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Research Article

Toxicological Investigations of *Aristolochia longa* Root Extracts

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Aristolochia longa L. (Aristolochiaceae) is an herbaceous plant recognized in alternative medicine for its many therapeutic virtues. The aim of this study was to determine the pharmacotoxicological effects of this plant in order to ensure safe clinical use. The oral toxicity of the aqueous extract of *A. longa* roots was performed *in vivo* on Wistar rats at doses of 0.8, 1.25, 2, 2.5, and 5 g/kg/day for 21 days. Clinical signs were observed throughout the experimental period, followed by measurement of body weight change, while selected biochemical parameters, as well as relative organ weights and the histology of liver, kidney, and intestinal tissues, were evaluated after 6, 11, and 16 days and then at the end of 21 days of daily administration. At repeated doses for 21 days, the extract contributed to significant weight gain, in both control and treated rats. The global analysis of hepatic and renal biomarkers showed a significant increase between control and different doses of the extract, from the first to the third week of treatment, indicating the likely toxic effect of the extract on liver and kidney function. Organ toxicity was confirmed by histopathological examination, which revealed greater renal and hepatic parenchymal changes in animals treated with a high dose beyond the 16th day. At the end of the treatment, relatively small size of intestinal villi was also observed. It was concluded that ALAE has a low toxicity potential in nonprolonged oral administrations. However, at high chronic oral doses, *A. longa* appears to have significant toxicity on the organs tested.

1. Introduction

Traditional medicine based on the use of medicinal plants for the prevention and treatment of many diseases continues to be used, and over the past decade, its popularity has also increased. This widespread use usually comes from the belief that medicinal plants have very low toxicity due to their natural origin. The practices of traditional medicine vary from one country to another and from one region to another. They are influenced by factors such as culture, history, and personal philosophies. According to the WHO, nearly 80% of people in developing countries use natural products [1, 2], which are practically the only therapeutic arsenal available to traditional healers [3]. These medicinal plants can therefore constitute important resources for new substances with therapeutic potential and low cost [4]. However,

many herbs used in phytotherapy are known to be toxic. In some cases of intoxication, unsuitable formulations, falsifications, underestimation of the risks of interaction between plants and drugs, and ignorance of the doses used in drug preparation and administration result in therapeutic accidents and adverse reactions that may be tragic [5].

Aristolochia longa, belonging to the Aristolochiaceae family, is a plant frequently required in Moroccan traditional medicine and known locally as "Barraztam." It is a widespread species in North Africa, Europe, and Asia [6]. It has been reported that *A. longa* has many uses and virtues, and it is recommended to be used in weight loss diets, arthritis prevention [7], healing [8], and also used as anti-inflammatory and analgesia [9]. However, the most widespread use of *A. longa* in Morocco concerns the treatment of cancer. There were other preparations, suggested by traditional healers, mixing *A. longa* root powder with salted butter for the treatment of multiple abnormalities such as skin infections, gangrene [10, 11], upper respiratory tract infections, and abdominal pain [12–14]. Despite the importance of this herb in improving or healing pathological processes, it contains aristolochic acid (AA) [15, 16] which is the toxin responsible for the development of clinical syndromes called Chinese herbal nephropathy (CHN) and endemic nephropathy (EN) [17].

In view of the wide use of this plant, it is important to highlight its toxicological properties, in order to optimize its use. For this reason, the toxic effect of rhizomes of *A. longa* on the biochemical and histological parameters was evaluated over time.

2. Materials and Methods

2.1. Plant Material

2.1.1. Plant Collection. The roots of *A. longa* were collected in the province of Al Haouz in Morocco during the month of April 2016. After collection, the plant material was authenticated and a voucher specimen "RAB 110969" has been deposited in the Herbarium of Botany Department of the Scientific Institute of Rabat, Morocco. Fresh roots of the plant were first washed with water, dried at room temperature (under shade in a dry place), and ground into powder.

2.1.2. Preparation of Aqueous Extract. With a magnetic stirrer, 50 g of powder was macerated in 500 mL of distilled water for 24 hours. The macerate was then centrifuged and filtered using a filter paper (Whatman). The filtrate obtained was finally evaporated to dryness at 50°C by a rotary evaporator. The aqueous extract obtained was conserved at 4°C until other uses.

2.2. Animals. The toxicity test was performed on albino Wistar rats of both sexes selected at random (140–174 g). The animals were housed in cages containing sawdust and acclimatized under standard culture conditions of light/dark cycle (12/12 h), temperature (22–24°C), and air changes. They had free access to food and water during the experiment. They were used in accordance with the Guide for the Care and Use of Laboratory Animals [18].

2.3. Toxicity Test. This study was performed by gavage using an intragastric probe on rats of both genders. The rats were randomly distributed into 6 groups of 8 animals each (males and females), and they are marked for individual identification.

The control group received the vehicle only (distilled water), while the other five lots received daily increasing doses of the *A. longa* aqueous extract (ALAE), respectively (0.8, 1.25, 2, 2.5, and 5 g/kg body weight) for 3 weeks divided into 4 periods; the 1st week of treatment, the 11th day, the 16th day, and the 21st day of the beginning of the experiment. The volume of solution administered daily in a single dose was 1 mL/100 g of body weight. The behavior of rats was observed regularly and their weight measured daily.

