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Acute toxicity of the aqueous extract of *Aloe barbadensis* miller gel

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ABSTRACT

Aloe vera (*A v*) is a medicinal plant used since ancient times. Itsmucilage contains no less than 75 active ingredients with multiple properties. The present research aims to study the acute toxicity of the aqueous extract of *A v* gel (AEAVG) and to determine itsno-toxic effect dose (NTED). After the aqueous extraction of *A v* gel, an acute toxicity study was conducted in Swiss mice for 14 days by oral administration of AEAVG in a single dose (0, 250, 500, and 1000 mg/kg BW). Acute toxicity evaluation affected mouse behavior, weight gain, food and water intake, serum biochemical parameters (transaminases, urea, and albumin), and organ histology (liver and kidneys). These results show that the NTED is lower than 500 mg/kg BW. AEAVG has toxic effects at doses equal to or greater than 500 mg/kg BW, raising the alarm about the therapeutic use of AEAVG.

Keywords: AEAVG; Acute toxicity; Swiss mice; Oral dministration; NTED; Histological sections

Introduction

Aloe vera (*Aloe barbadensis* Miller) is a native species to South Africa, which has been widely distributed in the continent of Europe from where they have spread to almost the entire world (Pistelli and Laxmipriya, 2015). It was named after botanist Miller who discovered and registered it in the registry of medicinal herbs. In nature, it grows only in warm and dry climates, such as the Caribbean and Mexico. It has saber-like, pointy leaves, which in its form remind of a rose and grow close to the ground. There are more than 250 species worldwide (The *A v* taxonomy is shown in Table I), and only four have a healing effect, predominantly *Aloe barbadensis* Miller. It was used as medicine 6000 years ago in ancient Egypt, and back then, it was known as “the herb of immortality." The most frequently used part of the plant is its gel, jelly-like mass from the inside of the *A v* leaf has a nutritious effect on every cell of the human organism, and because of its nutritional value and exceptional healing abilities, it is often called as "the queen of medicinal herbs”. The gel contains more than 240 nutritious and healing ingredients: Vitamins A, B1, B2, B3, B6, B9, B12, C, and E, more than 20 minerals (magnesium, manganese, zinc, copper, chrome, calcium, potassium, iron), and 20 types of amino acids (Lejla, 2020). The community widely uses *A v* species because it has many advantages: the size of the leaves is large, pest resistant, and safe to use for cosmetics, and many contain good nutrients for the body, as shown below (Azirah, 2019).

The toxicological assessment of any medicinal plant has as its primary objective to identify the adverse effects that may be associated with its use and to determine the exposure limits at which such effects occur, thus avoiding possible adverse effects when used as a medicinal product (Subramanian *et al.* 2018). In this work, we are interested in the acute toxicity of this miracle gel which has a long history of being used for medicinal purposes.

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Table I. Taxonomic classification of *A v* (Sánchez-Machado *et al.* 2017)

Rank Scientific	Name and Common Name
Kingdom	Plantae
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Liliopsida
Subclass	Liliidae
Order	Liliales
Family	Aloaceae
Genus	Aloe L
Species	*Aloe barbadensis* Mill. or *Aloe vera* (L.) Burm. F

Materials and methods

AEAVG preparationand its extraction yield

The A v was harvested in northwest Algeria (Oran). After recovery of the plant and its identification, the leaves of *A v* are weighed and measured (length and release).

Fig. 1. *Aloe barbadensis* miller

About 12 *A v* leaves (Figure 1), over three years old, were cleaned with distilled water (DW), cut into halves, and placed upside down for half an hour to allow drainage of yellow latex. Leaves were peeled, the clear inner gel was cut into small pieces and blended with an equal volume of DW then filtered through cloth, then stored in the freezer (-70°C) overnight (Akev *et al.* 2015) after was lyophilized (CHRIST lyophiliser, ALPHA 1- 2 LD, Germany) (Rajasekaran *et al.* 2006). Freezing the lyophilized extract at -20°C is necessary to keep the extracted molecules intact .

The AEAVG extraction yield is calculated by the formula given by Falleh *et al.* (2008).

$$Y\ (\%) = 100\ Mext / Msam.$$

Where: Y is the yield in %; Mext, is the mass of the lyophilized extract expressed in mg, and Msam, is the mass of the plant sample (gel) in mg.

Acute toxicity of AEAVG

The AEAVG acute toxicity was carried out on Swiss mice (25.912 ± 4.895), males (25.425 ± 4.543), and females (26.4 ± 5.295) around 2 to 2.5 months old devised into 04 groups. The general guidelines for the care and use of laboratory animals recommended by the Council of European Communities (CEC, 1987) were followed, and all the experimental protocols involving the use of laboratory animals were approved by the Institutional Animal Ethics Committee of Oran 1 Ahmed Ben-Bella University (Reg. No. 13/355/2015).

The Swiss mice came from the Algeria Institut Pasteur and were housed in an air-conditioned room [(25 ± 1)°C] with a 12-h light/12-h dark cycle. The animals were treated according to the guidelines recommended by the Committee for the Purpose of Control and Supervision of Experiments on Animals and were acclimatized to the environment before practical use by supplying water and fed with a standard laboratory diet ad libitum.

One of the groups was used as control receiving DW, while the other groups were each treated by oral route with a single dose of AEAVG (250, 500, and 1000 mg /Kg BW) dissolved in DW and adjusted to 10 ml/kg BW per dose) (Tahraoui *et al.* 2010). All the animals have free access to water and food four hours after the administration.

The animals were subjected to continuous observation for 3 hours (in order to note the clinical signs and the number of deathsafter the oral administration of AEAVG), then were observed every 14 days (in order to note the number of deaths) (Gopalsamy *et al.* 2012). During this period, the measurement of food and water intake and Swiss mice weight was carried out every two days.

After 14 days, individual blood samples were collected from the retro-orbital venous plexus, and serum was prepared by centrifugation (3500 rpm for 15 min at 4°C) and then stored at −70°C until biochemical analysis. The liver and both kidneys of each mouse were removed for histological study.

Mice body weight

The initial BW and the Swiss mice growth are monitored. The weight measurement is carried out on fasting mice.

The weight change ratio (WCR*)* is expressed as a percentage (%) and calculated as follows:

$$WCR\ (\%) = ((Wd_x - Wd_1) / Wd_1) * 100$$

Wd_x: weight day xet Wd_1: weight 1st day.

The Weight gain (WG) is expressed in grams (g), which is the difference between the weight of the last and the initial day.

