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Histopathological changes of mice liver induced by an *Aloe vera* whole leaf extract

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Abstract

Plants commonly used in traditional medicine are assumed to be safe. This safety is based on their long usage in the treatment of diseases according to knowledge accumulated over centuries. One such plants is *Aloe vera* which has been used medicinally for centuries. Recent widespread importance of commercial *Aloe vera* has encouraged scientists to scientifically assess these products since it contains the anthraquinones which associated with considerable risks. In present study oral administration of **20 µl** of *Aloe vera* extract **(**experimental group) (G) was given for 21 days to immature male Swiss Webster mice at weaning period. While the control groups (C) were given by the same dose and rout of administration with normal saline only. After six weeks (around puberty) the male were sacrificed to get their liver, then fixed with 10% formalin, and histological sections with a thickness of 5 microns were prepared. *Aloe vera* whole leaf extracts treatment resulted in liver necrosis, hepatocellular dissociation with lymphocytic cell aggregation and increase of cytoplasmic vacuolation. The sinusoids of animals fed *Aloe vera* whole leaf extracts were found to be widened. This study was designed to assess the histopathological changes of liver tissues of male mice administered low dose of fresh whole leaf *Aloe vera* extract.

Keywords: *Aloe vera*, liver, anthraquinones, histopathological changes of liver, whole leaf extract.

التغيرات النسجية المرضية لكبد الفئران الناجمة عن مستخلص نبات الصبار

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الخلاصة:

عادة ماتعتبر النباتات التي تستخدم في الطب التقليدي آمنة. تستند هذه السلامة لاستخدامها الطويل في علاج الأمراض وفقا للمعرفة التي تراكمت على مر القرون. تستخدم بعض هذه النباتات كمضافات غذائية. نبات الصبار واحد من هذه النباتات التي استخدمت في الطب لفترات طويلة مضت. وللاهمية التجارية واسعة النطاق لنبات الصبار دفعت العلماء لتقييم هذا المنتج نظرا لأنه يحتوي على الانثراكوينونيس الذي يرتبط بمخاطر كبيرة. في هذه الدراسة تم اعطاء ذكور الفئران غير البالغة بعمر الفطام 20 ميكرولترمن مستخلص نبات الصبار عن طريق الفم (المجموعة التجريبية) لمدة 21 يوما. في حين اعطيت مجموعة السيطرة نفس الكمية وبنفس طريق الإعطاء محلولا ملحيا فقط. بعد ستة أسابيع (عند سن البلوغ) شرحت هذه الحيوانات للحصول على الكبد. ووضعت الاكباد بمحلول مثبت (10٪ من الفورمالين) لاغراض الدراسة النسجية وليتم تحضير مقاطع نسجية بسمك (5) مايكرون. اشارت نتائج هذه الدراسة بان تعاطي مستخلص نبات الصبارنتج عنه نخر و تفكك الخلايا الكبدية وزيادة ظهور الفجوات في سايتوبلازم الخلايا واتساع الجيوب الدموية لاكباد الحيوانات المختبرية مع تراكم الخلايا اللمفاوية. هذه التغيرات النسجية تشير الى التهاب الكبد الحاد. تم تصميم

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هذه الدراسة لتقييم التغيرات النسجية المرضية لاكباد ذكور الفئران المعالجة بجرعة منخفضة من مستخلص نبات الصبار .

Introduction

The expense of conventional medications, especially in developing countries, has pushed researchers to focus on the healing potential of plants and plant extracts which are readily available in the communities [1]. Plants have been selected and used empirically as drugs for centuries, initially as traditional preparations then as pure active principles [2]. Medicinal plants are plants which contain substances that could be used for therapeutic purposes or which are precursors for the synthesis of useful drugs [3].

One plant that has received a lot of attention for centuries and in different cultures is *Aloe vera*. As reviewed earlier, *Aloe vera* has many tested and untested medicinal attributes affecting all the body systems. It was originally classified in the family *Liliaceae*, but according to Reynolds, it has now been designated its own family, known as *Aloaceae* [4]. *Aloe vera* is a very popular plant which was used for alternative medicine [5]. Most of the biologically active constituents of *Aloe vera* are found in the leaves which are composed of the rind, juice and a gel like substance [6]. It has been shown that some constituents of *Aloe vera* have similar biological activities to amino acids, vitamin C, polysaccharides, anthraquinones, barbaloins (glucosides) and growth factors [7]. *Aloe vera* also contains several potentially bioactive compounds such as salicylates, magnesium lactate, acemannan, lupeol, campestrol, β- sitosterol, γ-linolenic acid, aloctin A, anthraquinones, *Aloe*-emodin, resins, and chromone derivatives [8]. *Aloe* is widely used to treat several diseases as it is known to exhibit antioxidant, antibacterial, anti-inflammatory, antipyretic, antimicrobial, antihypertensive and analgesic properties [9, 10]. Furthermore *Aloe vera* has been used for several medicinal purposes such as wound and burn healing, treatment of diabetes and treatment of cancer [11].

