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Research Article

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Toxicological Evaluation of *Aristolochia Albida L.* Used in Traditional Medicine in Morocco: Histopathological and Biochemical Evidence

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ABSTRACT

Aristolochia albida 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.

Keywords: *Aristolochia albida*; Acute toxicity; Sub-acute toxicity; Histopathological profile; Biochemical profile

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INTRODUCTION

Aristolochia albida L. (Aristolochiaceae) locally called "Beroustoum" is a species commonly used in Morocco traditional medicine [1]. It has multiple applications and virtues; it is recommended for ovarian failure [2], healing diuretic, analgesic, anti-inflammatory, anti-mitotic [3] etc. The powder of roots of *Aristolochia albida* L. with salted butter is used to treat skin infections and gangrene [4], as anti-cancer [5], especially in case of sclerosis, uterine and nasal cancer [6]. Despite their virtues, *Aristolochia albida* L. contains aristolochic acid (AA) [7], AA-I and AA-II acids are mutagenic in bacteria, mice and mammalian [8]. The ingestion of herbal remedies of this plant is associated with the development of a syndrome indicating aristolochic acid nephropathy [9], which is characterized by chronic renal failure, tubulointerstitiale fibrosis and urothelial cancer. Following these dangerous health consequences, a decision was taken to prohibit the use of any natural remedy containing this acid. The aim of this work is to highlight the toxicity of rhizomes of *Aristolochia albida* 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.

MATERIALS AND METHODS

Plant Material

The roots of *Aristolochia albida L.* plants were harvested in May 2012, 11 km from Medea (West of Algiers) [9]. 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 et al [10] 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 [11]. 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 [12]. The measured serological parameters: Creatinine and urea. Fluctuations of mice weight were considered.

RESULTS

Acute Toxicity

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 (Figure 1). Thus the aqueous extract of *Arsitolochia albida* 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 (Figure 1-6).