At the end of each period, animals were anesthetized and euthanized by decapitation. The blood of each animal was collected in vacuum tubes containing anticoagulant lithium heparin and centrifuged at 4000 rpm for 10 minutes. The serum obtained was stored at −20°C for biochemical analysis. Then, the organs (liver, kidney, and intestine) were removed, rinsed with saline solution (0.9%), and weighed. The relative weight of the liver and kidneys was calculated.

2.3.1. Behavioral Changes. During the 3 weeks of study, clinical symptoms of morbidity and the general behavior were observed daily and individually in all animals, after administration of the solution. All observations were systematically recorded and focused on mobility, sensitivity to noise, breathing, convulsions, vomiting, diarrhea, salivation, sleep, and coma. Moreover, all signs of toxicity or death appeared were recorded.

2.3.2. Body Weight. Throughout the exhibition period, we followed the individual weight of each rat by daily weighing. The changes are calculated and presented in Figure 1.

2.3.3. Biochemical Parameters. Renal function was determined by plasma creatinine and urea, while the liver function was determined by the enzymatic activity of the aspartate aminotransferase (AST) and alanine aminotransferase (ALT). These biochemical analyses were performed by standard methods using an automated biochemical analyzer of the type COBAS INTEGRA® 400 plus (Roche Diagnostics GmbH, Mannheim, Allemagne).

2.3.4. Histopathology. After weighing, the organs were sectioned into small slices and preserved in 10% formalin for 48 hours, then dehydrated by serial alcohol solution, cleaned with toluene, and immersed in liquid paraffin. Sections of 4 microns were performed using a microtome and stained with hematoxylin-eosin (HE). The slides were observed using a light microscope, and photomicrographs were recorded [19, 20].

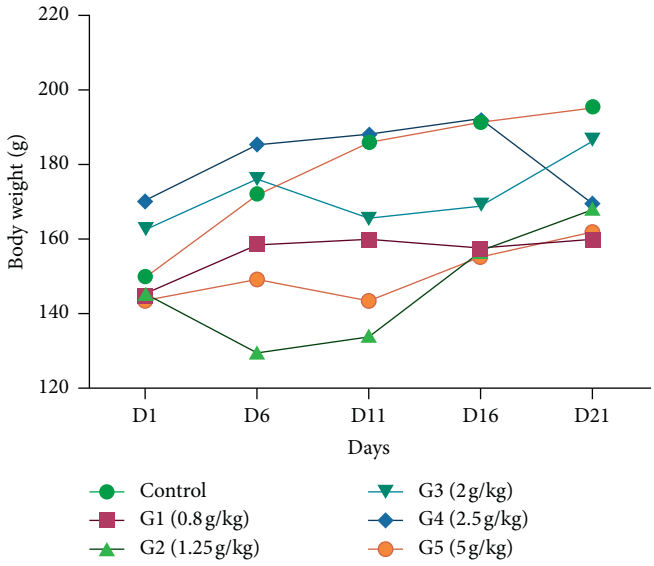


Figure 1: Changes in the body weights in rats treated with the aqueous extract of *Aristolochia longa*.

2.4. Statistical Analysis. Data are expressed as means ± SD. Statistical significances between control and treated groups were determined by one-way analysis of variance (ANOVA), followed by Dunnett's post hoc test. GraphPad Prism version 6.0 for Windows was used for statistical analysis. The differences were considered statistically significant at $p < 0.05$.

3. Results

3.1. Behavior of Animals. The behavior of rats was observed, counting mortality for 21 days of administration of the aqueous extract or solvent (control). Doses lower than 2 g/kg did not give serious signs of intoxication. However, on the 4th day of treatment, 2 g/kg of the extract caused in one animal swelling of the right side under the shoulder, respiratory distress, and slowing of the movements. Concerning the dose of 2.5 g/kg on the 15th day, one rat vomited one hour after administration. On the 3rd day of the highest dose, abnormalities of coordination and a decrease in motility of rats were noted. The day that follows, all the signs were observed to be disappeared. Effectively, all changes observed occurred at least 2 hours after gavage and disappeared depending on the dose of the extract.

3.2. Body Weight. In comparison with the body weight of the first day of the experiment, a significant gain in weight was noted, after 21 days, in both control and treated rats. However, at the end of the experiment, a difference is observed between the groups, and the weight of the treated animals remains lower than that of the control animals. These observed weight changes between controls and treated rats are statistically significant ($p < 0.05$). However, during the treatment period, all groups recorded significant decreases ($p < 0.05$ to $p < 0.001$) in body weight gain compared to their counterparts in the control group (Figure 1).

3.3. Relative Organ Weight. For the liver, no statistically significant difference was found between the control and the different doses of the extract, except for the 21st day when a decrease was observed for all doses (Figure 2). Also, the kidneys removed showed no significant variation in their weight, except an increase recorded on the 11th day at a dose of 1.25 g/kg (Figure 3).

3.4. Biochemical Parameters. Evaluation of biochemical parameters allowed us to reveal the probable effects of *A. longa* roots on the liver and kidney. As shown in Table 1, the overall analysis of hepatic biomarkers expressed a significant increase between the control and the different doses of the extract, from the first to the third week of treatment. The highest dose of the extract resulted, at the end of treatment, in a marked and significant increase in AST (189.5 IU/L ± 2) and ALT (87.3 UI/L ± 2.1) as compared to the controls (AST: 93.3 UI/L ± 6.6; ALT: 32.7 UI/L ± 1.1; $p < 0.05$).

