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Full Length Research Paper

Immunostimulatory potential of *Aristolochia longa* L. induced toxicity on liver, intestine and kidney in mice

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***Aristolochia longa* L. (AL) (Aristolochiaceae) is used by Moroccan patients suffering from cancer during the course of their specific treatment. This practice has remained till date without scientific validation. To validate evidence for or against its continued use as antitumor product and afford data for further studies of this plant, the present investigation was carried out to evaluate the safety of an aqueous extract of *A. longa* by determining its potential of toxicity after oral administration in mice. To explain the mode of action of *A. longa*, the immunomodulatory activity test was equally carried out. For acute toxicity study, aqueous extract of *A. longa* given to adult 'Swiss albinos' mice in single dose of 2.5 g/kg/day did not produced any visible toxic signs or deaths. While in the sub-chronic toxicity study, the *A. longa* extract at doses of 1.25 and 2.5 g/kg/day for 3 and 6 weeks induced atypical locomotion, anorexia, asthenia, ataxia, diarrhea and urination for the higher dose used. The histopathological examination showed that *A. longa* extract at 1.25 g/kg was not toxic, while at 2.5 g/kg it caused a significant toxicity on the liver, intestine and kidney. A gradual regression of hepatic and intestinal lesions was observed during 30 days recovery period. However, in the kidney tissue persistent interstitial nephritis was noted with no significant recovery. The high number of lymphocytes noted in the different organs indicated that it was an immune activity. In fact, when tested against SRBC, there was a statistically significant increase of "haemagglutinating antibody titer" and insignificant increase in "delayed type hypersensitivity" response in mice treated by the non toxic dose of *A. longa* (1.25 g/kg) compared to control group. We conclude that administration of the aqueous extract of *A. longa* at saturation limit dose (2.5 g/kg) produced severe and irreversible renal toxic effects in mice induced by a high immunostimulation activity.**

Key words: *Aristolochia longa* L., animal toxicity, traditional medicine, immunomodulatory activity.

INTRODUCTION

Aristolochia longa L. (Aristolochiaceae) locally known as "Barraztam" is a plant species communally used in Moroccan traditional medicine. One of *A. longa* medicinal uses is the topical application of a mixture of its rhizome powder with salted butter for the treatment of skin Merzouki et al., 1997; Gadhi., 1999). The ingestion of a little amount infections (Charnot, 1945; Bellakhdar, 1997; of this powder with either honey or salted butter is also used by several traditional healers for the treatment of abdominal pain and infections of the upper respiratory tract (Bellakhder et al., 1991; Benchaabane and Abbad, 1997; Bellakhder, 1997). In addition, the Greek doctors recommended it against ovarian failure (Blanc-Daire, 1978) and in Egypt; it was applied against snakebites (Schenenerg, 1977). In Morocco, the most widely uses of *A. longa* are in cancer treatment. The traditional healers recommended its use with care and always for short treatment periods (Benchaabane and Abbad, 1997). In

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2005, two cases of death and five cases of medullar aplasia were reported by the department of Oncology in the university hospital center of Casablanca, Morocco. In patients with malignancies who used *A. longa* during cancer treatment, it was reported that it causes 16% of renal failure (Statistics of Anti-poison center of Morocco, 2010). The genus of Aristolochiaceae contains aristolochic acids (AAs). Ingestion of herbal remedies containing AAs is associated with the development of a syndrome, designated aristolochic acid nephropathy (AAN), which is characterized by chronic renal failure, tubulointerstitial fibrosis and urothelial cancer (Pozdzik, 2010). Although the toxicities of AAs have been well studied (Mengs et al., 1982; Mengs, 1987, 1988; Schmeiser et al., 1988; Mengs and Stotzem, 1993), Aristolochia species are still used as alternative medicines. In 1993, there was several case reports of interstitial nephritis developed after a slimming regimen consisting of Aristolochia species (Vanherweghem et al., 1993; Vanhaelen et al., 1994). The US Food and Drug Administration advised consumers to discontinue using herbal products which contain AAs (Arlt et al., 2002). In Morocco, the National Anti-poison Center of Morocco has published an article (Skalli, 2010) to advertize the use of "Barraztam". At present, herbal research is limited mostly to *in vitro* studies and not to clinical use. The toxic effect of plant *in vivo* and its immunomodulatory potential has never been investigated before. The present study was carried out to determine the immunomodulatory activity and the histopathological toxicity of an aqueous extract of *Aristolochia longa* L. rhizomes after acute and sub-chronic oral gavages in mice at different doses, with the aim to obtain information on its toxic effects and the mode of action on several organs during and after the treatment.

MATERIALS AND METHODS

Plant material

Aristolochia longa L. Subsp. *Aristolochia paucinervis* Pomel (Maire, 1961; Fennane et al., 1986) was collected in May 2009 at 30 km south of Marrakech City, Morocco. The plant was authentified and a voucher specimen has been deposited (No 61318) in the Herbarium of Scientific Institute of Rabat, Morocco.

