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ORIGINAL ARTICLE

Toxicological Study of the Effect *in vivo* and *in vitro* of *Artemisia herba-alba* Aqueous Extract in Rats

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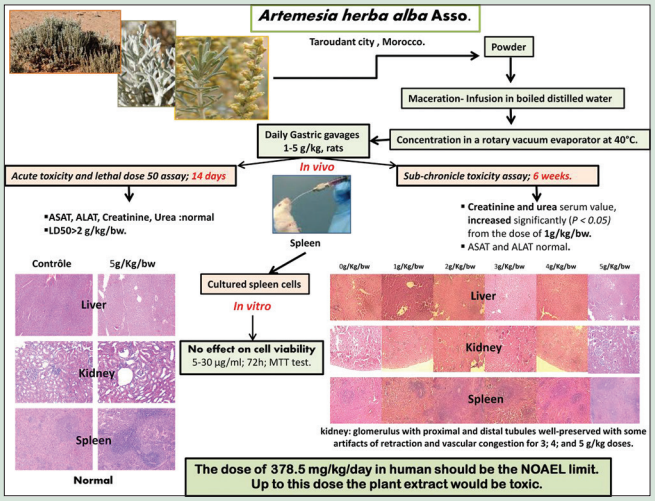
ABSTRACT

Background: *Artemisia herba-alba* (AHA) is largely used in folk medicine in different countries. However, rare studies provided toxicological evaluation regarding their safety on human health. **Objective:** This study investigated the safety of the standardized aqueous extract of AHA, like used by patients, to evaluate their toxicity *in vivo* and *in vitro*. **Materials and Methods:** For toxicological evaluation *in vivo* we used acute (during 14 days) and sub-acute oral gavages in Wistar rats (rats treated daily for 42 days at 1–5 g/kg bw) and the 3-[4, 5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide assay was performed to determine the level of cell viability and the degree of cytotoxicity *in vitro* (0–30 µg/ml) on cultured spleen cells. **Results:** The LD_{50} was up to 2 g/kg. Signs of mortality and toxicity were observed after single doses and no-observed-adverse-effect levels in the sub acute toxicity was up to 2 g/kg bw. Compared to the control, the treatment did not produce any statistically significant changes on alanine aminotransferase and aspartate aminotransferase serum titer. However, for creatinine and urea serum value, a significant increase ($P < 0.05$) was observed. The histological observations of liver and spleen tissues have shown well-preserved normal cells. Indeed for kidney tissues some artifacts of retraction and vascular congestion were noted for 3–5 g/kg doses after sub-chronic treatment. The addition of plant extracts to the spleen cells did not show any sign of toxicity for all doses tested. **Conclusion:** We conclude that AHA aqueous extract at the dosage up to 2g/kg bw will be toxic and can affect mainly the kidney tissues.

Key words: *Artemisia herba-alba,* biochemical parameter, histopathology, *in vivo* and *in vitro* toxicity, standardized aqueous extract

SUMMARY

- The safety of the standardized aqueous extract of *Artemisia herba-alba*, like used in folk medicine by patients was evaluated for their acute and sub acute toxicity *in vivo* and on spleen cells *in vitro* in rats. The LD_{50} and NOAEL were up to 2 g/kg body weight. We observed a significant increase ($P < 0.05$) for creatinine and urea serum value and no significant changes on ALAT and ASAT serum titer. The histological observations of liver and spleen tissues have shown for kidney tissues some artifacts of retraction and vascular congestion for 3-5 g/kg doses after sub-chronic treatment. The addition of plant extracts to the spleen cells did not show any sign of toxicity for all doses tested. We conclude that the aqueous extract at the dosage up to 2g/kg will be toxic and can affect mainly the kidney tissues.



Abbreviations Used: AHA: *Artemisia herba-alba;* NOAEL: No-observed-adverse-effect levels; LD 50: Lethal dose for 50% of the animals; FBS: Fetal bovine serum; ALAT: Alanine aminotransferase; ASAT: Aspartate aminotransferase; MTT: 3-[4, 5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide; DMSO: Dimethyl sulfoxide.

