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# Toxicological evaluation of Aristolochia longa L. extract in mice

KEYWORDS: Aristolochia longa L., acute toxicity, sub-acute toxicity, histological examination


| Cherif H.S | Saidi F | Guedioura A |
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| Département de Biologie, Faculté des Sciences Agronomiques-Vétérinaires et Biologiques Université Saad Dahlab, Route de Soumâa BP 270, Blida-Algérie | Département de Biologie, Faculté des Sciences Agronomiques-Vétérinaires et Biologiques Université Saad Dahlab, Route de Soumâa BP 270, Blida-Algérie | Département de Biologie, Faculté des Sciences Agronomiques-Vétérinaires et Biologiques Université Saad Dahlab, Route de Soumâa BP 270, Blida-Algérie |


**ABSTRACT** *Aristolochia longa L. belongs to the Aristolochiaceae family. Although it is a plant known for its toxicity due to aristolochic acids (AA) which it contains, it is widely used in traditional medicine. This work focuses on toxicological evaluation of A. longa L. aqueous extract of the roots. The study of the aqueous extract of the roots acute toxicity in male and female mice, administered orally, has verified its safety to a singles doses of 1g/kg to 5g/kg.. Just as, the evaluation of the sub acute toxicity of the aqueous extract in mice for 28 days at doses of 1.5, 2.5, and 3.5g/kg body weight, showed a slight decrease in body weight and other signs morbidity and a disturbance of some hematological parameters. We also noted a mortality rate that increases with doses. Biochemical parameters studied in this evaluation showed a significant increase in the concentration of urea and plasma creatinine. Histological examination showed alterations of the renal parenchyma and the liver which are greater in animals treated with high dose.*

**Introduction**

Aristolochia longa L. (Aristolochiaceae) locally called "Beroustoum" is a species commonly used in Algerian traditional medicine (Cherif *et al.*, 2011). It has multiple applications and virtues; it is recommended for ovarian failure (White-ary, 1977), healing (Valnet, 1983), diuretic (Bellakhdar, 1997), analgesic, anti-inflammatory, anti-mitotic (Iserin, 2001; Gounet *et al.*, 2002), etc.

The powder of roots of Aristolochia longa L. with salted butter is used to treat skin infections and gangrene (Gadhi & al, 1999; Gadhi & al, 2001), as anti-cancer (Wu *et al.*, 2004; Caude & Jardy, 2007), especially in case of sclerosis, uterine and nasal cancer (Wu & al., 2004).

Despite their virtues, Aristolochia longa L. contains aristolochic acid (AA) (Menges & Stotzen, 1993; Vanhaelen &al., 1994). AA-I and AA-II acids are mutagenic in bacteria, mice and mammalian cells (Arlt &al, 2002; Kohara *et al.*, 2002; Mei & al., 2006).

The ingestion of herbal remedies of this plant is associated with the development of a syndrome indicating aristolochic acid nephropathy (NAA) (Arlt &al., 2002; Cosyns, 2003) which is characterized by chronic renal failure, tubulointerstitiale fibrosis, and urothelial cancer (Pozdzik & al., 2010). Following these dangerous health consequences, a decision was taken to prohibit the use of any natural remedy containing this acid (Lewis & Alpert, 2000; FDA, 2001; Jian &al., 2007).

The aim of this work is to highlight the toxicity of rhizomes of Aristolochia longa L. To do so, acute and sub-acute toxicity were studied. Different doses of aqueous extract of rhizomes are administered by gavages to mice and biochemical parameters were measured.

**Materials and methods**

**Plant material**

The roots of Aristolochia longa L. plants were harvested in May 2012, 11 km from Medea (West of Algiers). The species was identified at the Department of Biology, University of Blida.

The roots were first washed with water and dried at room temperature (in the shade in a dry and ventilated place), the same procedure described by Benzacour& al. (2011) was used.