$WG = Weight\ d_{l5} - Weight\ d_1$

Wd_{l5}: Mouse sacrifice days.

Relative organ weight

The relative weight of each organ (ROW) was calculated according to the formula of Etame *et al.* (2017):

$$RWO\ (g / 100\ g) = (Wo / Wm) * 100$$

Wo: organ weight (g); Wm: mice weight (g).

Food and water intake

The food and water intakes (in g per kg of BW) of Swiss mice were recorded every two days.

Evaluation of biochemical parameters

The serum biochemical parameters "Aspartate transaminase (AST), Alanine transaminase (ALT), Urea and Albumin"are analyzed using standard assay kits (SPINREACT, Spain).

Histopathology study

The liver and kidney were isolated and washed continuously with phosphate-buffered saline to remove the debris. The samples were then fixed with 10% formalin and processed further by routine histopathological methods. Tissues were then embedded in molten paraffin wax, and a 4.5-5µm thickness section was obtained in each case. The sections were stained with (*H&E*) stain. Histopathological tissue morphology was observed under the optical microscope.

Statistical analysis

Results were expressed as means ± Standard Error (ES), and $p \leq 0.05$ were considered statistically significant. Obtained data were statistically analyzed using STATISTICA (AXXF307CO20802FA) 2006.

Results and discussion

In Africa, people use medicinal plants as part of their culture and civilization, which were recognized before the introduction of conventional medicine (Kayombo *et al*. 2013). There is a solid belief in people on medicinal plants that they do not induce toxic side effects. However, scientific reports have proven the contrary when herbal medicines are used above a certain tolerance level (Patel *et al.* 2012). Different scholars have also reported on some medicinal plants exhibiting higher toxicity levels harmful to human and animal life (Botha and Penrith, 2008; Wagstaff, 2008; Frohne and Pfänder, 2005).

The first part of this study is devoted to the aqueous extraction of *A v* gel from its leaves which have the following characteristics: Weight = 325.91 (± 146.49) g; Length = 64.2 (± 4.56) cm and Width = 8.4 (± 0.84) cm.

Extraction process and its yield

The substance had a viscous white appearance, and the hue remained the same after lyophilization. Aqueous extractions of *A v* gel allowed us to produce an extract with a yield of 0.58 (0.077)% because water is the most often utilized solvent for the increased recovery of phenolic components. This result verifies the gel's high water content of roughly 99.5% and demonstrates a low yield relative to the entire gel weight (Eshun and He, 2004). The lack of research on this extraction makes it challenging to compare these results to those in the literature, mainly since yield is relative and dependent on several factors, including genetic characteristics, geographic origin, storage conditions and duration, harvest, solvents used, and extraction methods used.

Acute toxicity in the Swiss mice

Acute toxicity refers to those adverse effects following oral or dermal administration of a single dose of a substance, multiple doses given within 24 hours, or an inhalation exposure of 4 hours. The oral toxicity of AEAVG in mice showed no lethality or Visible Pathological changes.

Clinical signs

The clinical signs observed after gastric gavage of AEAVG (0, 250, 500, and 1000 mg/kg BW) in mice are shown in Table II, and Figure 2 shows some signs of acute toxicity at 1000 mg/kg BW.

A v toxicity remains a taboo subject and little studied. This plant, known as a miracle remedy from a history seen under

Table II. Clinical signs of the AEAVG acute toxicity in mice (M: males; F: females and n= 5 mice in each group)

Dose (mg/kg BW)	Major toxicity signs
0 (M)	No signs
0 (F)	No signs
250 (M)	No signs
250 (F)	No signs
500 (M)	Straightening reflex, hypoactivity then Irritability, They scratch, drowsiness. Isolation and drowsiness with hypoactivity, slight tremor. They woke up but with hyoactivity and are still scratching, permanent drowsiness. Scratching, tremors, Irritability then Isolation and anorexia.
500 (F)	Hypoactivity and Isolation. After they started to scratch with slight Tremors and straightening reflex. Marked drowsiness, they fell asleep, and then woke up, they scratch Hypoactivity, drowsiness and Tremors, they scratch. Hyoactivity after tremor.
1000 (M)	Scratching, straightening reflex, hypoactivity, slight body tremors, drowsiness in isolation. Scratching and isolation. More marked scratching and isolation. After this abnormal behavior, they eventually fell asleep. Always sleeps deeply with slight body tremors and straightening reflex. Hypoactivity and scratches then sleeping. Waking up with tremors. Isolation and scratching. Sleeps deeply. After wakes up and take back activity with more marked scratching. Isolation and scratching, then take back normal activity.
1000 (F)	Itching, straightening reflex, hypoactivity, slight tremors, drowsiness and isolation. After scratch a lot in isolation. Continue to scratch with marked drowsiness and isolation. After this abnormal behavior, they ended up falling asleep. Always sleep deeply with slighttremors and straightening reflex. Woke up but with hypoactivity, scratching and abnormal behavior. Hypoactivity with straightening reflex, sometimes scratching but not too much. Still sleeping deeply and then woke up with tremors. After itches with isolation and slight tremors.Scratch with isolation. Then sleep deeply, wake up and take back activity after they started to scratch and then scratch too much. They take back their normal activity.

a prism, is perfect for marketing. The discovery of specific toxicity could then have the effect of a bomb on the cosmetics industry. Today, only 8% of publications are on the toxicity of *A v,* and very few on *A v* gel, and there is a wide disparity between the results found. This can be explained by many factors, including the plant's living conditions (season, localization, irrigation, etc.) and differences in the gel's preparation (Guo and Mei, 2016).

Observation of clinical signs indicates that 250 mg/kg BW is a dose tolerated by mice, and no extract-related clinical signs were observed at this dose. In fact, in mice given the respective doses of 500 and 1000 mg/kg BW and immediately after gavage, a clinical map characterized by various symptoms was observed, which was more severe in mice given 1000 mg/kg BW of AEAVG. Oral administration of AEAVG, therefore, has a dose-response effect, and it concluded that the NTED dose was lower than 500 mg/kg BW.