Preparation of the aqueous extract of *Aristolochia longa* L. rhizomes

The vegetal material was washed with water, and then dried at room temperature for 48 to 92 h. The aqueous extract was prepared by adding 500 ml of distilled water to 50 g of *A. longa* dry rhizomes powder. After 24 h of maceration under magnetic stirring at room temperature, the mixture was centrifuged, filtered and then concentrated in a rotary vacuum evaporator. The extracted material (yield of approximately 10% w/v) was dissolved in 0.9% NaCl solution and stored at – 20°C in the dark until further use.

Experimental animals

Male and female adult mice (Swiss albinos) with an average weight of 23 g, obtained from the animal colony of our department, were divided into five groups, each group containing 6 mice. The animals were acclimatized in cages under standard environmental conditions of light/dark cycles (12/12 h), temperature (23 ± 1°C) and air changes. Animals had free access to tap water and standard pellet diet. Animals have been deprived of food for 12 h before the beginning of the experiment.

Toxicological evaluation of the *Aristolochia longa* L. extract

Acute toxicity studies in mice

The aqueous extract was administered by gavages to a group of mice (n = 6) at dose 2.5 g/kg body weight (saturation limit of the plant), while the control group received 0.9% NaCl only. The volume administered was 25 µl/1 g of corporal weight. The general behavior of mice and signs of toxicity were observed continuously for 1 h after the oral treatment, and then intermittently for 4 h and thereafter over a period of 24 h (Twaij et al., 1983). The mice were further observed once a day up to 14 days following treatment for behavioral changes and signs of toxicity.

Sub-chronic toxicity studies in mice

Mice in both treated groups (n = 6 in each group) received *A. longa* extract daily by oral gavages for 3 or 6 weeks at dose of 1.25 or 2.5 g/kg body weight, while the control group received the vehicle only. During 3 and 6 weeks, animals were submitted to observations. Toxic manifestations were monitored daily (Brock et al., 1995).

Measurement of biochemical parameters in blood

Creatinine, ALT and AST were determined enzymatically by standard methods using biochemical automat (KoneLab 20 Thermo).

Histopathological assessment

At the end of the experiment, mice were sacrificed by cerebral dislocation. Liver, intestine and kidney of sacrificed animals were removed, examined and carefully dissected. Small slices of these freshly harvested tissues were fixed in buffered formaldehyde solution (10%), dehydrated in a series of alcohols, cleared in toluene and then enclosed in Paraplast. Micrometer sections, cut by a microtome, were dewaxed, rehydrated in a series of alcohols and stained with hematoxylin-eosin (HE), periodic acid-Schiff reagent (PAS), Masson's trichrome stain or Reticulin method. The slides were examined and photographed by a pathologist using a light microscope (Luna, 1986; Thomson et al., 1998).

Immunomodulatory investigation

Antigen

The sheep red blood cells (SRBCs) (purchased from the national laboratory of veterinary research, Casablanca, Morocco) were washed three times in a large volume of pyrogen-free sterile normal saline by centrifugation at 3000 × g for 10 min on each occasion. The washed SRBCs were adjusted to a concentration of 10^9 cells/ml of normal saline and used for immunization and challenge (Dan et al., 1990).

Determination of haemagglutinating antibody (HA) titer (humoral-mediated immune response)

The method of Bin et al. (2001) was used to determine the antibody level resulting from sensitization with SRBC. Briefly, mice were divided into two groups with 7 mice in each and were immunized by intraperitoneal (i.p) injection of 0.2 ml of SRBCs suspension (30% v/v in PBS) on day 0. One group was treated with the *A. longa* aqueous extract orally at 1.25 g/kg body weight (limit dose with no toxic effect) 3 days prior to immunization and continued once daily for 7 days after challenge. The other group was referred as control. The mice were sacrificed by decapitation and blood samples were collected from each mouse on day 7 for serum preparation. The blood was incubated for 1 h at 37°C, centrifuged and supernatants pooled. The sera were incubated for 30 min at 56°C in order to inactivate complement and stored at – 20°C until use. The primary antibody titer was determined by hemagglutination technique (Sharma et al., 1996). Briefly, 25 µl of serum was serially diluted twofold in 96-well microtiter plates in PBS. The diluted sera were challenged with 25 µl of 1% (v/v) SRBCs in the plates and then incubated at room temperature for 2 h. The highest dilution showing visible hemagglutination was taken as antibody titer. Titers of sera were determined as the reciprocal of the maximal dilution presenting positive hemagglutination.

Determination of foot pad swelling (delayed type hypersensitivity (DTH) cellular-immune response)

The antigen-specific cellular immune response was measured by determining the degree of DTH response using the footpad swelling test (Benencia et al., 2000). For sensitization, seven animals per group (control and treated) were immunized on day 0 by an i.p injection with 0.2 ml of a SRBC suspension (30% v/v in PBS). Seven days later (day +7), animals were injected subcutaneously (sc) with 50 µl of 1% a SRBC suspension in PBS into the right hind footpad for elicitation of the DTH reaction. A footpad swelling was measured on day +8. The difference between the mean thickness of right and left hind footpad was used for group comparisons. To establish the effect of the *A. longa* extract on this immune response, a daily dose of 0.7 ml of aqueous extract of *A. longa* at 1.25 g/kg body weight in PBS was administered orally at different stages of the reaction: previous to sensitization (days $-2 \rightarrow 0$) and at the induction phase ($0 \rightarrow +7$). Simultaneously, another group of animals (controls) was inoculated in the same conditions with 0.7 ml of PBS.