The aqueous extract was prepared by adding 500 ml of distilled water to 50 g of dry roots powder of A. longa L. After 24 hours of soaking and magnetic stirring at room temperature, the mixture was centrifuged, filtered and then concentrated in a rotary vacuum evaporator. The extracted material was dissolved in a solution of 0.9% of sodium chloride (NaCl).

**Animal material**

A number of 76 male and female mice (Swiss Albino NMRI) weighing between 18 and 22g were provided by the animal laboratory of Pharmacology and Toxicology (Antibiotical Saidal, Medea). The animals were acclimated to standard culture conditions; 12:12h light-dark cycle, temperature (22-24 °C), ventilation system, and free access to water.

**Study of acute toxicity**

The animals were divided into six groups of six mice each (3 males and 3 females), including a control group, and were deprived of food 24h before testing. They were weighed during the test. Five batches of the aqueous extract were administered orally successively: 1g/kg, 2g/kg, 3g/kg, 4 g / kg and 5g/kg. The control group received only saline. The general behavior of mice and clinical symptoms of toxicity (morbidity) were registered. Animals were observed individually every hour during the first day (intermittently for 8 hours), then every day for 14 days (Twaij &al., 1983).

**Study of sub-acute toxicity**

The mice were divided into four groups; three treatment groups and a control one containing 10 mice each. In the treated groups the mice received daily oral doses of aqueous extract of A. longa for 4 weeks at doses of 1.5g/kg, 2.5g/kg and 5g/kg body weight. However, the control group received normal saline. During the four weeks the animals were observed and toxic manifestations were considered (Brock & al., 1995).

The measured serological parameters: Creatinine and urea. Fluctuations of mice weight were considered.

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**Statistical analysis:**
The statistical significance of the data was determined by the Kruskal-Wallis test using STATISTICA software. Values were considered significant when $p < 0.05$.

## Results

### Acute toxicity of the aqueous extract of A. Longa

During the first three days of treatment, the animals were characterized by strong convulsions, diarrhea and a slight decrease in weight, even at low doses. After administration of the aqueous extract to different groups of mice, we observed mortalities only at doses of 4g/kg (1/6 mortalities) and 5g/kg (2/6 mortalities). Depending on the scale of Viala (1998), the extract tested is considered non-toxic or very little to a single dose.

### Sub-acute toxicity of aqueous extract of A. Longa

The behavior of the treated mice and control mice was observed, taking into account mortality during 28 days of feeding the aqueous extract or solvent (control). The lowest dose (1.5g/kg) has not given serious signs of intoxication; however, from the dose (2.5g/kg) signs of morbidity became increasingly important.

### Effect of aqueous extract on biochemical parameters - Weight

The weight of the mice in lot 1 was equivalent to that of the control mice until the seventh day, a decrease was observed at day 28. The average weight of the mice in lot 2 and 3 has a decreasing trend after the fourth day, compared to the control mice which had a growing weight ( see Fig. 1). Thus the aqueous extract of Aristolochia longa L. induces a more pronounced decline of body growth in the group treated with a high dose group, as in the treated medium and low dose groups compared with the control.

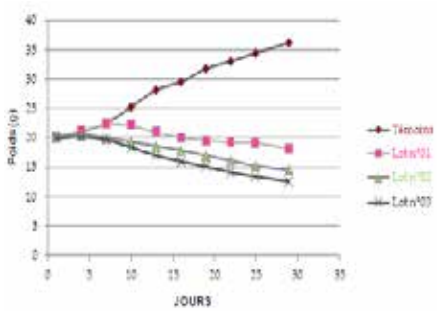



**Fig. 1. Evolution of the weight of control mice and mice treated under the conditions of the test sub-acute toxicity.**

### Plasma creatinine

The determination of plasma creatinine is performed to assess kidney function. Treated mice (Lot No. 01, 02 and 03), plasma creatinine increased on day 28 of 12.49%, 35.26% and 58.48% respectively compared to controls ( see Fig. 2).