Serum parameters of renal function assessment presented in Table 2 showed that, at each period of the experiment, urea concentrations increase significantly in treated animals ($p < 0.05$) compared to the control. Quantification of creatinine revealed that, regardless of the treatment period, there was a significant increase in rates outside the 16th day for which an increase was noted but remained insignificant. At the end of the experiment, there was a clear proportionality between the dose and the creatinine concentration with high levels of 5.31 mg/L ± 0.13 and 5.40 mg/L ± 0.03 for doses 2.5 and 5 g/kg, respectively, relative to the control (3.07 mg/L ± 0.12) (Table 2).

3.5. Histopathological Changes. The photomicrographs of liver sections showed that, during the first six days, the architecture remains preserved with hepatocytes of quite normal appearance. However, at a dose of 5 g/kg, there was a slight centrilobular sinusoidal dilatation (Figure 4). On the 11th day, the stability of the already described aspect was observed, with an accentuation of the sinusoidal dilatation (Figure 5) with some images suggesting hepatocyte suffering, such as clarification, ballooning of the cytoplasm, the presence of rare macro- and microvesicles (Figure 6), and discrete nuclear retraction (Figure 7). Beyond the 16th day, there was a tendency towards the aggravation of lesions. Indeed, we noted the presence of dissociation of hepatocyte cords and the signs of hepatocyte suffering seemed even more obvious with some foci of liquefaction necrosis (Figure 8). At the highest dose, we have noticed the presence of some macrophage cells of perivascular seat corresponding to hypertrophied Kupffer cells (Figure 9). Moreover, the presence of sometimes extensive hemorrhagic foci, images of edema, and congestion of the parenchyma, pyknotic nuclei in places, and paradoxically the absence of extensive inflammatory infiltrates have been recorded (Figure 10).

The histological aspect of the kidneys of the euthanized rats showed a lesion progression similar to that of the liver previously described. Indeed, for the first six days, no lesion was noted (Figure 11). On the 11th day, the Malpighian

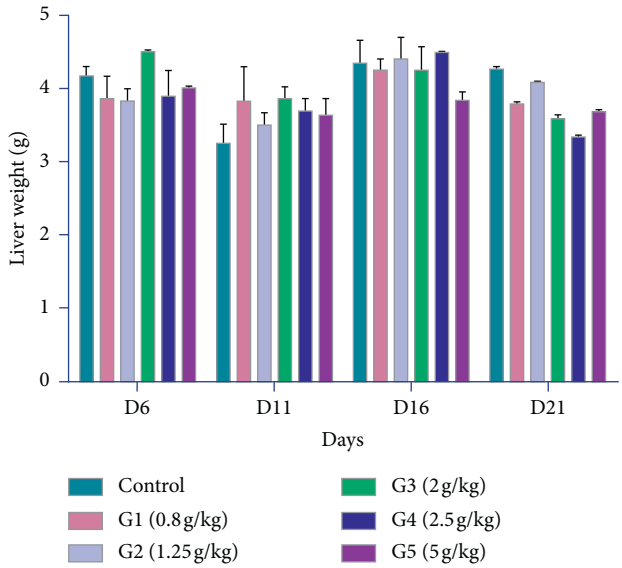


Figure 2: Effects of ALAE on the relative weight of the livers. The values are the mean of two determinations ± SD.

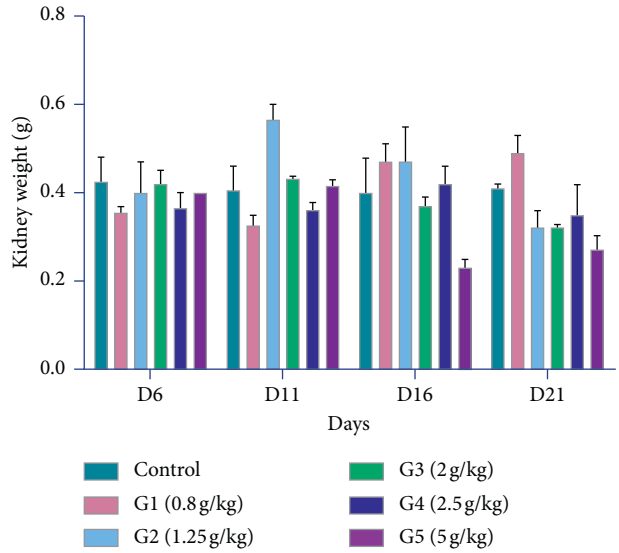


Figure 3: Effects of ALAE on the relative weight of the kidneys. The values are the mean of two determinations ± SD.

Table 1: Effect of ALAE on liver function (AST and ALT) in rats.

Groups	AST (UI/L)				ALT (UI/L)			
	Day 6	Day 11	Day 16	Day 21	Day 6	Day 11	Day 16	Day 21
Control	100.8 ± 6.4	101.32 ± 5.12	80.0 ± 3	93.3 ± 6.6	22.8 ± 1.5	25.6 ± 3.1	32.2 ± 3.3	32.7 ± 1.1
G1	134.6 ± 5.4**	107.9 ± 2.5	93.2 ± 0.4	227.4 ± 6.2***	36.6 ± 0.5	37.7 ± 2.5*	44.6 ± 3.90*	86.3 ± 2.1***
G2	145.6 ± 1.30***	123.3 ± 0.2*	87.7 ± 2.4	130.8 ± 3.5*	25.3 ± 2.60	31.3 ± 2.1	29.7 ± 0.9	45.9 ± 1.4**
G3	122.5 ± 4.6*	139.4 ± 2.9***	106.0 ± 5.2	117.1 ± 5.8	28.7 ± 1.1	35.4 ± 0.3	33.5 ± 1.7	34.9 ± 1.3
G4	100.8 ± 2.9	144.8 ± 5.3***	94.8 ± 0.9	107.1 ± 4.7	29.6 ± 0.6*	34.2 ± 2.4	43.3 ± 2.1	33.4 ± 1.6
G5	124.1 ± 3.4*	172.5 ± 3.8****	92.0 ± 4.4	189.5 ± 2****	32.2 ± 1.9*	44.0 ± 1.6**	23.7 ± 2.0	87.3 ± 2.1****