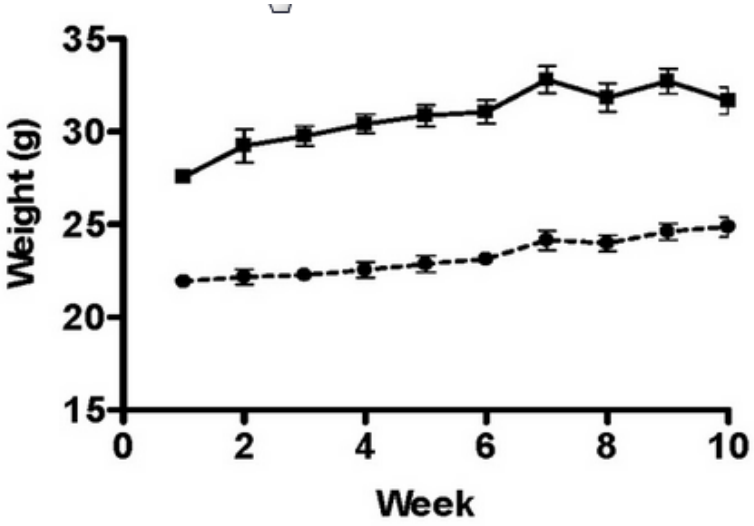

Figure 1: Effect of the plant extract on adminstred mice of weight and TCTHA

Effect of aqueous extract on biochemical parameters

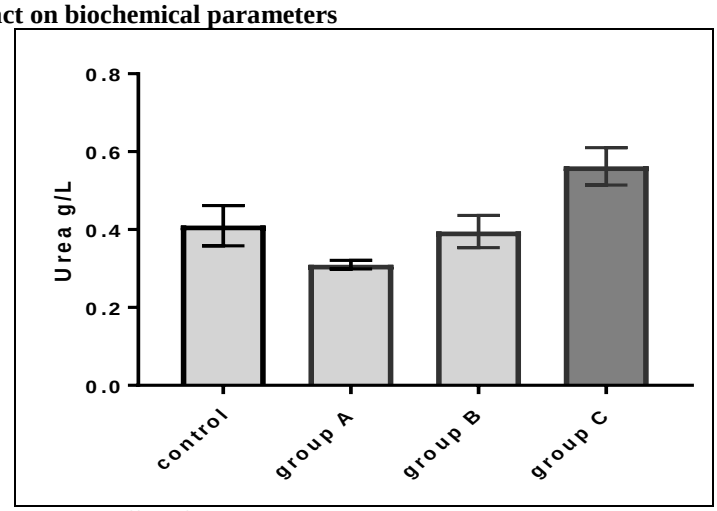

Figure 2: Effect of the plant extract on the FAS against urea transaminases

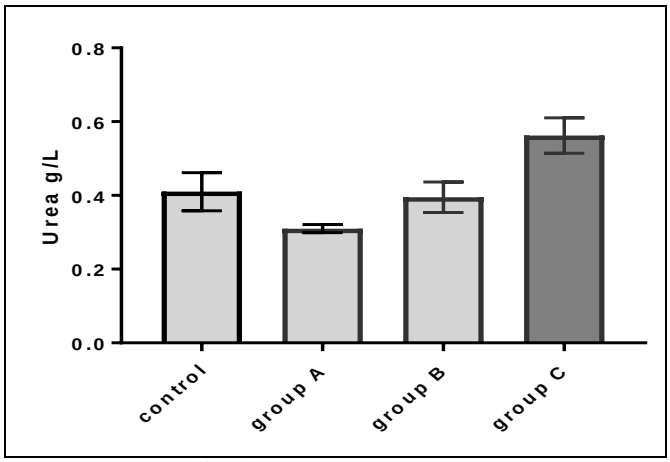

Figure 3: Effect of the plant extract on the SH vs urea

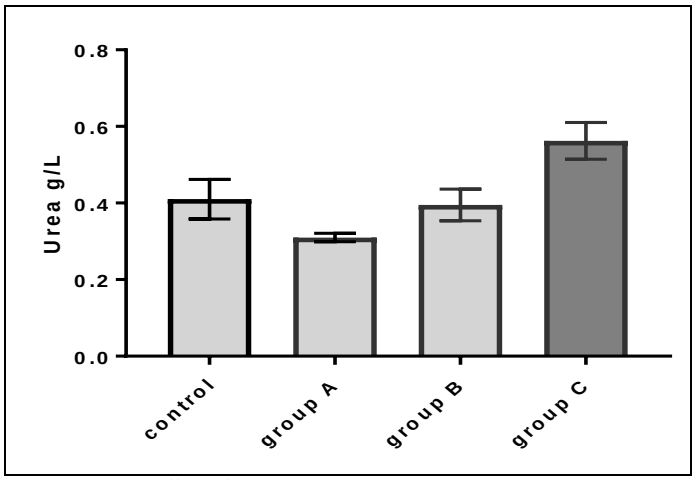

Figure 4: Effect of the plant extract on gonadostumline vs urea

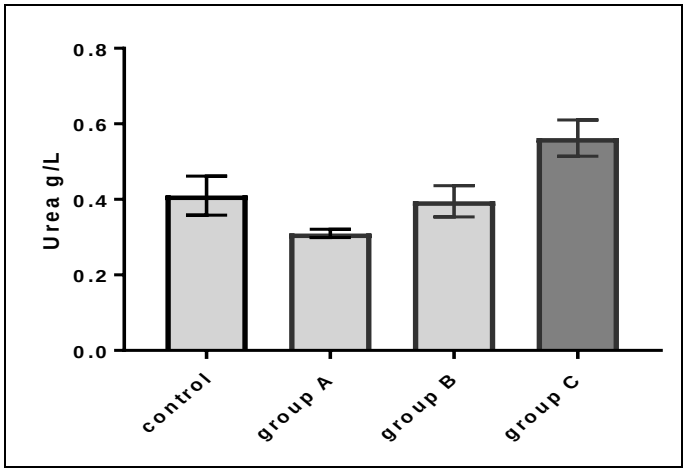

Figure 5: Effect of the plant extract T3 hormone vs the urea

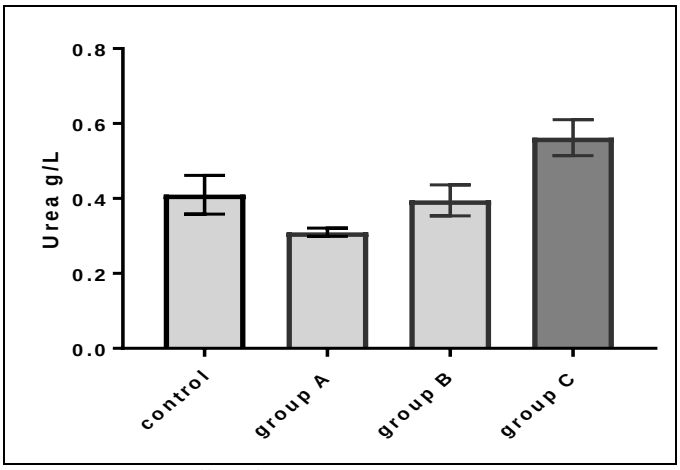

Figure 6: Effect of the plant extract on the GH vs urea

Histopathological changes

Kidney

Hematoxylin-eosin staining on sections of kidney tissu showed that control mice have normal renal parenchyma structure is preserved proximal tubules and the epithelial membrane is well defined (Figure 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 (Figures 7AB and 10AB). 