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Acute Oral Toxicity of Ethanol Extract of *Adenium obesum* Stem Bark in Female Rats

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ABSTRACT

Aims: *Adenium obesum* is a known medicinal plant thereby creating the need for the evaluation of its toxicity and histopathological effects on the liver of female Wistar rats orally administered ethanol extract of the plant's stem bark.
Place and Duration of study: Department of Veterinary Pathology, Ahmadu Bello University, Zaria, Nigeria, between January 2011 and January 2012.
Methodology: Ethanol extraction of *A. obesum* stem bark was performed prior to screening it for its phytochemical constituents. Female rats per group were orally administered pre-defined doses (300 $mgkg^{-1}$, 2000 $mgkg^{-1}$ and 5000 $mgkg^{-1}$) of the extract separatively in a stepwise procedure and observed for signs of toxicity. Control rats were administered distilled water placebo.
Results: The extract contained some alkaloids, saponins, tannins, flavonoids, glycosides, steroids and triterpens with no anthraquinones. Exposed rats did not show signs of toxicity and neither was there any mortality. Changes in aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase activities were non-significant ($p>0.05$). Congestion and fatty degenerative changes were seen in the liver of the exposed rats, which were not significantly ($p>0.05$) different in exposed rats compared to the control.
Conclusion: *Adenium obesum* did not cause major hepatic damage and therefore, it is a safe oral medicinal plant within the extract dose and exposure period used in the study.

1. INTRODUCTION

Medicinal plants are used to treat variety of ailments worldwide [1, 2]. This is because medicinal herbs are integral parts of alternative therapy [3]. The World Health Organization (WHO) estimated that about 80 % of the world population presently uses herbal medicine for some aspects of their primary health care needs while plant products also play important roles in the health care system of the remaining 20 %, who mainly reside in developed countries [4]. No wonder herbal medicine has attracted public attention over the past 20 years especially as this type of medicine is easily accessible in some regions [5]. However, prolonged use of these plants is associated with toxic effects [6, 7] especially as most are used indiscriminately without adequate information on their safety or toxicity risk [8]. This

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calls for the continuous evaluation of their toxicity in attempts to elucidate on possible risks associated with the practice.

Adenium obesum is a deciduous pachycaul shrub with half buried and distinctly swollen base along with twisted branches that bears sparse leaves, which are shed prior to the appearance of its characteristic pink "showy" flowers [9, 10]. Although the plant grows mostly within the Sahel to Sudanese savannahs in Africa and in also Arabia [11, 12], it is equally found worldwide where it's cultivated for ornamental purposes [13]. The bark of the plant is chewed as an abortifacient to produce miscarriages or induce abortions [14, 12] even as its latex is used to treat decaying teeth, boils and septic wounds [13 15]. Similarly, the latex and bark of the plant is used to treat bone dislocation, rheumatism, sprains, paralysis, swellings and wounds [16]. There is therefore, a need to investigate the toxicity of this medicinal plant in order to optimize its dosage, especially as pharmacology is simply toxicology at a lower dose [17] and vice versa. The fact that herbal toxicity represents a serious human health threat further makes the study very imperative [18]. Therefore, the study evaluates the toxicity and histopathological implications of the oral administration of ethanol extract of *A. obesum* stem bark in the liver of exposed Wistar rats as animal models for predicting possible effects in humans.

2. MATERIAL AND METHODS

2.1 Plant Extraction

The stems of *Adenium obesum* were gathered from the open fields of Rurum town, Rano Local Government Area, Kano State, Nigeria between the months of January – April, 2011. These were authentication with Voucher No. 1386 at the Herbarium, Department of Biological Sciences, Ahmadu Bello University (A. B. U.), Zaria, Nigeria by Mallam Musa Mohammed. The barks were removed from the stems, sun-dried and pounded into powder before soaking 3.95 kg of it in 21 L of ethanol (96.0 % vol. Sigma-Aldrich® Inc., St. Louis, MO 63178, USA) over a 72-h period. The method of Abu-Dahab and Afifi [19] was used to concentrate the filtrate to dryness in an evaporation dish at room temperature until constant weights were obtained. Preliminary screening of the extract for its phytochemical constituents was performed using the methods of Trease and Evans [20] and Harborne [21].

2.2 Wistar Rat Toxicity Bioassay

Female rats (169 - 189 g) were obtained from the Animal Unit of National Veterinary Research Institute (NVRI), Vom, Plateau State, Nigeria. The rats were acclimatized for seven days in a well ventilated room under natural photo-period (12/12-h) while being housed in clean metal cages. Fresh drinking water was provided *ad libitum* along with NVRI pelletized rat feed (crude protein - 19.97 %, crude fibre - 6.89 %, Ash - 7.60 %, nitrogen free extract - 59.21 % and moisture - 12.98 %) during the period.

The toxicity bioassay was performed as described in the OECD guideline No. 423 [22] using the pre-defined doses of the extract (300 $mgkg^{-1}$, 2000 $mgkg^{-1}$ and 5000 $mgkg^{-1}$) separately in a stepwise procedure with the use of three female rats per step based upon the presence or absence of morbidity and/or mortality from preceding dose. The unexposed control rats were given distilled water placebo. Exposed rats were observed for signs of toxicity during the first 30 minutes and daily thereafter throughout the 14-day observational period. The LD_{50} of the extract was established based on the OECD guideline No. 423 [22]. Similarly, changes in their body weights were used as a measure of toxicity [23].

2.3 Biochemical Analyses

Two millilitres of blood were collected from the exposed rats via vene-section under light chloroform anaesthesia at the end of the 14-day post administration of the extract. These were dispensed into sample tubes that were not containing EDTA anticoagulant and centrifuged at 1,006 *g* for 10 minutes to obtain the serum after allowing them to clot. The

Reference method by International Federation of Clinical Chemistry [24] was used to determine the aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities using an autoanalyzer (Bayer Express Plus, Model 15950, Germany.). Enzymatic hydrolysis method as described by King and Armstrong [25] was used to determine the alkaline phosphatase (ALP) activity.

2.4 Histopathological Analyses

The liver of experimental rats was harvested after sacrificing them under light chloroform anaesthesia at the end of the 14-day observational period. The harvested tissues were fixed in 10 % neutral buffered formalin prior to paraffin embedding, sectioning at 5 µm and staining with haematoxylin and eosin [26, 27]. These were examined under light microscopy for histopathological lesions.