Values are expressed as mean ± SD, $n = 2$. * $p < 0.05$ compared to the control group. ** $p < 0.01$ compared to the control group. *** $p < 0.001$ compared to the control group. **** $P < 0.0001$ compared to the control group.

corpuscles at the highest doses showed an enlargement of the capsular spaces, and the urinary tubules looked normal, with a balloon cell epithelium in some places (Figure 12). From the 16th day, the lesions worsen with a distortion of corti-comedullary structure, signs of hemorrhage, congestion, and edema (Figures 13(a) and 13(b)). In the Malpighian

Table 2: Effect of ALAE on renal function (urea and creatinine) in rats.

Groups	Urea (g/L)				Creatinine (mg/L)			
	Day 6	Day 11	Day 16	Day 21	Day 6	Day 11	Day 16	Day 21
Control	0.19 ± 0.01	0.26 ± 0.02	0.23 ± 0.02	0.23 ± 0.03	2.48 ± 0.04	2.51 ± 0.04	3.16 ± 0.09	3.07 ± 0.12
G1	0.20 ± 0.01	0.40 ± 0∗	0.20 ± 0.02	0.34 ± 0.04	3.94 ± 0.16∗ ∗ ∗	3.36 ± 0.27∗	3.26 ± 0.12	3.82 ± 0.08∗ ∗
G2	0.34 ± 0.02∗ ∗ ∗	0.32 ± 0.03	0.26 ± 0.01	0.40 ± 0.03∗	3.57 ± 0.05	4.97 ± 0.17∗ ∗ ∗ ∗	3.13 ± 0.16	3.13 ± 0.09
G3	0.29 ± 0.02∗ ∗	0.29 ± 0.02	0.41 ± 0.02∗	0.32 ± 0.03	3.76 ± 0.38∗ ∗ ∗	4.51 ± 0.03∗ ∗ ∗	3.49 ± 0.12	3.51 ± 1
G4	0.25 ± 0.01∗	0.25 ± 0.03	0.27 ± 0.03	0.29 ± 0.02	3.33 ± 0.21∗ ∗	3.21 ± 0.09∗	2.72 ± 0.08	5.31 ± 0.13∗ ∗ ∗ ∗
G5	0.37 ± 0.03∗ ∗ ∗	0.29 ± 0.02	0.26 ± 0.06	0.33 ± 0.03	3.67 ± 0.09∗ ∗ ∗	3.90 ± 0.08∗ ∗	3.33 ± 0.17	5.40 ± 0.03∗ ∗ ∗ ∗

Values are expressed as mean ± SD, $n = 2$. ∗ $p < 0.05$ compared to the control group. ∗ ∗ $p < 0.01$ compared to the control group. ∗ ∗ ∗ $p < 0.001$ compared to the control group. ∗ ∗ ∗ ∗ $P < 0.0001$ compared to the control group.

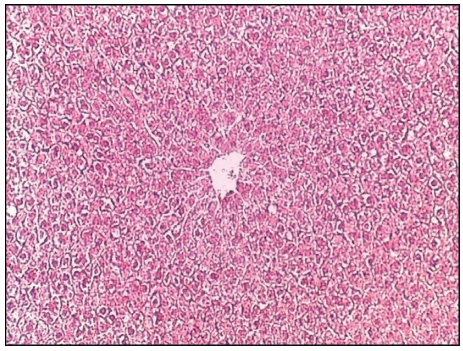


Figure 4: The 6th day of treatment at a high dose. Centrilobular sinusoidal dilatation with macro- and microvesicles (magnification ×20).

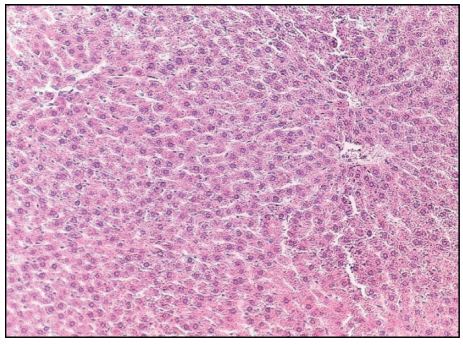


Figure 5: 11th day of treatment at a concentration of 2.5 g/kg. Accentuation of sinusoidal dilatation (magnification ×20).

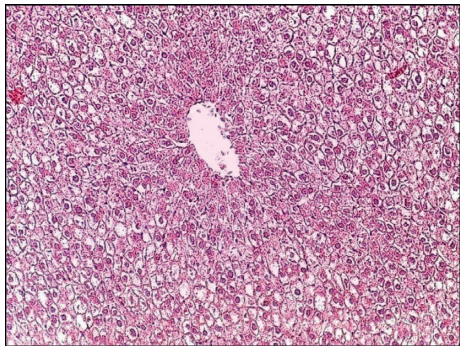


Figure 6: The 11th day of treatment at a concentration of 2 g/kg. Macro- and microvesicles with clarification and ballooning of the cytoplasm (magnification ×20).