On the other hand many of plants contain some bioactive principles which have not been fully discovered. Most research studies on biological system are based on the use of plant extracts which do not seem to provide adequate and accurate analyses with respect to the bioactive compounds responsible for the observed effect [2]. Moreover in the active ingredients of plant extracts are chemicals that are similar to those in purified medications, and they have the same potential to cause serious adverse effects. Whilst the literature documents severe toxicity resulting from the use of herbs, on many occasions the potential toxicity of herbs and herbal products has not been recognized [12]. However, scientific research has shown that many plants used as food or in traditional medicine arc potentially toxic, mutagenic and carcinogenic [13]. The toxicity benchmarks for herbal drugs depend on purity, herbs containing toxic substances, bioavailabilily, and reported adverse effect [14}. Due to the increasing popularity of complementary and alternative medicine, several new substances have been suspected to induce liver injury. These include among others ingredients from herbal products [15]. There are limited studies reported toxic effects of *Aloe vera* in the literature. In previously conducted two studies reporting the toxic effect of *A. vera* on liver. A male patient after the use of *Aloe vera* for 20 days came to hospital with whole body of a deep yellow and darkness in his urine was diagnosed with toxic hepatitis [16]. Another patient also used *Aloe.* Supplements as directed by the manufacturer was also diagnosed with toxic hepatitis [17]. It was revealed that *Aloe vera* gel has cytotoxic effect on not only tumor cells but also normal cells [18]. According to Avila *et al.*, (1997) [19] the green shoot of *Aloe vera* is very rich with anthraquinone. These are phenolic compounds that are found exclusively in the plant sap [20], which could have an adverse effect on cell growth. Low molecular weight compounds such as aloin is held responsible for this cytotoxicity [19]. Moreover use of *Aloe vera* as a laxative during pregnancy may pose potential teratogenic and toxicological effects on the embryo and fetus [21]. However, the ALP levels were significantly elevated in the rats exposed to the high dose of alcohol extracts of *Aloe vera*. ALP elevations are usually associated with bile duct damage/bile stasis. Previous study by [22] suggests that continuous consumption of *Aloe vera* whole leaf extract result in histopathological changes of the renal tissue in mice. Ordinary people recommend the medicines to others without safety considerations. Compared to synthetic drug treatments, the amount of information on the relative safely of herbal remedies is limited [23].

The liver is one of the largest glandular organs in the body that serves as a metabolic powerhouse for the processing of nutrients, absorption of lipids and glycogen storage to maintain energy levels

[24]. Other liver functions, including the bioregulation of fats, carbohydrates, amino acids, proteins, blood coagulation and immunomodulation, metabolism [25], oxidation, methylation and conjugation required for inactivation or detoxification of various substances before their excretion from the body [26]. Serious drug-induced liver injury is the leading single cause for withdrawal of approved drugs from the U.S. market. It also accounts for more than 50% of the cases of liver failure in the united states today [27]. Little documentation exists on the long term toxicity of herbs. There is a lack of use of standard/measured doses, and the large volumes of the doses used are difficult to manage. Most herbal medicines are classified as dietary supplements, and together with nutritional based therapies they are not subjected to the safety, quality assessment and standardization which we associate with conventional drugs supplied by the pharmaceutical industry [28]. In addition herbal medicines often contain several active ingredients, and composition of the product (quantity and purity) is often poorly described [29]. Due to vital function of liver, the present study was, therefore, designed to investigate the histopathological changes of liver tissues of male mice at weaning time administered low dose of fresh *Aloe vera* whole leaf extract.

Materials and Method:

1- Preparation of fresh *Aloe vera* whole leaf extract:

Fresh leaves of plant having a length of approximately 25 to 50 cm were weighed, cut and washed thoroughly with water. Fresh leaves were cut from the middle; the whole leaf extract was separated by scratching with a spoon. The obtained substance is then divided into different pieces, divided into 2 ml volume tubes, The extract was kept at 4°C overnight, before being used [30].

2-Animal and experimental design:

Forty immature Swiss Webster male mice (3) weeks old at weaning period were divided into two equal groups: experimental (G) and control (C), twenty animals in each. These immature male mice obtained from animal house of the High Institute for Infertility Diagnosis and Assisted Reproductive Techniques/ Al-Nahrain University were randomly selected. The mice weighed 15-18 g and were about 3 weeks old were housed under constant temperature (25 ± 2°C), humidity (55%) and light/dark conditions (12/12 h). They were fed on mice pellet and free access to drinking water *ad libitum* [31, 32]. The experimental group (G) was given 20 µl of *Aloe vera* whole leaf extract orally for 21 days. While the parallel control group was given normal saline by the same rout and dose as that used in the experimental group.

3-Histopathological study:

After six weeks (around puberty) the male were sacrificed to get their liver, fixed with 10% formalin, and histological sections with a thickness of 5 microns were prepared using the routine histological technique [33].

Results:

Light microscopic study

Control mice

The histological section of control mice illustrates hepatic lobules. Central veins are located at the centers of lobules. From the central vein radiate the plates of hepatocytes toward the lobule periphery. Located between the plates of hepatic cells are the blood channels called hepatic sinusoids. The portal area of the hepatic lobule contains the branches of the portal vein, hepatic artery and normally bile duct Figure-1.

Histopathological changes:

The sections of *Aloe vera*-treated liver demonstrate degenerative changes in most of its entire structure in comparison with control. The hepatocytes are arranged haphazardly with congested central veins and distraction of parenchymal tissue resulting in loss of hepatic tissue structural pattern Figure-2. Some hepatocytes have small pyknotic nuclei and are probably necrotic, others have undergone necrosis and disappeared. Figure-3. Focal interlobular necroses with lymphocytes infiltrations are present in some liver sections, the hepatocytes are swollen with numerous fat-containing vacuoles of widely varying size, because the fat has dissolved in reagents, the vacuoles seem to be empty Figure-4 and Figure-5. Within the hepatic lobules the cords of hepatocytes are irregular, heavily infiltrated with small deeply basophilic inflammatory cells (mostly lymphocytes, neutrophils and plasma cells) which is dense and concentrated to a large extent. Several degenerated hepatocytes in the form of acidophilic bodies are also evident Figure-6. The portal tract is enlarged with an aggregation of inflammatory cells which have eroded the limiting plate of hepatocytes that show (ballooning

degeneration). Connective tissue stains demonstrated an increase of fibrous tissue in portal tract Figure-7 and Figure-8. In other sections the destruction may encompasses zones 1 and 2 making liver's tissue almost unrecognizable Figure-9.