The nature and severity of lesions in the liver of the exposed rats were noted and determined semi-quantitatively based on the adaptation of the degree of tissue changes (DTC) method by Poleksic and Mitrovic-Tutundzic [28] and Simonato *et al*. [29]. This involved the progressive classification of liver alterations in stages of tissue damage where the sum of the number of lesion types within each of the three stages is multiplied by the stage coefficient to give the numerical values of the DTC using the formula: DTC = (1 x $\sum$ *I*) + (10 x $\sum$ *II*) + (100 x $\sum$ *III*). Liver alterations that did not alter the normal functioning of the tissue were tagged Stage *I* alterations. Similarly, alterations that were more severe and impaired the normal functioning of the liver were tagged stage *II* alterations while those that were very severe and induced irreparable liver damage were tagged stage *III* alterations, respectively. The grading and interpretations of the results were as follows: 0 – 10 (normal liver); 11 – 20 (slightly damaged liver); 21 – 50 (moderately damaged liver); 50 - 100 (severely damaged liver); >100 (irreversibly damaged liver).

2.5 Statistical Analyses

GraphPad Prism (GraphPad Prism, version 4.0, San Diego, California, USA.) was used to analyse the data (mean ± SEM) where a one-way analysis of variance (ANOVA) was performed for statistical significance at $p<0.05$, including Tukey's multiple comparison test to compare the differences between the various means. Differences between the DTC in the liver of the exposed groups and the control group were compared for statistical significance ($p<0.05$).

3. RESULTS AND DISCUSSION

3.1 Preliminary Phytochemical Screening

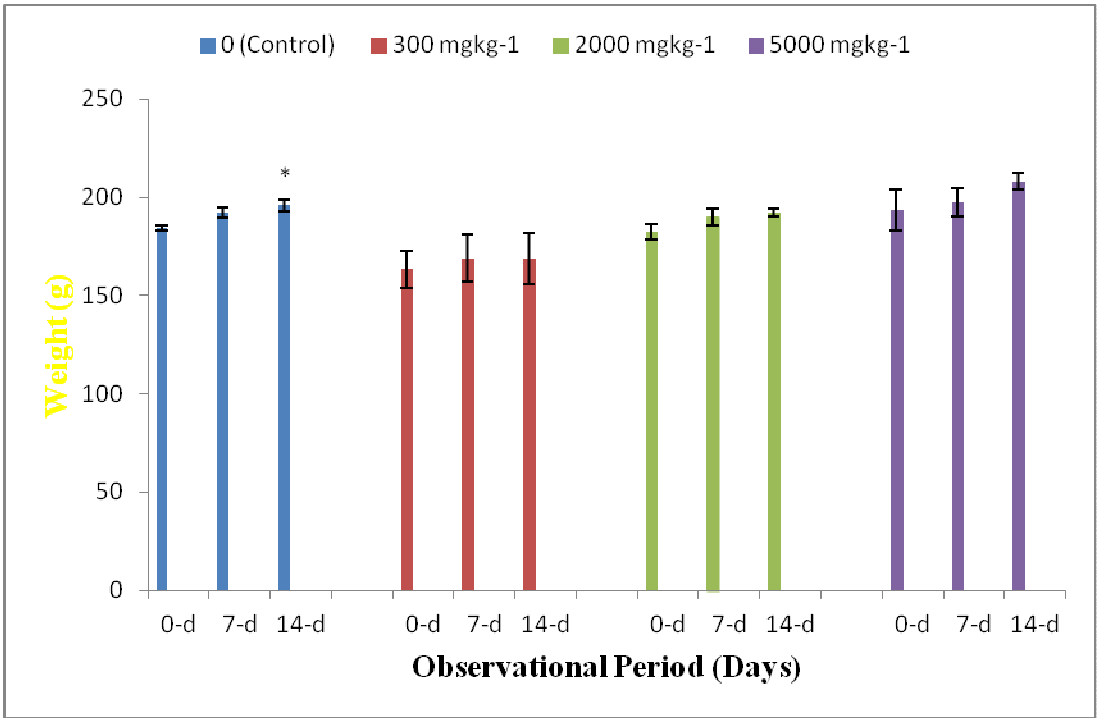
The extractive yield of the extract was 6.58 %w/w. Preliminary phytochemical screening of the extract revealed the presence of alkaloids, tannins, saponins, flavonoids, glycosides, steroids and triterpenes but no anthraquinones, which are of pharmacological and toxicological importance. Similar phytochemical constituents were reported from the aqueous and methanol extracts of *A. obesum* stem bark [30, 31].

3.2 Toxicity Bioassay

There were no obvious changes in the skin and fur, eyes and mucous membranes of the exposed rats and neither were there changes in their behavioural patterns. Similarly, no obvious signs of tremors, salivation, diarrhoea, sleep, convulsions and coma were seen in the exposed rats, including the absence of mortality. The LD_{50} of the extract was therefore, greater than 5000 $mgkg^{-1}$ or ∞ (unclassified) based on the fixed LD_{50} cut off values [22]. The absence of obvious signs of toxicity, including mortality was indicative of the very low toxicity of the extract in the exposed rats leading to very high LD50 value of > 5000 $mgkg^{-1}$ or ∞ (unclassified) based on the fixed LD_{50} cut off values [22]. This is in spite of the fact that the

plant is a potent arrow poison [13, 32]. The toxicity of the plant might be influenced by the route of administration as animals are normally exposed parenterally when the plant is used as arrow poison unlike the oral route of administration of the present study. This in addition to the fact that the toxicity of the plant is influenced by the age and parts of the plant used, genetic variation between species, climatic conditions and the soil profile of where the respective plants are found [33, 34].

There were gains in body weights of experimental rats but this was significant ($p<0.05$) only in the unexposed control rats as shown in Fig. 1. Therefore, the extract did not considerably affect the growth of exposed rats, indicative of its very low toxicity in the exposed rats. This is because toxic chemicals or drugs adversely affect growth or weight gain in exposed animals [23].



* Significantly ($p<0.05$) different from its control

Fig. 1: The effects of oral administration of ethanol extract of *Adenium obesum* stem bark on body weights of the exposed Wistar rats.