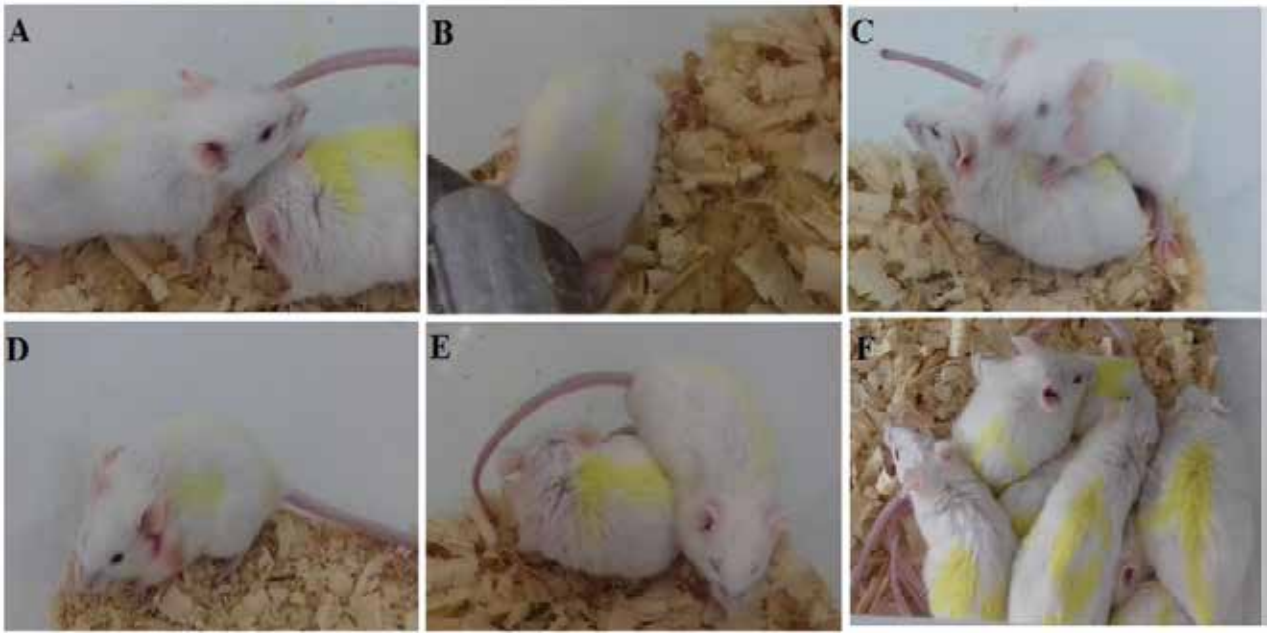

Fig. 2. Some signs of AEAVG acute toxicity (1000 mg/kg BW) in Swiss mice

A: Scratching; B: Hyperactivity; C: Abnormal behavior; D: Isolation; E: straightening reflex and F: Grouping.

The oral administration of the AEAVG at various doses has altered the behavior and physical activity of mice to diverse degrees, however, The toxicological profile of AEAVG has not been studied .

In the literature, the toxicological studies of *A v* gel in rats, Swiss mice, and dogs have shown that acemannan has minimal systemic toxicity following intraperitoneal or intravenous administration (Heggers *et al.* 1996). In contrast, *A v* gel is usually non-toxic and completely harmless (Donadieu, 2000).

A v gel (in freeze-dried form) is non-toxic in rats, either acutely or chronically, at 1 to 24 mg/kg BW twice daily by the oral route. In mice or rats, fresh or freeze-dried gel administration did not cause any toxicity, even at doses up to 20 g/kg BWorally or intravenously acutely or 5 g/kg BW orally for 45 days (Natacha, 2013). Another study has shown that *A v* gel taken orally had no genotoxic effect on Swiss mice, either as an acute or chronic treatment (Sehgal *et al.* 2013).

Physiological measurements

The evolution of the animal's body weight (BW), food intake, and general behavior must be examined in order to evaluate the toxic effect of a chemical on an animal model because these factors are the early indicators of toxicity (Mbaka *et al.* 2010; Almança *et al.* 2011 and Panunto *et al.* 2010).

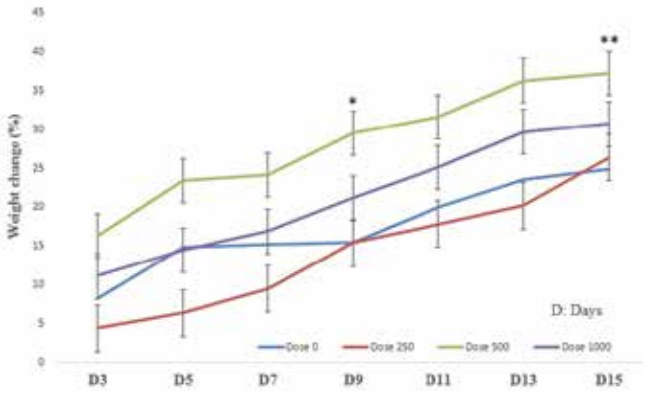

Fig. 3. Weight change ratio (WCR) of Swiss mice during AEAVG acute toxicity study (0, 250, 500 and 1000 mg/kg BW) for 14 days

Statistical difference between the negative control and other groups (*: p≤ 0.05 and **: ≤0.01).
n = 10 (05 Males and 05 Females). M: Males and F: Females.

Animal's BW

The effect of AEAVG oral administration on the WCR (Figure 3) of both sexes during two weeks showed no significant variation for the two doses, 250 and 1000 mg/kg

BW, compared to the controls, but the dose of 500 mg/kg BW showed a significant change. Its high fiber content (Morine, 2008) could explain this, and the high fiber content of Aloe preparations has an appetite suppressant effect at high doses (1000 mg/kg BW) and has no effect at low doses (250 mg/kg BW). In conclusion, all groups of mice showed an overall rise in WCR over time. Animals given AEAVG at a dose of 500 mg/kg BW experienced a more significant increase.

Table III. WG of mice during AEAVG acute toxicity (WG: Weight gain and n= five mice in each group)

Dose (mg/kg BW)	WG (g)	
	Males	Females
D 0	6.8 ± 1.643	5 ± 2.45
D 250	7.4 ± 7.021	5.4 ± 0.894
D 500	9.8 ± 0.836	7.6 ± 2.302
D 1000	7.5 ± 3.335	6.6 ± 3.664

The results are expressed as an average ± ESM (n = 10).

It should be emphasized that the change in BW is utilized as a gauge for chemical substances' negative impacts. WG of mice given AEAVG is represented in Table III. In male mice treated with both doses (250 and 1000 mg/kg BW) of AEAVG, the WG was not significantly different from controls but significantly different (**) from that of controls in the mice treated with 500 mg/kg BW dose. The exact significance was found for the second sex (females) when comparing the treated with the controls 250 (ns), 500 (**), and 1000 mg/kg BW (ns). The study's WG between the two sexes of the same dose showed no significant difference. We can conclude that the AEAVG at the two doses, 250 and 1000 mg/kg BW did not affect the animal's WG, while it affected the animal's BW at the dose of 500 mg/kg BW.