Reversibility/Irreversibility of toxic effects

First, mice were received aqueous extract of *A. longa* daily by gavages for 6 weeks at dose 2.5 g/kg body weight. The mice were further observed once a day up to one month without giving any treatment for behavioral changes and signs of toxicity. At the end of the experience, mice were sacrificed by cervical dislocation and histopathological examination was performed as previously described for liver, kidney and intestine.

Statistical analysis

The statistical significance of the data was determined by Student's *t*-test. *p*-values less than 0.05 were taken as significant.

RESULTS

Acute toxicity of aqueous extract of *A. longa* in mice

Over a period of 24 h, single oral administration of aqueous extract of *A. longa* at 2.5 g/kg body weight did not produce any visible toxic effects compared with the control.

Sub-chronic toxicity of aqueous extract of *A. longa* in mice

Clinical observations of intoxication

The main behavioral signs of toxicity observed from the 10th day of daily oral administration of aqueous extract of *A. longa* at 2.5 g/kg were atypical locomotion, anorexia, asthenia, ataxia, diarrhea and urination. Asthenia, hypoactivity and urination were noticed immediately after gavages and were more pronounced and persisted until the end of experimentation. For the dose of 1.25 g/kg no visible toxic effects were observed.

Effects of aqueous extract of A. longa on biochemical parameters

Serum levels of ALAT and ASAT and concentration of 'creatinine' were markedly and significantly increased in mice treated by 2.5 g/kg of aqueous extract of *A. longa* (AST: 45 U/L ± 0.01; ALT: 80 U/L ± 0.01; creatinine: 4 mg/L ± 0.001) as compared to the controls (AST: 15 U/L ± 0.01; ALT: 20 U/L ± 0.01; creatinine: 2 mg/L ± 0.001; $p<0.05$). The activities of AST and ALT are indicators of liver functions and the level of creatinine is an indicator for kidney activity. Therefore, *A. longa* L. induced serious kidney and liver injury.

Histopathological changes

For the lower (1.25 g/kg) dose used of the aqueous extract of *A. longa*, no histopathological changes were observed in kidney, liver or intestine tissues between the controls and the treated mice. For the groups treated with 2.5 g/kg of the aqueous extract, the histopathological results are summarized in Figures 1 to 3. In the liver, lobular hepatocellular necrosis, centrilobular macrovesicular steatosis and centrilobular lymphocytic inflammatory infiltrations were observed for mice group treated for 3 weeks. The damages were more pronounced and characterized by centrilobular cholestasis, hypertrophy of Kupffer's cells, centrilobular sinusoidal lymphocytic inflammatory infiltration and hepatocellular necrosis after 6 weeks of treatment. Some blood vessels were disrupted causing foci of hemorrhage (Figure 1).

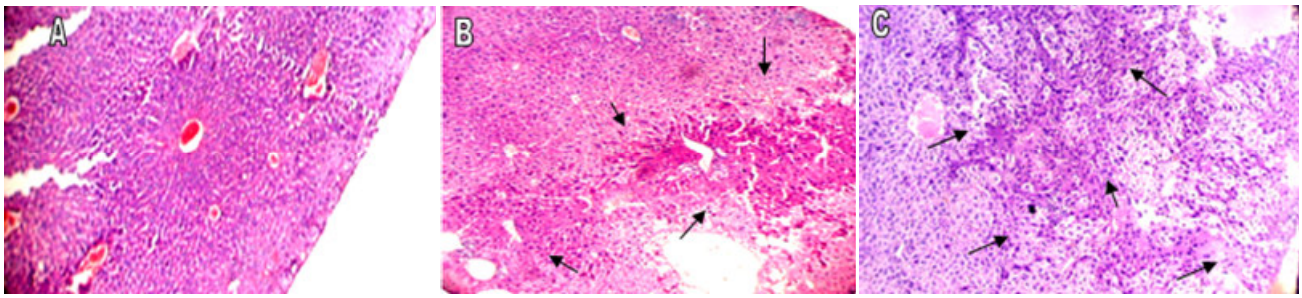

Figure 1. Histopathological changes in the liver's mice given 2.5 g/kg of *Aristolochia longa* L. extract orally. Hematoxylin and Eosin stain (magnification x 200). Panel (A) control mice; Panel (B) mice treated with *A. longa* for 3 weeks: marked hepatocellular necrosis, centrilobular macrovesicular steatosis and centrilobular inflammatory infiltration and Panel (C) mice treated with *A. longa* for 6 weeks: centrilobular sinusoidal inflammatory infiltration and hepatocellular necrosis.