3.3 Biochemical Analyses

Changes in some biochemical parameters of the exposed rats are as shown in Figs. 2. Although there were decreases in AST and ALP activities with increased ALT activity in the exposed rats, these changes were non-significant ($p>0.05$) and also not concentration-dependent. The non-significant ($p>0.05$) changes in ALT activity showed that the extract did not cause considerable damage in the liver of the exposed rats. This is because serum enzymes are cytoplasmic and are only released into circulation in cellular damage [35] where ALT activity is more hepato-specific than AST activity [36]. Similarly, the non-significant ($p>0.05$) changes in ALP activity showed that the extract did not cause hepatobiliary problems. The toxicological importance of the decreased AST activity is unknown [37] and well less understood compared to the significance of its increased activity [38]. Mgbojikwe [30] reported similar non-significant ($p>0.05$) changes in the AST, ALT and

ALP activities of Wistar rats topically exposed to the aqueous extract of *A. obesum* stem bark.

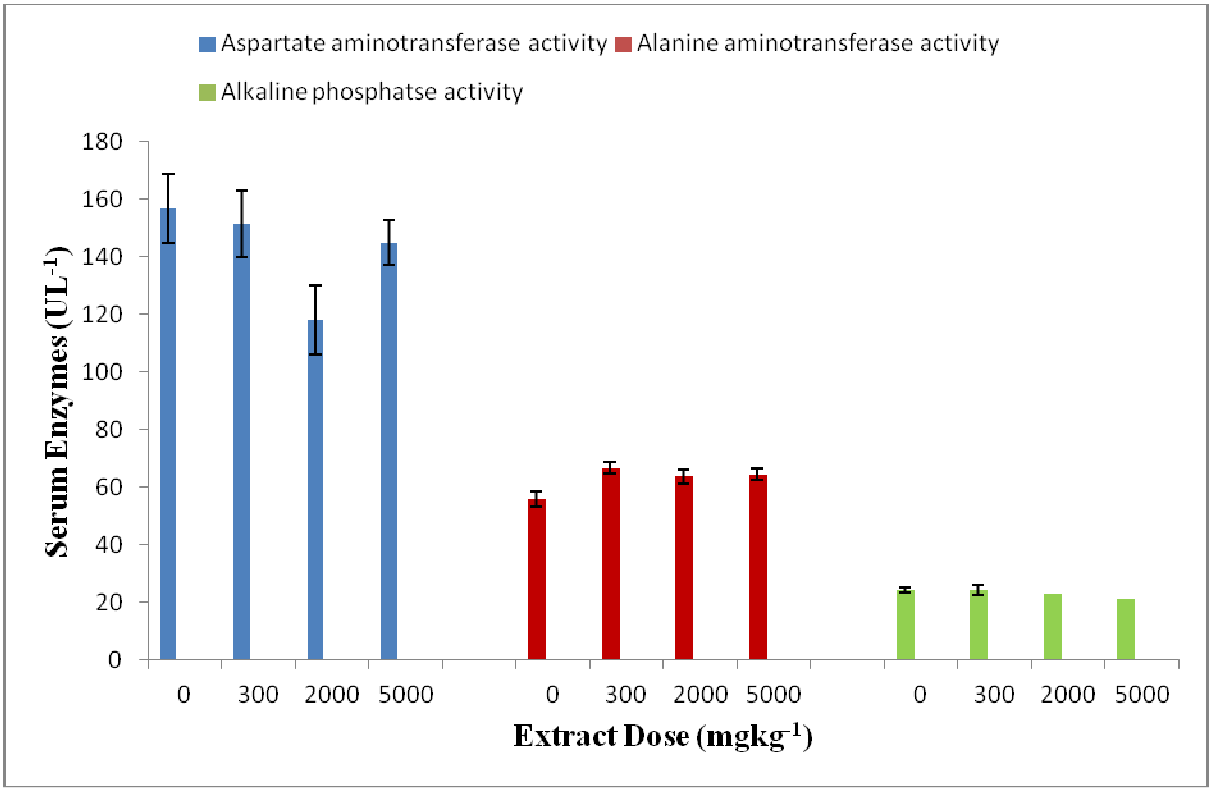


Fig. 2: The effects of oral administration of ethanol extract of *Adenium obesum* stem bark on aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase activities of the exposed Wistar rats.

3.4 Histopathological Analyses

Histopathological lesions were seen in the liver of the exposed and unexposed rats but these were comparatively to a lesser extent in unexposed rats. The liver of the exposed rats showed congestions of the central vein along with hepatic fatty degenerations as shown in Figs. 3 - 6 with a cumulative DTC value of 3.33 ± 1.67, indicative of the normal functioning of the liver based on the DTC grading. The incidence of histopathological lesions in the liver of the exposed and unexposed rats is as shown in Table 1. However, there were no significant ($p>0.05$) differences between the DTC in the liver of the exposed groups and the unexposed control group.

The observed congestion and fatty degenerative changes might be due to the unique vascular, secretary, synthetic and metabolic features of the liver [39, 40]. This is because of its ability to degrade toxic compounds but can easily be overwhelmed by elevated concentrations of these compounds resulting in its structural damage [41]. The hepatic fatty degeneration is indicative of metabolic disturbance, which is a normal feature of toxic exposures [42]. These changes are usually reversible except in some extreme cases where the functional efficiency of the affected liver might be compromised [43]. Similar congestion and fatty degenerative changes were reported in the liver of Wistar rats exposed to extracts of *Sorghum bicolor* leaf sheath [44]. The fact that the DTC in the liver of exposed groups compared to the unexposed control group was non-significant ($p>0.05$) showed that the extract did not cause considerable liver damage or hepatotoxicity in the exposed rats.

