifuged (Safstar Desktop Centrifuge 80-2) at 5000 rpm and aspartate aminotransferase (AST), alanine aminotransferase (ALT), and albumin levels were evaluated from the serum spectrophotometrically according to the manufacturer's instructions.

5.6 Oxidative Stress Markers

Part of the liver was homogenized in normal saline centrifuged and malondialdehyde (MDA), superoxide dismutase (SOD), catalase, and reduced glutathione (GSH) activities were estimated from the supernatant as described in a previous study carried out [21,22].

5.7 Histology and Histochemical Study

Part of the liver was fixed in neutral buffered formalin, dehydrated in graded alcohol an routinely processed for light microscopy and stained with hematoxylin and eosin (H&E), micrographs were photographed at

X200 and X400 magnifications using using an Amscope light microscope (MBJX-ISCOPE, Los Angeles) fitted with a digital camera (M500, X 64, version 3.7). While another part The tissue was frozen at -80°C. It was sectioned and mounted on pre-cleaned Superfrost Plus microscope slides. Immediately after mounting the liver tissue, the tissue were frozen again to preserve the chemical structure of the tissue. The sections were stained with hematoxylin Carrazi to demonstrate the nucei of the heepatocytes, then washing in water and 77 % ethanol, and after which the sections were stained with the Oil red O solution for 15 minutes to demonstrate lipid droplets. Finally the sections were quickly washed in 77 % ethanol and in water again for about 10 minutes, and the sections were mounted in aquamount. Liver sections taken at regular intervals for each mouse was interpreted by two researchers and micrographs were taken x200 and x400 magnifications.

5.8 Mitotic Division

The number of hepatocytes undergoing mitosis (telophase stage) were counted in 5 fields of each liver (3 slides per group) at x600 magnification. The telophase stage of cell division is when a hepatocyte has two nuclei that are either partially or completely separated from each other.

5.9 Statistical Analysis

The data were analyzed with GraphPad Prism 10.0 (San Diego, USA). One-way ANOVA followed by Sidak multiple comparison was carried out and statistical significance was considered at $P < .05$.

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