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DOI: 10.4103/pr.pr_4_20

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INTRODUCTION

Artemisia herba-alba (AHA) belongs to the *Asteraceae* family, called "desert wormwood," or "Chih" in Morocco, is widely consumed and used in traditional medicine in different countries.[1,2] Phytochemical studies showed that the genus of *Artemisia* contains in large quantities terpenoids, flavonoïds, coumarins, acetylenes, sterols and essential oils.[3-13] All these compounds have multiple beneficial bioactivities. Indeed, numerous studies have shown the antibacterial activities of different extracts of AHA on Gram-positive and Gram-negative bacteria and fungal strain.[4-9]

A cytotoxic effect of AHA essential oil extracts on murin mastocytoma and hamster kidney carcinoma cell lines was reported as antitumor activity.[10] Using network pharmacology-based analysis on North African plants, AHA was found to have the largest number of constituent that have been associated with different cancer related pathway like cell cycle arrest or inhibition of cellular proliferation or apoptosis.[11]

Cite this article as: Lahna A, Benjelloun N, Seddik N, Farida M, Naya A, Oudghiri M. Toxicological study of the effect *in vivo* and *in vitro* of *Artemisia herba-alba* aqueous extract in rats. Phcog Res 2020;12:207-11.

Submitted: 04-Feb-2020 **Revised:** 09-Apr-2020
Accepted: 10-Jun-2020 **Published:** 14-Aug-2020

Other bioactivities of AHA extract were described as anti-malarial, antioxidant , anti-coagulant, anti-pyretic, anti-ulcerogenic, anti-hepatitis and anti-spasmodic .[12-15] Recent studies have shown that the aqueous extract of AHA reduce the metabolic syndrome induced in rodent and ameliorates hyperglycemia, hyper-lipidemia in alloxan-induced diabetes in rats.[14,15] However it was reported by a Moroccan and others studies, that 50.7% among patients with kidney disease use herbal medicine and AHA was the most used.[16,17] However although all these studies have explored different bioactivities of AHA, rare studies provided toxicological evaluation for the different type of extract of a plant regarding their safety on human health.

The purpose of the study was to explore and to examine the safety of the standardized aqueous extract of AHA like used and prepared by Moroccan patients to evaluate their toxicity *in vivo* and *in vitro*.

MATERIALS AND METHODS

Plant material

The stems and leaves of AHA was collected at the end of may from "Ighram," an area of Taroudant city and was authenticated by Professor Zidane Lahcen, a plant taxonomist at the Faculty of Sciences of Ibn Tofail University of Kénitra. A voucher specimen of AHA was previously deposited in Scientific Institute of Rabat under the number 43,130.

Preparation of the aqueous extract of *Artemisia herba-alba*

Extraction of bioactive substances contained in the aerial part of AHA was carried out by maceration-infusion in boiled distilled water like prepared by patients.[1,2] After grinding the aerial part; 25 g of the powder obtained were added to 250 ml of boiled distilled water and then left for 30 minutes for infusion with stirring. The resulting mixture was then filtered and concentrated in a rotary vacuum evaporator at 40°C. The residue was collected in a volume of distilled water suitable for a 50% final solution (5 g/ml) which was stored at −20°C until use.

Animals

Young Adult Male Wistar rat (210–230 g), from the animal house of Faculty of Sciences, University Mohammed V; Morocco were kept in plastic cages in environmental conditions (22°C –24°C, 12 h: 12 h dark/light cycle) with frequent air changes and allowed to drink water *ad libitum* and standard pellet diet. They were deprived of food 16–18 h prior the experiments. An adaptation period of 2 weeks was allowed before each experiment.

Toxicological evaluation *in vivo* of the aqueous extract of *Artemisia herba-alba*

Acute toxicity and lethal dose 50 assay

The assessment of acute toxicity was performed according to the World Health Organization (2000) and the Organization of Economic Co-operation and Development guideline for testing of chemicals 420 (OECD 2001).