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 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 (Figures 7-11).

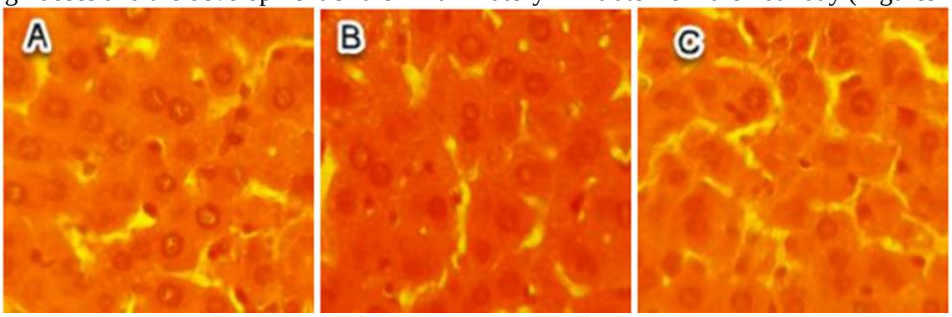

Figure 7: Histologic section of kidney tissue of control mice. (Section of normal parenchyma stained with H&E, x 28)

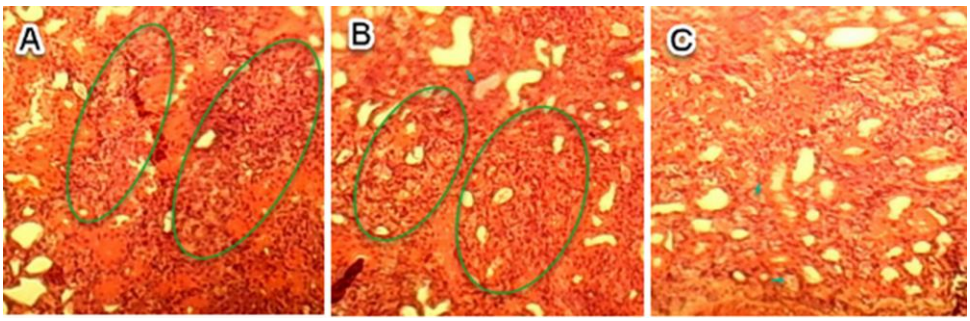

Figure 8: Histologic section of kidney tissue of mice treated with 1.5 g / kg/day. (group B, Section stained with H&E, x 28). (A) Inflammatory infiltrate, (B) inflammatory infiltrate, (C) tubular degeneration (arrow) and inflammatory infiltrate (circle)

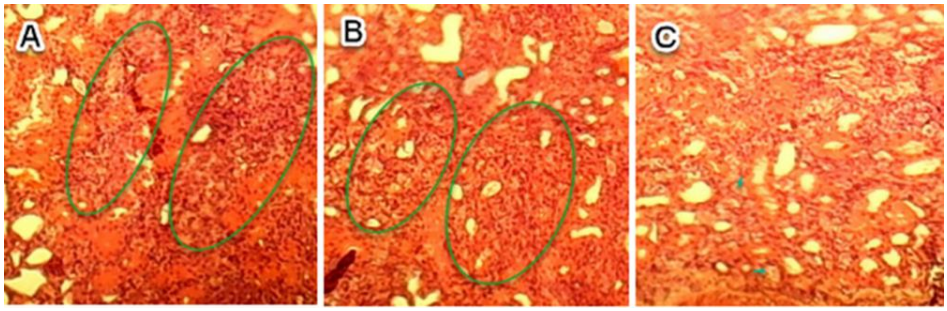

Figure 9: Histologic section of kidney tissue of mice treated with 2 g / kg/day. (group C, Section stained with H&E, x28). (A) Inflammatory infiltrate, (B) tubular necrosis (arrow), and inflammatory infiltrate (circle), (C) tubular degeneration