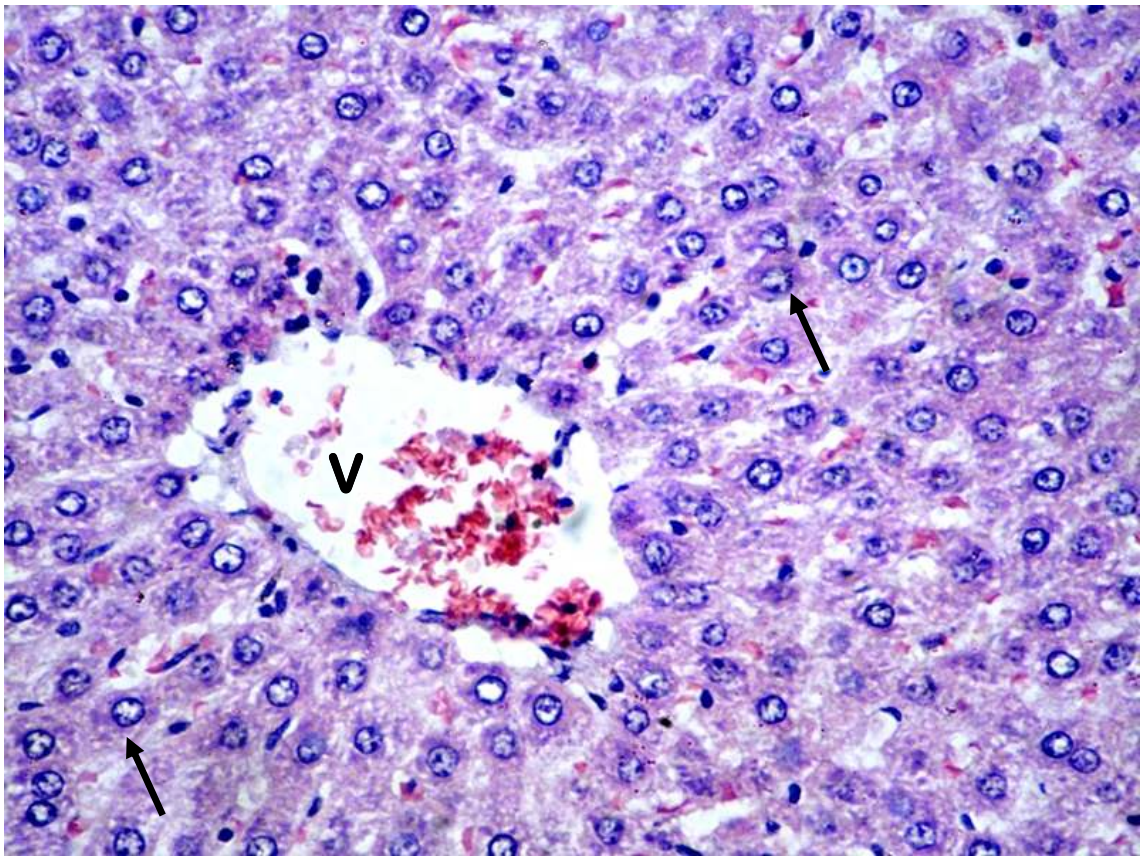


Fig. 3: Photomicrograph of the liver of the Wistar rats administered distilled water placebo (unexposed control). Note the central vein (V) and the hepatocytes (arrows). H & E x 397.

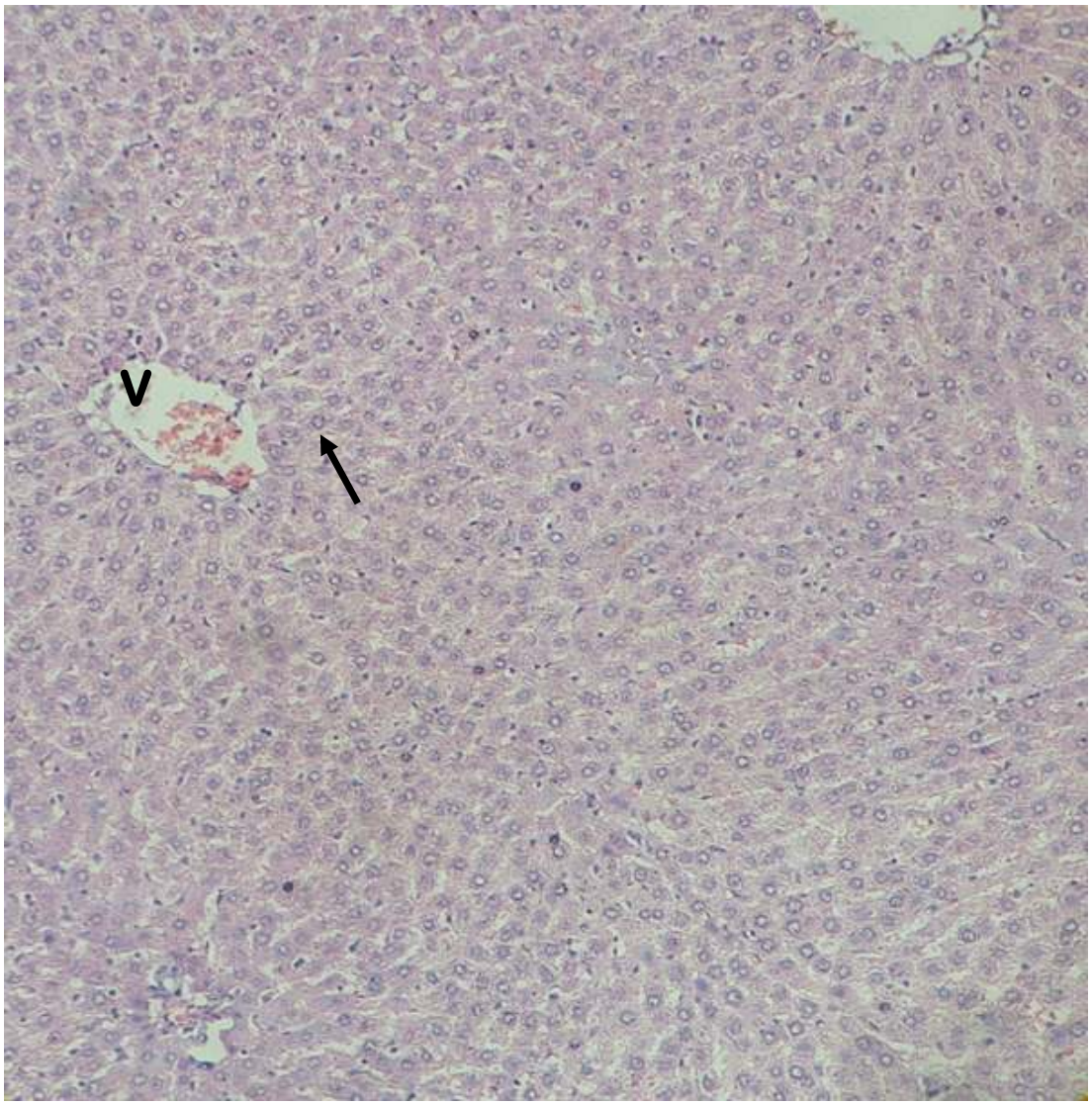


Fig. 3: Photomicrograph of the liver of Wistar rats administered distilled water placebo (unexposed control). Note the central vein (V) and the hepatocyte (arrow). H & E x 64.