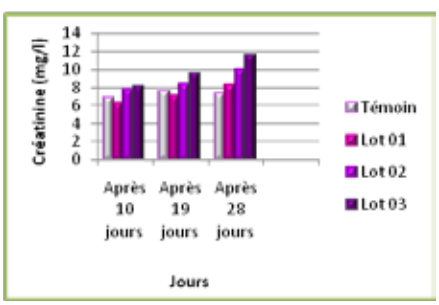



**Fig. 2. Parameters serum assessment of renal function (creatinine) treated mice and control mice in the test conditions of the sub-acute toxicity.**

### Urea

The results (see Fig. 3) show an increase in concentrations of urea from the 19th day treated mice (group 1, 2 and 3) and continues until the 28th day. In control mice, the concentration of urea is stable. We see that there's a clear proportionality between the dose and the concentration of urea.

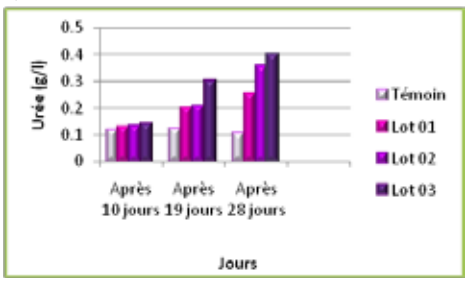


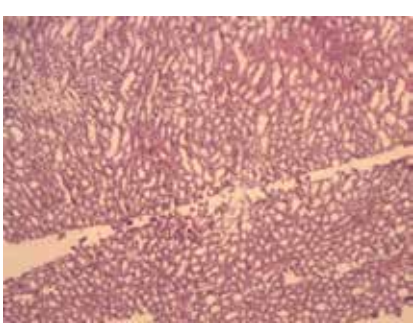

**Fig. 3. Serum assessment of renal function (urea)-treated mice and control mice in the test conditions of the sub-acute toxicity.**

### Histological observations

#### Renal

Hematoxylin-eosin staining on sections of kidney tissue showed that control mice have normal renal parenchyma structure is preserved proximal tubules and the epithelial membrane is well defined (see Fig. 4). Whereas the treated mice, this staining allowed us to identify early and evolving over time anomalies. Indeed, cell vacuolation which is a sign of suffering and cellular homes tubular necrosis were observed as early as 10 days and this even for mice treated with low dose ( see Fig. 5 AB; see Fig 6 AB;. See Fig.7 AB). In addition, we observed the 19th day tubular degeneration, congestion of the renal parenchyma and a structural deformation of the tubular epithelium that develops gradually from the 10th day (see Fig5 C; see Fig 6 C; see Fig7 C). Also, we noted hyaline a cellular areas of necrosis, extent of coagulation more severe in mice treated with high doses and the development of the inflammatory infiltrate from the 10th day until the 28th day.

These observations are consistent with early acute tubular necrosis detected from the 10th day of administration of aqueous extract massive becoming the 28th day (see Fig5. D; see Fig6 D E; see Fig 7 DE).



GX100

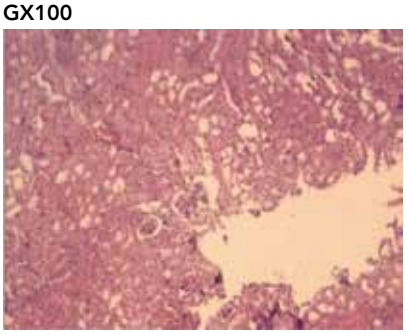


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**Fig. 4. Histological sections of kidney tissue of control mice (normal parenchyma).**

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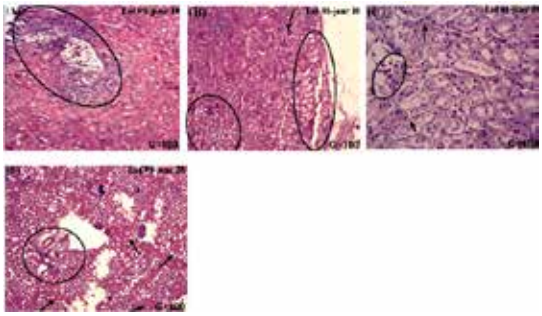


