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In vivo anti-psoriasis, anti-inflammatory activities and oral toxicity studies of aqueous extract from seeds of *Ammi visnaga* L.

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ABSTRACT

Ammi visnaga L (A.V.) is used in the traditional medicine for the treatment of kidney stones, diabetes and vitiligo. An *in vivo* anti-psoriasis, anti-inflammatory activities and oral toxicity studies of aqueous extract from seeds of *Ammi visnaga* L. was investigated. In the acute toxicity, the extract was administered orally in a single dose to rats (0- 2000-5000 mg/kg) and in the sub-acute toxicity daily for 28 days (0-300-600-1000 mg/kg/day). The symptoms of toxicity and mortality have been recorded daily and during 14 days of recovery with an examination of liver, kidney, hematologic, biochemical and histological analysis at the end of treatment. The anti-inflammatory activity was evaluated by induction of oedema and the anti-psoriasis by induction of a psoriasiform-like skin phenotype by UV-B radiations. No mortality was observed after single gavages by a dose up to 5000 mg/kg and no signs of toxicity noted. Clinical and biochemical examination during 28 days of gavages at all doses showed no significant difference compared to control group, while a significant reduction in MCV (mean corpuscular volume) and P-LCR (platelet large cell ratio) ($p < 0.05$), PDW (platelet distribution width) and MPV (multi-purpose vehicle) ($p < 0.01$) was observed and histopathological examinations showed slight inflammation in the liver and kidneys for the higher dose. Percentage of inhibition of the oedema was near the positive control 50% for all doses tested. Treatment with A.V. extract had decreased the thickness of the skin induced by UV-B irradiation. In conclusion, the LD50 was estimated greater than 5000 mg/kg; therefore A.V. can be classified as non-toxic but if used in the long term can induce a slight toxicity dose dependant with high anti-psoriasis and anti-inflammatory activities.

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INTRODUCTION

The Mediterranean countries have a long medical tradition and developed traditional knowledge into herbal medicines (Leonti and Verpoorte, 2017). In Morocco, 70% of the population of rural areas has resorted to medicinal plants. Still, the majority of famous medicinal plants practices come from secular oral culture and do not have a scientific reference (Lahsissene and Kahouadji, 2010). *Ammi visnaga* L, Umbelliferae (Apiaceae) species, is one of the abundant medicinal plants

in the North of Morocco and throughout the Mediterranean (El-Hilaly *et al.*, 2003). This plant presents a great reservoir of potentially beneficial compounds attributed to secondary metabolites, including furanochromes, pyranocoumarins, furanocoumarins, flavonoids, volatiles and proteins (Ammor *et al.*, 2017). The quality and quantity of these secondary metabolites depend on the part of the plant analysed, the growing conditions and the presence of bioregulators. These substances have a great diversity of chemical structure, which confers to *Ammi visnaga* (A.V.) in an extensive range of pharmacological activities. It is used as anti-asthma, as a powerful antimicrobial treatment, as anti-diabetic, as cytotoxic on some cancer cell lines for urinary lithiasis prevention for vitiligo and as potent antioxidants (Ammor *et al.*, 2017). The herbicidal activity was observed for khellin and visnagin, two main constituents of A.V. (Khalil *et al.*, 2020). The decoction or powdered plant has been traditionally used for the treatment of psoriasis. When applied topically, it has been found useful in the recovery from psoriasis (Abdel-Fattah *et al.*, 1983).

However, scientists reported some adverse effects. Indeed overdose or more prolonged use of the A.V. can lead to queasiness, dizziness, loss of appetite, headache, and sleep disorders. Similarly, side effects like pseudo-allergic reactions, reversible cholestatic jaundice and elevated activities of liver transaminases and γ-glutamyl transferase have been observed with the use of A.V. or its constituents. Intake of A.V. is not recommended at all along with blood thinners such as anti-hypertensive drugs like calcium channel blockers or other drugs that lower blood pressure (Zar and Das, 2013). During treatment, the exposure to sun or other sources of ultraviolet light should be avoided, minimise photosensitivity. Few data are available on the toxicity (Koriem *et al.*, 2019), on the effect on inflammation (Lee *et al.*, 2010) or psoriasis (Abdel-Fattah *et al.*, 1983) of A.V. and its different extracts. Psoriasis is a skin disease with massive skin cell proliferation (accelerated by releases of pro-inflammatory cytokines and growth factors), inflammatory cell infiltrates, generation of new blood vessels, modifications in lymphatic structure, and impaired differentiation of the epidermis. Then, growth of skin cells, accumulate and form thick red patches on the skin of diverse parts of the body. Therapies for treating psoriasis are systemic agents (methotrexate, cyclosporine, acitretin, biologics like adalimumab, etanercept), topical agents (retinoids, Vitamin D-3 derivatives, corticosteroids, or anthralin), and phototherapy (Kim *et al.*, 2017). No single treatment gives a complete and satisfactory cure, and most of them have adverse effects. As an alternative to these drugs, phytoconstituents have been widely used. These have better therapeutic value and have fewer side effects. The present work has been carried out to evaluate the acute and sub-acute toxicity of an aqueous extract of A.V. seeds and its effect on an induced inflammation and psoriasis-form-like skin phenotype.

MATERIALS AND METHODS

Plant material

The collection of the umbels of *Ammi visnaga* L was made in July 2016 in the province of Sidi Kacem, Morocco (34 ° 23 ’15 ”North 5 ° 30’ 14” West), these whole umbels have been preserved in anti-humidity obscure bags until the time of their use. A plant taxonomist authenticated them; Pr. Najat el khyati; at the department of biology of faculty of sciences, Hassan II University of Casablanca, Morocco.

Preparation of seed aqueous extracts of A.V.

Ammi visnaga L seeds, previously washed and dried, have been reduced to powder; the extraction solvent (distilled water) was versed on a quantity of this powder (10%). The intermixture was tempered in a water bath at 40 °C for 30 min with magnetic agitation, filtered with Whatman paper (0.45 mm), and then evaporated by a rotary evaporator set at 40-50 °C under reduced pressure. The solution obtained was stored at -20 °C.

Animals

The experiments were carried out on adult Wistar rats with bodyweight between 250-320g. They were obtained from the Faculty of Science of Mohammed V University of Rabat and were housed in the study laboratory seven days before the experiments.