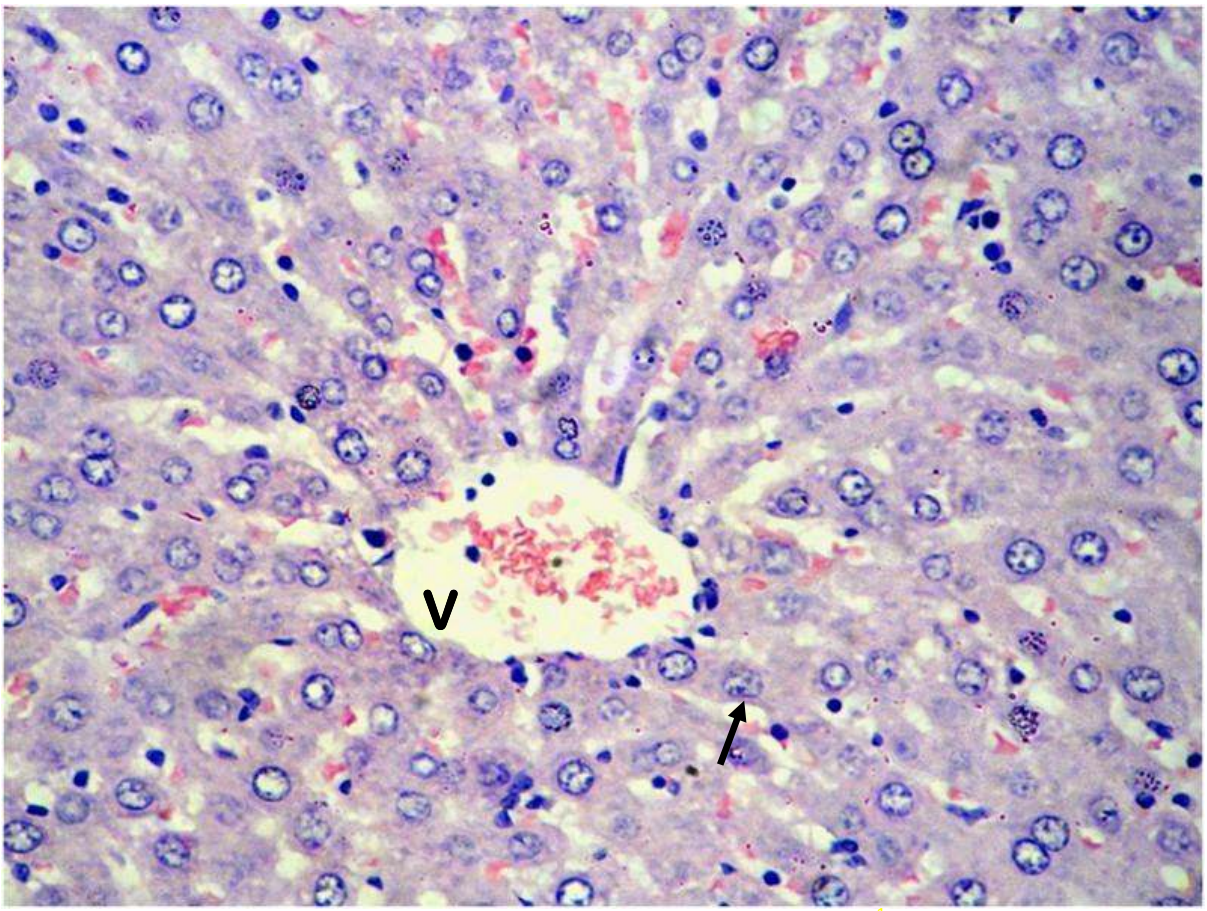


Fig. 4: Photomicrograph of the liver of Wistar rats administered 300 $mgkg^{-1}$ of ethanol extract of *Adenium obesum* stem bark. Note the central vein (C) and the hepatocyte (arrow). H & E x 397.