Rats were divided into 2 lots as follows: Lot 1 (4 rats): "Rats Controls'" received by gastric gavages 1 ml of distilled water for 14 days and Lot 2 (4 rats): "Treated rats" received by gastric gavages 1 ml of the AHA aqueous extract at a single dose of 1, 2, 3, 4 or 5 g/kg.

The animals were then placed in individual cages for observation. These observations concerned the behavior and general condition of the animals and the batch mortality during the 14 days after administration for the acute toxicity assay and the first 72 h to determine the lethal dose (LD_{50}); the amount of a drug or extracts given at once which causes the death of 50% population of test animals. Body weight was measured before and after administration on days 4, 7, 10 and 14. At the end of the 14th day treatment period, the rats were anaesthetized; a cardiac puncture was performed to collect blood for biochemical evaluations, and organs for histopathology assay.

Sub-chronicle toxicity assay

The rats were divided into 6 groups (4/group) in separate cages during the study. The aqueous extract was given daily by gavages (1 ml) at doses of 1, 2, 3, 4 or 5 g/kg body weight for 6 weeks. The control group received the vehicle only. The general animal behavior, food and water intake and clinical signs of toxicity observed continuously for 1 h after oral intake treatment, then intermittently for 4 h, and thereafter over a period of 24 h.[18] Body weight has been recorded every 7 days. At the end of the treatment period, the rats were anaesthetized; a cardiac puncture was performed to collect blood for biochemical evaluations, and organs for histopathology assay.

Toxicological evaluation *in vitro* of the aqueous extract of *Artemisia herba-alba*

A suspension of rat spleen Cells were plated at a density of 5×10^3 per well, in 96-well plates, in RPMI containing 10% fetal bovine serum and 1% penicillin/streptomycin, in a 37°C incubator under 5% of CO_2 saturation. The cell viability assay was determined by an 3-[4, 5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) assay. Cells received plant extracts (0, 5, 10, 15, 20, 25, and 30 µg/ml) incubated for 24, 48 and 72 h, then MTT and 3h after Dimethyl sulfoxide was added. The absorbance was measured at 570 nm, and the percentage of the growth inhibition power was calculated.

Serum biochemistry analysis

The serum biochemical parameter evaluation was done in all surviving animals at the end of the experiment. The collected blood with anticoagulant allowed to stand for 60 min at room temperature and then centrifuged at 3200 rpm for 10 min. The serum recovered was analyzed for creatinine, blood urea nitrogen concentrations, for the activity of liver enzymes: alanine aminotransferase (ALAT) and aspartate aminotransferase (ASAT). They were determined by standard methods with a biochemical automat (Konelab 20 Thermo).

Histopathological analysis of the liver, kidney and spleen tissues

The liver, kidneys and spleen were dissected out. Small slices of tissues were fixed in buffered formaldehyde solution (10%), dehydrated in ascending series of ethanol solution and then embedded in paraffin. Micrometer sections (4–5 µm) of each tissue were stained with hematoxylin-eosin and examined under a light microscope; photomicrographs of the samples were recorded and interpreted by a pathologist.

Statistical analysis

All values were expressed as mean ± standard error of measurement and the statistical significance between control and experimental groups were analyzed by means of Student's *t*-test. Statistical significance was assigned at $P < 0.05$.

RESULTS

Toxicological evaluation *in vivo* of the aqueous extract of *Artemisia herba-alba*

General signs and mortality during the acute toxicity and lethal dose 50 assay

No deaths were recorded within 72 h in LD_{50} assay after administration of the extracts at 1 and 2 g/kg. For the doses between 3 and 5 g/kg, we

observed a decrease in feed and water intake and mobility, abnormal stools and behavior with mortality in rats treated by a single dose during the 72 h and up to 14 days of observations [Table 1]. The LD_{50} seem to be >2 g/kg bw.

General signs and mortality during the sub-chronicle toxicity assay

The evolution of body weight gain during the 6 weeks of treatment is represented in Figure 1.

Compared to the control, AHA extract given by oral route to Wistar rats for 42 consecutive days have had no effects on body weights or body weight gains. However, all signs of toxicity were observed during the 6 weeks of daily treatment from the dose up to 2 g/kg/bw [Figure 1].