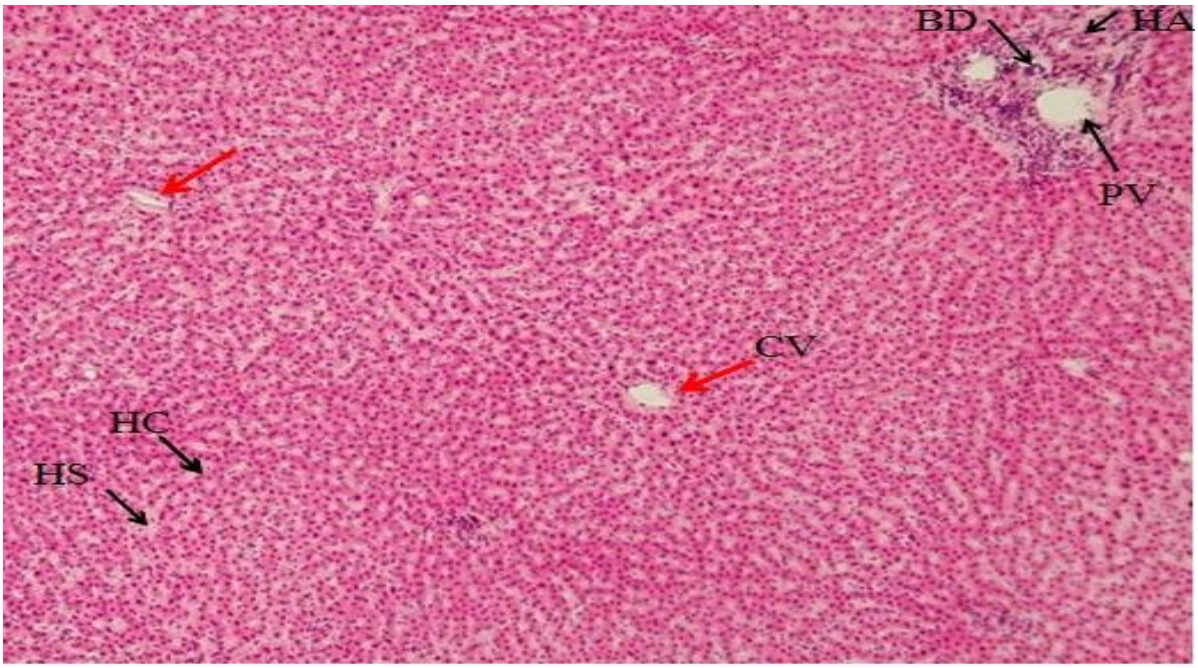

Figure1- A lower-magnification photomicrograph of normal structure of liver illustrates hepatic lobules, central vein (CV), hepatocytes (HC), hepatic sinusoids (HS), The portal area of the hepatic lobule contains the branches of the portal vein (PV), hepatic artery (HA), normal bile duct (BD) (H&E,400X).

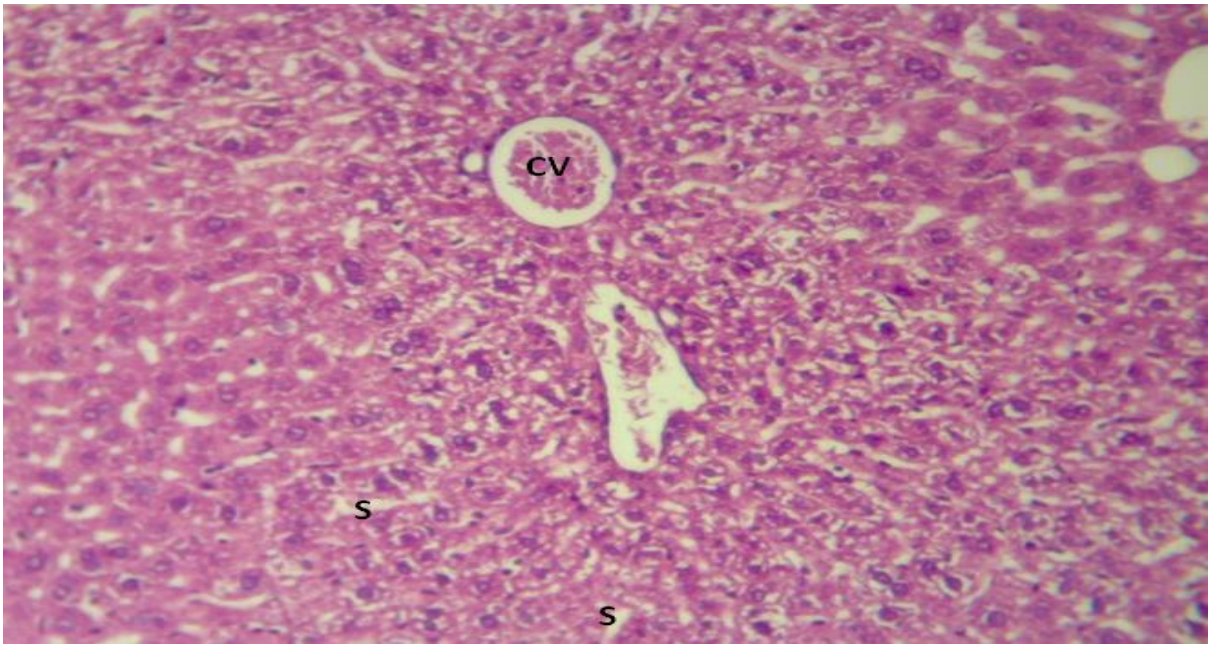

Figure 2-Micrograph of mouse liver treated with **20 µl** *Aloe vera* demonstrated congested central vein (CV). The loss of hepatic tissue structural pattern was clearly observed. (S) Sinusoids (H&E, 100X).