Relative organ weight

The ROW is a suitable parameter indicating whether a drug has targeted the organ. Generally, alterations in the ROW reflect toxicity after exposure to a toxic substance, with the heart, liver, kidney, spleen, and lung being the first organs affected by the metabolic response caused by the toxicant (Jothy *et al.* 2011). Often altered organs have abnormal atrophy (Hor *et al.* 2012). ROW results (liver and kidney) are shown in Figure 4 (by dose) and Table IV (by sex).

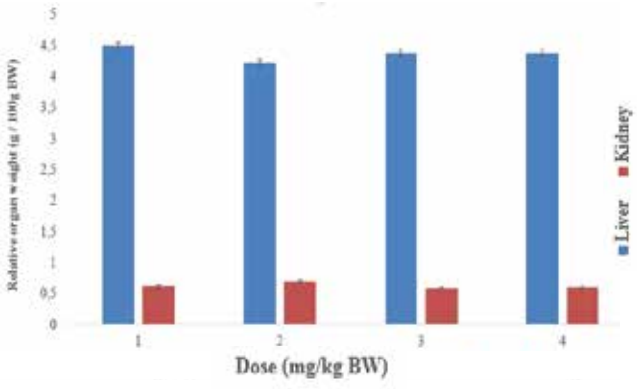

Fig. 4. Histogram of liver and kidney weights per 100g of Swiss mice BW in AEAVG acute toxicity (0, 250, 500 and 1000 mg/kg BW). Each kidney value is an average of the two kidneys

Table IV. Effect of AEAVG oral administration on ROW of Swiss mice (g) according to the sex. (ROW: relative weight organs and and n= 5 mice in each group)

Organ	ROW							
	0 (mg/Kg BW)		250 (mg/Kg BW)		500 (mg/Kg BW)		1000 (mg/Kg BW)	
	Male	Female	Male	Female	Male	Female	Male	Female
Liver	4.551 ± 0.111	4.449 ± 0.193	4.227 ± 0.422	4.210 ± 0.389	4.554 ± 0.657	4.181 ± 0.562	4.668 ± 0.382	4.063 ± 0.361
Kidney	0.669 ± 0.056	0.562 ± 0.045	0.658 ± 0.089	0.728 ± 0.131	0.689 ± 0.087	0.457 ± 0.056	0.716 ± 0.030	0.467 ± 0.067

The liver and kidneys of the three groups receiving AEAVG at different doses had no significant change in their ROW compared to the control, but the statistical analysis by sex revealed the following results:

Table V. Variations in food and water intake by sexes after AEAVG oral administration in Swiss mice acute toxicity. (D: Days, M: Males, F: Females and n= 5 mice in each group)

Dose (mg/kg BW)	Sex	Food intake (g/kg BW)						
		D3	D5	D7	D9	D11	D13	D15
0	M	308.99 (±22.63)	282.196 (±13.40)	287.366 (±15.40)	229.87(±14.27)	265.645 (±15.79)	255.718 (±17.96)	264.425 (±34.04)
	F	235.590 (±42.52)	208.134 (±32.14)	215.072 (±33.22)	215.240 (±27.39)	236.068 (±27.57)	251.264 (±30.03)	215.296 (±28.52)
250	M	495.826 (±103.9)	544.808 (±90.99)	561.992 (±65.18)	555.078 (±68.86)	454.952 (±40.03)	543.272 (±47.73)	554.311 (±21.10)
	F	600.287 (±56.27)	632.088 (±48.91)	401.840 (±15.46)	614.158 (±35.46)	329.371 (±8.63)	472.662 (±18.90)	466.392 (±23.18)
500	M	561.280 (±49.71)	484.018 (±64.79)	454.572 (±49.01)	494.274 (±78.22)	389.009 (±38.05)	363.922 (±33.99)	401.485 (±36.89)
	F	467.269 (±49.46)	538.857 (±41.46)	426.250 (±19.44)	343.288 (±18.27)	421.447 (±27.15)	487.905 (±23.19)	481.233 (±59.79)
1000	M	240.011 (±18.41)	226.862 (±16.31)	261.844 (±18.13)	219.789 (±10.92)	240.759 (±8.77)	295.769 (±14.88)	213.905 (±11.47)
	F	257.498 (±79.22)	266.385 (±6.71)	284.250 (±66.70)	295.002 (±62.25)	269.137 (±45.33)	263.703 (±47.11)	256.601 (±43.12)
		Water intake (g/kg BW)						
0	M	633.439 (±46.39)	458.569 (±21.78)	490.625 (±26.29)	466.717 (±28.98)	398.472 (±23.69)	383.588 (±26.95)	361.156 (±46.49)
	F	464.041 (±83.76)	506.461 (±78.23)	485.648 (±75.01)	416.593 (±53.02)	370.964 (±43.33)	330.61 (±39.51)	347.044 (±45.98)
250	M	630.408 (±132.1)	592.479 (±98.95)	656.709 (±76.17)	413.225 (±51.26)	742.291 (±65.31)	421.903 (±37.07)	803.186 (±30.57)
	F	573.607 (±53.77)	479.056 (±37.07)	476.759 (±18.34)	388.549 (±22.43)	435.019 (±11.40)	342.058 (±13.68)	362.75 (±18.03)
500	M	510.255 (±45.19)	423.516 (±56.69)	427.832 (±46.13)	385.148 (±60.95)	363.5 (±35.55)	363.922 (±33.99)	347.554 (±31.93)
	F	489.879	444.193	447.563	363.885	287.654	292.743	351.17
1000	M	713.177 (±54.72)	513.777 (±36.95)	537.117 (±37.19)	413.72 (±20.56)	418.161 (±15.24)	422.528 (±21.26)	348.359 (±18.69)
	F	647.648 (±199.2)	705.358 (±191.5)	582.712 (±136.7)	514.539 (±108.5)	485.76 (±81.82)	514.542 (±91.93)	438.1 (±73.62)

No significant difference was found between the liver's RW of the two sexes of the same dose in all groups;

In all groups of Swiss treated with AEAVG (0, 250, 500 and 1000 mg/kg BW), significant differences (different degrees) were found between the kidneys of both sexes of the same dose. These results showed that AEAVG does not affect the liver and two kidneys RW at doses up to 1000 mg/kg BW. This result requires another method to test doses higher than 1000 mg/kg BW to understand the AEAVG effect on these two organs.

control throughout the study period, especially in the first group (250 mg/kg BW). This increase demonstrates that AEAVG has an impact on food intake.

