Effects of Chronic Administration of Hydroethanolic Stem Bark Extract of *Anthocleista djalonensis* A. CHEV (Loganiaceae) on Liver Parameters in Wistar Rats

Serge David Dago Attemene*[1,5], Sylvain Beourou[5], Philippe Sansan Kambou[2], Joseph Alico Djaman[3,4], Seraphin Kati-Coulibaly[1]

[1]*Laboratory of Nutrition and Pharmacology, Faculty of Biosciences, University of Felix Houphouet Boigny, Côte d'Ivoire. 22 PO Box 582 Abidjan 22 (Côte d'Ivoire).*

[2]*Laboratory of Medical Biochemistry, Faculty of Medical Sciences, University of Felix Houphouet Boigny, Côte d'Ivoire. , PO Box 240 Abidjan 01(Côte d'Ivoire).*

[3]*Laboratory of Biochemistry and Pharmacodynamy Faculty of Biosciences, University of Felix Houphouet Boigny, Côte d'Ivoire, 22 PO Box 582 Abidjan 22 (Côte d'Ivoire).*

[4]*Laboratory of fundamental biochemistry, Institut Pasteur of Cote d'Ivoire, 01 PO Box 490 Abidjan 01.*

[5]*Laboratory of malariology, department of parasitology and mycology, Institut Pasteur of Cote d'Ivoire, 01 PO Box 490 Abidjan 01.*

ABSTRACT

Anthocleista djalonensis A CHEV (Loganiaceae) has been widely used to treat various ailments in traditional medicine. The aim of this study was to investigate potential liver toxicity of the hydroethanolic stem bark extract of *Anthocleista djalonensis* A CHEV. The acute toxicity of the extract was assessed in groups of mice. For the chronic toxicity, five groups of 10 rats including a satellite group were orally administered the extract at doses of 400mg/Kg, 800mg/Kg and 1200mg/Kg-bw daily for 180 days except for the control group which received distilled water. Results showed that the extract LD_{50} was found to be 17,79g/Kg-bw in male and 15,84g/Kg-bw in female Swiss mice. For the haematological and biochemical parameters, the extract decreased blood platelets in female rats and lower white blood cells in male rats. Moreover, a significant increase of alkaline phosphatase was recorded in both sexes of rats at the dose of 1200mg/Kg-bw. At this same dose, an increase of Aspartate Amino Transferase and Gamma Glutamyl Transferase was respectively noticed in both female and male rats. However, a decrease of conjugated bilirubin was noticed in both sexes of animals at a dose of 1200mg/Kg-bw. The histopathology examination of liver showed abnormalities in tissues in both sexes of rats at any dose. This study showed that the extract might induce thrombocytopenia in female rats and weaken male rats body defense at the doses of 800mg/Kg and 1200mg/Kg body weight. To sum up, the hydroethanolic stem bark extract of *Anthocleista djalonensis* A CHEV at studied doses may be toxic to liver by affecting its functioning.

INTRODUCTION

For years immemorial medicinal plants have been involved in treating various diseases [1] and about 80% of people living in developing countries are still relying on them to tend to their ailments [2]. Medicinal plants are known to contain a variety of substances [3] such as alkaloids, glycosides, essential oils, tannins which are responsible for its therapeutic effects. The many xenobiotics it contains are not risk free [4] and can be harmful to liver hepatocytes by causing damages to the body integrity [5]. *Piper methysticum* rhizoma (Kava kava) for instance used for its relaxing and anxiolytic properties was reported to be responsible for acute cytolytic hepatitis [6] as well as the seed extract of *Azadirachta indica* which caused microvesicular steatosis [7]. Liver, being the major organ for detoxication and removal of endogenous compounds, is permanently exposed to xenobiotic that can lead to its functions impairment [8, 9].

Anthocleista djalonensis A. CHEV which belongs to the family of Loganiaceae, is a medium-sized tree of West tropical Africa, 30 to 45 feet-high, with blunt spines on the unbranched, pale grey trunk and wide spreading crown [10, 11]. The plant is used for its powerful purgative and diuretic virtues [12]. Leaves and roots decoction of the plant is used to treat dermal mycosis, filariasis and infectious jaundice [13]. Moreover, the ethanolic roots extract showed antidiabetic activity [14]. The aim of this study was to investigate the effect of chronic intake of hydroethanolic bark extract of *Anthocleista djalonensis* A. CHEV on liver functions in Wistar rats.

MATERIAL AND METHODS

Plant material

The stem bark of *Anthocleista djalonensis* A. CHEV was collected in Abidjan in February 2015. Barks were carefully washed with tap water, air dried and shade for 3 weeks and grounded into powder. Plant sample was authenticated at the National Floristic Center, University of Felix Houphouet Boigny, (Abidjan, Côte d'Ivoire).

Experimental animals

9 weeks aged Swiss mice of both sexes with body weight ranging from 22g to 25g and 11 weeks Wistar rats of both sexes weighing 110g to 120g were used for this study were

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obtained from the animal husbandry of the Department of Nutrition and Pharmacology, Faculty of Biosciences, University of Felix Houphouet Boigny (Abidjan, Côte d'Ivoire). Animals were housed in plastic cages in a temperature and light controlled room with 12h dark and 12h light cycle. They were fed with pellets and given water ad libitum. Males were separated from females to avoid copulation during experiment. All experiments in this study were conducted in accordance with the international standards of animal welfare as recommended by the European Union on animal care (CE Council 86/609).

Methods

Preparation of extract

The hydroethanolic bark extract of *Anthocleista djalonensis* A. CHEV (HEAd) was carried out according to the method described by Zirihi et *al* [15]. 100g of *Anthocleista djalonensis* A. CHEV stem bark powder was macerated with 70% ethanol (1.5 L) at room temperature and was frequently shaken for 72 h and filtered through cotton sieve, then on whatman filter paper for 24 h. The filtrate was collected and concentrated by evaporation to dryness in a drying oven at 40°C using evaporating dish for 48 h to obtain a dry extract which was stored at 4°C for further use.