Acute toxicity test

The acute toxicity of *Ammi visnaga* L seeds aqueous extract was evaluated according to the Organization for Economic Co-operation and Development (OECD, 1997) Guideline 423 on rats; World Health Organization (WHO) guideline (WHO, 1999); and the Organization of Economic Co-operation and Development (OECD) guideline for testing of chemicals 420 (OECD, 1997) with a limit dose of 5000 mg/kg (David and Enegide, 2013; Olson *et al.*, 2000). The test was done on three groups of three rats each. The plant extract was orally administered to rats only once, at a dose of 0 mg/kg (control group), 2000 mg/kg or 5000 mg/kg (treated groups), after 12 h of fasting. The animals were observed for any toxic effects during the first 4 hours after treatment and daily for 14 days. Observations included changes

in the skin, fur, eyes, mucous membranes, secretions, stereotype, excretion and autonomous activity. Changes in movement, posture and response to manipulation, as well as the presence of clonic or tonic movements, or bizarre behaviours have been recorded. The bodyweight of all animals was measured on days 1, 7 and 14. At the 14th day, the rats were anesthetised with chloroform; blood has been collected in dried tubes by cardiac puncture and centrifuged (400g for 10 min). The serum was collected for biochemical studies included activity of ASAT (aspartate aminotransferase), ALAT (alanine aminotransferase), urea (UREA) and creatinine (CREA) level. The animals were then quickly dissected, and the organs (liver, spleen, lungs, kidney and heart) were removed and microscopically examined, cleaned and weighed. Livers and kidneys were stored in 10% formalin for histological examination.

Subacute toxicity test

Subacute toxicity was also realised by the Organization for Economic Co-operation and Development (OECD) guideline 407, with slight modifications. Male and female rats were randomly divided into four groups of 10 rats and given daily by oral gavages of A.V. extract at 0-300- 600 and 1000 mg/kg body weight (bw) during 28 days. Rats were observed daily for the same physical and behavioural signs of toxicity used for the acute toxicity study. Bodyweight recording and dose adjustment were made after every seven days of treatment. On day 22 of the study, five animals for each group were anaesthetised with chloroform and blood collected by cardiac puncture with or without anticoagulant. Subsequently, kidneys and liver were removed, weighed, subjected to macroscopic examination and rapidly conserved in 10% formalin for a histological study. Blood without anticoagulant was allowed to coagulate before centrifugation (400g for 10 min) to obtain serum, which was used for the evaluation of Urea, Creatinine ASAT and ALAT activities. Anti-coagulated blood samples were analysed for the hematologic parameters: WBC, RBC, HGB, HCT, MCV, MCH, MCHC, PLT, LYM, RDW-SD, PDW, MPV, P-LCR, P-LCR, and PCT. The residual rats (5 of the group treated with 0-1000mg/Kg) treated were observed for another 14 days without any treatment to evaluate the recovery.

Edema in the right hind paw of the rat was induced by an injection of 0,02ml of formaldehyde at (1 à 2%), subcutaneously in the plantar side 1 hour after intramuscular injection of the A.V. extract or the standard anti-inflammatory drug; the Diclofenac (Troge; Germany) (6 mg/kg body weight) or NaCl 0.9% in rats treated ten days by oral gavages of A.V. extracts (0-60-80-100 mg/kg bw). The evaluation of the oedema was done by measure the size of oedema and the evaluation of the percentage of inhibition of oedema was calculated using this formula,

% inhibition of edema = (Mean edema increase in control group - Mean edema increase in treated group $\times$ 100) / (Mean edema increase in control group)

Induction of psoriasis in rats

The induction of a psoriasis-form-like skin phenotype was done by irradiation with a UV-B lamp (280-315 nm). It was kept at a vertical distance of 20 cm from a 1,5$\times$ 2,5 cm shaved skin during 3h. Twelve hours after irradiation and during 14 days; rats were treated once day by oral gavages with AV extract (300 or 600mg/kg bw) or retinoic acid (0,5 mg / kg bw) or NaCl 0.9% (10ml/kg bw). The animals were sacrificed two hours after the last treatment. The area of the skin treated was removed by surgical incision and fixed in a 10% formalin solution for histopathological examination. Psoriasiform-like skin phenotype includes an increased epidermal thickness in duplicate an absence of stratum granulosum and movement of neutrophils towards the epidermis.

Statistical analysis

Statistical significance between control and treatment groups was determined by the analysis of variance (ANOVA) followed by the Dennett post-hoc test, and the differences were considered statistically significant at $p < 0.05$. All data are expressed as mean $\pm$ standard deviation (S.D.)

RESULTS

Acute toxicity: Clinical observation

Rats treated with plant extract at doses of 2000 mg/kg and 5000 mg/kg showed no morphological or behavioural changes, and very similarities with the control group were observed during the entire observation period. Therefore, the LD50 was estimated to exceed 5000 mg/kg.

Bodyweight change and macroscopic study and relative organ weights

Evaluation of body and organ weight in toxicology studies is an essential endpoint for identification of potentially harmful effects of chemicals (Bailey *et al.*, 2004). The bodyweight of all rats was increased gradually throughout the study period. Figure 1 indicates that the evolution of this weight is similar between the different groups. Statistical analysis of body weight gain indicated no significant differences between the treated groups (2000 mg/kg and

Table 1: Relative organ weights g/100g body weight of rats, 14 days after a single oral gavage with *Ammi visnaga* L extract.

Doses mg/Kg bw Organs	0	2000	5000
Liver	3,700±0,053	3,733±0,227^{ns}	4,037±0,251^{ns}
Kidneys	0,640±0,021	0,607±0,039^{ns}	0,560±0,011^{ns}
Lungs	0,582±0,044	0,610±0,037^{ns}	0,573±0,021^{ns}
Heart	0,355±0,003	0,380±0,021^{ns}	0,367±0,011^{ns}
Rate	0,300±0,000	0,313±0,021^{ns}	0,357±0,004^{ns}

Mean values ± SD, n = 3 rats/group. ns: no significant differences

Table 2: Biochemical analyses of rat serum, 14 days after a single oral gavage with *Ammi visnaga* L aqueous extract

Doses of extract mg/Kg bw	0	2000	5000
ASAT (UI/L)	138,80±7,50	127,10±12,90	147,35±30,75
ALAT (UI/L)	75,25±7,45	58,65±0,15	63,25±12,75
UREA (g/L)	0,37±0,03	0,31±0,06	0,36±0,02
CREAT (g/L)	4,03±0,72	4,96±1,62	4,78±0,61

Values expressed in mean ± SD, n = 3 rats / group.