Evaluation of biochemical parameters after acute and sub-chronic toxicity test

The Figure 2 represents the effect of a one dose administration of AHA (5 g/kg body weight) on biochemical parameters after 14 days. Compared to the control, the treatment did not produce any statistically significant changes on Urea, ALAT and ASAT. For creatinine serum value we found a significant increase ($P < 0.05$).

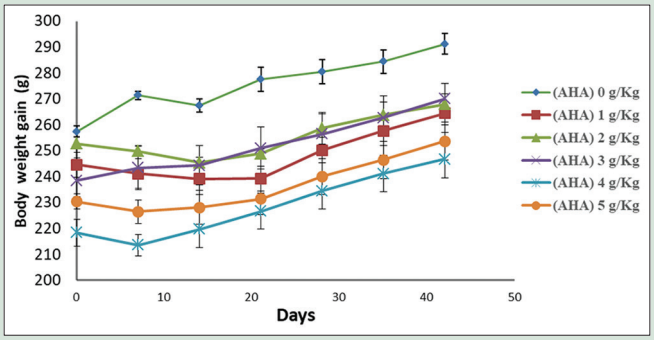

Figure 1: Body weight gain curves of Wistar rats treated orally with aqueous extract of *Artemisia herba-alba*. Rats were treated by gastric gavages at doses of 1, 2, 3, 4, or 5 g/kg/body weight for 6 weeks. Data are expressed as mean ± standard error of measurement ($n = 4$)

For the sub-chronic toxicity studies, the extract of AHA was given daily by gavages at the following doses: 1, 2, 3, 4, or 5 g/kg body weight for 6 weeks. The results of biochemical parameters value are on Table 2. The treatment did not produce any statistically significant changes on ALAT and ASAT. For creatinine and urea serum value, we observed a significant increase ($P < 0.05$) from the dose of 1 g/kg/bw [Figure 2].

Toxicological evaluation *in vitro* of *Artemisia herba-alba* extract

To determine the degree of cytotoxicity of plant extracts on spleen cells we used a MTT assay. Unfortunately no effect on cell viability was noted in the conditions of experimentation described.

Histopathological examination of the liver, kidney and spleen tissues

The Figure 3 represent the histological sections of liver, kidney and spleen tissues of rats treated with one dose of the AHA (5 g/kg) showing well-preserved normal cells prominent cytoplasm, nucleus and nucleolus and venous after 14 days [Figure 3].

The Figure 4 represents the histological sections of the three tissues after daily oral gavages (1–5 g/kg) with AHA for 6 weeks. No differences were observed with the control for the hepatic tissue. For the kidney we observed a normal architecture showing glomerulus with proximal and distal tubules well-preserved with some artifacts of retraction and vascular congestion for 3; 4; and 5 g/kg doses. No differences were observed for spleen tissues between treated and no treated rats [Figure 4].

DISCUSSION

The acute toxicity study highlighted that AHA causes mortality at the dose up to 2 g/kg. The no-observed-adverse-effect levels (NOAEL) in the sub acute toxicity was also up to 2 g/kg bw. These doses were selecting first, at the base of the previous studies for estimation of starting dose and secondly by calculating the equivalent dose used by Moroccan population like described in the ethno botanical studies and then calculating the dose conversion from human to animal studies.[1-2,14,15,19]