Toxicological studies

Acute toxicity study

The acute toxicity study of stem bark of *Anthocleista djalonensis* A CHEV was performed on Swiss mice by using OECD guidelines 420 [16]. Fifty (50) Swiss mice of both sexes were used for the test. Mice were gathered in 5 groups of 10 animals each (5 males and 5 females) and were kept 18h fasting prior to extracting administration [17]. A single dose of the extract range from 12 to 24 g/Kg body weight of HEAd was administered by oral route to each group of Male and female mice. After administration of extract, animals were observed individually for signs of toxicity and mortality the first four hours and therefore daily for 15 days [18]. LD_{50} of the extract was determined by using both the graphic Method of Miller and Tainter [19] and the arithmetic method of Karber and Berhens [20].

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Liver toxicity assessment

50 healthy Wistar rats of both sexes divided into five (5) groups of 10 animals each (5 males and 5 females) were used for this study, male and female rats were separated during experimentation and were orally administered a single dose of extract daily for 180 days.

Group 1: received distilled water, **Group 2**: Received 400mg/Kg bw of HEAd extract, **Group 3**: Received 800mg /Kg bw of HEAd extract, **Group 4**: Received 1200mg/Kg bw of HEAd extract. **Group 5** (Satellite): Received 1200mg/Kg bw of HEAd extract. On completion of experimental period, blood was collected from rats of group 1 to 4 for hematological and biochemical parameter analyses to investigate liver functions. As for group 5, 28 days without treatment was added to the experimental period before blood collection.

Body weight measurements

Individual body weight of each group of rats was recorded prior to the administration of extract and monthly throughout experimental periods.

Blood sampling

At the end of the experimental period, rats of each group were anesthetized using diethyl ether and blood was collected by tail incision according to the modified method described by **Flutter et *al*** [21]. The tails of anesthetized rats were cut using scissors. The rat was vertically held, for the tail to be directed downwards into the collecting tube. The tail of the animal was slightly massaged with both thumb and index to allow blood to adequately flow into the collecting tubes duly identified. After collection, the tip of the tail was cleaned with cotton soaked with ethylic alcohol 90 ° and the animal was put back to its original cage.

Hematological analyses

Blood samples were collected in EDTA tubes and the hematological analyses were performed using an automated hematological analyzer (Sysmex) at the hematology department of Cocody teaching hospital (Abidjan, Côte d'Ivoire). The following parameters such as Reb blood cell (RBC), Erythrocyte count, Hemoglobin, Hematocrit, Mean corpuscular hemoglobin concentration (MCHC), white blood cell (WBC), lymphocytes, eosinophils, neutrophils, monocytes and platelet counts were determined.

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Biochemical analyses

Blood samples were collected in 4cc dry tubes without anticoagulant and were centrifuged at 3000rpm for 5 minutes. Serum samples were removed and kept in Eppendorf tubes and stored at -20°C. Serums were further analysed using automated biochemistry analyzer at the hematology department of Cocody teaching hospital (Abidjan, Côte d'Ivoire) to determine the level of Alanine amino transferase, Aspartate amino transferase, Alkaline phosphatase, Gamma glutamyl transferase, Total bilirubin, Conjugated bilirubin, Triglycerides, Total proteins, Total cholesterol, HDL, LDL and albumin.

Histopathological examination

At the end of treatment, animals were sacrificed and livers were collected and preserved in 10% buffered formalin and then dehydrated with progressively increasing concentration of ethanol. After being impregnated with paraffin, thin section of tissue organs was cut and stained with hematoxylin and eosin were examined under the microscope (LEICA).

Statistical analyses

Graphics were performed using Graphpad Prism 5 software (Microsoft, San Diego California, USA).

All values were expressed as mean ±SD. Data analysis were performed using one way analysis of variance (ANOVA), followed by Tukey-Kramer multiple comparisons test using graph pad instat® software. Values were statistically significant at P< 0.05.

RESULTS AND DISCUSSION

Results

Acute toxicity

After the oral administration of the hydroethanolic stem bark extract of *Anthocleista djalonensis* A. CHEV (HEAd) several clinical signs were observed according to received dose. 2 hours after extract ingestion, mice were motionless, sleepy and abdominal writhing was noticed. Moreover, they refused to drink and eat and death was recorded in all groups of animals. The LD_{50} of the hydroethanolic stem bark extract of *Anthocleista djalonensis* A.

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CHEV (HEAd)was 17.782g/Kg in male mice and 15.848 g/Kg in female mice (Figure1A and 1B).

Liver toxicity assessment

Evolution of body weight

Body weight of rats treated with HEAd extracts was assessed before and during experiment. A weight gain in all treated animals including controls was observed regardless of sex, figure (2A and 2B). In male rats treated with HEAd, a weight gain was observed in all groups compared to control. At doses of 800mg/kg and 1200mg/kg a highly significant weight gain (P < 0.001) were observed respectively with $113.8 \pm 9.81\%$ and $120.04 \pm 8.76\%$. However, at a dose of 400mg/Kg, a nonsignificant weight gain (P >0.05) as compared to the control (figure3A) was observed. In female rats, at doses of 400mg/Kg and 800mg/Kg, a nonsignificant weight gain (P >0.05) was recorded compared to control. Whereas at a dose of 1200mg/Kg there was a highly significant weight gain (P < 0.001) with a value of (74.22 ±6.66%) compared to control. (Figure 3B)

Relative organs weight

In male rats treated with HEAd a non-significant (P >0.05) decrease of liver relative weight was recorded at the doses of 800mg/Kg and 1200mg/Kg. Whereas in female rats a significant (P < 0.01) increase of this organ weight was observed at the dose of 1200mg/Kg (Figure 4A and B).

Haematological parameters

The haematological indices observed after 180 days HEAd administration in rats was tabulated in table (2). In male and female rats treated with HEAd, a non-significant rate difference (P>0.05) was observed for the following hematological parameters such as hemoglobin, red blood cells, hematocrit, MCV, MCHC, MCH, neutrophils, eosinophils, lymphocytes and monocytes compared to the control. However, a highly significant decreased (P <0.001) of blood platelets in female rats was recorded at the doses of 800 mg/Kg and 1200mg/Kg with values of $952 \pm 33.05.10^3/\mu L$ and $930 \pm 13.92.10^3/\mu L$ respectively (table 2B). Furthermore, a highly significant decrease (P <0.001) level of white blood cell count (WBC) with values of $13.63\pm1.93.10^3/\mu L$, $11.33\pm0.76.10^3/\mu L$ and

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$13.92 \pm 0.07.10^{3}/\mu L$ was observed for doses of 800 mg/Kg, 1200mg/Kg and in males satellite group respectively.