5000 mg/kg) and control group (0 mg/kg). Macroscopic diagnosis of all organs (liver, spleen, kidney, heart, and lungs) at the end of the acute toxicity test showed that all these organs are expected, and do not present any visible pathological signs. The relative weight of these organs of rats receiving plant extract at 2000 mg/kg or 5000 mg/kg is very nearly equal to that of rats in the control group (Table 1).

Oral administration of *Ammi visnaga* L seed aqueous extract did not induce any significant change in the relative weight of organs (P>0.05).

ALAT: alanine aminotransferase. ASAT: aspartate aminotransferase, CREA: creatinine and UREA: blood urea.

WBC, white blood cell count; RBC, red blood, cell count; HGB, haemoglobin concentration; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; PLT, platelet 341 counts; LYM, lymphocytes number; RDW, Red blood cell distribution width; PDW, platelet distribution width; MPV, multi-purpose vehicle; P-LCR, platelet large cell ratio; PCT, pro-calcitonin.

Serum biochemical parameters

Table 2 shows the results of the biochemical analysis of the serum of rats, 14 days after single gavages with an aqueous extract of *Ammi visnaga* L. No significant differences in the levels of alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), creatinine and blood urea were observed in rats treated compared to the control group (P>0.05).

Liver and kidney histopathology

No significant differences were observed between the rats treated and the control (Figure 2) after 14 days.

Liver and Kidney sections of normal control rats (A**;** 0 mg/Kg**)** and rats treated with 2g/kg (B) or 5 g/kg (C) (HE x 40), showing typical architecture without lesions and hepatic cells with well-preserved cytoplasm with some vacuolisation post mortem.

Sub acute toxicity

Clinical observation

The subacute toxicity test was conducted according to OECD guideline 407. Daily observation during the entire treatment (28 days) and recovery (14 days) period showed no change when comparing the groups treated with the control group on both behavioural level and morphological symptoms of toxicity. Also, no cases of mortality were recorded.

Bodyweight change

In the first week, weight gain was noted in all rat groups. After that, weight decreased in the group

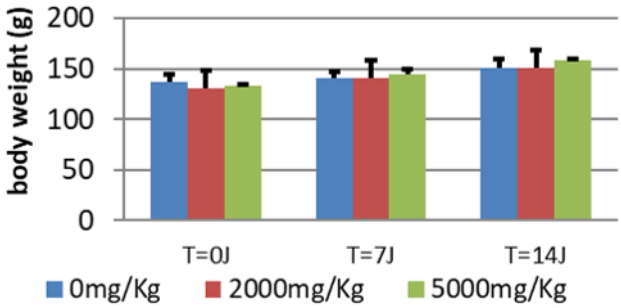


Figure 1: Body weight of rats treated with a single oral gavage of *Ammi visnaga* L aqueous extract. Values expressed in mean ± SD, n = 3 rats / group.

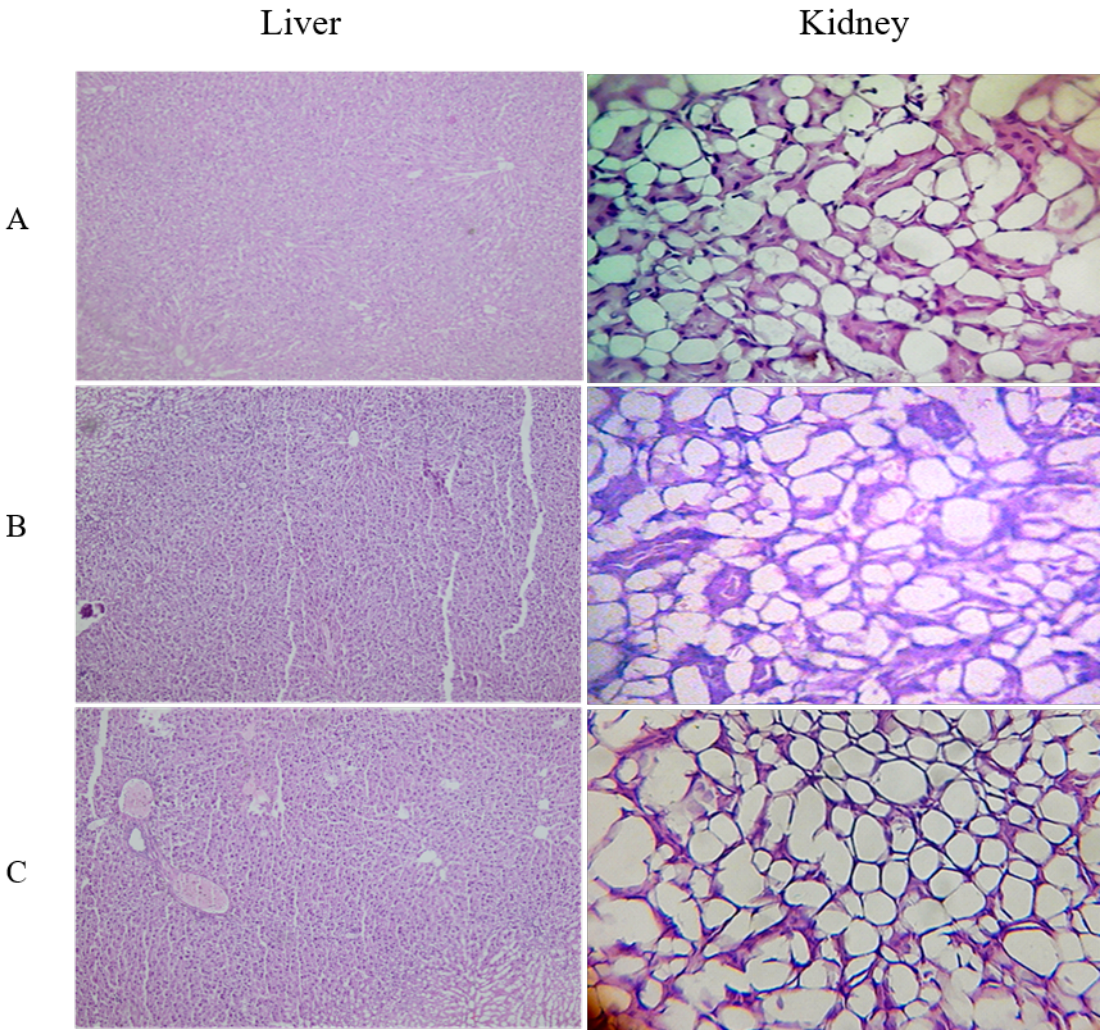


Figure 2: Histopathological sections of the liver and kidney of rats, 14 days after a single oral gavage with *Ammi visnaga* L extract. (x40 for liver and x400 for kidney).