**Fig. 5. Histopathological changes in renal tissue of mice treated with a dose of 1.5 g / kg of the aqueous extract to the 10th, 19th and 20th day.**

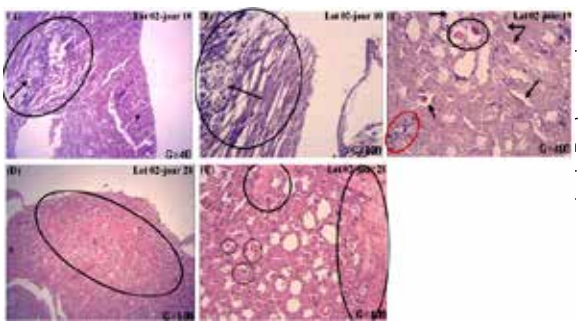


**Fig. 6. Histopathological changes in renal tissue of mice treated with dose 2.5 g/kg of the aqueous extract to the 10th, 19th and 20th day.**

**Representative morphological lesions observed tubulointerstitial sector are:**
(A) tubular necrosis (arrow) and inflammatory infiltrate (circle), (B) inflammatory infiltrate (circle) + tubular necrosis (arrow), (C): nercose tube (black circle) + inflammatory infiltrate (red circle), altered tissue architecture (arrows), (D): hyaline acellular beaches extended tubular necrosis (E): coagulation necrosis extended (circles).

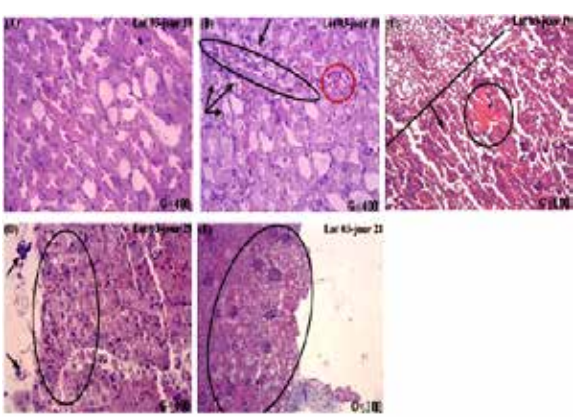


**Fig. 7. Histopathological changes in renal tissue of mice treated with dose 3.5 g/kg of the aqueous extract to the 10th, 19th and 20th day.**

**Representative morphological lesions observed tubulointerstitial sector are:**
(A) altered tissue architecture (B) tubular necrosis (arrows), inflammatory infiltrate
(Black circle), congestion (red circle), (C) congestion (circle), the line of demarcation between normal and arichitecture loss (arrow), (D) tubular necrosis (circle) and inflammatory infiltrate (arrows) (E) vacuolation and tubular degeneration.

**- Liver**

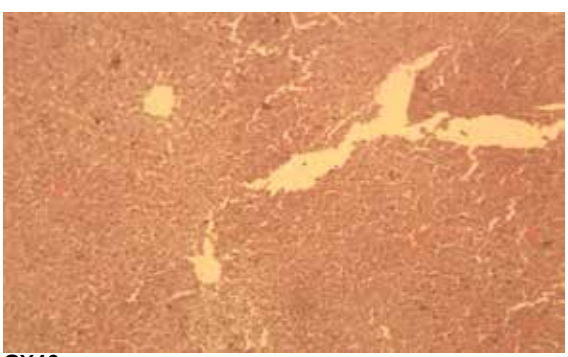
Hematoxylin-eosin staining on sections of liver tissue shows that control mice have a normal parenchyma (Fig. 8). Whereas mice treated from the 10th day of treatment with the aqueous extract, sinusoidal dilatation and a hepatocyte necrosis are observed and this for all doses.