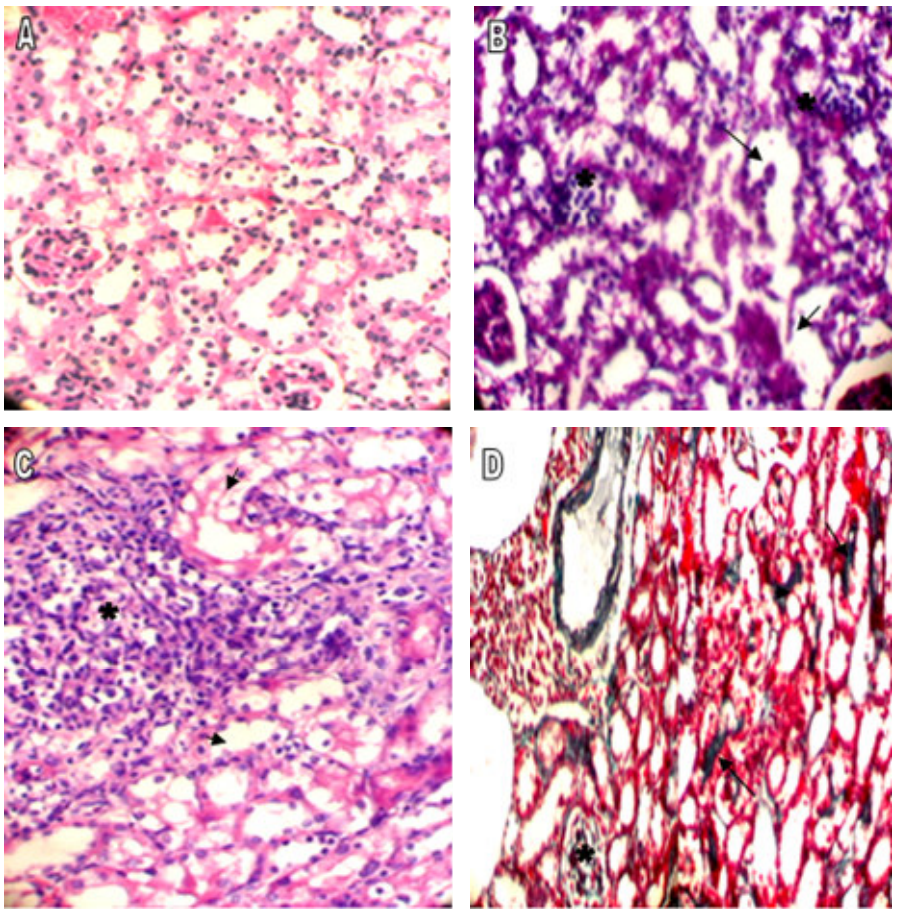

Figure 2. Histopathological changes in the Kidney of mice given 2.5 g/kg of *Aristolochia longa* L. extract orally (magnification x 400). (A) Control mice. Hematoxylin and Eosin stain; (B) Mice treated for 3 weeks: slight tubular atrophy (arrows) and a few scattered lymphocytes (*) periodic-acid Schiff stain; (C) Mice treated for 6 weeks: tubular necrosis, atrophy (arrows) and lymphocytic infiltrates (*). Hematoxylin and Eosin stain and (D) Mice treated orally for 6 weeks: interstitial fibrosis (arrows) and Glomerular lesions (*) Masson's Trichrome stain.

In the kidney, slight tubular atrophy and a few scattered lymphocytes were observed after 3 weeks of treatment. Significant increases in severity were observed after 6 weeks of treatment with tubular necrosis and atrophy, as well as lymphocytic infiltrates surrounded by slight interstitial fibrosis. Glomerular lesions were absent in mice group treated for 3 weeks but appeared after 6 weeks of treatment (Figure 2). In the small intestine, we