Table 3: Foods intakes by rats treated with *Ammi visnaga* L extract during and after treatment

Doses mg/Kg bw	0	300	600	1000
W1	11,83±1,47	12,50±1,76	11,83±2,42	10,41±3,13
W2	12,33±0,91	12,83±1,08	14,16±1,12*	13,00±1,37
W3	11,20±0,69	11,45±1,96	13,16±1,98	12,83±0,75
W4	12,50±1,84	12,16±1,72	12,75±0,61	11,00±1,09
W5	11,80±1,25			12,00±1,69
W6	12,32±1,00			13,20±2,58

W: Week, W1-W4: treatment period; W5-W6: recovery period
Values expressed in mean ± SD. * p<0.05.

Table 4: Relative organ weights in g/100g body weight of rats during and after treatment

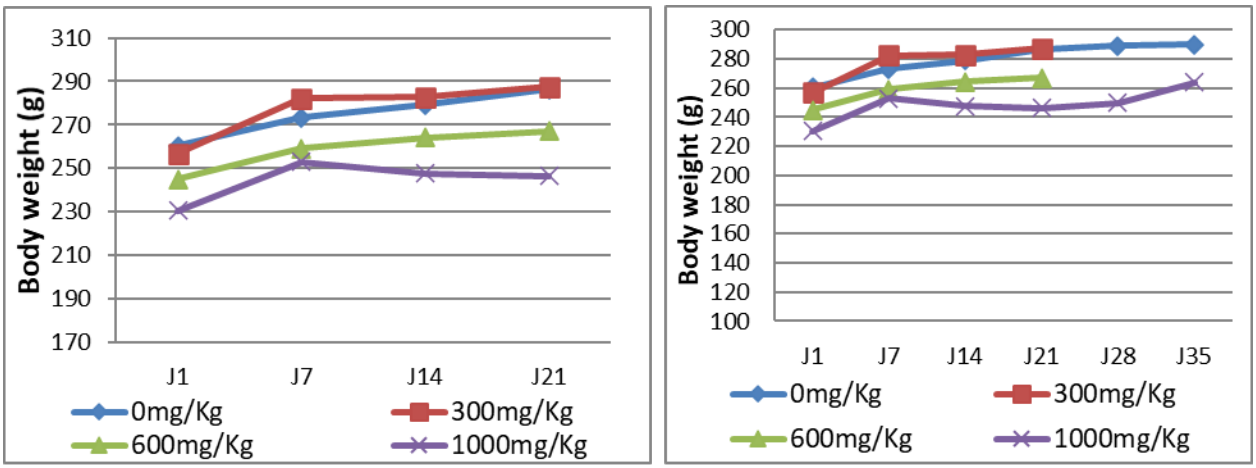
Doses	Treatment period				Recovery period	
mg/Kg bw	0	300	600	1000	0	1000
Liver	3,51±0,21	3,69±0,48	4,09±0,58	3,76±0,31	3,28±0,24	3,45±0,73
Kidney	0,65±0,07	0,70±0,07	0,65±0,17	0,65±0,04	0,64±0,05	0,62±0,04
Spleen	0,33±0,04	0,38±0,07	0,28±0,01	0,31±0,05	0,28±0,05	0,37±0,06

Values expressed in mean ± SD

Table 5: Biochemical analyses of rat serum, after 28 days of oral gavages with AV extract and after 14 days of recovery period

Doses	Treatment period				Recovery period	
mg/Kg bw	0	300	600	1000	0	1000
ASAT (UI/L)	134,04±6,50	119,10±18,82	118,91±14,77	122,58±7,60	115,00±6,30	172,01±6,32
ALAT (UI/L)	86,09±6,20	74,00±12,60	85,41±5,36	82,33±2,18	69,01±3,56	95,55±4,31
UREA (g/L)	0,41±0,05	0,40±0,03	0,39±0,04	0,38±0,06	0,22±0,02	0,37±0,01
CREAT (g/L)	4,96±0,46	4,78±0,37	4,61±0,20	4,73±1,02	4,62±0,32	4,74±0.56

Values expressed in mean ± SD. ALAT: alanine aminotransferase, aspartate ASAT: aminotransferase, CREA: creatinine and blood urea



A B

Values expressed in mean ± SD, A: 28 days period of treatment, B: 14 days recovery period

Figure 3: Body weight variation of rats treated with *Ammi visnaga* L extract by oral gavages during and after treatment.

Table 6: Hematological analyses ofthe blood of rats, After 28 days of oral gavages with *Ammi visnaga* L extract.

Parameters	Doses (mg/Kg bw)			
	0	300	600	1000
WBC ($10^3/\mu$l)	8,90±2,12	9,40±1,90	9,65±2,41	9,27±2,99
RBC ($10^6/\mu$l)	6,86±0,47	7,65±0,66	6,99±0,70	6,78±0,29
HGB (g/dl)	12,45±0,56	12,97±0,63	12,52±1,27	11,47±0,57
HCT (%)	39,32±2,41	41,75±2,75	38,97±4,75	36,25±2,11*
MCV (fl)	57,35±0,67	54,72±2,38	55,65±1,74	53,45±2,05
MCH (pg)	18,20±0,46	17,07±1,41	17,95±0,85	16,92±0,72
MCHC (g/dl)	31,67±0,48	31,17±1,38	32,22±1,30	31,67±0,46
PLT ($10^3/\mu$l)	842,25±142	1020,25±64,11	985,50±167,95	1064,75±240,00
LYM%	65,35±5,65	79,83±23,71	80,87±11,83	53,30±4,93
LYM ($10^3/\mu$l)	5,82±1,43	6,46±1,24	6,73±0,52	4,82±1,23
RDW-SD (fl)	33,55±3,46	31,20±1,30	31,55±2,48	31,55±0,57
RDW-CD %	15,87±2,58	15,42±2,61	14,77±1,88	15,87±1,41
PDW (fl)	11,40±1,12	10,55±1,95	8,77±0,33**	9,65±0,80**
MPV (fl)	8,37±0,24	7,95±0,55	7,50±0,27	7,77±0,33
P-LCR %	15,60±2,17	12,55±3,65	8,55±1,77**	10,87±2,22*
PCT %	0,70±0,10	0,83±0,10	0,74±0,14	0,82±0,16

Values expressed in mean ± SD, n = 5 rats / group.*: $p < 0.05$, **: $p < 0.01$.