We also noticed the presence of inflammatory infiltrates and congestion the hepatic parenchyma in mice treated with high doses (2.5 g / kg and 3.5 g / kg) (see Fig. 9 AB;see Fig 10 AB; see Fig 11 AB.).

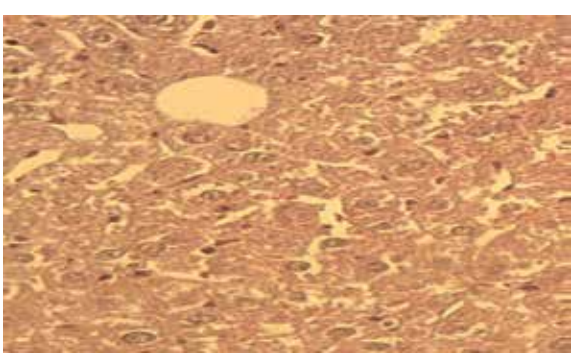
From the 19th day of feeding the aqueous extract at a dose of 1.5 g / kg, only extensive necrosis and vacuolization of cells were observed, whereas the dose of 2.5 g / kg and 3.5 g / kg,

We noticed the presence of an inflammatory infiltrate, extensive necrosis, dilated sinusoids and loss of tissue architecture (see Fig. 9 CD; see Fig 10 C; see Fig 11 CD). Finally, at day 28 we noted dilated sinusoids, cellular vacuolization, hepatocyte necrosis and the disappearance of the inflammatory infiltrate (Fig. 9 EF; Fig 10 DE; Fig 11 E).

We noticed also that liver damage is more severe than the 19th day 28th day and this can be explained by the ability of liver regeneration in mice.



**GX40**



**GX40**
**Figure 9: histopathological changes in liver tissue of mice treated with dose1.5 g / kg of the aqueous extract to the 10th, 19th and 20th day.**

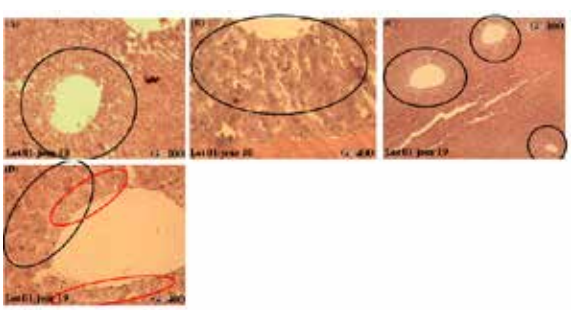


**Fig. 9. Histological sections of liver tissue of control mice**

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**(normal parenchyma).**

(A): sinusoidal dilatation (B) necrosis (C): centrilobular necrosis extended (circles),(D) cell vacuolation (red circles) + centrilobular necrosis (black circle), (E)

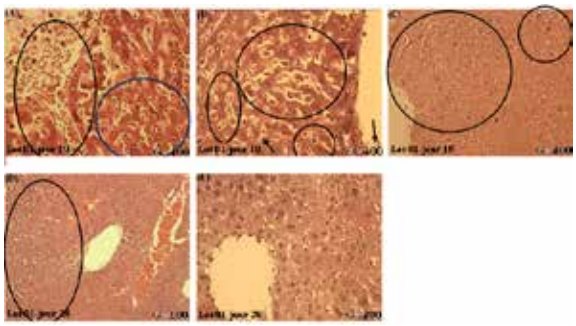

**Fig. 10. histopathological changes in liver tissue of mice treated with dose 2.5 g / kg of the aqueous extract to the 10th, 19th and 20th day.**

(A): pericentrolobulaire necrosis (blue circle) + inflammatory infiltrate of neutrophils (black circle) and dilatation of sinusoids (B): centrilobular necrosis (circles) + inflammatory infiltrate of neutrophils (arrows) and dilatation of sinusoids (C): extensive necrosis and loss of tissue architecture (circles), (D): dilatation of sinusoids + necrosis (circle), (E): cell necrosis.