Biochemical parameters

The biochemical test results obtained after 180 days oral administration of HEAd to rats were shown in tables (3). We noticed a significant increase of AST, ALAT, ALP and GGT in both sexes of rats. A significant increase (P <0.001) of AST level at the dose of 1200mg/Kg in male and female rats with respective values of $261,5 \pm 4,72$ IU/L and 268 ± 6.73 IU/L. As for ALAT, a significant increase of this parameter in male and female rats a dose of 1200mg/Kg with values of 70.25 ± 4.99 IU/L and 67.75 ± 6.44 IU/L respectively. ALP rate for both sexes of rats was highly significant (P <0.001) with values of 196 ± 4.6 IU/L and 125.6 ± 10.23 IU/L at a dose of 1200mg/Kg respectively for male and female rats. As for GGT, a significant increase was observed at a dose of 1200mg/Kg in both sexes of rats with values of $8,5 \pm 0,57$ IU/L and $9,25 \pm 0,95$ IU/L for male and female respectively. However, for conjugated bilirubin, a highly significant decrease was recorded in both sexes of animals at the dose of 1200mg/kg with a respective values of $2,75 \pm 0,57$ mg/L and $6,75 \pm 0,95$ mg/L for male and female rats (table 3). Furthermore, no significant difference (P>0.05) was observed for the level of total cholesterol, HDL, LDL, Triglycerides and total proteins, albumin, glucose in both in male and female rats at experimental doses (Table 3).

Histopathological examination

Notable abnormalities related to extract administration were noticed in experimental animals. Light microscopic observation of rat's organs treated with HEAd at doses of 400mg/kg, 800 mg/kg and 1200 mg/kg showed histopathological changes in liver compared to the control group (Figures 4 and 5). The liver of the control rats showed characteristic features showing normal central vein lined by endothelial cells, radiating hepatic cells and hepatic sinusoids (Figure 4A). However at doses of 400mg/kg and 800mg/Kg hepatocyte necrosis and lesions of hepatocyte degeneration were observed in the liver of both male and female rats (Figures 4B, 4C, 5B and 5C). At the dose of 1200 mg/kg bw cell degeneration lesions followed by the dilation of sinusoidal capillaries were observed in treated rats. 28 days after discontinuation of treatment, the liver of satellite rats treated with HEAd at the dose of 1200mg/Kg bw showed hepatic peliosis lesion with sinusoidal cavities dilatation containing a serohematic substance (Figures 4D, 5D and 4E, 5E).

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DISCUSSION

The assessment of acute toxicity consists of recording various adverse effects that appear after administration of a tested substance [22]. The LD_{50} of HEAd was estimated to be 17.782 g/Kg body weight in male Swiss mice and 15.848 g/Kg bw in Swiss female mice. Those results were different from those obtained by Bassey et *al* [23] who observed a LD_{50} of 2230mg/Kg with the ethanolic extract of *Anthocleista djalonensis* A CHEV by intraperitoneal route. The observed difference could be linked to the route of administration [24]. Through oral route, drugs may be destroyed by gastric acid and digestive juices and this sometimes make drugs inefficient and only a part is absorbed. In the intraperitoneal route, drugs do not go to the stomach so could not be destroyed by enzymes and stomach acids but directly reach the bloodstream and could entail greater risk of adverse effects [25]. According to the chemical labeling and classification defined by Gosslin et *al.* [26]. Natural extract with LD_{50} greater than 15000mg/kg bw is considered non-toxic. According to those authors, HEAd is almost non-toxic in Swiss mice. The LD_{50} of HEAd could be comparable to some plants of the pharmacopoeia such as *Stachytarpheta indica* with a LD_{50} of 19837.5mg/Kg bw [27] and *Anredera cordifolia* TEN. V. STEENIS with a LD_{50} of 15000 mg/Kg [28]. This study showed that HEAd is considered non-toxic in Swiss mice by oral route.

In this study, a body weight gain was recorded in all experimental animals notwithstanding the sex. Variation in body weight gain for laboratory animals is an indicator of adverse effects of drugs and chemicals [29, 30]. This body weight gain could be related to food and water intake which is considered to be the fundamental elements of any growth [31] and by the accumulation of fat in those animals [32]. This observation demonstrates that the extract did not have any anti-nutritional effect in treated rats and might not interfere with the normal metabolism of all treated animals. The observed effect of HEAd on weight gain in test animals is similar to that of *Phyllanthus amarus* SCHUM THONN [33] which entailed body gain weight as well. However, the slight decrease in the body weight gain noticed in the female satellite group after discontinuation of treatment could be linked to a loss of appetite.

In this study, an increase in the liver relative weight was recorded in female rats at the dose of 1200mg/Kg and in the satellite group. The liver is a target organ for toxic chemicals on account of their functions in the body detoxification and excretion and is sensitive to harmful compounds [34]. This increase in organ weight may be due to the bioaccumulation of some phyto-compounds in this organ or to inflammation [35].

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As for male rats, a non-significant decrease of liver relative weight was recorded at doses of 800 mg/Kg, 1200mg/ Kg body weight and in the satellite group and this may be due to the cellular construction ability of the extract. Moreover, the difference observed in the liver relative weight of both genders could be explained by the fact that some chemicals may be more toxic to one gender than the other [36].

The hematological parameters assessment can be used to determine the harmful effects of plant extracts through animals' blood [37] by providing information on the bone marrow activity [38].

The administration of HEAd did not produce any significant change in red blood cells and hemoglobin rate in rats regardless of sex. HEAd did not affect the erythropoietic system of rats at experimental doses. However, at doses of 800mg/kg and 1200mg/Kg, HEAd led to a significant reduction of platelets level in female rats but the observed decrease tend to come to normalcy after discontinuation of extract intake in the satellite group. Blood platelets play a key role in homeostasis and its decrease in blood is called thrombocytopenia [40] and is associated with chronic liver disease [41, 42].