Table 7: Percentage of inhibition of edema after induction of inflammation

	Treatment mg/Kg body weight			
	Diclofenac	AV	AV	AV
	6	60	80	100
T=0 min	0	0	0	0
T= 30 min	37,50±12.50	25,00±11.25	47,75±11.32	22,50±8.25
T= 60 min	40,00±15.75	30,00±17.35	55,00±13.25	30,00±11.25
T= 120 min	42,00±10.25	40,00±12.25	55,00±17.75	40,00±15.25
T= 180 min	48,00±15.50	50,00±22.50	62,50±12.35	58,33±11.23

Values expressed in mean ± SD, n = 5 rats / group for 5 repeated experiences. ($p < 0.05$)

treated with plant extract at 1000mg/Kg; however, weight gain was continued in the other groups (Figure 3A). Analysis of these changes in body weight (α=0, 05) shows that they are not significant compared to the control group (P>0.05).

In terms of body weight during the recovery period, Figure 3B shows that rats in the control group continued to increase in weight; rats treated returned to its initial state after a slight decrease in weights between the 7^{th} day and 28^{th} day.

Food intakes

As shown in Table 3, the group of rats treated with *Ammi visnaga L* extract at 600 mg/kg was the only group that consumed significantly more than the control group (0 mg/kg) during the second week. No other significant differences in food intakes were observed between the treated and control groups, neither during the gavages period (W1-W4) nor during the recovery period (W5-W6).

Macroscopic study and relative organ weights

Repeated oral administration of *Ammi visnaga* L aqueous extract (300; 600 and 1000 mg/kg) produced a slight dose-dependent increase in liver weight, but statistical analysis showed that these variations and those observed in kidney weight were not significant (P>0.05) compared to rats in the control group (Table 4). The microscopic examination showed that the organs of the treated and control rats were similar, and no pathological signs were observed. For the recovery period, comparison of relative organ weights shows that differences between rats in the control group and those treated with plant extract at 1000 mg/Kg disappeared for

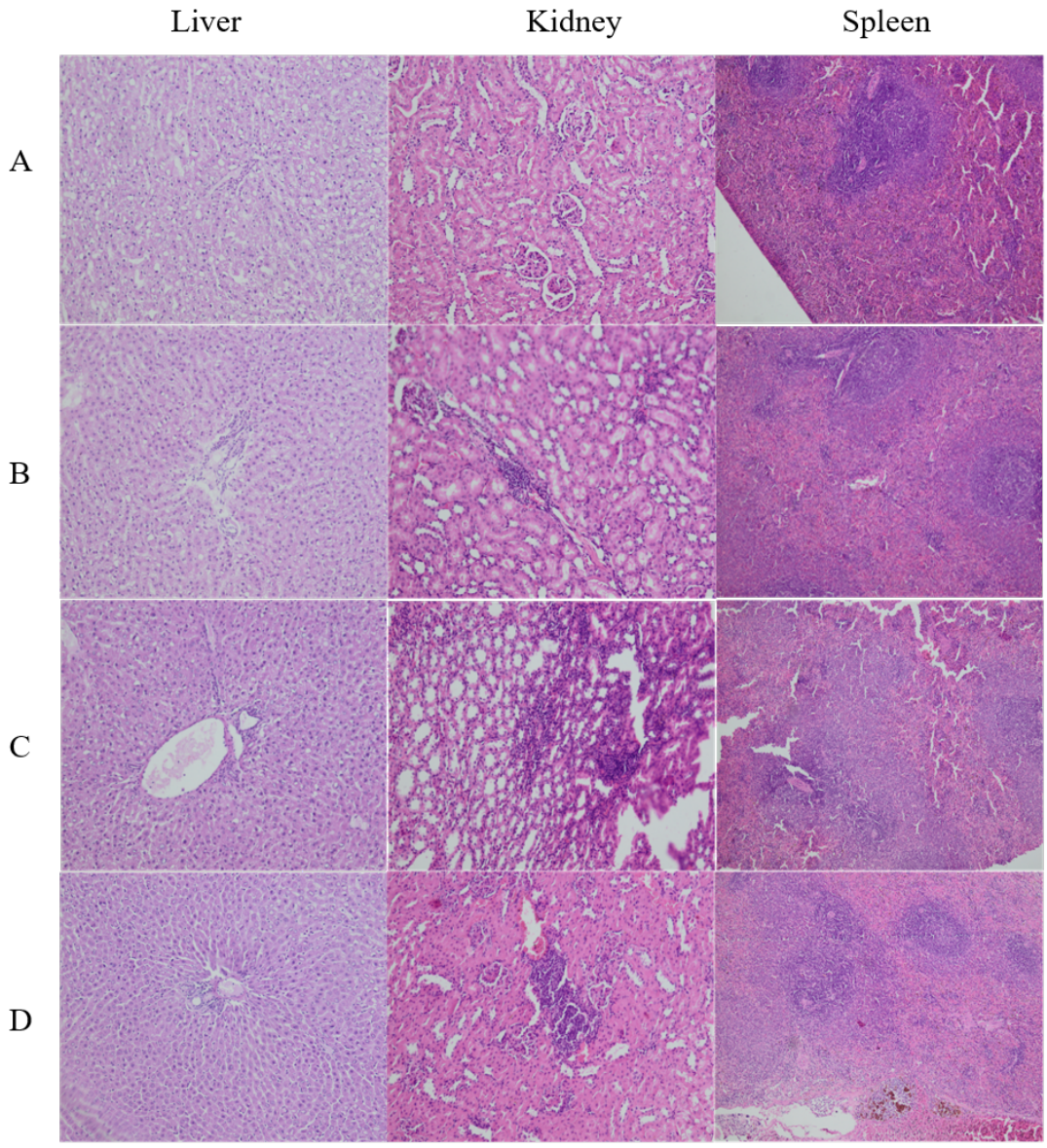


Figure 4: Sections of the liver, kidney and spleen (X40) of rats, after 28 days of oral gavages with *Ammi visnaga* L extract.

the kidney and decreased for the liver.