No significant difference in food intake between the groupsthat received 1000 mg/kg BW of AEAVG and the control group (Figure 5 A). This decrease in consumption compared to the other AEAVG-treated groups (250 and 500 mg/kg BW) is due to the anorexic effect probably exerted by this dose and is likely to be induced by an increase in satiety.

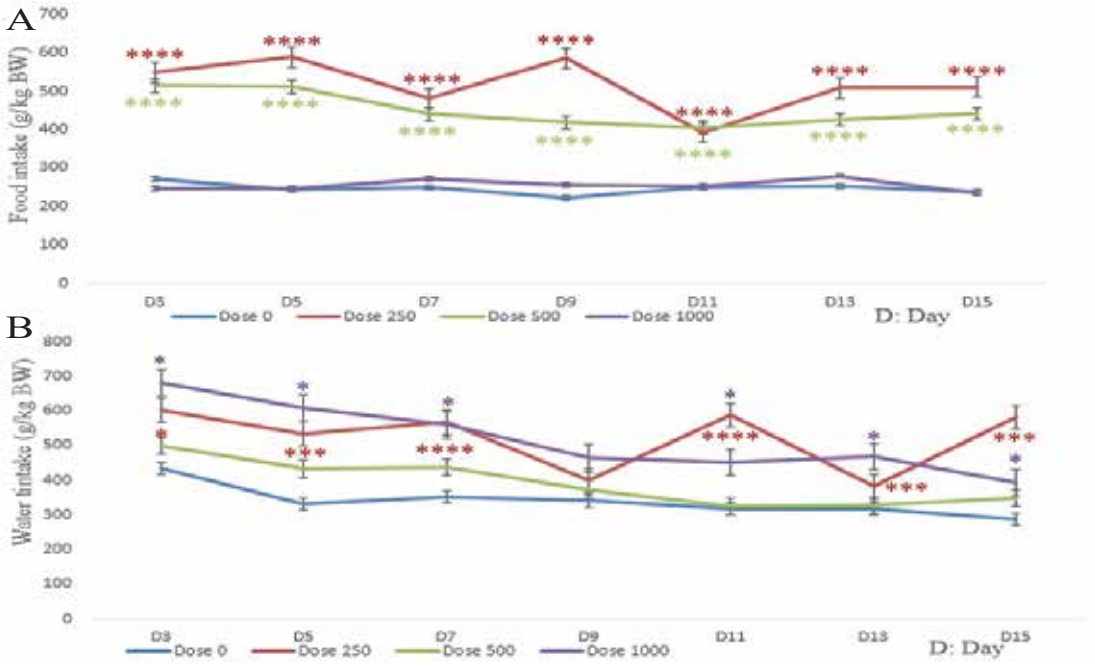

Fig. 5. Food (A) and water (B) intake by Swiss mice in acute toxicity after AEAVG oral administration (0, 250, 500 and 1000 mg/kg BW). M: Males, F: Females and D: day. (**p ≤0.0001; ***p ≤0.001; *p ≤0.05)**

Generally, the change in organ weight indicates toxicity after exposure to a toxic substance (Raza *et al.* 2002; Teo *et al.* 2002). Researchers have shown that the toxic effect is sex-dependent as Baliga *et al.* (2004).

Food and water intake

The food and water intake for each group are shown in Figure 5 and for each sex in Table V.

Food intake

The food intake of the groups receiving 250 and 500 mg/kg BW of AEAVG increased significantly compared to the

The comparison of food intakes between males and females of the same dose of all the groups showed no significant difference throughout the experimental period (Table V).

Water intake

The results of the effect of AEAVG oral administration on the water intake of gavaged mice compared to controls; we observed (Figure 5 B):

- The mice that received 1000 mg/kg BW show differences (*);
- Swiss gavaged by 250 mg/kg BW demonstrate differences

generally ranged from very significant (***) to highly significant (****);

- The consumption of the Swiss received 500 mg/kg BW is comparable to that of the controls.

In conclusion, the mice that received the 250 mg/kg BW of AEAVG have a greater thirst than the other groups; this dose is the only one that does not show any toxicity signs.

The statistical comparison between the two sexes of the same dose (Table V) demonstrated:

- Dose 0: No significant difference between the two sexes except on the third day (*).
- Dose 250: Differences between the two sexes onthe 7th day (*), 11th day (***), 13th day (**), and 15th day (****).
- Dose 500: Differences between the two sexes on the 11th day (*) and the 13th day (**).
- Dose 1000: No significant difference between the two sexes except on the last day (*).

We can conclude that the water intake bythe male was increased compared to that of femalemice of the same dose.

The food and water intake results showthat the mice who received 250 mg/kg BW of AEAVG were more hungry and thirsty than the other groups. Nevertheless, the water intake of rodents is strongly linked to their food intake (75 to 80% of total water intake occurs during meals) (Fitzsimons and Magnen, 1969; Kissileff, 1969).

Biochemical parameters

In this part of our study, four parameters were determined: GOT, GPT, Urea, and Albumin (Figure 6).

Transaminases (ALT and AST) are enzymes with significant metabolic activity within cells,and the increases in their serum levels reflect cellular injury, particularly in the liver (Lazare *et al.* 2011).

GOT

It is an enzyme found in high levels in the cytoplasm and mitochondria of various tissues, including the liver, heart, skeletal muscle, kidney, and brain (Gad *et al.* 2013). AST is an enzyme considered a good indicator of liver function (Hilaly *et al.* 2004) and a biomarker to predict eventual toxicity (Rahman *et al.* 2001).

AST's statistical comparison of the six AEAVG-fed groups (3 males and three females) with that of the two control groups (Figure 6 A) showed the same significance (a non-significant increase) except for the two sexes received 1000 mg/kg BW of AEAVG which showed significant increases (*) probably due to the AEAVG effect in high doses.

AST's statistical comparison between males and females of the same dose demonstrated:

- A significant increase (*) was observed in the males AEAVG-fedat 250 mg/kg BW;
- Insignificant increases in the males AEAVG-fed the other doses (0, 500 and 1000 mg/kg BW). Therefore, it is a physiological difference between the two sexes since the ASTs of males are higher than those of females, even in the negative control group.

It was concluded that an increase in AST activity was observed at 1000 mg/Kg BW in both sexes.