Decreased production of platelets may be due to the extract ability to cause a hyperproliferation of megakaryocytic cell line entailing a deficient thrombopoietin [43] (Turgeon, 2005) or by a diffuse intravascular coagulation [44]. Thrombocytopenia could also be mediated by formation of platelets antibodies, HEAD at quoted doses could stimulate formation of platelets antibodies as well as chlorothiazides and its various congeners [45] lowering production of platelets in bone marrow by reducing thrombopoiesis. Normalcy of platelets observed after discontinuation of the extract may be due to a progressive recovering of thrombopoiesis in the bone marrow. The extract might have induced higher production of oestrogenic hormones in female rats which appear to affect platelet kinetics, by facilitating reticuloendothelial phagocytosis and by impairing thrombopoiesis [45].

The role of white blood cells is to help the body to fight against infections and any invasion of foreign body [3]. In this study, HEAd did not show any significant difference in rate of white blood cell in female rats but a significant decrease of this parameter was registered at doses of 800mg/kg and 1200mg/Kg in male rats and did not reverse in the satellite group. This decrease of white blood cells called lymphocytopenia could be due to the alteration of white blood cells production by causing a progressive suppression of leucopoiesis in the bone

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marrow [46, 47]. This suggests that HEAD at doses of 800mg and 1200mg/kg may be deleterious to male rats by weakening their body immune system.

Liver plays a key role in the metabolism, detoxification and secretory functions in the body and its alteration or destruction could be deleterious with no effective remedies [48, 49].

In this study, the rate of Aspartate amino transferase increased significantly in both male and female rats at a dose of 1200mg/kg. Likewise the rate of alanine aminotransferase increase in both male and female but significantly in male rats. The observed increase of these enzymes in animal serums may be due to pathological changes, causing an increase in the permeability of cell membranes entailing a leakage of these enzymes from the periportal region and its release into the bloodstream [50]. HEAD at higher dose may damage hepatocytes and the endoplasmic reticulum in Wistar rats.

As for Alkaline phosphatase, an increase at a dose of 1200mg/Kg both in male and female rats treated with HEAd was noticed as well as the level of Gamma Glutamyl Transferase at a dose of 1200mg/Kg in male rats. The high level of ALP in plasma could be due to cholestasis or biliary obstruction which increases synthesis and a release of ALP in blood [51, 52]. Moreover, Gamma Glutamyl Transferase is a membrane enzyme that is an indicator of hepatobiliary toxicity [53]. An increase of both Gamma Glutamyl Transferase and alkaline phosphatase usually points out to hepatobiliary injury [54]. HEAd could increase pressure on biliary gall at a dose of 1200mg/Kg in rats.

The administration of HEAd showed a decrease of conjugated bilirubin at higher dose, Conjugated bilirubin is low, maybe because the extract did not reduce the ability of the hepatocytes to conjugate bilirubin.

The level of total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, total proteins, albumin and blood glucose did not vary significantly according to the control group in this study. HEAD did not have any deleterious effect on those biochemical parameters in Wistar rats.

Histopathological analysis is mostly used to determine morphological changes in organs and ascertain possible effect of xenobiotics on tissues [58]. Histology findings from this study at experimental doses highlighted lesions in liver rats such as hepatic necrosis, hepatic degeneration and hepatic peliosis confirming the raise of liver enzymes in experimental

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animals due to tissue leaking allowing those enzymes to flow in the bloodstream [59]. These results were similar to the methanolic leaves extract of *Pterospernum acerifolium* L Willd at doses of 1000mg/Kg and 2000mg/Kg in rodents [18]. At a dose of 1200mg/Kg bw an hepatic peliosis lesion was observed in rats. Peliosis is a pathological entity characterized by the gross appearance of multiple cyst-like, blood-filled cavities within parenchymatous organs and has been related to hematological malignancies, intravenous drug abuse, chronic alcoholism and to oral intake of contraceptives or steroids [60]. In addition, this study showed that discontinuation of HEAd treatment with the highest dose to rats did not lead to reversibility of the observed hepatotoxic effects within twenty eight days. HEAd at tested doses might induce liver tissue damage.

CONCLUSION

To sum up, this study showed that HEAd might cause thrombocytopenia in female rats at higher doses and may also weaken male rats body defense. Moreover, this plant extract may entail liver toxicity in Wistar rats and could not be safe for a long term usage. Therefore, caution should be taken in a prolonged intake of this medicinal plant.

Conflict of interest

The authors declare no competing interests.

Appreciations

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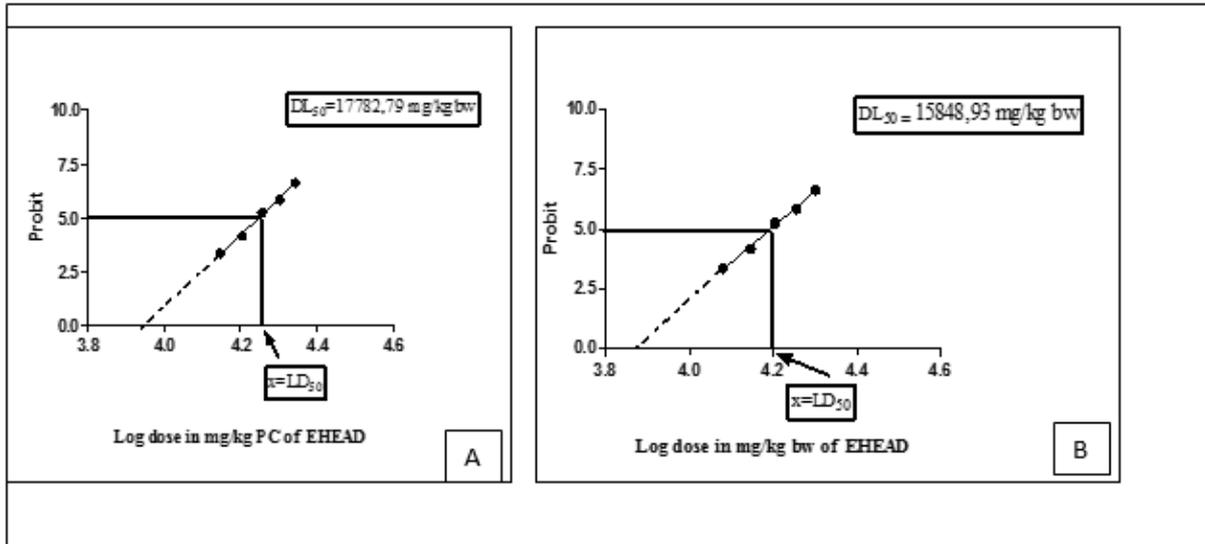
A: male mice   B: female mice