Serum biochemical parameters

Serum biochemical analysis including ASAT, ALAT, Urea and Creatinine after 28 days of gavages by aqueous *Ammi visnaga L* extract (Table 5) showed no significant difference between each treated group (300, 600 and 1000 mg/kg) and the control group (0 mg/kg).

Haematological parameters

Compared to the control group, a significant reduction in HCT and P-LCR was observed at the end of the treatment period in rats treated with 1000 mg/kg ($p < 0.05$) and PDW and P-LCR ($p < 0.01$) in rats treated with 600-1000 mg/kg. Besides, a slight increase in PLT (300mg/Kg and 1000mg/Kg) and a dose-independent difference in LYM were noted in the treated groups. But these last two differences were not significant (Table 6).

Liver, spleen and kidney histopathology analysis

Histopathological evaluation of organs (liver, kidney and spleen) showed no profound macroscopic or histological changes in animals treated with *Ammi visnaga L* aqueous extract at a limit dose of 1000 mg/kg (Figure 4). The liver had a standard architecture with no sign of damage, the kidney had adequate glomerulus and normal tubules, and the spleen was found with pulp similar to those in the

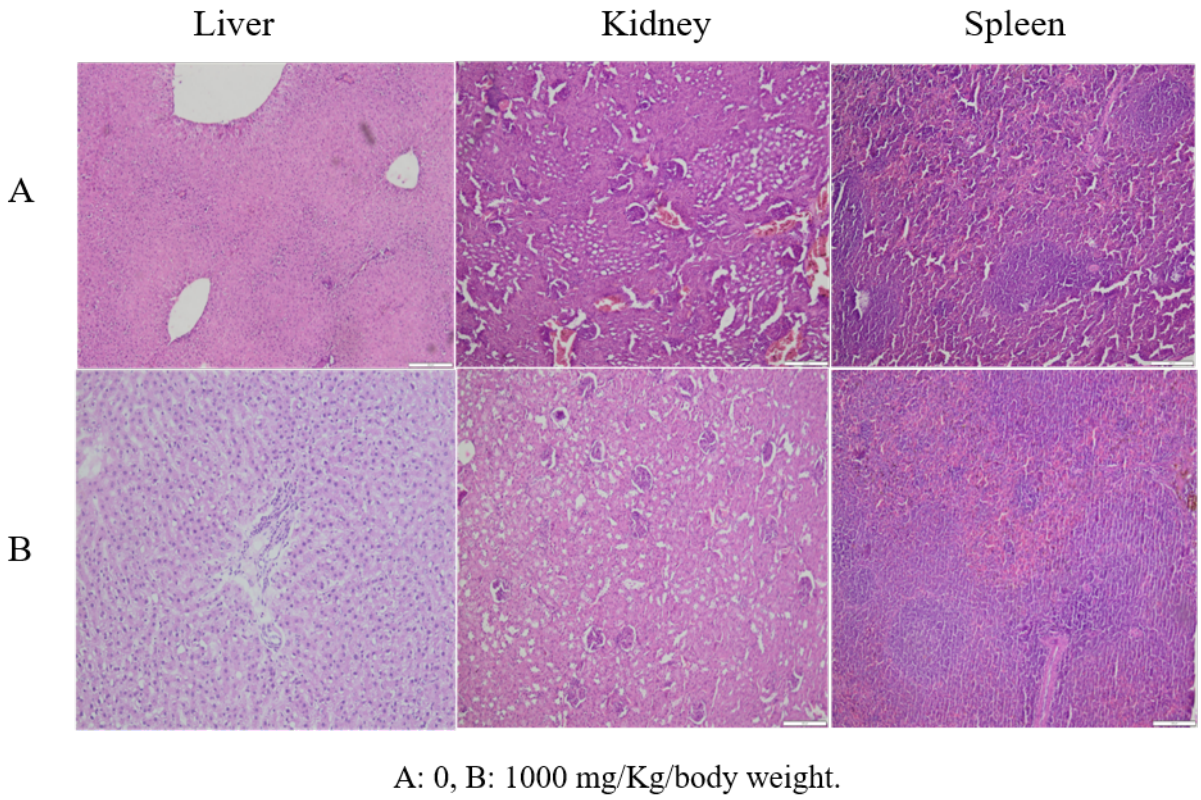


Figure 5: Sections of the liver kidney and spleen (x40) of rats, after 28 days of oral gavages with *Ammi visnaga* L extract and 14 days of recovery period.

control group.

However, small inflammation, too focal between cells, was observed in these groups of rats as infiltration of immune cells (lymphocytes and plasmocytes) into some portal veins and the few kidney glomeruli. These small signs of inflammation were present also in some specimens in the control group. The same histopathological test, 14 days after stopping treatment (recovery period), showed that these inflammations disappeared utterly, and the liver and kidneys returned to their normal state (Figure 5).

Anti-inflammatory studies

Compared to the standard anti-inflammatory drug, Diclofenac, the extract has shown a percentage of inhibition of inducted oedema of 50, 62 and 58% after 180 min for the doses of 60, 80 and 100 mg/kg respectively (Table 7). The inhibition began 30 min after the oedema induction and was of 47.75% for the rats treated by A.V. at 80 mg/kg vs 37.50% for the standard control and increased with time for all doses tested.