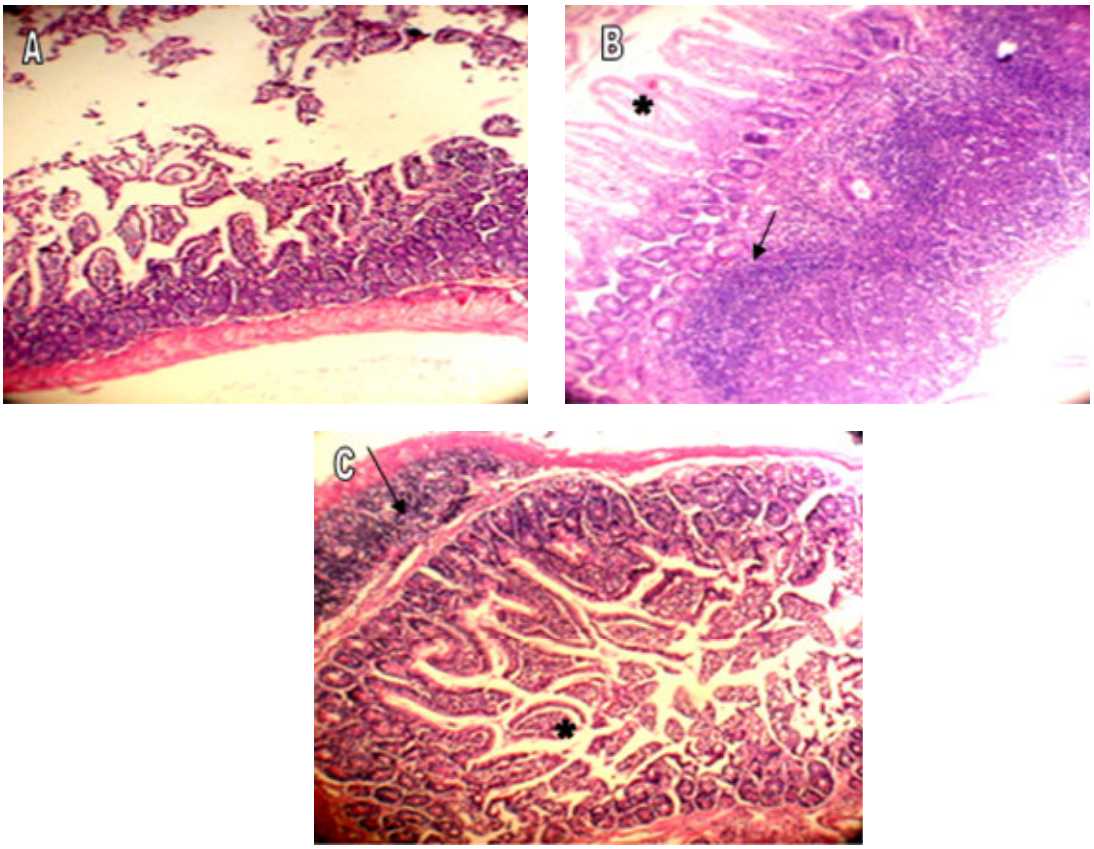

Figure 3. Histopathological changes in the small intestine of mice given 2.5 g/kg of *Aristolochia longa* L. extract orally. Hematoxylin and Eosin stain (Magnification x 200). (A) Control mice; (B) Mice treated for 3 weeks: edema of intestinal villosity (*) and inflammatory lymphocytic infiltrates (arrows) and (C) Mice treated for 6 weeks: Edema of intestinal villosity (*) and inflammatory lymphocytic infiltrates (arrows).

observed a very severe edema of intestinal villosities with inflammatory lymphocytic infiltrates for the two periods of treatment (Figure 3).

Effect of *A. longa* extract on immune functions in mice

Effect of A. longa extract on humoral immunity

The effect of *A. longa* extract treatment on the production of hemagglutinating antibody titers in mice was tested. As shown in Figure 4, a significant ($p<0.05$) increase in primary titer values of antibodies at limit dose of no toxic effect (1.25 g/kg) was observed as compared to control.

Effect of A. longa extracts on the delayed-type hypersensitivity (DTH) reaction

A. longa extract at dose tested of 1.25 g/kg elicited an insignificant increase in DTH response in comparison to control animals ($p<0.05$) (Figure 5).

Changes after withdrawal

During 30-days treatment-free rest period, the mice still showed asthenia and hypoactivity. However, the diarrhea and the other signs have disappeared from the 7th day after withdrawal of aqueous extract of *A. longa.* The histopathological examination showed that the recovery was pronounced at 30th day after withdrawal indicating gradual reversal of hepatic lesions. The centrilobular cholestasis and microvacuolar steatosis disappeared and the cellular renewal was seen to appear gradually (Figure 6). Edema in the intestinal villosity was scanty (Figure 7). Moreover, a few scattered lymphocytic infiltrates were persistent in hepatic and intestinal tissue. In kidney tissue, the severity of changes was pronounced in the 30th day of withdrawal. A persistent interstitial nephritis was noted. Tubular atrophy, lymphocytic infiltrates and foci of necrosis were still present on day 30 of withdrawal of treatment. The interstitial fibrosis initially localized (at the end of 6 weeks of treatment) was subsequently generalized (30-days after withdrawal) characterized by thickened reticulin fibers. The Glomerular extensive lesions were also seen indicating gradual alterations with