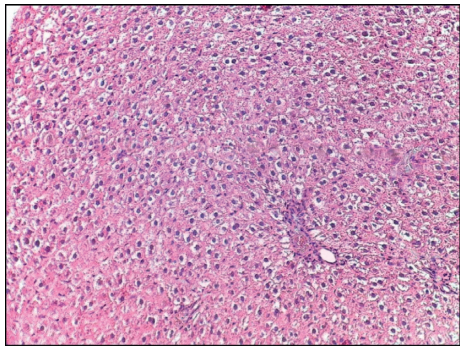


Figure 7: The 11th day of treatment at a concentration of 2 g/kg. General necrosis and significant nuclear retraction (magnification ×20).

corpuscles, the retraction of the glomeruli is even more obvious and corresponds to glomerular atrophy (Figure 14). The urinary tubules showed a tendency to luminal collapse with cells that appeared ballooned reminiscent of osmotic nephrosis (Figure 15). Otherwise, there was an inflammatory infiltrate that seemed unspecific, and the signs were dose-dependent (Figure 16). At the highest doses, extensive hemocorticomedullary lacunae were recorded.

In the intestine, whatever the dose administered on both the 6th and the 11th days, no abnormalities were observed (Figures 17(a) and 17(b)). On the 16th day, the number and height of the villi as well as the Lieberkühn glands appeared normal (Figure 18). The intravillous inflammatory infiltrate appeared to be substantially normal, and the proportion of enterocyte/mucus cells seems respected. On the 21st day, the villi were more fragile of relatively small size (Figure 19(a)). However, the inflammatory infiltrate was more abundant and signs of hemorrhage were evident (Figure 19(b)). There has also been a slight rupture of the enterocyte/mucus cell ratio in favor of a relative increase in the number of mucus cells.

4. Discussion

About 85% of the population in developing countries uses medicinal plants for their primary health care [21]. This widespread use may be due to the low price and the belief

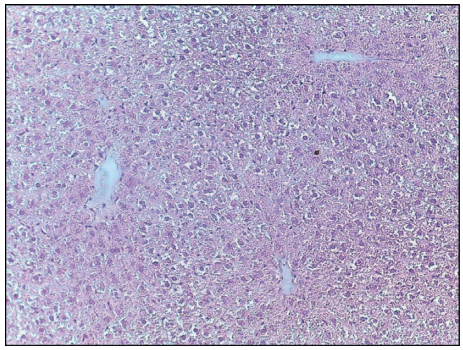


Figure 8: The 16th day of treatment at a concentration of 0.8 g/kg. Liquefaction necrosis (sign of liver suffering) with congestion and dissociation of hepatocyte cords (magnification ×20).

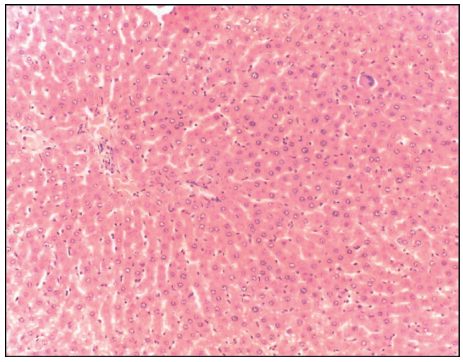


Figure 9: The 16th day of treatment at a high dose. Giant cell (Kupffer) (magnification ×20).

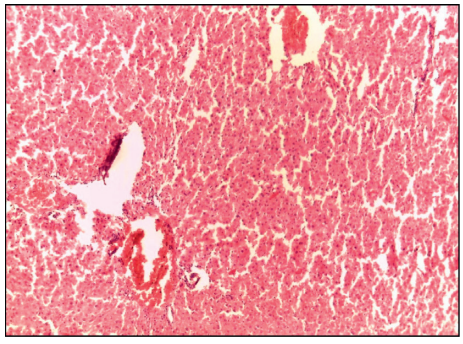


Figure 10: The 21st day of treatment at a high dose. Presence of hemorrhagic foci, edema, parenchymal congestion, dissociation of hepatocyte cords, and extensive necrosis (magnification ×10).

that they have no ill effects [22]. *A. longa* contains bioactive principles that may be beneficial and/or harmful. In fact, Aristolochia herbs are rich in nitrophenanthrene carboxylic acids, mainly aristolochic acid [23] which is a combination of structural acids (AA I and AA II) [24] with a toxic effect [17]. A formal toxicological evaluation of this plant is necessary in order to define its intrinsic toxicity, its effects in case of overdose [25], and to be able to select a safe dose in humans by assessing the toxicity limits of the plant on animals.

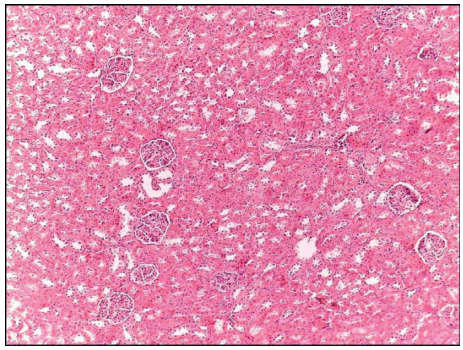


Figure 11: The 6th day of treatment at a concentration of 2.5 g/kg. Normal structures (magnification ×10).