Psoriasis

The irradiation of the depilated rat skin with ultraviolet radiation produces biphasic erythema with two phases: -

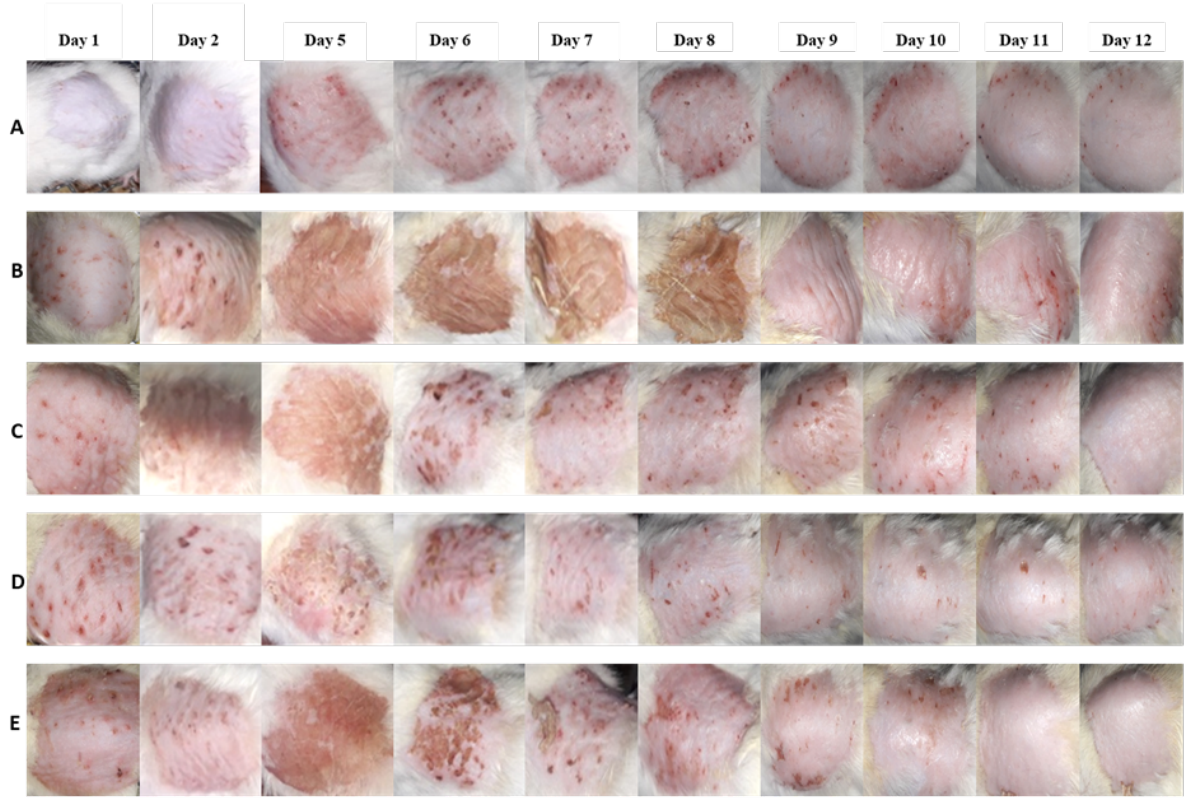
1. First phase: slight erythema appears immediately after irradiation and disappears within 30 minutes.

2. The second phase: erythema begins 6 hours after irradiation and gradually increases, peaking between 24 and 48 hours. This reaction is confined to the exposed area and has a transparent border. It develops a brownish-red colour. From 48 to 72 hours, white silvery scales appear on the erythematous lesion. These scales are relatively thick and begin to fall beyond 72 hours (Figure 6).

U.V. irradiation allows increasing the thickness of the skin by creating ridges and micro-abscesses in some cases (Figure 7B).

The comparative histology studies show that treatment with retinoic acid can decrease the thickness of the skin (Figure 7C).

Similarly, treatment with the aqueous extract of the plant (300 and 600 mg/kg) affects comparable to that of retinoic acid or much more positive (Figure 7D and Figure 7E).



A- Normal skin: oral gavage with saline solution without UV irradiation.
B- Negative control: oral gavage with saline solution with UV irradiation.
C- Positive control: oral gavage with retinoic acid (0, 5 mg / kg/bw) with UV irradiation.
D- UV irradiation and oral gavage with AV aqueous extract 300mg/kg/bw.
E- UV irradiation and oral gavage with AV aqueous extract 600mg/kg/bw.

Figure 6: Photos of skin treated with UV-B radiations and AV extracts

This decrease was observed since the 7^{th} day of the treatment, and all micro-abscesses and ridges disappeared between the 9-12^{th} day of treatment with A.V. extract.

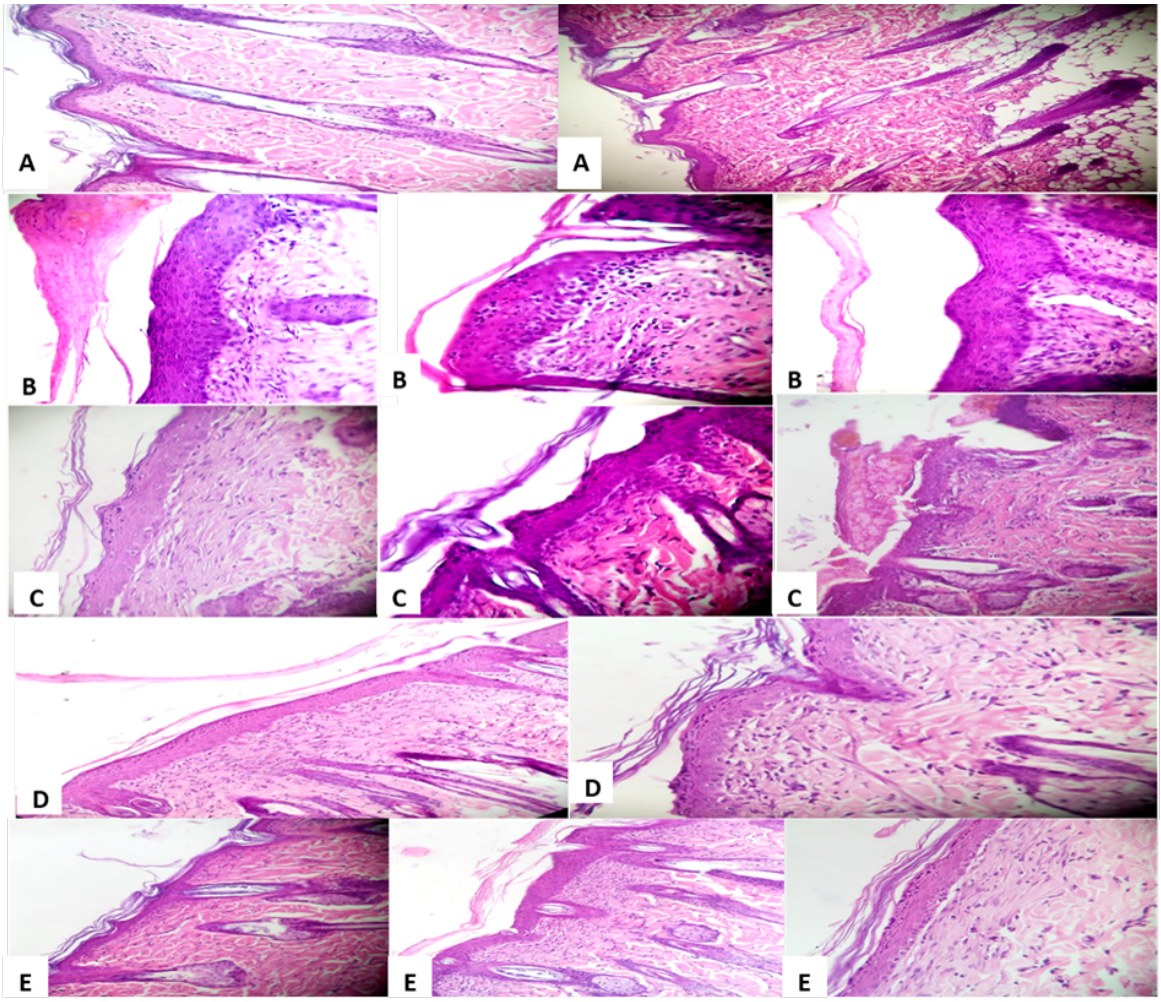
The induction of a psoriasiform-like skin phenotype in rat was done by UV-B lamp (280-315 nm) radiation treatment; kept at a vertical distance of 20 cm from 1,5 × 2,5 cm shaved skin during 3h. 12 hours after irradiation and during 14 days rats were treated once day by oral gavages with AV extract (300 or 600mg/kg/bw) or retinoic acid (0,5mg/kg/bw) or NaCl 0.9% (10ml/kg/bw).