Transaminase elevation in the blood generally indicates liver parenchymal cell damage (Wolf *et al.* 1972). Furthermore, AST found in serum is of mitochondrial and cytoplasmic origin, and any increase can be taken as the first sign of cellular damage, which leads to the release of the enzyme into the serum (James *et al.* 2010).

GPT

ALT is a cytoplasmic enzyme found in very high concentrations in the liver, and a serum increase in this enzyme suggests hepatocellular damage (Gad *et al.* 2013). The analyses performed showed an increase in ALT in the six AEAVG-fed groups (3 males and 3 females) compared to the two control groups (Figure 6 B) but without significance except for the males AEAVG-fedat 1000 mg/kg BW(*). The statistical study between the two sexes of the same dose showed only a significant increase in serum ALTs of the males AEAVG-fed at 1000 mg/kg BW compared to the females; this increase demonstrates the possible toxic effect of AEAVG at1000 mg/kg BW on the liver.

GPT and GOT are concentrated in the cytoplasm, and liver injury results in the leakage of these enzymes into the extracellular medium, leading to increased levels of these parameters in the serum (Mitra *et al.* 1998; Dar *et al.*

2012). An increase in these enzyme activities reflects active liver damage and hepatocellular inflammatory disorders (Foreston *et al.* 1985; Hultcrantz *et al.*1986).

Urea

Urea is a 60-dalton molecule and represents the main form of nitrogen elimination, synthesized during protein catabolism by the liver, it is one of the first markers used to measure glomerular filtration rate. Urea passes through the nephrons regardless of its concentration in the blood: it is a substance without a threshold (Chanton and Paniel, 1966). The urea concentration of each sex of the mice given AEAVG showed a not significant elevation compared to the two control groups except for the female Swiss given 1000 mg/kg BW of AEAVG (*) (Figure 6 C).

Statistical comparisons of urea concentrations between the two sexes of the same dose did not show any significant difference. This increase in urea in female mice given 1000 mg/kg BW of AEAVG could be explained by an increase in the degradation of protein compounds and impairment of renal function. Piva *et al.* (1997) reported similar results from work on pigs, and probably functional renal failure was noted (elevated urea and creatininein a person who voluntarily got intoxicated with another plant, D*atura* (Montériol *et al.* 2007).

Elevated urea usually indicates glomerular damage, but the concentration may also be altered by inadequate nutrition or hepatotoxicity, which is common with many toxicants (Franck, 1992).

Albumin

Albumin is the main plasma protein synthesized and secreted by the liver, representing about 50% of total hepatic protein production. The half-life of albumin in human blood plasma is about 20 days, representing 60% of total plasma protein. Normal blood albumin levels are 40 to 50 g/l or 0.5 to 0.7 mmol/l (Valdiguié, 2000). A comparison of the serum albumin concentration of each sex with the controls shown in Figure 6 D indicated a non-significant decrease except in female mice receiving 500 mg/kg BW of AEAVG (*). Another statistical study between the two sexes of the same dose showed no significant difference.

According to Gaw *et al.* (2004), severe acute liver injury can also result in hypoalbuminemia, a characteristic of advanced chronic liver disease. However, due to the long half-life of albumin, it typically remains normal in the early stages of acute hepatitis (Marchall and Bangert, 2005).

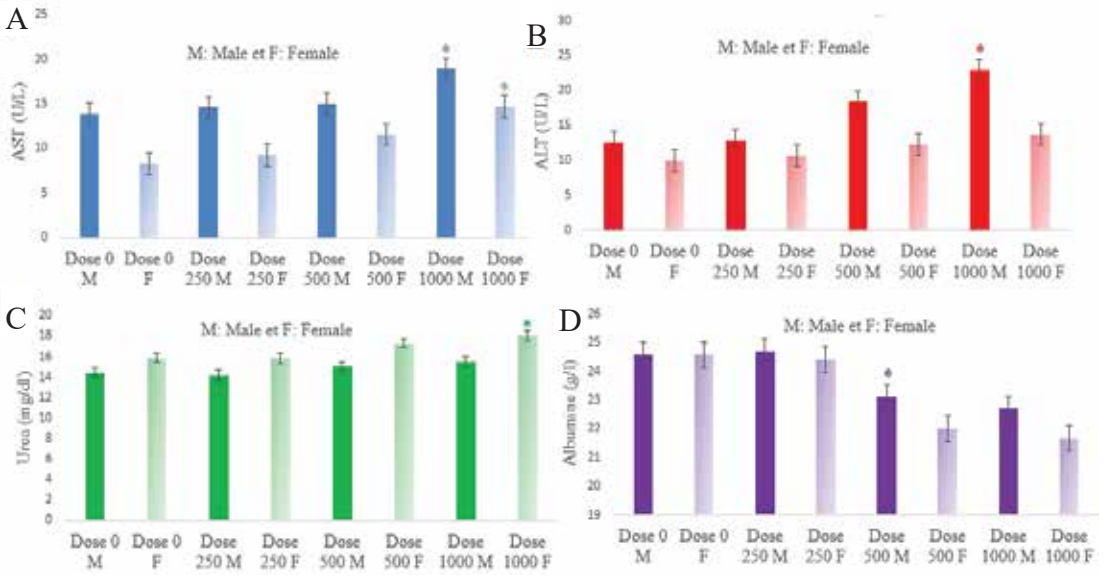

Fig. 6. Effects of AEAVG oral administration on serum biochemical parametersin acute toxicity study. Values are expressed as mean ± SEM (n=5 Swiss mice) by comparing each group with negative controls (*p ≤0.05).

In conclusion, the biochemical screening showed:

- Assessment of serum transaminases showed an increase in ASTs activity in male and female mice given 1000 mg/Kg BW of AEAVG and ALTs in male mice given the same dose, which could clinically signify liver damage in these mice.

- Renal function tests showed high serum urea levels in female mice receiving 1000 mg/kg BW of AEAVG and normal albumin levels in both sexes at the same dose, so this is probably mild renal damage. This toxicity may be due to various active organic constituents present in AEAVG, such as toxic alkaloids.

Histological sections

The liver works with the kidneys to remove toxic substances from the blood (Tulsawani, 2010). They are organs that usually regulate metabolism and excretion and are particularly sensitive to potentially toxic agents, and their function should be monitored in toxicological studies (OMS, 2000). The macroscopic examination of these two organs showed no visible abnormality.