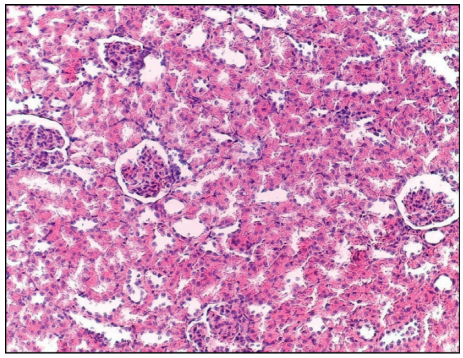
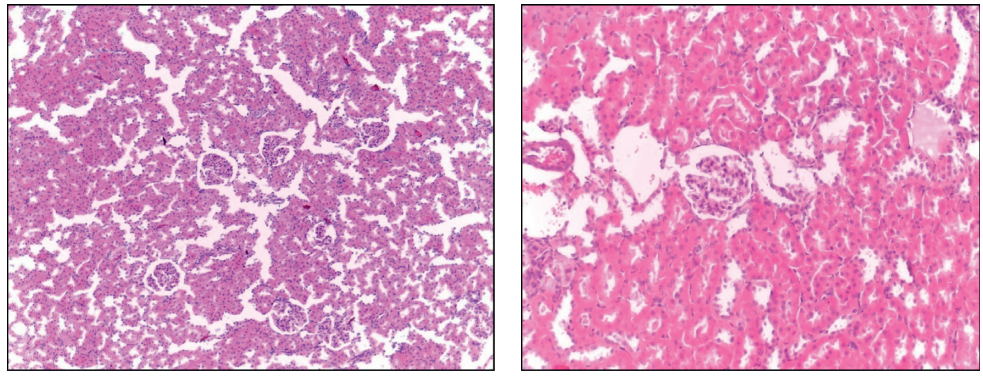


Figure 12: The 11th day of treatment at a high dose. Retraction of glomeruli and enlargement of capsular spaces (magnification ×20).

The change in body weight is used as an indicator necessary to check the evolution of body weight as well as the general behaviors because they are the first signs of toxicity [26, 27]. Indeed, ingestion of ALAE at high doses resulted in transient and low-intensity behavioral changes, and this is consistent with the results of Guinnin et al. [28] who recorded clinical signs of short duration after administration of the ethanolic extract of *A. albida* to animals. At the last weighing (21st day), the extract favored the weight growth of all the rats. This could be due to the increased food consumption of animals by stimulating their appetite through the extract [29]. On the other hand, weight loss observed during treatment could be related to several factors such as animal stress, dose/absorption interactions, and poor food assimilation by the presence of polyphenols [28]. A similar decrease in body weight was noted in mice given 5 g/kg of aqueous extract roots of *A. longa* for 4 weeks [30]. From these results, it can be deduced that ALAE delayed the biological growth of rats over time.

The liver and kidneys are among the first organs affected by the metabolic reaction caused by a toxic substance [31]. Regarding our study, there was a significant decrease in relative liver weight in all ALAE-treated rats during the last treatment period. This attenuation would be due to a probable toxic effect of the extract [32]. Usually, alterations in body weight gain and internal organ weight reflect toxicity after exposure to a toxic substance [33, 34].



(a) (b)

Figure 13: (a) The 16th day of treatment at a concentration of 0.8 g/kg. Distortion of the corticomedullary architecture with congestion and edema (magnification ×10). (b) The 16th day of treatment at a concentration of 1.25 g/kg. Hemorrhage and luminal collapse (magnification ×20).

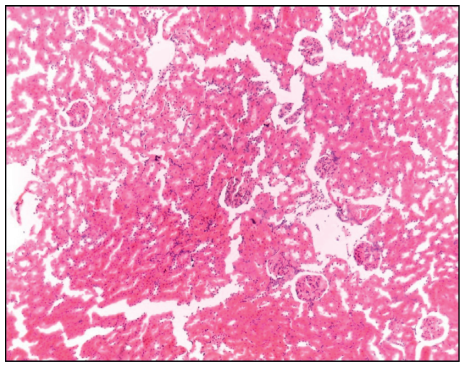


Figure 14: The 16th day of treatment at a dose of 2 g/kg. Distortion and glomerular retraction (magnification ×10).

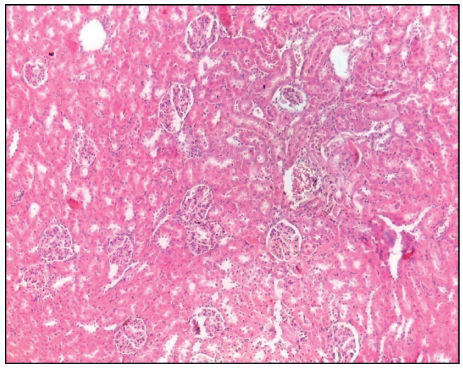


Figure 15: The 16th day of treatment at a dose of 2.5 g/kg. Hemorrhage and ballooning of the cells of the urinary tubules (magnification ×10).