Liver

Hematoxylin-eosin staining on sections of liver tissue shows that control mice have a normal parenchyma (Figure 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) (Figures 9AB,10AB,11AB.). 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 (Figures 9C,10C,11C). Finally, at day 28 we noted dilated sinusoids, cellular vacuolization, hepatocyte necrosis and the disappearance of the inflammatory infiltrate (Figures 9-11). 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

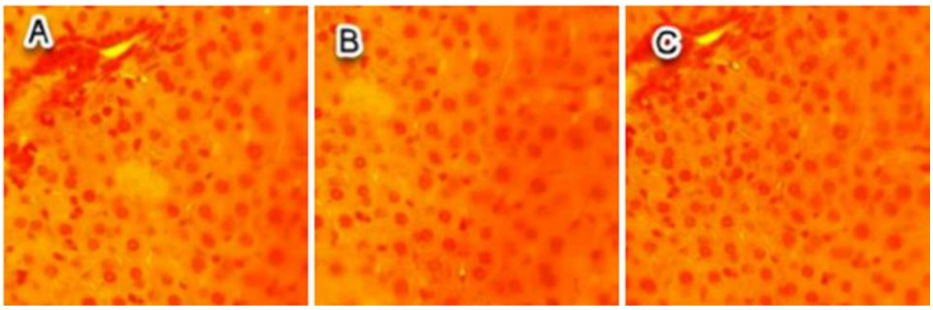

Figure 10: Histologic section of liver tissue of control mice. (Section of normal parenchyma stained with H&E, x28)

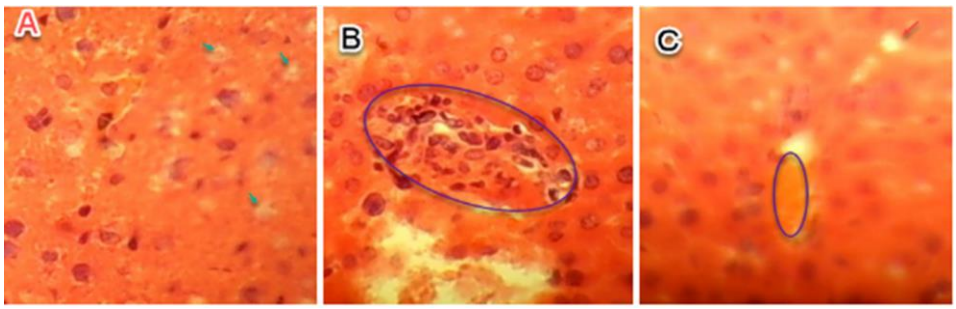

Figure 11: Histologic section of liver tissue of mice treated with 2 g /kg/day (group C, Section stained with H&E, x28). (A) hepatic necrosis, (B) inflammatory infiltrates, (C) hepatic cholestasis

DISCUSSION

The study of acute toxicity of aqueous extract of *Arsitolochia albida* 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 [13], 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 Carlini et al [9], 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" [14].

In the test conditions of the sub-acute toxicity of aqueous extract of *Aristolochia albida* 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 [2], or else to cytotoxicity and apoptogenic activity of aqueous extracts [12]. 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 Griffiths et al. [15], respectively can be explained by renal dysfunction and glomerular disease. This increase was already reported by several authors. Used AA at a dose of 2.5 mg/kg by injection and [13] 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 [16], inflammatory infiltrate and foci of necrosis becoming massive at day 28, the kidneys. A similar result was reported by several authors such as [13] 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 which showed tubular atrophy and inflammatory and 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 [17], dilated sinusoids, hepatocellular necrosis, inflammatory infiltrate and a congestion of the liver parenchyma. This result is consistent with that of [18], which showed the same lesions after three weeks of feeding [5].

CONCLUSION

On the basis of our results, the aqueous extract of the studied plant of *Arsitolochia Albida*.the toxicity of aqueous extract in mice showed that the extract was dangerous viruses many vital organs such as kidney and liver for this reason we encourage the people throughout the world de not eat or use this plant without knowing some information of the safety from the Moroccan government headed by sad eddine otmani.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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