**Figure 1: Curved expressing the acute toxicity of HEAd according to Miller and Tainter (1944).**
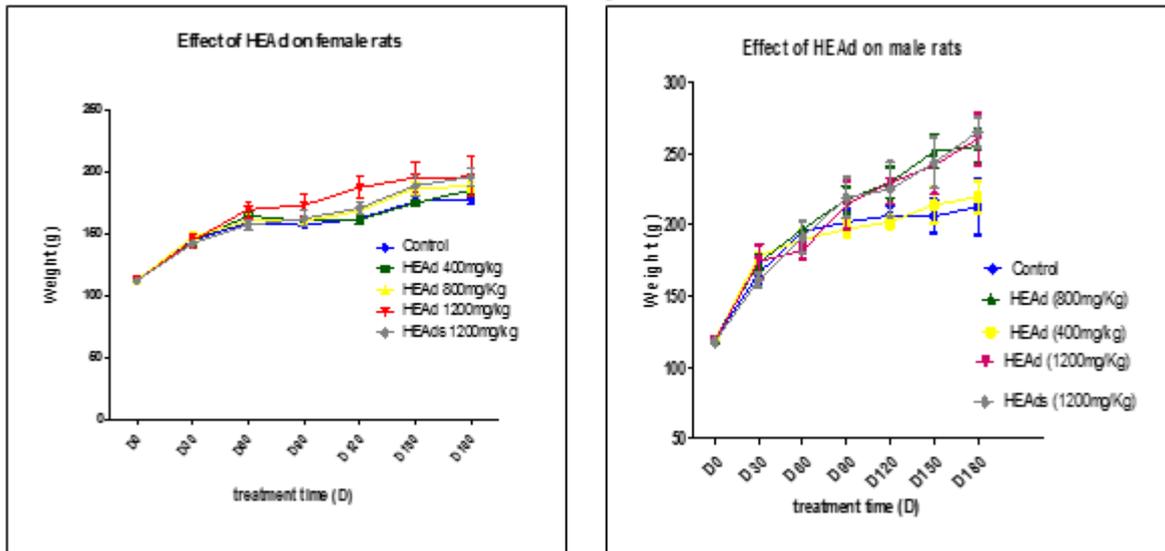
Figure 2: Evolution curve of rats body weight after 180 days treatment with HEAd Heads: Satellite group treated at a dose of 1200mg/Kg
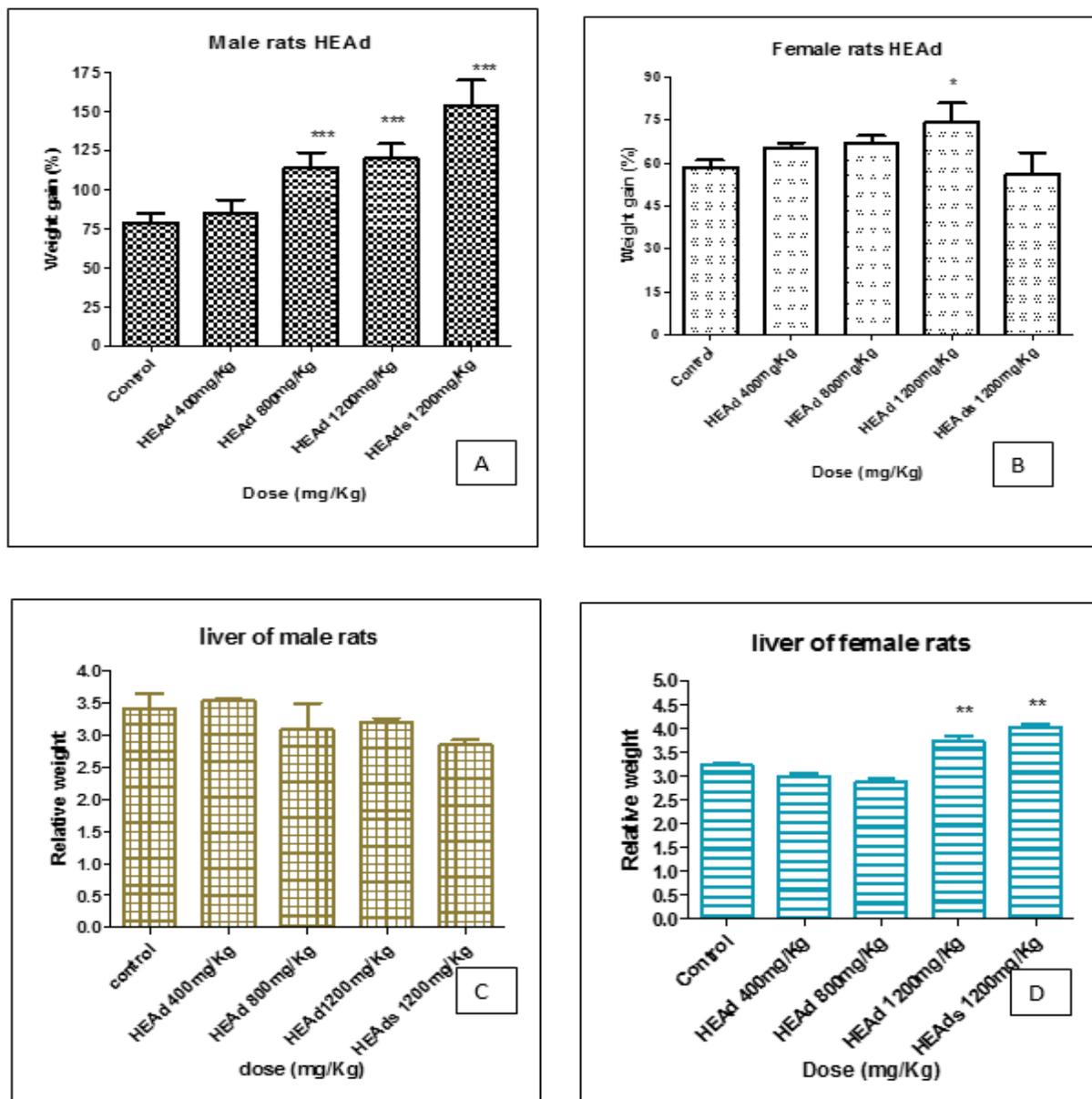
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Each value represents the mean ± Standard deviation; (n = 5); values are statistically different from control at *p<0, 05, **p<0, 01 and ***P<0,001. One way analysis of variance (ANOVA) and Tukey-Kramer multiple comparisons test