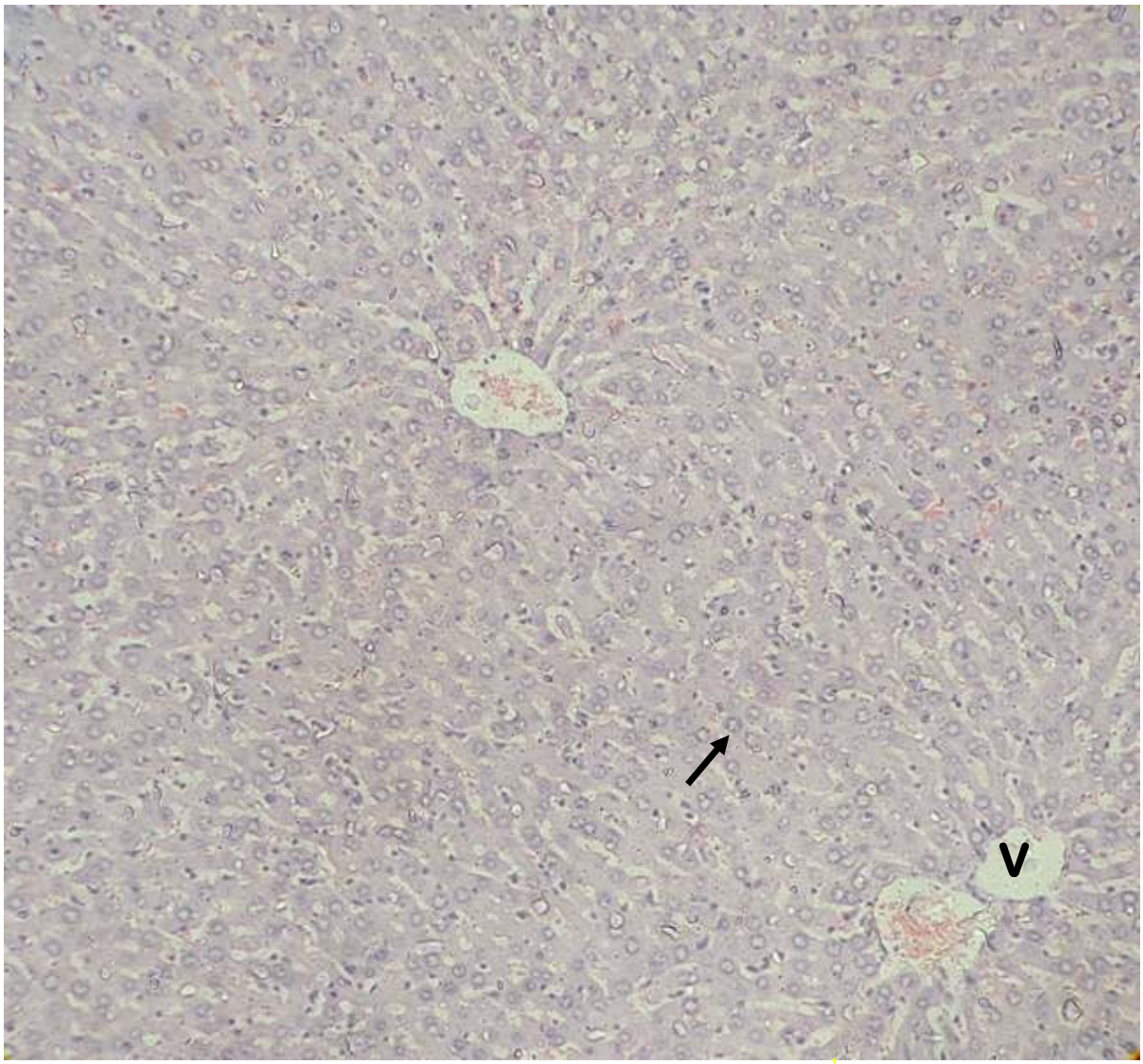


Fig. 4: Photomicrograph of the liver of Wistar rats administered 300 $mgkg^{-1}$ of ethanol extract of *Adenium obesum* stem bark. Note the central vein (C) and the hepatocyte (arrow). H & E x 64.

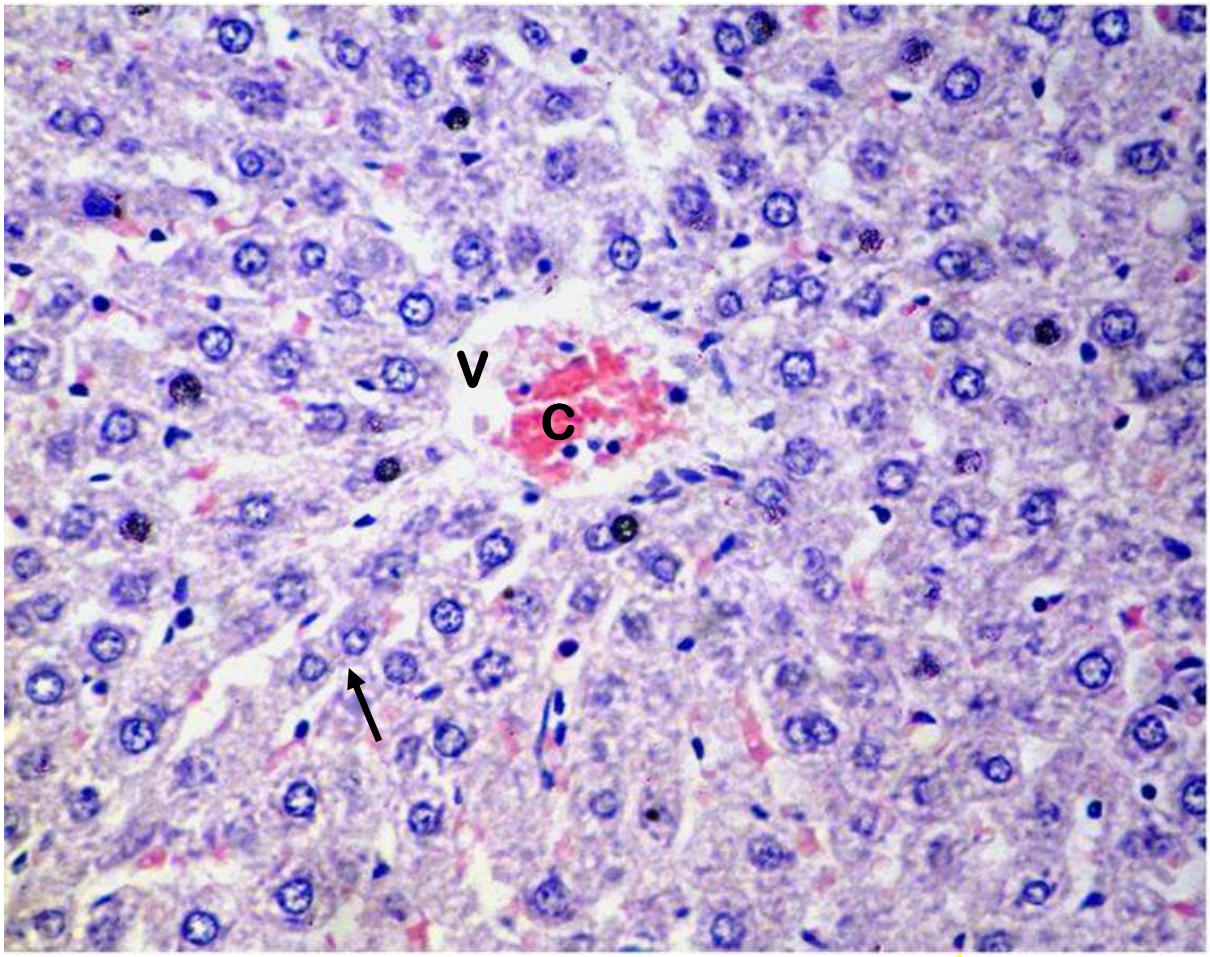


Fig. 5: Photomicrograph of the liver of Wistar rats administered 2000 $mgkg^{-1}$ of ethanol extract of *Adenium obesum* stem bark. Note the central vein (V) with congestion (C) and the hepatocyte (arrow). H & E x 397.