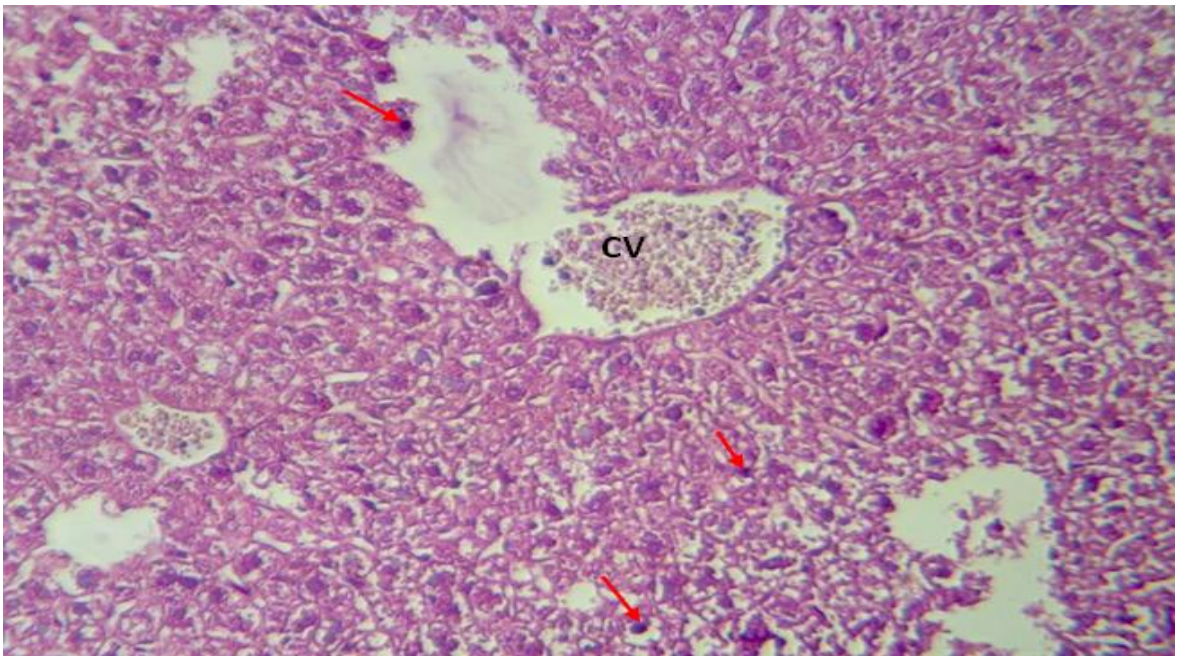

Figure3- The histological section of *Aloe vera*-treated with **20 µl** *Aloe vera* liver showed parenchymal necrosis in almost all hepatocytes. The cytoplasm of some hepatocytes stained dark acidophilic, the nuclei are small, pyknotic and dark stained (red arrows). Dilated and congested central veins (CV) were noticed along with sloughing of necrotic areas (H&E, 100X).