A: Body weight gain of male rats treated with HEAd   B: Body weight gain of female rats treated with HEAd

C: Relative organ weight of male rats treated with HEAd   D: Relative organ weight of female rats treated with HEAd

Heads: Satellite group treated at a dose of 1200mg/Kg

Figure 3: Effect of chronic oral administration of HEAd for 180 days on body gain weight and relative organ weight in Wistar rats

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Table 1: Effect of chronic oral administration of HEAd for 180 days on the haematological parameters in wistar male rats

Parameters	Control	*Anthocleista djalonensis A.CHEV* (HEAd)			
		400mg/Kg	800mg/Kg	1200mg/Kg	1200mg/Kgs
Hemoglobin (g/dl)	14.85±0.89	16.47±0.66	15.37±1.37	15.22±0.68	16±0.14
Red blood cells (10^6/µl)	6.2±0.55	7.13±0.31	6.2±0.53	6.60±0.31	6.68±0.04
Hematocrit (%)	44.5±2.38	49±0.81	45±4.08	45.5±2.38	47.5±0.70
MCV (fl)	60.25±1.89	62.5±5.32	60.25±1.5	58±1.63	60.5±2.12
MCHC (g/dl)	34.5±1	34.5±0.57	34.75±0.95	34.25±1.5	34.5±2.12
MCH (pg)	20.15±2.4	21±0.81	20.75±1.5	20±0.81	21±1.41
Platelets (10^3/µl)	1102.25±52	1017.25±19	1090±41.87	1190.25±43.74	1008.5±9.19
Leucocytes (10^3/µl)	18.40±1.08	18.37±0.56	13.63±1.93***	11.33±0.76***	13.92±1.7**
Neutrophils (10^3/µl)	26.5±4.12	24.5±4.04	27±2	21.25± 2.1	17.5±1.70
Eosinophils (10^3/µl)	3.75±0.95	3.75±0.95	2.75±0.5	3.25±0.5	3±0.8
Lymphocytes (10^3/µl)	62.5±7.59	64±4.24	64.25±4.02	66.25±3.5	75±7.25
Monocytes (10^3/µl)	7.25±0.95	7.75±1.5	6±0.81	7.5±0.57	5±1.41

Each value represents the mean ± Standard deviation; (n = 5); values are statistically different from control at *p<0, 05, **p<0, 01 and ***P<0,001. One way analysis of variance (ANOVA) and Tukey-Kramer multiple comparisons test.

1200mg/Kgs: Treated satellite group of rats treated at a dose of 1200mg/Kg followed by 28 days without treatment.

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Table 2: Effect of chronic oral administration of HEAd for 180 days on the haematological parameters in wistar female rats

Parameters	Control	*Anthocleista djalonensis* A.CHEV			
		400mg/Kg	800mg/Kg	1200mg/Kga	1200mg/Kgs
Hemoglobin (g/dl)	15.12±1.18	15.32±0.63	14.85±0.88	15.75±0.47	15.92±0.65
Red blood cells (10^6/µl)	6.45±0.67	6.49±0.47	6.48±0.47	6.44±0.51	6.59±0.45
Hematocrit (%)	45.25±3.2	46.5±1.29	45.75±1.70	45.75±2.63	48.25±2.5
MCV(fl)	63.75±2.63	64.5±1.73	64.5±1.91	62.5±1.91	64.25±2.06
MCHC (g/dl)	36±0.81	35±1.2	35.5±0.57	35.25±0.5	35±0.81
MCH (pg)	23±0.81	22.75±0.5	23.25±0.5	22.25±0.95	22.75±0.95
Platelets (10^3/µl)	1190.75±63.3	987.25±10.34	952±33.05**	930±13.92**	1060±39.14*
Leucocytes (10^3/µl)	11.92±2.33	12.96±4.66	14.72±5.21	11.81±4.28	12.59±1.70
Neutrophils(10^3/µl)	18.25±1.5	17.75±2.63	18.25±1.25	19.75±0.5	20.31±3.5
Eosinophils(10^3/µl)	2.25±0.5	2±0.87	1.75±0.5	2.5±0.57	2.25±0.5
Lymphocytes (10^3/µl)	75±2.44	75.75±3.77	76.25±3.77	74.25±5.5	69±2.70
Monocytes (10^3/µl)	4.5±0.57	4.5±0.57	3.75±0.95	4±1.15	3.5±0.57

Each value represents the mean ± Standard deviation; (n = 5); values are statistically different from control at   *p<0, 05, **p<0, 01 and ***P<0,001. One way analysis of variance (ANOVA) and Tukey-Kramer multiple comparisons test.

1200mg/Kgs: Treated satellite group of rats treated at dose of 1200mg/Kg followed by 28 days without treatment.