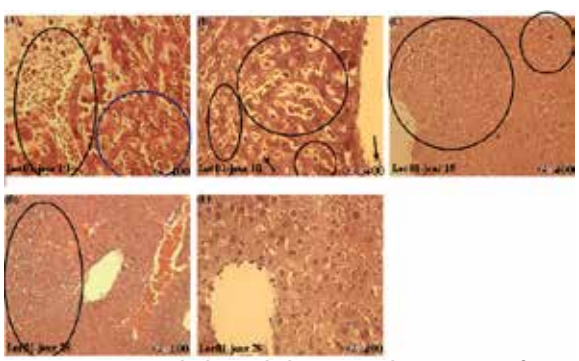

**Fig. 11. Histopathological changes in liver tissue of mice treated with dose 3.5 g / kg of the aqueous extract to the 10th, 19th and 20th day.**
(A): centrolobullaire necrosis (circle) + dilatation of sinusoids, (B): centrilobular congestion point (red circle) + centrilobular necrosis (black circle), (C) expansion of sinusoids (circles), (D) early inflammatory infiltration (circles) + expansion of the sinusoids (arrows) (E): hallo clear péricentrolobulaire (cellular vacuolations) (circle).

Regarding the stomach, lungs, heart and spleen, no lesions were detected.

**Discussion**
The study of acute toxicity of aqueous extract of Aristolochia longa L. (1 g /kg to 5 g/kg orally in mice) allowed us to classify this sample as non or very low toxicity to a single dose. A similar result was reported by Benzakour & al (2011) who tested a dose of 2.5 g / kg of aqueous extract, administered orally in NMRI mice and this dose did not produce any signs of disease.

According Bruneton (2005), there seems to be no cases of acute human poisoning by species of the genus Aristolochia and animal poisoning are infrequent. However, these plants are particularly dangerous when ingested over a long period, especially because they cause a so-called "Chinese herbal nephropathy" nephropathy "AA nephropathy."

In the test conditions of the sub-acute toxicity of aqueous extract of Aristolochia longa L. administered orally to albino NMRI mice at a dose of 1.5 g / kg, we recorded only a slight decrease in body weight and diarrhea. As against the doses of 2.5 g / kg and 3.5 g / kg, produced toxic effects. Indeed, we observed some clinical signs (diarrhea, fatigue and severe weight loss, it could be explained by anorexia or diet may be related to properties of the plant (Vanherweghem &al, 1993; Depierreux &al, 1994), or else to cytotoxicity and apoptogenic activity of aqueous extracts (Benarba & al, 2012).

On plasma level we have noticed an increase in plasma creatinine and urea over time compared to controls and this increase is proportional to the dose administered. According to Frank (1992) respectively can be explained by renal dysfunction and glomerular disease. This increase was already reported by several authors. Shibutani & al (2007) used AA at a dose of 2.5 mg / kg by injection and Benzakour & al (2011) who worked on the aqueous extract administered orally.

The biochemical results (disturbance of biochemical parameters) are confirmed by the results of the histological examination of the kidneys and liver have been the subject of another study where we recorded a cell vacuolation, congestion parenchyma, loss of tubular architecture, inflammatory infiltrate and foci of necrosis becoming massive at day 28, the kidneys. A similar result was reported by several authors such as Mengs (1988) who noted the presence of acute tubular necrosis of the epithelium after administration of AA by gavage at a dose of 5 mg / kg for three weeks to NMRI mice; Benzakour & al. (2011) which showed tubular atrophy and inflammatory and Shibutani &al (2007) found that after ten days of treatment with AA, the presence of severe tubular necrosis and even inflammatory infiltrates.

In the liver we observed a cell vacuolation, dilated sinusoids, hepatocellular necrosis, inflammatory infiltrate and a congestion of the liver parenchyma. This result is consistent with that of Benzakour & al (2011) which showed the same lesions after three weeks of feeding.

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