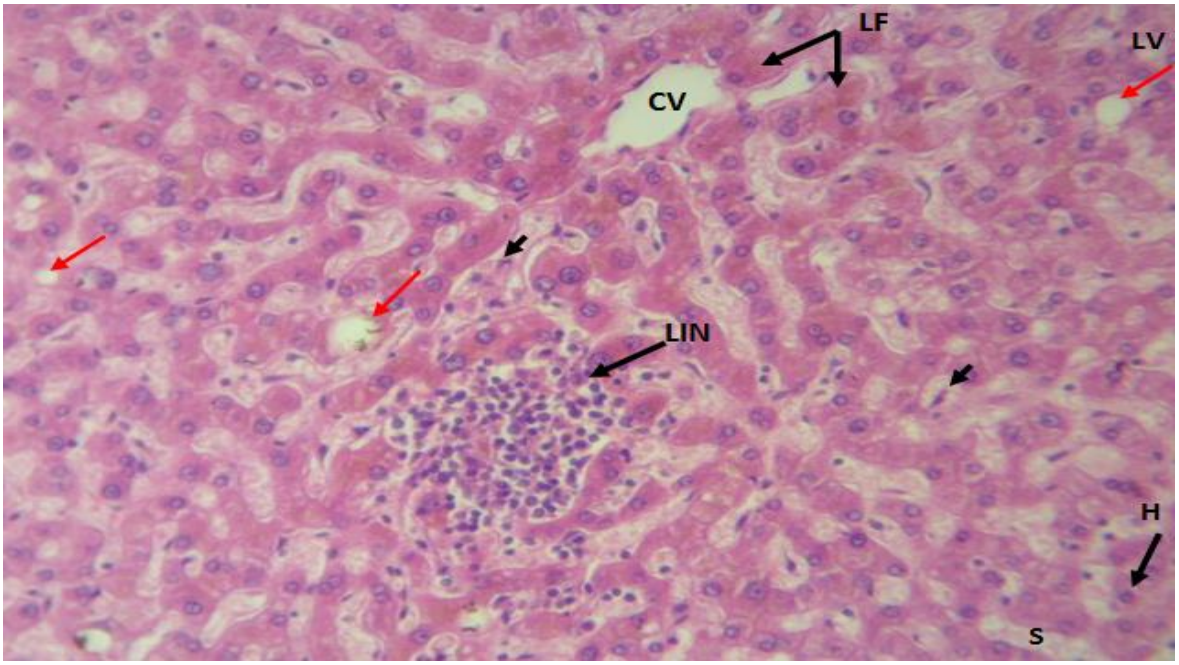

Figure 4- Section of *Aloe vera*-treated liver for 21days demonstrating focal lymphocytes inflammation (LIN) in the middle zone of liver lobule. The pale brown pigment in some hepatocytes is lipchrome (lipofuscin) (LF). Scattered fatty change (Lipid vacuoles) (LV) (red arrows) was observed in some hepatocytes (H). The sinusoidal Kupffer cells were prominent (Head arrows) (H&E, 200X).

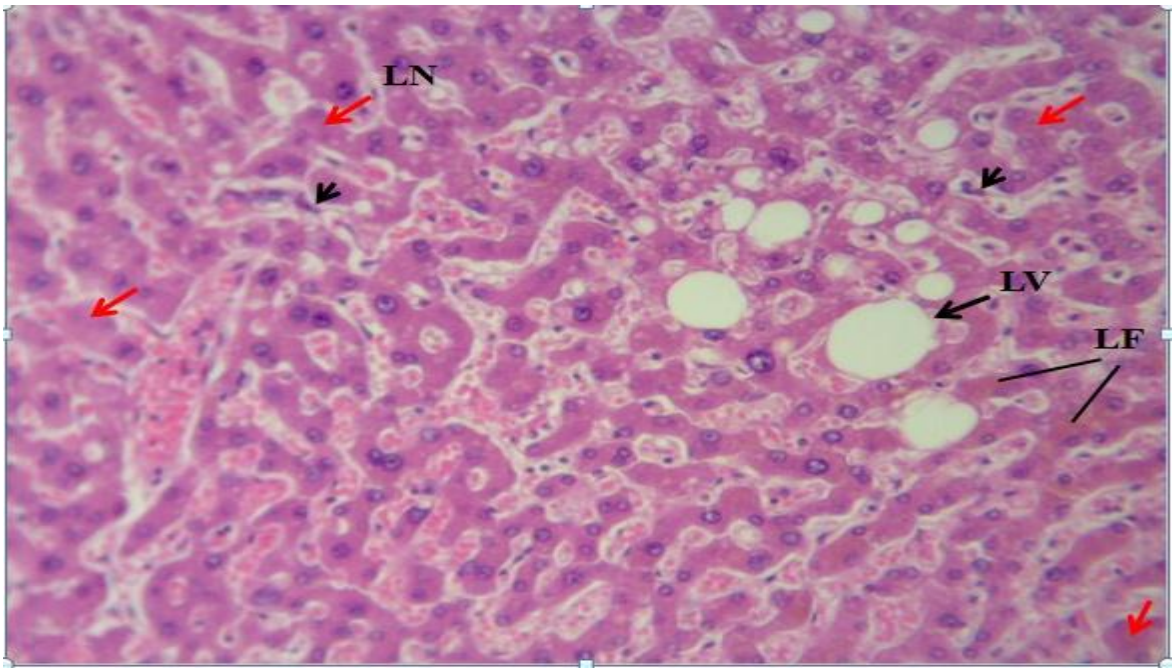

Figure 5- Section of *Aloe vera*-treated liver for 21days demonstrating lipid vacuoles (LV) within hepatocytes. Kupffer cells were highly infiltrated in sinusoids (head arrows). Few hepatocytes with partially lysed nuclei (LN) (red arrows) were observed. Dilated and congested sinusoids were noticed and lipofuscin (LF) pigment in some hepatocytes was also visible (H&E, 200X).