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Table 3: Effect of chronic oral administration of HEAd for 180 days on the biochemical parameters of male Wistar rats

Parameters	*Anthocleista djalonensis A chev*				
	Control	400mg/Kg	800mg/Kg	1200mg/Kg	1200mg/Kgs
Aspartate amino transferase (UI/L)	226,25±3,5	232,5±4,5	237,62±6,62	261,5±4,72**	233±4,24
Alanine amino transferase (UI/L)	51±2.12	62.5±6.45	65±2.16	70.25±4.99*	55.25±5.90
Alkaline phosphatase (UI/L)	150,5±4,9	133±6,48***	164,25±2,9	196±4,6***	157±5,09
GGT (UI/L)	5,75±0,95	5,5±0,57	6,85±0,95	8,5±0,57*	5±0,1
Total bilirubin (mg/L)	7±0,81	6,14±0,81	5,75±0,95	3±0.2***	4±1,41**
Conjugated bilirubin (mg/L)	8±2,16	7,5±1	6,59±0,57	2,75±0,57***	4,5±0,70*
Total cholesterol (g/L)	0.63±0.1	0.61±0.03	0.69±0.08	0.68±0.07	0.67±0.05
Cholesterol HDL (g/L)	0.32±0.05	0.35±0.08	0.35±0.08	0.35±0.04	0.25±0.01
Cholesterol LDL (g/L)	0.18±0.09	0.16±0.07	0.23±0.06	0.11±0.07	0.12±0.02
Triglycerides (g/L)	0.63±0.06	0.64±0.09	0.65±0.03	0.67±0.21	0.64±0.06
Total proteins (g/L)	75.5±5.91	73±1.41	74.25±2.87	74.25±3.30	75±1.41
Albumin (g/L)	26,25±1,25	25,25±0,95	27,25±1,89	25,75±1,25	26,5±0,70
Glucose (g/L)	0,74±0,24	0,87±0,09	0,85±0,06	0,93±0,26	0,88±0,06

Each value represents the mean ± Standard deviation; (n = 5); values are statistically different from control at *p<0,05, ** p<0, 01 and ***P<0,001. One way analysis of variance (ANOVA) and Tukey-Kramer multiple comparisons test.

1200mg/Kgs: Treated satellite group of rats at dose of 1200mg/kg followed by 28 days without treatment.

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Table 4: Effect of chronic oral administration of HEAd for 180 days on the biochemical parameters of female wistar rats

Parameters	*Anthocleista djalonensis A Chev*				
	Control	400mg/Kg	800mg/Kg	1200mg/Kg	1200mg/Kgs
Aspartate amino transferase (UI/L)	212±9.41	227±6.24	231±11.16	268±6.73***	229.25±11.95
Alanine amino transferase (UI/L)	57.25±5.9	59.8±13.25	62.5±5.80	67.75 ± 6.44*	58.25±9.53
Alkaline phosphatase (UI/L)	66.87±7.33	83±4.32	95±5.68	125.6±10.23***	81.5±13.91
GGT (UI/L)	7,5±1	7,85±0,45	8,2±0,83	9,25±0,95*	7,25±0,95
Total bilirubin (mg/L)	6.75±1.70	5.2±1.09	7.5±1.73	4.5±0.5	5.5±0.57
Conjugated bilirubin (mg/L)	12±1,63	12,5±2,08	9±2,73	6,75±0,95**	6,01±1,25**
Total cholesterol (g/L)	0.57±0.05	0.55±0.07	0.68±0.09	0.59±0.03	0.69±0.07
HDL (g/L)	0.41±0.12	0.37±0.08	0.4±0.11	0.37±0.05	0.4±0.14
LDL (g/L)	0.07±0.006	0.08±0.005	0.05±0.082	0.03±0.004	0.09±0.008
Triglycerides (g/L)	0.91±0.30	0.99±0.22	0.88±0.33	1±0.5	1.12±0.46
Total proteins (g/L)	79.25±6,44	78±2.23	79.23±5.31	80.5±3.87	85±20.19
Albumin (g/L)	27.5±2.08	26±1.58	27±0.81	27±6.37	27.75±1.5
Glucose (g/L)	1.32±0.09	1.23±0.28	1.25±0.29	0.99±0.32	1.16±0.27

Each value represents the mean ± Standard deviation; (n = 5); values are statistically different from control at   *$p<0,05$, ** $p<0,01$ and ***$P<0,001$. One way analysis of variance (ANOVA) and Tukey-Kramer multiple comparisons test.

1200mg/Kgs: Treated satellite group of rats at dose of 1200mg/Kg followed by 28 days without treatment.

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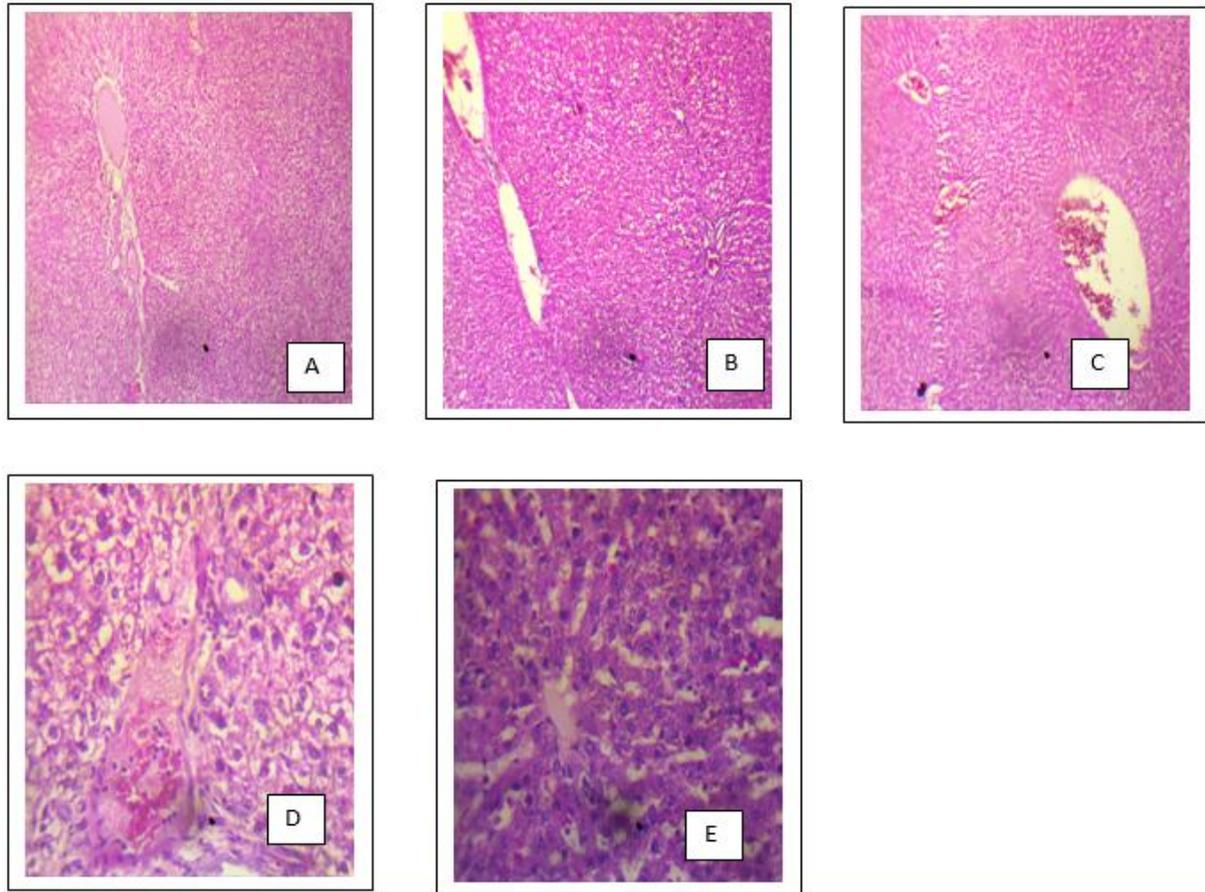
Figure 4: Histological sections of Wistar rat livers stained with hematoxylin and eosin (under ×100 magnification power) showing the effect of the hydroethanolic stem bark of *Anthocleista djalonensis* A Chev in a 180 days chronic toxicity study in male rats.