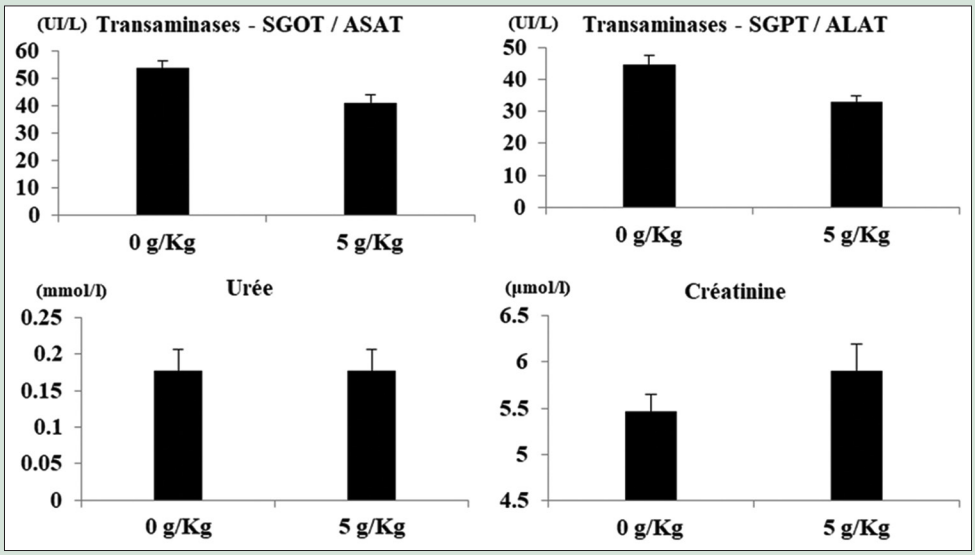

Figure 2: Effect of single oral administration (5 g/kg body weight) of aqueous extract of *Artemisia herba-alba* extract on biochemical parameters after 14 days. Data are expressed as mean ± standard error of measurement ($n = 4$). ALAT: Alanine aminotransferase; ASAT: Aspartate aminotransferase

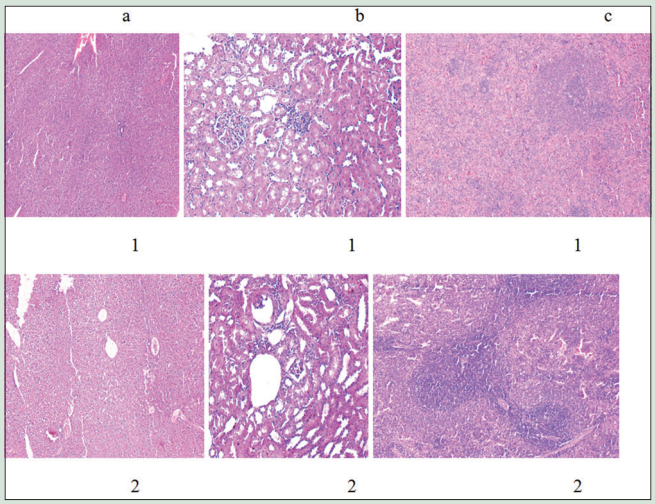

Figure 3: Histological observation of liver, kidney and spleen tissues after acute treatment with the *Artemisia herba-alba* extracts. (a) Liver sections of normal control rats (1) and rats treated with 5 g/kg (2) (H and E × 40), showing normal architecture without lesions and hepatic cells with well-preserved cytoplasm with some vacuolization post mortem. (b) Kidney sections of normal control rats (1) and rats treated with 5 g/kg (2) (H and E × 40) showing a normal architecture of the tissue with some lymphatic cellule's in the interstitial space similar to normal control. (c) Spleen sections of normal control rats (1) and rats treated with 5 g/kg (2) (H and E, ×40) showing a normal architecture similar to normal control

Therefore, calculation of human equivalent dose (HED) for a drug of NOAEL in rats is 2 000 mg/kg with an average weight of 220 g is as below:

HED (mg/kg) = 2 000 (mg/kg) $\left(\frac{7}{37}\right)$ × = 378.5 mg/kg in human.[19]

K^*_m value in rats (0.220 kg/0.031 m^2) = 7 k_m value in human (60 kg/1.62 m^2) = 37.

*Correction factor: the average body weight (kg) of species to its body surface area (m^2).

The dose of 378.5 mg/kg/day in human should be the NOAEL limit. Up to this dose the plant extract would be toxic.

Tested *in vitro* on normal spleen cells it didn't show any toxicity after 72 h for the doses between 0 and 30 µ/ml compared to the control.