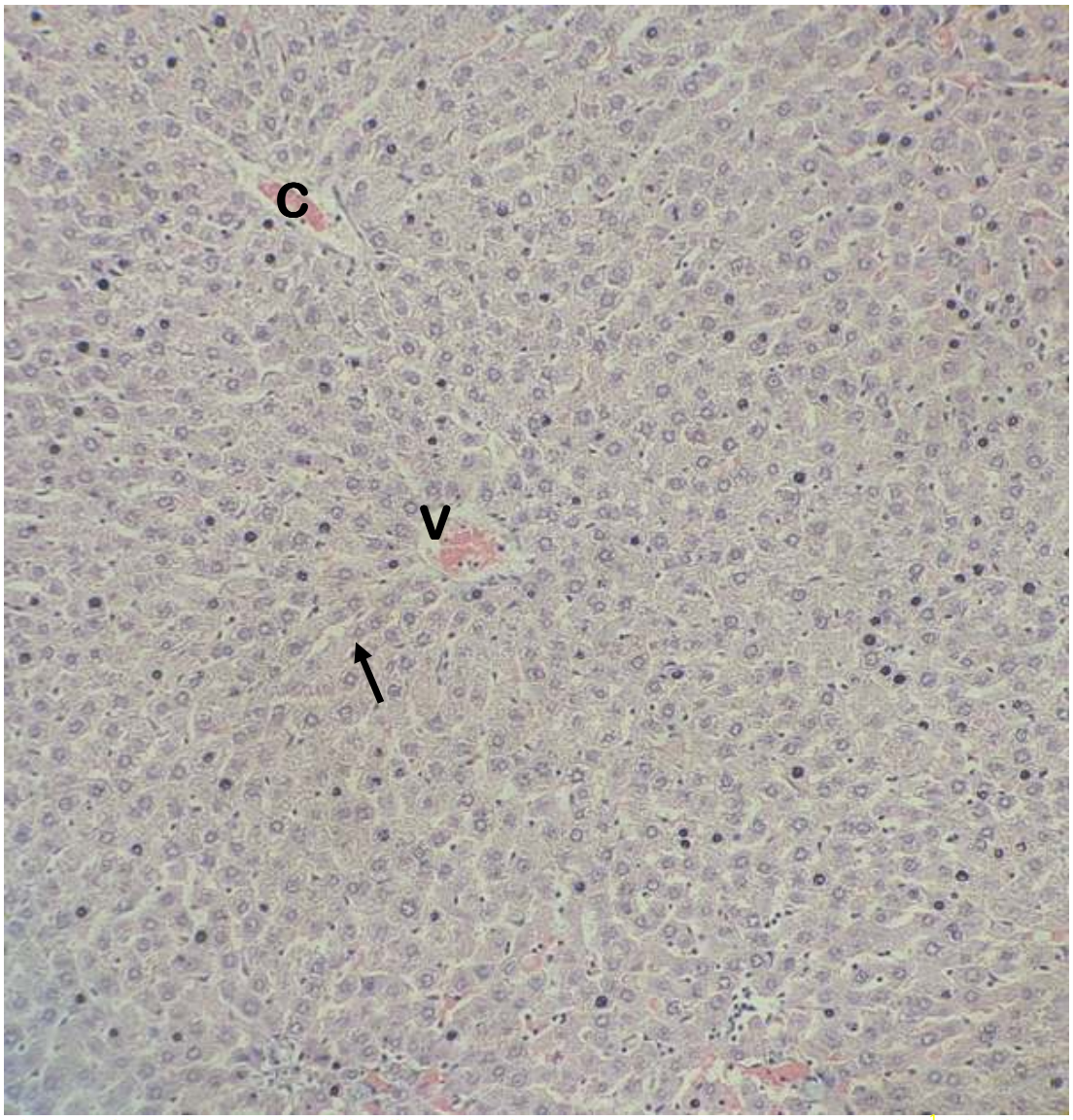


Fig. 5: Photomicrograph of the liver of Wistar rats administered 2000 $mgkg^{-1}$ of ethanol extract of *Adenium obesum* stem bark. Note the central vein (V) with congestion (C) and the hepatocyte (arrow). H & E x 64.

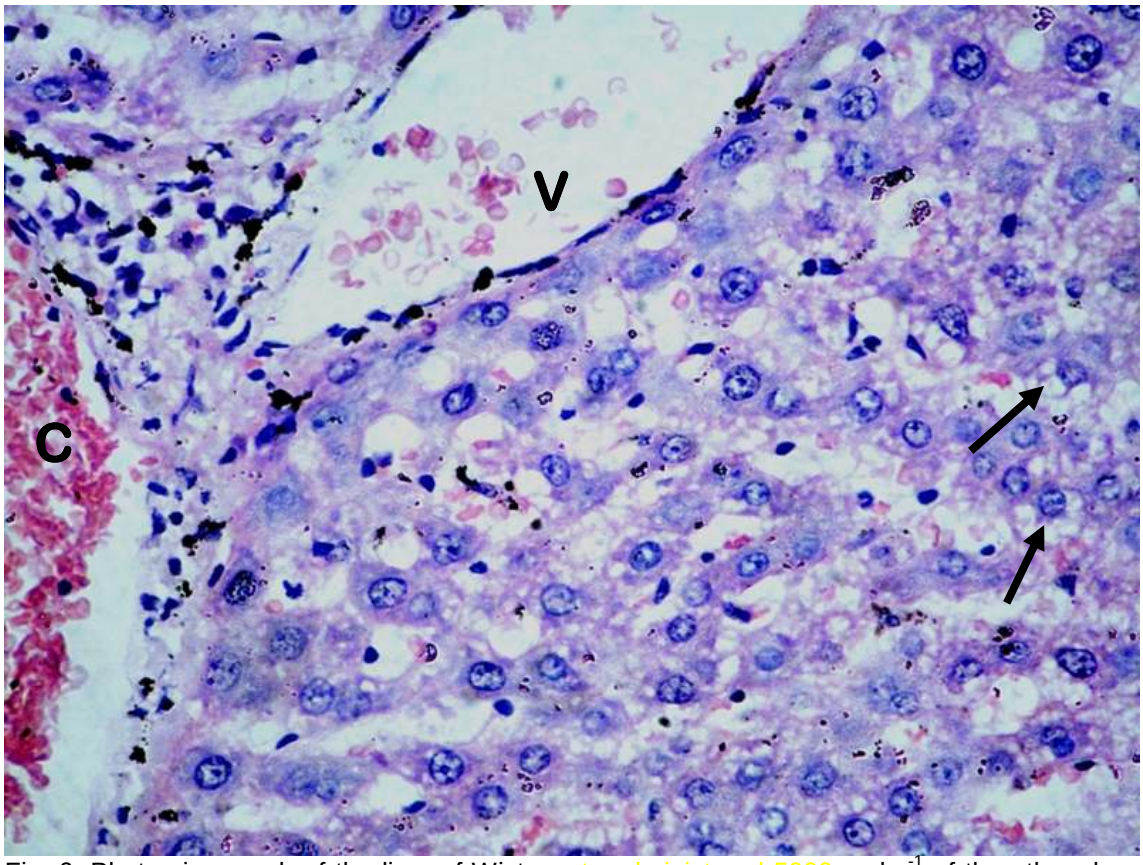


Fig. 6: Photomicrograph of the liver of Wistar rats administered 5000 $mgkg^{-1}$ of the ethanol extract of *Adenium obesum* stem bark. Note the central vein (V) with congestion (C) and vacuolation of the hepatic cells (arrows). H & E x 397.