The liver

The liver is the first target of toxicity and the first organ exposed to anything absorbed in the small intestine; it

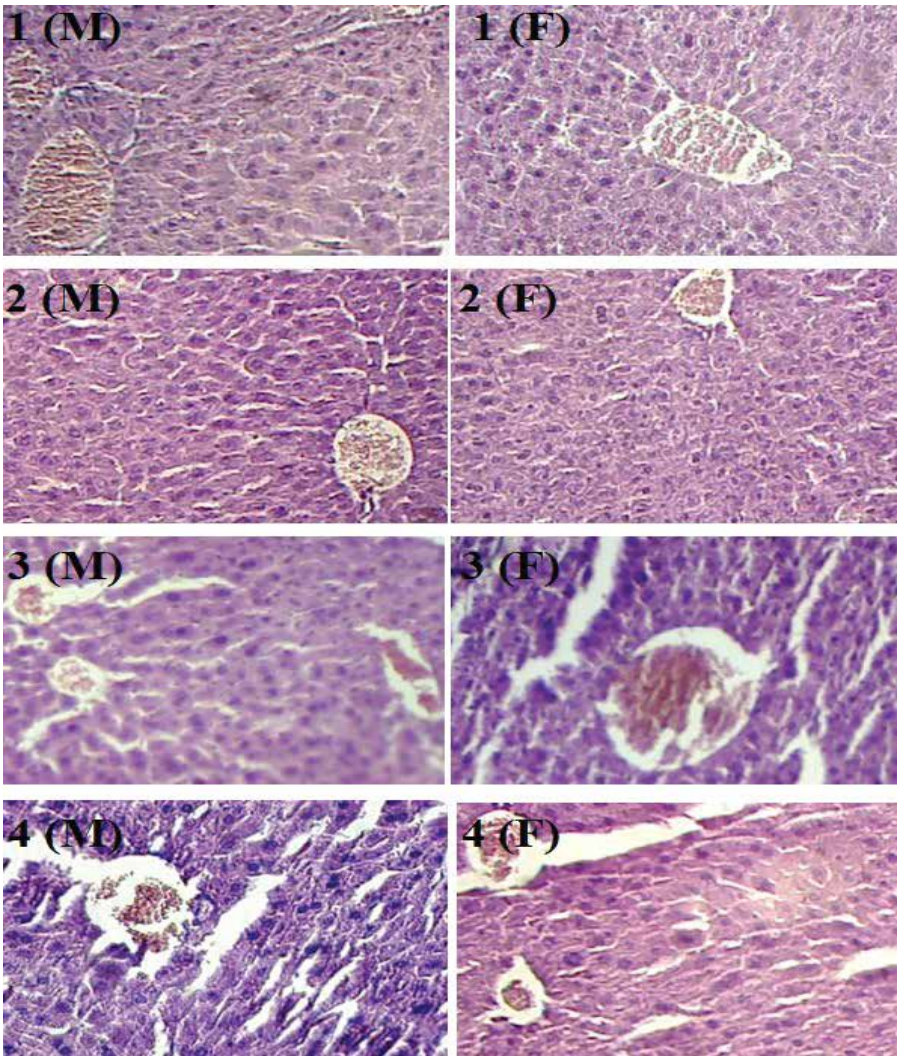

Fig. 7. Photomicrographs section of Swiss mice livers in acute toxicity study stained by *H&E* at a magnification (X400). 1: Dose 0; 2: Dose 250 mg/kg BW; 3: Dose 500 mg/kg BW; 4: Dose 1000 mg/kg BW; M: Male and F: Female