We evaluated ASAT and ALAT as are linked to liver function.[20] Our results have shown there were no differences in ASAT and ALAT values in treated rats compared to control rats. ALT is an enzyme mainly in the cytosol of hepatocytes and is a sign of hepatic cellular disturbance more than ASAT. ASAT is located in the cytoplasm and mitochondria of different type of cells as cardiac, hepatic cells or erythrocytes.[20] The variation in the values of these two enzymes is linked to possible toxicity. In our study there was no significant variation between the treated animals and the untreated. The histopathology assay confirmed the absence of toxicity of the liver tissue. However we found an elevated serum creatinine value for the treated rats and at a level of the kidney tissue, glomerulus showed proximal and distal tubules well-preserved but with some artifacts of retraction and some vascular congestion for 3–5 g/kg doses after 42 days of AHA aqueous extract treatment.

A period of 42 days matches with to that used by Moroccan patients for their treatment. However the kidney lesions observed will potentially be more important if we had continued the daily gavages for a longer period. These observations can be compared to the reported results of number of patients hospitalized for nephrology diseases and that use herbal medicine.

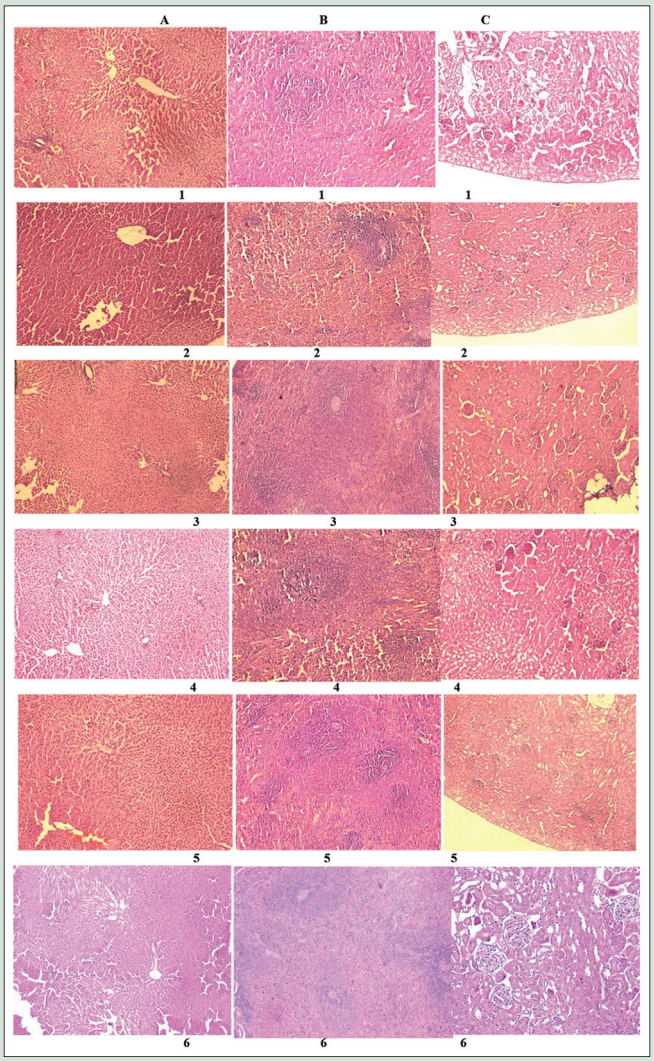

Figure 4: Histological observation of liver, kidney and spleen tissues after sub-chronic treatment with the *Artemisia herba-alba* extract. (A) Liver sections of normal control rats (1); rats treated with 1 g/kg (2); rats treated with 2 g/kg (3); rats treated with 3 g/kg (4); rats treated with 4) rats treated with 1 g/kg (2); rats treated with 2 g/kg (3); rats treated with 3 g/kg (4); rats treated with 4 g/kg (5) and rats treated with 5 g/kg (6) of extract for 6 weeks (H and E × 40) (5) and rats treated with 5 g/kg (6) of extract for 6 weeks (H and E, ×40) showing normal architecture without lesions with some artifact between hepatocyte trabecular. (B) Spleen sections of normal control rats (1) rats treated with 1 g/kg (2); rats treated with 2 g/kg (3); rats treated with 3 g/kg (4); rats treated with 4 g/kg (5) and rats treated with 5 g/kg (6) of extract for 6 weeks (H and E, ×40) showing normal architecture compared to controls. (C) Kidney sections of normal control rats (1) rats treated with 1 g/kg (2); with 2 g/kg (3); with 3 g/kg (4); with 4 g/kg (5) and with 5 g/kg (6) of extract for 6 weeks (H and E, ×40). A normal architecture showing glomerulus with proximal and distal tubules well-preserved with some artifacts of retraction and with some vascular congestion noted for 3; 4; and 5 g/kg