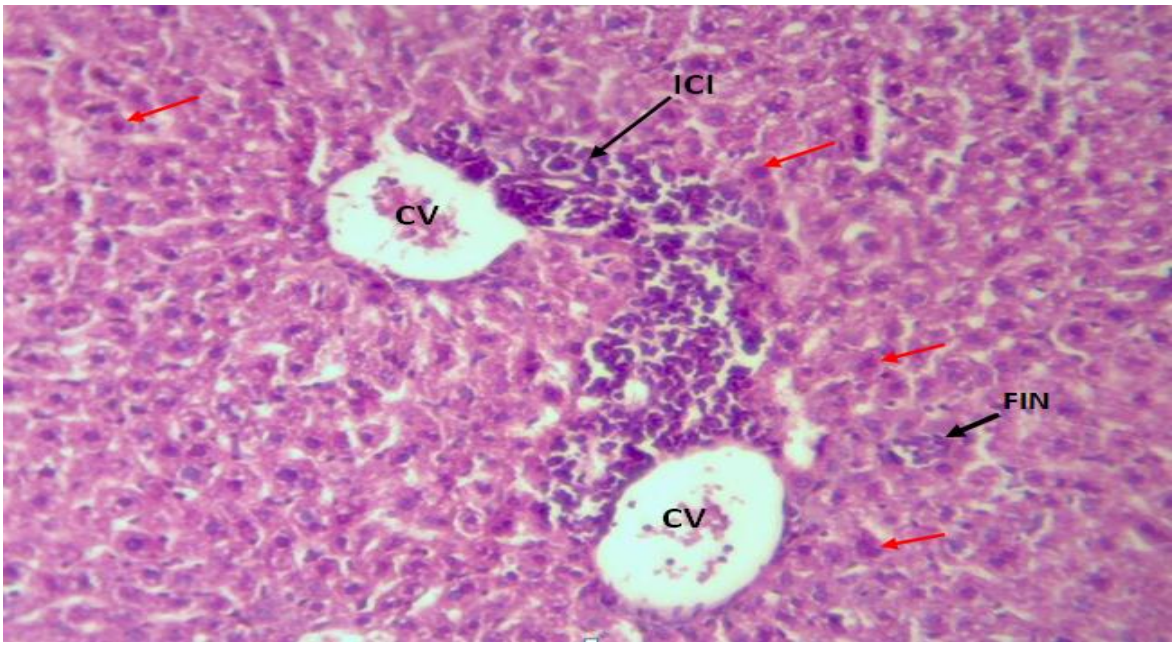

Figure 6- Section of *Aloe vera*-treated liver for 21days revealed inflammatory cells infiltration (ICI). Focal interlobular necroses (FIN) in the liver lobule. There was cytoplasmic eosenophilia in some hepatocytes (red arrows) (H&E, 200X).

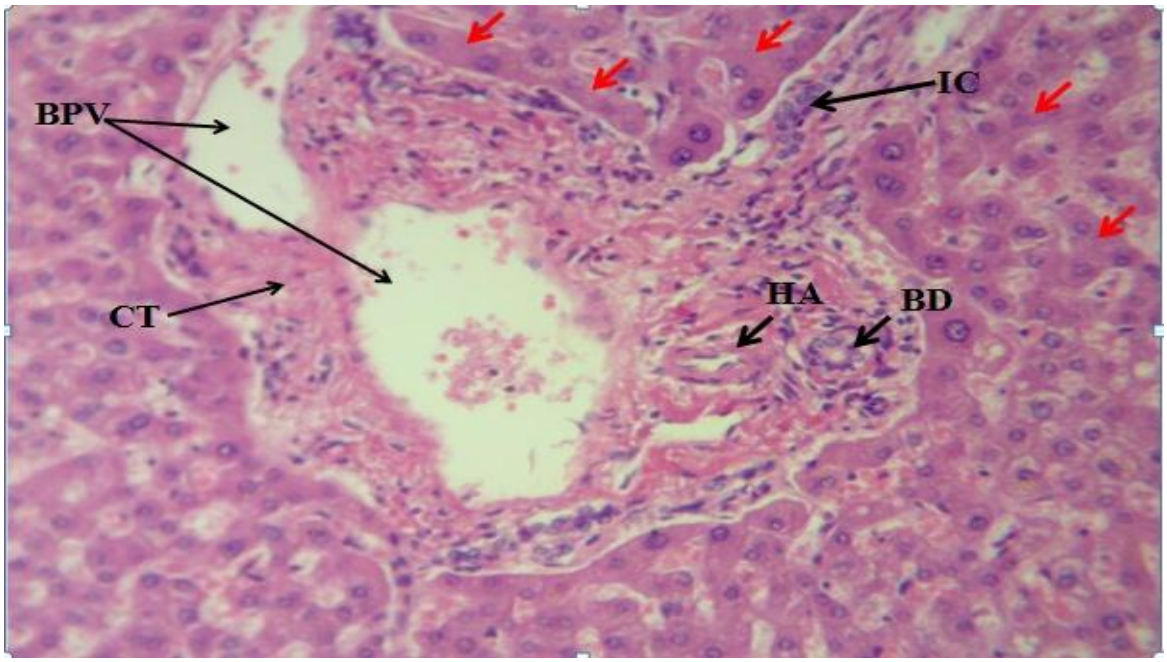

Figure 7- Section of *Aloe vera*-treated liver for 21days demonstrating an aggregate of inflammatory cells (IC) in the portal triads. The clearly visible swollen of hepatocytes with indistinct cell membrane (red arrows). Dilated Branches of portal vein (BPV) are obviously seen, hepatic artery (HA), bile duct (BD), connective tissue (CT) (H&E, 400X).