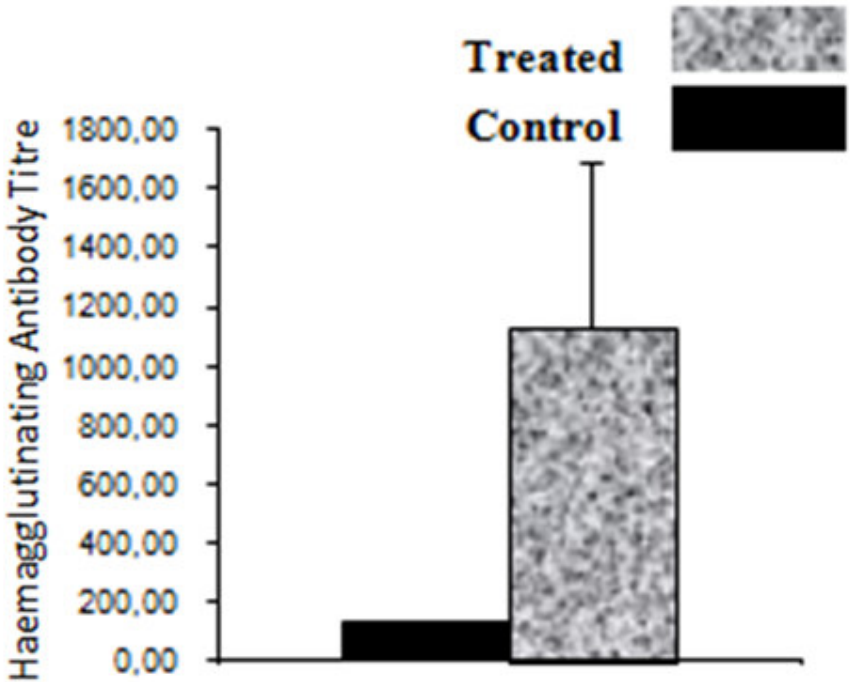

Figure 4. Effect of *Aristolochia longa* extract on antibody production. Two groups of seven animals for each were immunized with a 30% erythrocyte suspension in PBS, were inoculated by gavages with an extract concentration (1.25 g/kg; treated) in PBS or an equal volume of PBS (Control). The animals received a daily dose of the extract. The inoculation volumes were: 0.7 ml for the oral route. The animals were bled 7 days post-immunization and the hemagglutinating antibodies were determined. The day of immunization with erythrocytes was considered day 0. Titers were expressed as the reciprocals of the maximal dilution that produced positive heamagglutination. Statistical significance versus control: $p < 0.05$.

no significant recovery (Figure 8).

DISCUSSION

In the present study, we report that the aqueous extract of *A. longa* L. at dose of 1.25 g/kg was not toxic but at 2.5 g/kg (dose used by Moroccan patients corresponding to the saturation limit of the extract) produced toxic effects in mice after 6 weeks of treatment by oral gavages. Previous research has shown that consumption of phytomedicinals including the genus of Aristolochia can result in severe renal injury, including renal interstitial fibrosis (Tang and Eisenbrand, 1992; Vanherweghem et al., 1993). *A. longa* and 31 related species are known to contain Aristolochic acids (AAs), nitrophenanthrene carboxylic acids responsible for the nephrotoxic and genotoxic effects associated with nephropathy (Lord et al., 2001; Kumar et al., 2003). AA is a mixture of structurally acids, aristolochic acid-I (AA-I) and aristolochic acid-II (AA-II) being the major components. While one had not previously determined the hepatic-renal toxicity for the dose used in folk medicine in Morocco, in this study, we have now shown that this dose (2.5 g/kg) did not exhibit any signs of adverse effects in the acute toxicity study in mice. In all organs tested, the damages were more pronounced after 6 than 3 weeks particularly in renal tissue. The observed renal pathology was very similar to that noted in patients taking Chinese herb: nephropathy, major tubulo-interstitial lesions, essentially tubular necrosis and atrophy, lymphocytic infiltrates surrounded by slight interstitial fibrosis and Glomerular lesions were observed. The hepatic-renal toxicity effect of *A. longa* extract is also supported by marked elevation of serum transaminases (ALT and AST) and creatinine which are good indicators of liver and kidney functions respectively (Martin et al., 1981; Vijayalakshmi et al., 2000). The ALT is a cytoplasm enzyme found in very high concentration in the liver (Tennekoon et al., 1991) and an increase of the specific enzyme suggests hepatocellular damages. The lymphocytic infiltrates were very pronounced in all tissues examined suggesting an immunostimulating effect induced the toxicity observed. So we tested the immunomodulatory effect of the aqueous extract of *A. longa* in mice. It has equally showed an overall stimulatory effect on both humoral and cellular immune functions in mice. In HA titer test, the plant showed a

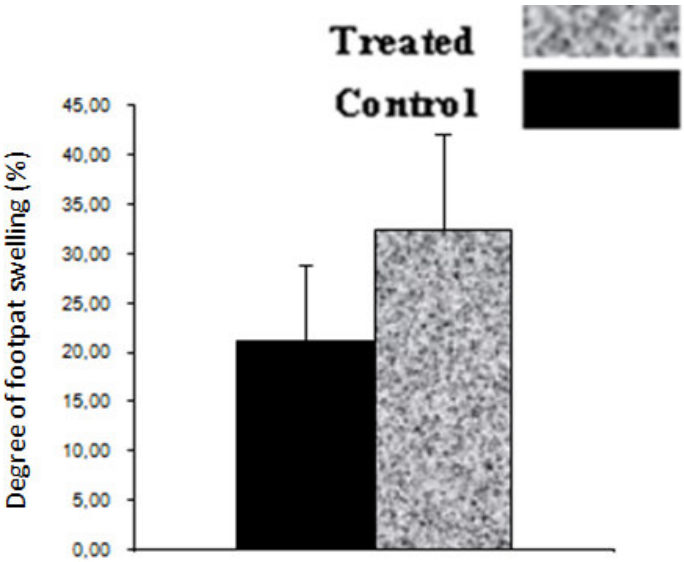

Figure 5. Effect of *Aristolochia longa* on the DTH response. Mice sensitized with an i.p injection of 30% erythrocyte suspension in PBS on day 0. The mice received a daily oral dose (1.25 g/kg) of *A. longa* extract (treated) or an equal volume of PBS (control) on days -3 → +7. On day +7, the DTH reaction was elicited with a sc injection of 1% erythrocyte suspension in PBS into the right hind footpad. The left hind footpad was referred as control. Footpad swelling was measured on day +8. The difference between the means of right and left hind footpad thickness gave a degree of footpad swelling. Statistical significance versus control: $p<0.05$.