AHA was cited among the most used plant because their belief in its efficacy and safety.[16] Other study reported cases of kidney toxicity related to herbs and dietary supplements where AHA was cited among 7 herbs.[17]

In regard to its several antimicrobial activities, an external use is more recommended; in example for periodontitis.[5,6,8]

Table 1: Clinical signs and mortality during acute toxicity and Lethal dose for 50% of the animals assay

	Clinical signs							
	Body weight	Feed and water intake	Mobility	Aggressive	Stools aspect	Behavior	Mortality	
AHA (mg/kg bw)	14 days	24 h-14 days	24 h-14 days	24 h-14 days	24 h-14 days	24 h-14 days	24 h-72 h	After 14 days
0.00	Normal	Normal	Normal	Normal	Normal	Normal	0/4	0/4
1.00	Normal	Normal	Normal	Normal	Normal	Normal	0/4	0/4
2.00	Normal	Normal	Normal	Normal	Normal	Normal	0/4	0/4
3.00	Normal	Decreased++	Decreased ++	++	Abnormal	Abnormal ++	2/4	2/4
4.00	Normal	Decreased++	Decreased ++	++	Abnormal	Abnormal ++	2/4	2/4
5.00	Normal	Decreased+++	Decreased+++	++++	Abnormal	Abnormal +++	2/4	-

Rats were divided into 2 lots as follows: lot 1 (4 rats): "Rats Controls" received by gastric gavages 1 ml of distilled water for 15 days and Lot 2 (4 rats): "Treated rats" received by gastric gavages 1 ml of the AHA aqueous extract at a single dose of 1, 2, 3, 4 or 5 g/kg bw. Observations concerned the behavior and general condition of the animals and the batch mortality were done every day. Each value is represented as mean±SEM (n=4). SEM: Standard error of mean; AHA: *Artemisia herba-alba*. ++: Medium; +++: Strong

Table 2: Effect of sub chronic treatment *in vivo* by *Artemisia herba alba* aqueous extracts on biochemical parameters

Doses AHA (g/kg)	Urea (g/L)	Creatinine (mg/L)	ASAT (UI/L)	ALAT (UI/L)
0	0.265±0.088	7.650±2.156	67.075±9.661	71.675±6.602
1	0.275±0.039	10.800±2.560	89.500±12.480	83.050±5.950
2	0.255±0.061	8.200±2.980	72.225±12.598	69.825±3.807
3	0.220±0.079	9.250±3.602	61.675±10.768	50.450±5.229
4	0.287±0.104	10.575±4.654	72.375±10.051	61.725±13.419
5	0.350±0.179	13.600±4.235	70.500±9.848	59.230±9.137

The extract of the plant was given daily by the oral route to groups of Wistar rats (n=4) at the following doses: 0, 1, 2, 3, 4, or 5 g/kg body weight for 6 weeks. Data are expressed as mean±SEM. (n=4). ALAT: Alanine aminotransferase; ASAT: Aspartate aminotransferase; SEM: Standard error of mean; AHA: *Artemisia herba alba*

CONCLUSION

In conclusion AHA aqueous extract will be toxic at the dose up to 2 g/kg bw. Their toxicity will affect mainly the kidney tissue. In view of the wide traditional use of AHA, recommendations are necessary in case of difficulty to control the doses administered.

Financial support and sponsorship

The authors thank the Moroccan Ministry of High Education and Hassan II University of Casablanca for their financial contribution.

Conflicts of interest

There are no conflicts of interest.

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