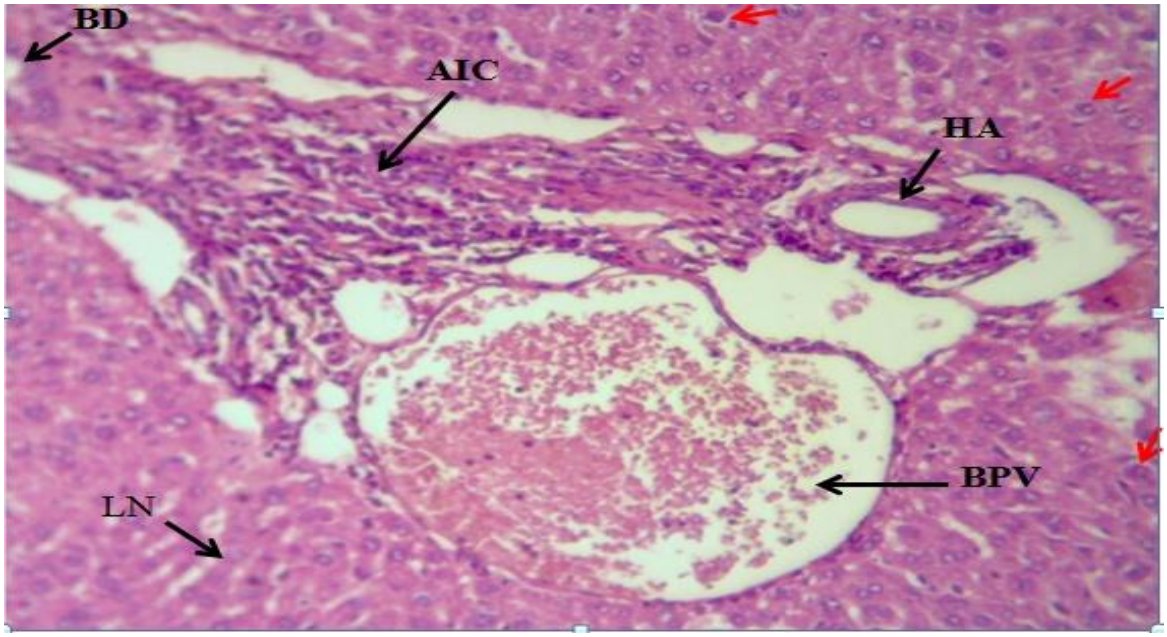

Figure 8- Section of *Aloe vera*-treated liver for 21days revealed aggregate of inflammatory cells in the portal triads (AIC). Liver parenchyma showed polymorphous necrotic hepatocytes with pyknotic nuclei (PN) (red arrows) with indistinct cell membrane, & partially lysed nuclei (LN). Dilated branch of portal vein (BPV) clearly congested. Hepatic artery (HA), bile duct (BD), (H&E, 400X).

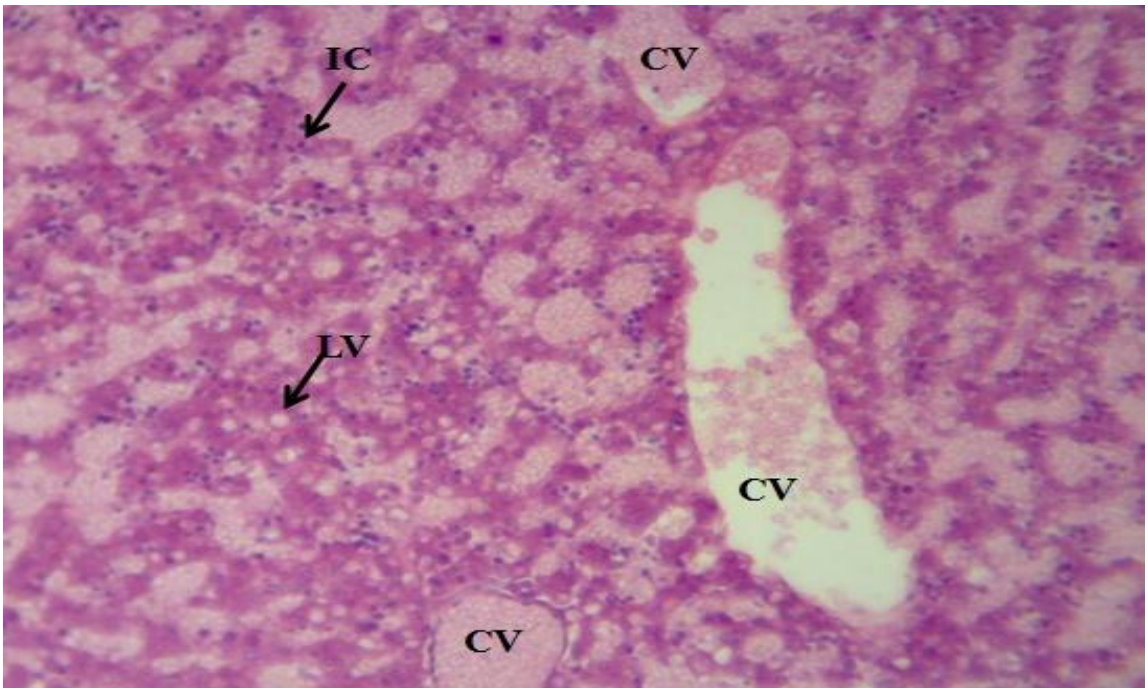

Figure 9- The histological section of *Aloe vera*-treated liver showed widespread necrosis of the liver with microvesicular steatosis (lipid vacuole) (LV), an aggregate of inflammatory cells (IC) also observed. The destruction encompasses zones 1 and 2 making this tissue almost unrecognizable (H&E, 200X).

Discussion

Liver is associated with metabolism and elimination of toxicant from the body and it's histological & biochemical parameters are considered as key points to elucidate toxicity of the chemicals. Evidences of changes in liver due to toxicants has been revealed by abnormal metabolic functions, reduced activity of detoxication reaction and altered structure of sub cellular organelles [34].

The toxicity exerted by *Aloe vera* whole leaf extract was confirmed from histological sectioning which indicated various degrees of hepatocellular necrosis in the treated group in comparison to control group. Increasing degree of hepatocellular necrosis and degenerative changes may relate to that the liver has a central role as a detoxifying organ towards xenobiotics and chemicals. However, biotransformation to less toxic substances can actually involve production of molecules that can induce liver injury [35].

The observed damage may also due to the fact that the liver is subjected to toxic injury more often than any other organ. Since the portal vein blood that drains the absorptive surface of the intestinal tract flows directly to liver. Thus, the liver is exposed to all ingested substances that are absorbed into the portal blood [36]. The toxic effect of *Aloe vera* whole leaf extract could be due to the generation of anthraquinones formed by oxidation of low molecular weight (LMWF) components such as aloin which are present in the plant leaves [37]. The first cell death pattern that was identified was cell necrosis results from an extreme disruption of cell balance dramatically affecting cell metabolism with a drastic decrease in cellular energy ATP, ion contents changes, increased mitochondrial and cell volume, and intracellular protcasc activation. This process ultimately leads to a disruption of cell membranes, and release of cell contents, which promotes a secondary inflammatory response [38]. The most frequent mechanism of hepatocellular injury involves production of injurious metabolites by the cytochrome P450 system. This family of enzymes is located in the smooth endoplasmic reticulum of hcpatocytcs primarily, although they are also found in many other cells of the body. A major role of cytochrome P450 enzymes is to metabolize lipid soluble chemicals into water-soluble compounds for excretion from the body in bile or urine [35]. However, in some circumstances, such as over dosage, the high-energy reactive metabolites can form adducts that form covalent bonds with other cellular constituents such as proteins and nucleic acids. In acute toxicity, adducts can be formed with essential cellular enzymes leading to cell injury or death [39]. Moreover it was proposed that mitochondria were primary target of the reactive metabolite. Indeed, some of the target proteins were localized in mitochondria fraction, including glutamate dehydrogenase, aldehyde dehydrogenase, carbamyl