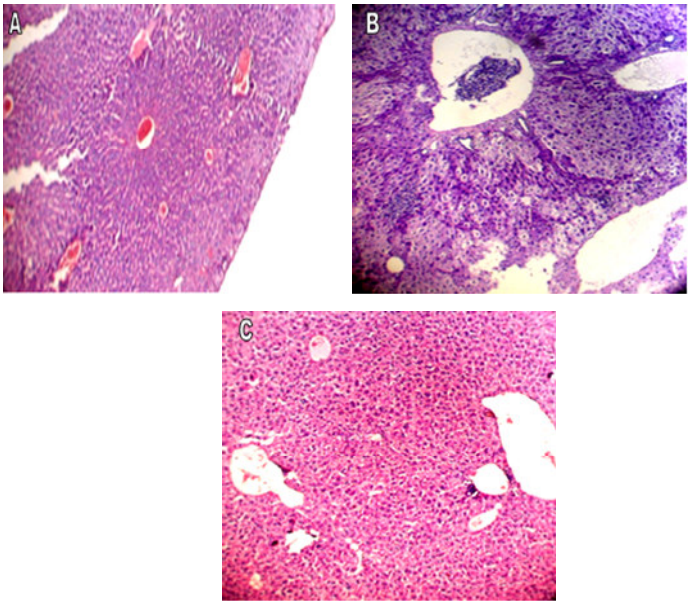

Figure 6. Histopathological changes in the liver before and after 30-day recovery period of treatment. Hematoxylin and Eosin stain (Magnification x 200). (A) Control mice; (B) Mice treated orally with 2.5 g/kg of *Aristolochia longa* L. for 6 weeks: centrilobular sinusoidal inflammatory infiltration and hepatocellular necrosis and (C) Mice after 30 days of treatment withdrawal: a significant regression of hepatic lesions with regeneration of hepatic parenchyma.

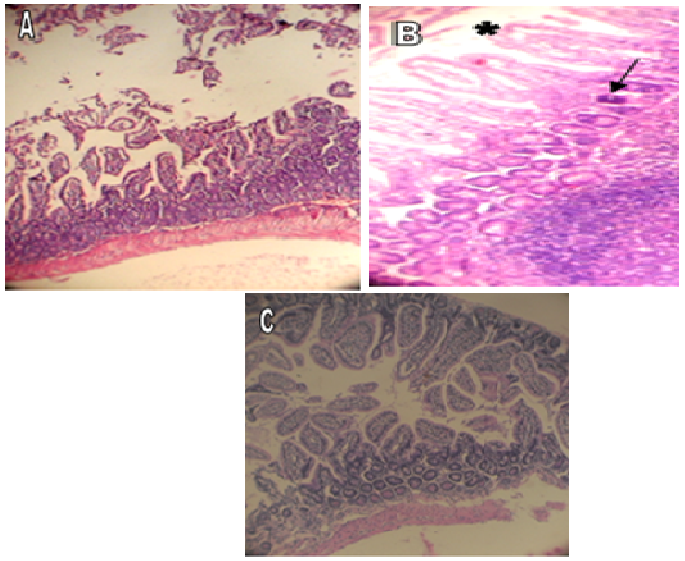

Figure 7. Histopathological changes in the small intestine before and after 30-day recovery period of treatment. Hematoxylin and Eosin stain (magnification x 200). (A) Control mice; (B) Mice treated orally with 2.5 g/kg of *Aristolochia longa* L. for 6 week: Edema of intestinal villosity (*) and inflammatory lymphocytic infiltrates (arrows) and (C) Mice after 30 days of withdrawal treatment: disappearance of villous edema.