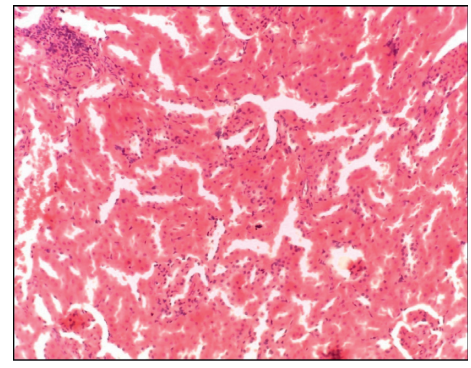
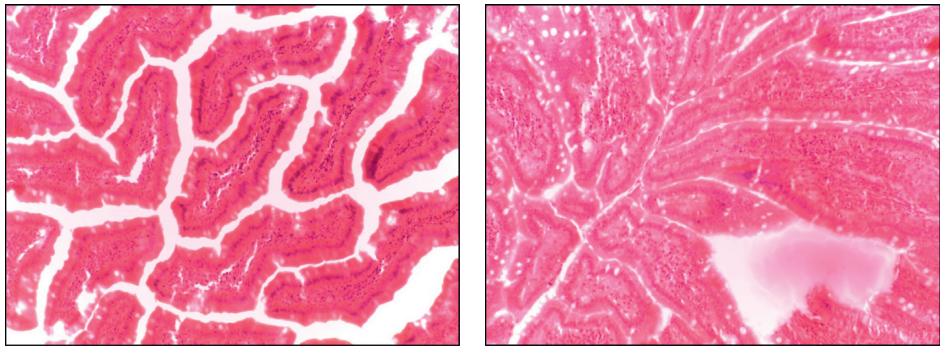


Figure 16: The 21st day of treatment at a dose of 0.8 g/kg. Non-specific inflammatory infiltrate with retraction of glomeruli (magnification ×20).

In this study, hepatorenal toxicity was studied by assaying a few biochemical parameters and by histopathological analysis of the kidney and liver. ALT is a cytosolic enzyme secreted in hepatocytes from which it is released into the blood in case of hepatic cell necrosis [35, 36]. It is a liver-specific enzyme, making it an important and very sensitive indicator of hepatotoxicity [37, 38]. AST is also an indicator of hepatocyte destruction, although in addition to the liver, it is found in the heart, skeletal muscles, lungs, and kidneys [36]. Transaminases (ALT and AST) are enzymes with significant metabolic activity within cells, and increased serum levels reflect hepatic damage caused by various reasons including hepatic cell necrosis, hepatitis, cirrhosis, and the hepatotoxicity of certain drugs [36, 37]. In the case of this study, the significant increases recorded suggest that administration of ALAE altered hepatic cells and consequently animal metabolism. These results corroborate those of Benzakour et al. [39] when the aqueous extract of *A. longa* roots at a dose of 2.5 g/kg body weight caused hepatocellular damage. This is also explained by the fact that the liver is the first organ exposed to all that is absorbed by metabolizing foreign substances that may be hepatotoxic. Equally, urea and creatinine analysis revealed that this extract resulted in significant increases. Indeed, these two parameters are considered the major markers of nephrotoxicity, although urea is often considered to be a more reliable predictor of renal function than creatinine [40], which is also a good indicator of renal function [41]. Moreover, the elevation of creatinine levels in the blood is a sign of functional nephrons



(a) (b)

Figure 17: (a) The 6th day of the treatment at a concentration of 0.8 g/kg. Normal structures (magnification ×20). (b) The 11th day of the treatment at a concentration of 1.25 g/kg. Normal structures (glands and villi) (magnification ×20).

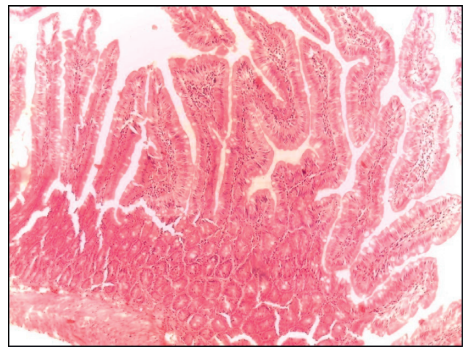
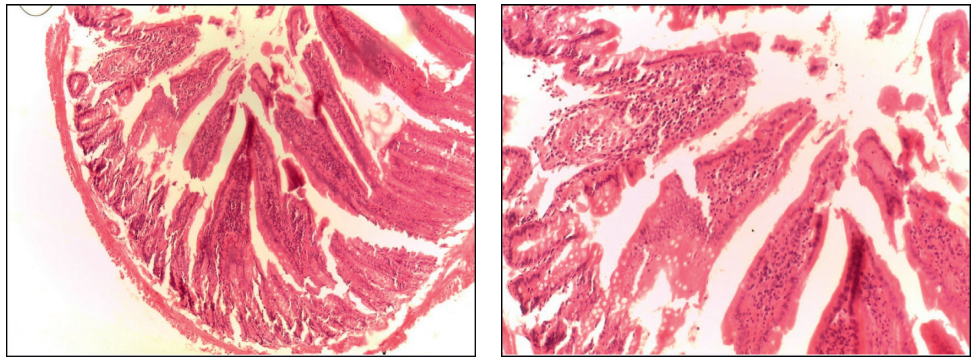


Figure 18: The 16th day of the treatment at a concentration of 2.5 g/kg. Normal structures (magnification ×10).