A: Control rat liver showing normal tissues. **B:** Male rat liver treated at 400mg/Kg showing lesions of hepatocyte degeneration. **C:** Male rat liver treated at 800mg/Kg, showing hepatocytic degeneration lesion showing intracellular edema with clarification and/or cytoplasmic vacuolation with vascular congestion. **D**: Male rat liver treated at 1200 mg/Kg showing lesions of cellular degeneration followed by sinusoidal capillary dilatation. **E:** Satellite male rat liver treated at 1200mg/Kg, showing disorganized hepatocyte plates associating lesions of hepatic peliosis (dilation of the sinusoidal cavities containing a serohematic crust).

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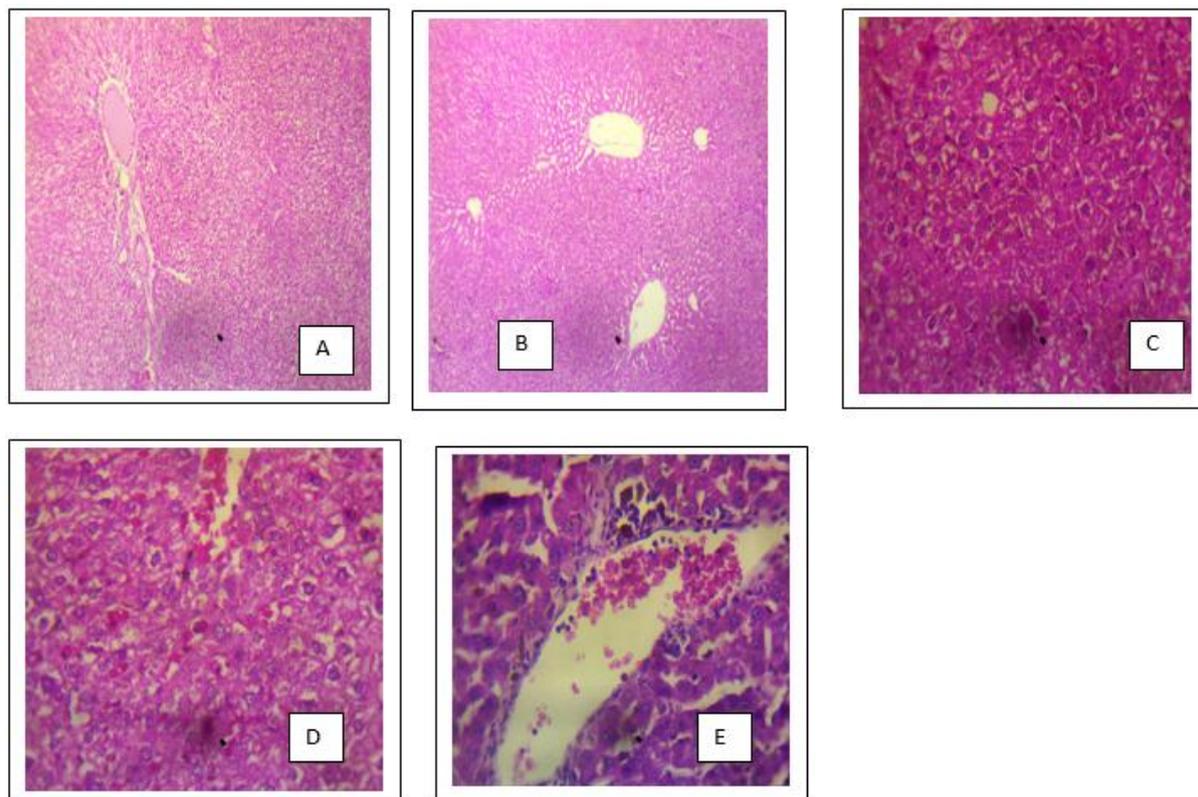
Figure 5: Histological sections of Wistar rat livers stained with hematoxylin and eosin) under ×100 magnification power) showing the effect of the hydroethanolic stem bark of *Anthocleista djalonensis* A Chev in a 180 days chronic toxicity study in female rats.

A: Control rat liver showing normal tissues. **B:** Female rat liver treated with 400 mg/Kg pc showing bridging hepatic necrosis. **C:** Female rat liver treated with 800 mg/kg, showing a lesion of hepatocyte degeneration followed by necrosis and disorganization of plates of hepatocytes. **D:** Female rat liver treated at 1200 mg/Kg, showing a lesion of hepatocyte degeneration with vascular congestion and microvesicular steatosis (hepatocytic fatty degeneration). **E:** Lesions of hepatic peliosis, dilation of the sinusoidal capillaries, containing fibrinous substances.shown in satellite female rat liver treated at 1200mg/Kg.

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