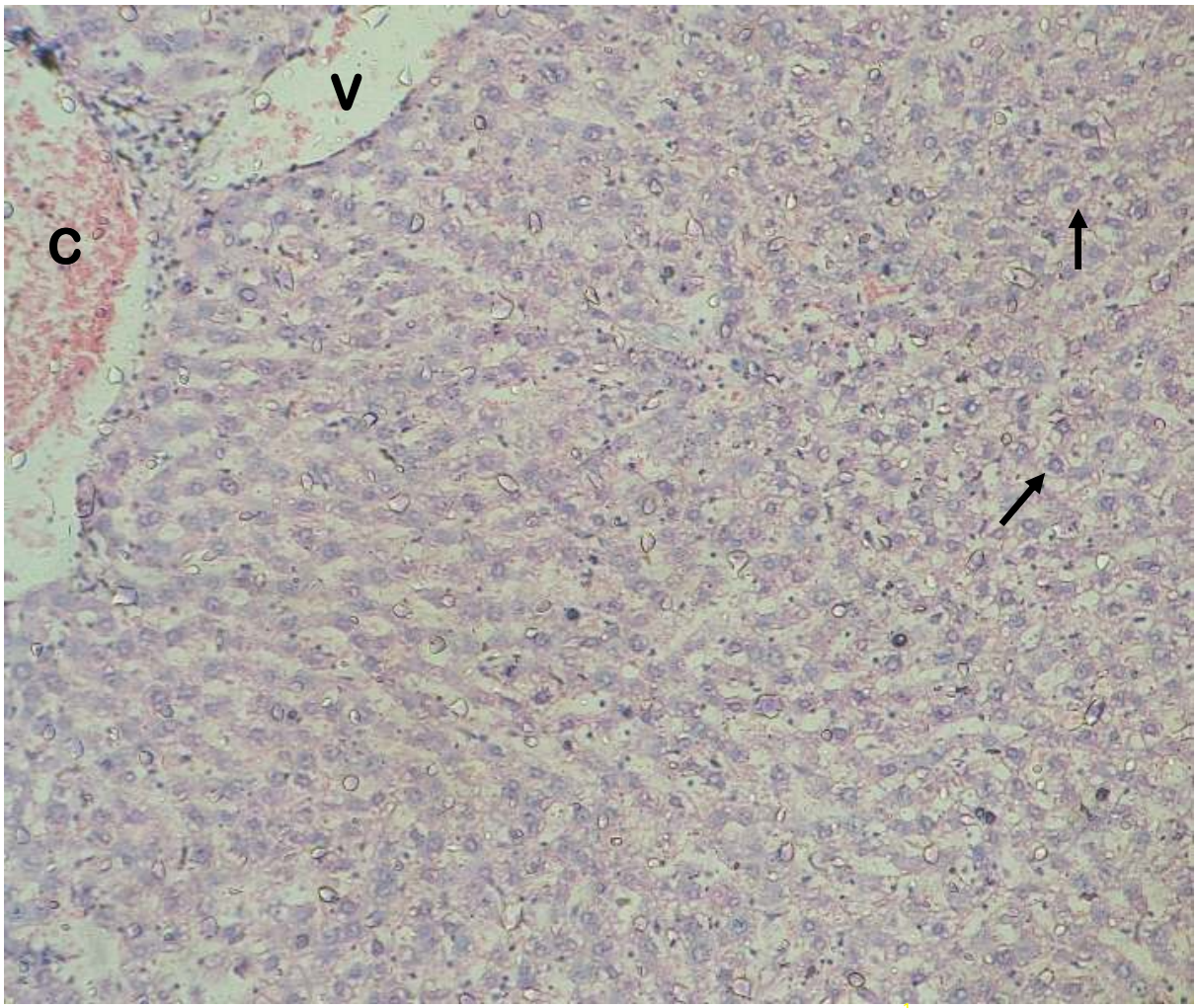


Fig. 6: Photomicrograph of the liver of Wistar rats administered 5000 mgkg^{-1} of the ethanol extract of *Adenium obesum* stem bark. Note the central vein (V) with congestion (C) and vacuolation of the hepatic cells (arrows). H & E x 64.

Table 1: The incidence of degree of tissue changes (DTC) in the liver of Wistar rats exposed to ethanol extract of *Adenium obesum* stem bark

Histopathological lesions	**DTC stage**	**Extract dose**			
		0 (Control)	**300 mgkg^{-1}**	**2000 mgkg^{-1}**	**5000 mgkg^{-1}**
Vacuolations	I	0	0	0	+
Congestion	II	0	+	+	+

(0) – absent; (+) – rare; (++) – low incidence; (+++) – high incidence

4. CONCLUSION

The ethanol extract of *A. obesum* stem bark did not cause major liver damage and therefore, is a safe oral medicinal plant within the limitations of the study's extract dose and exposure period. However, there is a need for further investigation over repeated and prolonged exposures.

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COMPETING INTERESTS

We declare the existence of no competing interests

AUTHORS' CONTRIBUTIONS

Author A was responsible for the conceptualization and design of the work in addition to data collection, analyses and interpretations with the manuscript preparations. Author B was involved with the study's conceptualization and design, and data interpretations. Author C partook in the design of the study and data interpretation. Author D was also involved with the design of the study and data analysis with interpretations. All authors read and approved the final document.

CONSENT (WHERE EVER APPLICABLE)

None

ETHICAL APPROVAL (WHERE EVER APPLICABLE)

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee

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DEFINITIONS, ACRONYMS, ABBREVIATIONS

Here is the Definitions section. This is an optional section.

Term: Definition for the term

APPENDIX