(a) (b)

Figure 19: (a) The 21st day of the treatment at a concentration of 0.8 g/kg. Fragility of the villi (magnification ×10). (b) The 21st day of the treatment at a concentration of 0.8 g/kg. Fragility and signs of hemorrhage with abundant inflammatory infiltrate (magnification ×20).

damage [42]. In this work, the increase in renal function parameters was evident and very significant from the first week which might suggest that the kidney was highly sensitive to the toxicity of ALAE, but thereafter there was a tolerance to this substance since the rates of these parameters tend to stabilize until the end of the experiment when we observed again a clear increase (Table 2). The same result was obtained by Yuan et al. [43] on rats treated for 28 days with *A. fructus* and honey-fried *A. fructus*, and also according to Liu et al. [44], using the same animal model, the administration of 4 g of *A. manshuriensis* and 4 mg of aristolochic acid per day developed acute renal failure. On the other hand, another work [45] which also investigated the ethanol extract of *A. manshuriensis* for 8 weeks found that the renal function of the mice tested was unaffected. The changes observed will interfere with the kidney's ability to perform normal excretory roles. This may have contributed to elevated levels of blood urea in rats treated with ALAE. In

addition, the kidney is very sensitive to toxic substances because a large volume of blood flows through the kidney and filters large amounts of toxins that can concentrate on the renal tubules [46]. Also, this organ is the main site of toxicity by AA [47]. Given the results obtained, we can deduce that our product was toxic for all the parameters tested. As a result, it influenced renal and hepatic function.

This hepatic renal toxicity has also been confirmed by the results of the histological examination. The majority of studies evaluating the tissue toxicity of plant extracts provide results at the end of the process [48–50]. The originality of our study was to be able to give results in a chronological order during the administration of the daily doses of ALAE. However, one study [30] published the results in a chronological order comparable to ours.

Regarding the liver and according to Cherif et al. [30], abnormalities that have been observed, such as sinusoidal dilatation, hepatocyte necrosis, and the presence of an inflammatory infiltrate whose extent was not specified appeared at the end of the first ten days for all doses administered (1.5, 2.5, and 5 g/kg). These results are virtually identical to ours, but they appeared earlier, at the end of the 6^{th} day, only for the dose of 5 g/kg. From the 3^{rd} week (16^{th} day), concerning our study, the anomalies tend to accentuate with a dissociation of hepatocyte cords, images of hepatocyte suffering starting from the centrilobular seat to extend to the rest of the parenchyma. All authors are unanimous to attest to this evolution. However, for Cherif et al. [30], the presence of an inflammatory infiltrate of unspecified nature that appears early is fading on the 28^{th} day, and this seems paradoxical since the liver tissue lesions and the sinusoidal dilation were always present; then in another side, the inflammatory infiltrate that we observed remained extensive for the duration of the experiment. The severity of the lesions described by Benzakour et al. [39] was impressive and was not described anywhere else by the authors who have conducted similar studies on *A. longa*. Indeed, in this work, *A. longa* showed overt toxicity beyond the 16^{th} day for high doses in a progressive manner, while the liver had a normal appearance at low doses.

At the renal level, we observed a progression of lesions as already marked for the liver; thus, around the 11^{th} day, we recorded the first discrete signs, namely, vascular congestion and a noticeable widening of the capsular space at high doses. These findings may be supported by the work of Liu et al. [44] attributing the nephrotoxicity of *A. manshuriensis* to its aristolochic acid. However, there was no evidence of tubular involvement as reported by most authors even at low doses [17, 44, 51]. With regard to the observed lesions, we share the same histopathological aspects concerning tubular damage and the atrophy of these cells but at high doses and beyond the 16^{th} day, as indicated by [15, 39]. Several authors have noted the presence of inflammatory infiltrate and interstitial fibrosis caused by the administration of Aristolochia species [39, 44, 45]. Furthermore, the consumption of plants of the genus *Aristolochia* can result in renal interstitial fibrosis [52, 53]. Effectively, for our case, we observed an inflammatory infiltrate that remained discrete, nonspecific, and dose-dependent. About fibrosis, we noted hemorrhagic lacunae sometimes extensive, as reported by Cherif et al. [30]. The observed nephropathy can be explained by the presence of AA responsible for the nephrotoxic [15, 51, 54] and genotoxic effects [55, 56] as well as by the presence of phenol [57, 58]. The originality of our study was the observation of an important enlargement of the glomerular capsular space and consequently glomerular retraction and these damages were not described elsewhere.

Concerning the intestine, the bibliographic data, in our possession, report only one observation in which a lesion was found in the small intestine [39]. Toxicity in the intestine can be manifested by intestinal atrophy with villous depletion and flattening [59], and the inflammatory contingent is also exaggerated [60]. In our case, we cannot affirm that we observed these same lesions, but nevertheless, we can note at the high doses some discrete signs which can suggest a villous atrophy.

The molecular mechanism of toxicity of the organs studied could be explained by the presence of AA, which is mutagenic and capable of forming covalent DNA adducts during metabolic activation in different organs *via* a cyclic nitrenium ion [61, 62], leading to DNA damage and cell cycle arrest [63].

Considering these data, it can be deduced that *A. longa* seems to be toxic, since it caused renal, liver, and intestinal toxicity. However, at the beginning of the experiment, the herb has not caused significant toxic effects even at high doses; this suggests that, in addition to the AA, there could be other major constituents in the rhizomes of *A. longa* dominating the intensity of toxicity, action speed, and possessing immunomodulatory properties [39] able to involve immune responses in induced lesions.

5. Conclusion

In conclusion, this study demonstrated that oral administration of ALAE for several weeks at high doses has a dose-response effect. Therefore, all species of Aristolochiaceae contain very different levels of AA; if used properly, the population can benefit without toxic effects. Further studies should be conducted to determine the effects of this plant on pregnant animals and their fetuses, over longer study periods, in order to complete the toxicity profile of this plant.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest to declare.

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