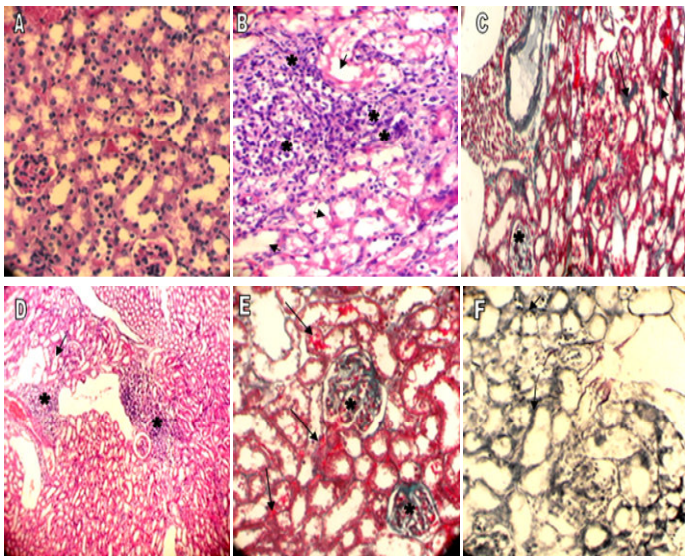

Figure 8. Histopathological changes in the kidney before and after 30-day recovery period of *Aristolochia longa* treatment (magnification x 400). (A) Control mice. (Hematoxylin-Eosin stain); (B) Mice treated orally with 2.5 g/kg of *A. longa* L. for 6 weeks: Tubular necrosis, atrophy (arrows) and lymphocytic infiltrates (*) (Hematoxylin-Eosin stain); (C) Mice group treated for 6 weeks: focal interstitial fibrosis (arrows) and Glomerular lesions (*) (Masson's Trichrome stain). Mice group after 30-days of recovery period treatment; (D) Hematoxylin and Eosin stain: A persistent interstitial nephritis (*) and Tubular atrophy (arrows); (E) Masson's Trichrome stain: Glomerular lesion (*), foci of necrosis (arrows) and (F) Reticulin stain: Interstitial fibrosis characterized by thickened reticulin fibers (arrows).

significant enhancement of antibody responsiveness to SRBC in mice as a consequence of both pre and post plant treatment which indicates the enhanced responsiveness of B-lymphocytes involved in antibody synthesis (Mungantiwar et al., 1999). In the DTH test, the DTH response, which directly correlates with cell-mediated immunity (CMI), was found to be the highest at dose tested (1.25 g/kg) in the extract. The mechanism behind this elevated DTH during the CMI responses could be due to sensitized T-lymphocytes. When challenged by the antigen, they are converted to lymphoblast and secrete a variety of molecules including proinflammatory lymphokines, attracting more scavenger cells to the site of reaction (Delves and Roitt, 1998).

An increase in DTH response indicates a stimulatory effect of the plant which has occurred on the lymphocytes and accessory cell types required for the expression of this reaction (Mitra et al., 1999). Increase in both, HA titer and DTH response indicated that *A. longa* potentiates humoral as well as the cellular immunity. This plant also is a rich source of terpenoids which may act as immunomodulatory, which could justify the high number of lymphocytes infiltrates found in all tissues examined (Raphael and Kuttan, 2003). Reactive metabolites or intermediates, formed through the bioactivation of herbal constituents, are associated with herbal toxicity, mutagenicity and carcinogenicity. However, the toxic effect of the *A. longa* extract on the liver may be due to the fact that it is the first target of acute toxicity. The hepato-renale toxicity may be due in one hand to both AA-I and AA-II which undergo reduction of the nitro group to form reactive cyclic nitrenium ions that are able to form covalent DNA adducts with the exocyclic amino groups of adenine and guanine (Pfau et al., 1990a, 1990b, 1991) leading to cell cycle arrest (Li et al., 2006; Chang et al., 2007). Several studies have established that AA is strongly nephrotoxic (Mengs, 1987; Mengs and Stotzem, 1993; Arlt et al., 2001) and genotoxic (Robisch et al., 1982; Zhang et al., 2004). AA-I was found to be more toxic than AA-II, and other structural analogues either have less overall toxicity or no toxicity (Balachandran et al., 2005). On the other hand, generalized tissue destruction may be caused by high and continues stimulation of the immune system. Interestingly, histopathological analysis after withdrawal of treatment showed that the recovery was pronounced in hepatic and intestinal tissues. It may be explained by the metabolic pathway of converting AA-I to AA-Ia (a major metabolite of AA-I) functions as the detoxification of AA-I (Shibutani et al., 2010). In contrast, the changes in renal tissue were pronounced and progress to renal interstitial fibrosis because adducts impair physiological processes at the transcriptional level (Schmeiser et al., 1996; Arlt et al., 2001) and the continuous action of the immune system. The presence of inflammatory cells in human AAN has been reported for four end-stage AAN patients. Besides the tubulointerstitial lesions usually seen in the kidney cortex, a massive infiltration of macrophages, T and B lymphocytes was detected by immunohistochemistry in the medullary rays and in the outer medullae with some extension to the upper cortical labyrinth. In parallel with histological findings reported in the mice model, inflammatory cells are present preferentially in the interstitium of the medullary rays and of the outer medulllae in renal interstitium from human AAN cases (Pozdzik, 2010). In conclusion, at the dose consumed empirically in traditional Moroccan medicine, *A. longa* appears to be relatively toxic. It can cause liver, intestinal and kidney toxicity. Considering these data, we could state that Aristolochia longa rhizomes possess immunomodulatory properties and suggests their involvement of immune responses in the toxic lesions.

Further studies should be undertaken to determine the respective roles of AA and adaptive immunity in the progression of AAN. Additional clinical toxicological evaluations need to be performed with AA-depleted extract of *A. longa* to determine if the anti-tumor action of the plant is reality or myth. Since, the toxicity studies in experimental animals cannot always be totally extrapolated to humans (VanMiert, 1989) and a reasonable estimate of the self-administered dose is difficult to make, and in view of the widespread traditional use of this plant, recommendations are necessary to protect the population from possible toxic effects of the plant especially in patients treated for cancer who are already taking cytotoxic treatments.

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