phosphate synthetase-I and ATP synthetase α-subunit [40,41].These enzyme activities in the mitochondrial fraction were decreased partially [42,43], probably as consequence of covalent binding. Cytotoxic metabolites can attack the organelle directly or through some pathways. Important pathological mechanisms are mitochondrial permeability transition (MTP) through opening of MPT pore in the membranes of the organelle, and activation of signals, death receptors and proapototic pathways. As a result acute necrosis, apoptosis and autophagic cell death can occur [44]. Furthermore chemicals that damage mitochondrial structure, enzymes or DNA synthesis can disrupt oxidation of lipids and oxidative energy production within the hepatocytes [45].

The cytotoxic effects could be masked also by the production of reactive oxygen species (ROS) by redox cycling induced by anthraquinones of the low molecular weight fraction (LMWF). Likewise, it is also possible that the inhibitor is present in *Aloe vera* extracts, although in different amounts. It is apparent that LMWF obtained from *Aloe vera* gel has cytotoxic activities [19]. In addition ROS can initiate protein and lipid peroxidation, and deplete antioxidant defenses like reduced glutathione (GSH), and modify sulphydryl (SH) groups on cellular components. These non-covalent reactions represent additional toxic mechanisms to covalent reactions. The subsequent increased cellular stress and uncontrolled increase in cytosolic Ca^{2+} concentration stimulate Ca^{2+}-activated degradative enzymes with further cellular injury [34]. Modification of cellular function by down or up-regulation of genes can also induce hepatic damage. The modifying role of several cytokines and other signal substances in pathways of necrosis, apoptosis or survival are emerging, as is the relevance of various risk factors for the clinical course of the injury [46].

Toxins also causing blood clotting in the blood vessels this leads to a lack of tissue blood supply (Hypoxia) or not to supplied (Anoxia) [47].This may explain the cellular necrosis around congested hepatic veins accompanied with destruction of parenchymal tissue observed in histological sections of this study. The degenerative alterations may be due as mentioned before to that anthraquinones, which are poorly absorbed from the GIT, are cleaved by gut bacteria to produce aloe-emodin, which is more readily absorbed and responsible for the purgative properties of these preparations [48]. The liver and kidney were the only organs that had higher concentrations of aloe-emodin than plasma [49].

Vesicular steatosis induced by *Aloe vera* whole leaf extract clearly illustrated in histopathological sections of the present work. These observation is in consonance with Ben Beya (2010) **[**50] findings on rats liver how cited that the rats exposed to alcohol *Aloe vera* extracts showed an accumulation of lipids in the liver and may explained by that fatty liver is a metabolic disorder that occurs when the rate of fatty acid uptake and esterification exceeds the rate of fatty acid depletion either through oxidation or export as triglyceride within very low density lipoproteins. Prolonged interruption of oxidation leads to micro vesicular steatosis within hepatocytes [35].

The histological findings also showed enlargement of the portal tract with aggregation of inflammatory cells. Hepatic injury may be significantly exacerbated by recruitment of inflammatory cells [51]. The main encountered inflammatory cells in the recent work were lymphocytes. Lymphocytes are predominant in intoxication, viral and protozoal diseases [52]. Adams et al., (2010) [53] also confirm that various types of immune cells, including lymphocytes, reside in the liver, and other leukocytes are distributed to this organ during inflammation. Moreover, Hepatitis, with or without cholestasis, is the most common histological pattern of liver injury [54]. The reason of accumulation of inflammatory cells is the secretion of attractions resulting from decaying cells, as the decomposition of liver cells leads to edit materials with the ability to chemical attraction to the defensive cells [55]. The affected liver cells liberate compounds such as Prostaglandin E1 which have ability to chemical attraction to immune cells. It may be possible that inflammation is due to hepatocytes releasing cytokines into the blood stream. Furthermore, IL-12, in combination with IL-18, causes inflammation via the activity of interferon-gamma (IFN-γ), which is produced by T-lymphocyte and NK cells [56].

Connective tissue stains illustrate an increase of fibrous tissue in portal tract of some histological sections of this study. The formation of fibrous tissue may be due to the fibrin material which comprise as a result of conversion of fibrinogen which is present in exudes fluid by interaction with thromboplastin in the tissues leading to a deposition of a network of fibrin material in the affected tissue [57].

In conclusion the histological sections have clearly demonstrated that consumption of *Aloe vera* whole leaf extract could lead to impairment of liver entire structure and that may confirm that the

toxins from herbal medicine and nutritional-based therapies potentially can damage the liver in much the same way as conventional drugs [58- 60]. Herbal medicines although being widely used, should be used cautiously and after thorough scientific interrogation of their active constituents as they can contain harmful compounds and metabolites. Thus the ingestion of *Aloe vera* as whole leaf by humans should be reviewed. It is recommended that further studies should be carried out to corroborate these findings.

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