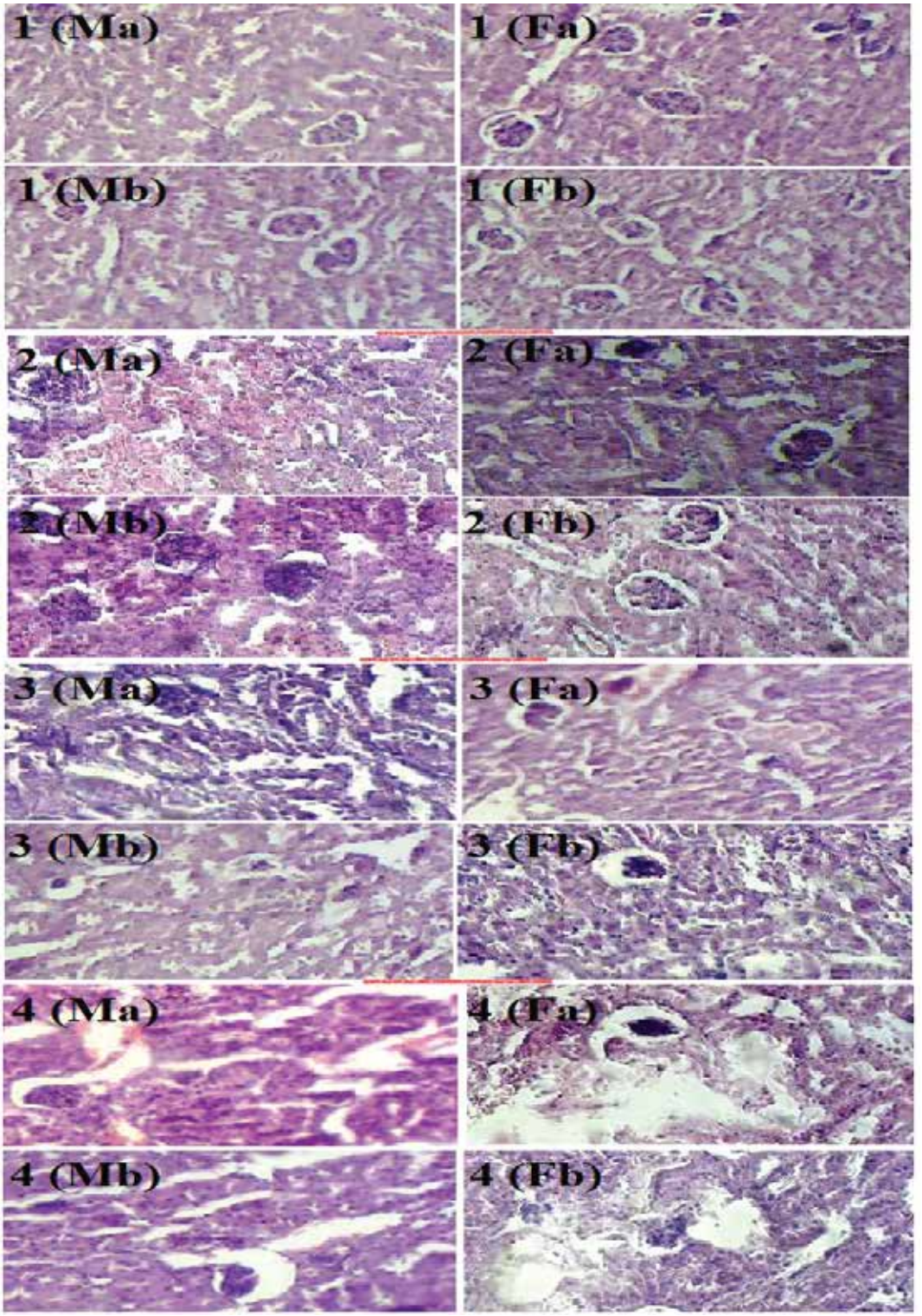

Fig. 8. Photomicrographs section of Swiss mice kidneysin acute toxicity studystained by *H&E* at a magnification (X400). 1: Dose 0; 2: Dose 250 mg/kg BW; 3: Dose 500 mg/kg BW; 4: Dose 1000 mg/kg BW; M: Male and F: Female

metabolizes foreign substances into compounds that may be hepatotoxic (Rhiouani *et al.* 2008). It is the leading site of biotransformation of xenobiotics; any liver disease modifies the metabolism and toxicity (Viala, 2007). The liver's histopathological examination inthis study is shown in Figure 7, which explains the following:

- The livers of control mice have a normal appearance without hepatic changes, with a lobed parenchyma architecture. Each lobe consists of hepatocytes arranged in Remak trabecular around a central vein called the centrilobular vein, which receives blood from the liver parenchyma in contact with the sinusoids. The Remak trabecular is made up of hepatocytes stacked in epithelial sheets one cell thick, while the sinusoids occupy the spaces between the trabecular (Figure 7.1 (M) and 1 (F));

- The livers of mice receiving 250 mg/kg BW of AEAVG showed a similar appearance to controls, with no hepatic changes (Figure 7.2 (M) and 2 (F));

- The livers of mice receiving 500 mg/kg BW showed dilated sinusoids, vascular congestion in the veins, and fatty degeneration (Figure 7.3 (M) and 3 (F));

- Degenerative changes are seen in the mice receiving 1000 mg/kg BW of AEAVG (Figure 7.4 (M) and 4 (F)); the architecture of the liver tissue is partially effaced, the sinusoids are dilated, the veins show vascular congestions and fatty degeneration is seen.

Rasheed *et al.* (2009) have shown similar cases in *Albino* rats after injection of *Eugenia jambolana* plant extract. However, the results indicate that there is no cell death yet, but perilobular necrosis initiation may lead to cell death in the long term. On the other hand, fatty degeneration and fibrosis are observed in female mice. Direct cytotoxicity is known to be the fundamental cause of liver damage in some cases, while in others, immunological mechanisms or even a mixture of cytotoxicity and immunogenicity may be involved (Ingwale *et al.* 2009).

The kidneys

The kidney is susceptible to various toxic compounds that may cause various types of damage and impair various organ functions (Cronin and Henrich, 2005). The photomicrographs of the mouse kidneys from this investigation are shown in Figure 8, which explains the following:

- The kidneys of control mice of both sexes (Figure 8.1 (Ma, Fa, Mb and Fb)) show a typical architecture with a conjunctivoadipuate capsule. In the cortical area, we observe small spherical masses, the glomeruli, surrounded by a capsule (the Bawman capsule). The glomeruli provide glomerular filtration of blood, passing water and low molecular weight compounds into the urinary tract, where a large proportion of the latter is reabsorbed, the remainder being the urine;

- The kidneys of mice given 250 mg/Kg BW of AEAVG did not show any changes in the renal parenchyma or renal glomeruli (Figure 8.2 (Ma, Fa, Mb and Fb));

- The kidneys of mice receiving 500 mg/Kg BW of AEAVG (Figure 8.3 (Ma, Fa, Mb and Fb)) showed tubular enlargement and congestion;

- The kidneys of animals receiving 1000 mg/kg BW of AEAVG show histopathological changes in both sexes characterized by tubular congestions and necrosis, enlargement of the tubular lumen, and tissue damage are more remarkable in females (Figure 8.4 (Ma, Fa, Mb and Fb)).

These tissue abnormalities could be due to certain toxic compounds in the aqueous extract, andthese toxic substances may be the alkaloids whose toxicity is increased by transformation into toxic reactive metabolites. The same liver and kidney damage was caused by other plants containing toxic alkaloids, such as *Atractylis gummifera*, *Callilepsis laureola* (Larrey, 1997; Peyrin-Biroulet *et al.* 2004).

It is insufficient to utilize a chemical in therapy simply because it is effective in pharmacology. A chemical is considered toxic if it has the potential to completely inhibit and kill a living thing by interfering with its normal functioning either instantly or over time, temporarily or permanently (Viala and Botta, 2007). Therefore, it is essential to specify the benefit-risk balance in each substance's therapeutic indication (Subramanion *et al.* 2011).

Conclusion

The aqueous extraction yield for *Aloe barbadensis* miller gel is 0.58 (0.077%). The mice that were given AEAVG at doses of 500 and 1000 mg/kg BW demonstrated a variety of toxicological symptoms, which were more prominent in the group receiving 1000 mg/kg BW but without a fatality, according to the EAGAV study on acute toxicity. At all doses, mice's WCR and WG typically increased with time, especially those receiving the AEAVG dose of 500 mg/kg BW. At dosages up to 1000 mg/kg BW, neither the relative weight (RW) of the two kidneys nor the weight of the liver changed. The mice consumed more food and fluids after receiving an AEAVG dose of 250 mg/kg BW, as seen by their increased consumption. Transaminases, urea, and albumin test results

indicate that a 1000 mg/kg BW dose of AEAVG may cause minor liver and kidney damage, and the anomalies seen in the histological sections supported these findings.

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Abbreviations

AEAVG: aqueous extract of *Aloe vera* gel
ALT: alanine aminotransferase
AST: aspartate transaminase
***A v*:** *Aloe vera*
DW: distillated water
GOT: glutamic oxaloacetic transaminase
GPT: glutamate-pyruvate transaminase
H&E: hematoxylin-eosin
LDH: Lactate dehydrogenase
NETD: no toxic effect dose
ns: not significant
RWO: relative weight organs
WCR: weight change ratio
WG: weight gain
ws: without significance

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