DISCUSSION

Over the centuries, human traditions have developed the knowledge and use of medicinal plants. The richness of plants in chemical substances with medical properties increases the risk of their undesirable reactions by the additive or synergistic effects of chemical interactions between these complex mixtures. Therefore, no one can deny the role of toxicity as a preliminary test in the orientation of the search for the pharmacological activity of plants (Olson *et al.*, 2000).

Hence, this study was conducted to evaluate, first, the toxicological profile of the aqueous extract of *Ammi visnaga* L using the acute oral toxicity test in rats for 14 days and the subacute oral toxicity test in rats for 28 days. Only one study has reported that Av seed ethanolic extract did not induce any toxic effects in acute and sub-acute toxicological studies in rats (Koriem *et al.*, 2019). In our study, we tested the aqueous extract like used by patients (El-Hilaly *et al.*, 2003).

Many previous toxicity studies have shown that a single dose of the extract of a medicinal plant is mostly sufficient to induce severe undesirable effects, this shows the great importance that an acute toxicity study must have in this type of traditional medication (Lee *et al.*, 2010).



A- Normal skin: oral gavage with saline solution without UV irradiation.
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D- UV irradiation and oral gavage with AV aqueous extract 300mg/kg/bw.
E- UV irradiation and oral gavage with AV aqueous 600mg/kg/bw.

Figure 7: Histological sections of skin treated with UV-B radiations and AV extracts.

In this context, the present study showed that the aqueous extract of *Ammi visnaga L* induced no mortality in rats after single oral gavage at doses up to 5000 mg/kg. Furthermore, no morphological or behavioural signs of toxicity were observed in rats treated with the plant. The body weight and relative weight of vital organs were very similar to those of rats in the control group, and no significant differences were recorded. Thus, according to this test, the estimated oral LD50 in female rats is more than 5,000 mg/Kg. Consequently, according to the Globally Harmonized System of Classification of Chemical Substances and Mixtures (GSH) adopted by the OECD, the aqueous extract of *Ammi visnaga* L could be classified as a Class 5 substance and considered as non-toxic substance.

The first signs of toxicity are manifested by mortality or behavioural and body changes in the other case. During the subacute toxicity test, the aqueous extract of *Ammi visnaga* L at doses of 300, 600 and 1000 mg/kg did not cause mortality of any rats in the experiment, and these rats showed no abnormal signs in the skin, fur and eyes, as well as respiration and autonomous activity.

The toxic effects of a chemical substance can induce changes in body weight. Therefore, this parameter is of considerable importance in toxicological studies (Tan *et al.*, 2008). Food intake and relative weight of vital organs complement the study of body weight, given that it has been confirmed that variations in body weight may be a normal physiological reaction related to changes in animal appetite (Bailey

et al., 2004). In this study, we observed only one significant difference in food intake between the 600 mg/kg dose group and the control group at week two only; however, this difference did not affect the rat's body weight or the relative weight of their vital organs (liver, kidney and spleen).

The evaluation of serological and haematological parameters is another approach to testing the toxicity of chemicals *in vivo*. At the end of daily gavage by the plant extract, no significant difference was found in AST, ALT, UREE and CREA levels, compared to the control group. This disclosure assumes that this extract does not alter liver and kidney functions. However, a significant decrease in PDW, MPV and P-LCR was observed in rats in the 600 mg/kg group, but these variations are not dose-dependent, and they may be incidental and not treatment-related.

Studies have shown that toxic substances have a significant impact on the hematopoietic system (Yalavarthi *et al.*, 2018). So it is advantageous to adopt these blood parameters as essential indicators for studying toxicity in animals, as long as it has a high significance on the prediction of toxicity for humans (Negash *et al.*, 2016). Mean platelet volume (MPV), platelet distribution width (PDW), and large cell platelet ratio (P-LCR) are a group of derived platelet parameters obtained as part of automatic complete blood count. These parameters are instrumental in discriminating between hyper destructive thrombocytopenia and hypo productive thrombocytopenia. So these indices are significantly higher in patients with hyper destructive thrombocytopenia (Negash *et al.*, 2016). The present study showed a significant increase in these parameters in rats after 28 days of gavage by plant extract at a dose of 600 mg/kg compared to the control group, leading to hypo-productive thrombocytopenia. But, the related parameters, such as WBC and PLT, are similar in both groups. As a result, this increase should be considered as an effect without significant toxicological indication, or it will be an indication to follow the effect of the extract for a more extended period (Raza *et al.*, 2002).

The histopathological study of the liver and kidneys showed some evidence of leukocyte infiltration. This condition refers to probable inflammation in both organs by the neutrophils and lymphocytes infiltration (Ramaiah and Jaeschke, 2007). A recovery period of 14 days after stopping treatment showed that these signs of an adverse effect had disappeared completely. Still, there is nothing to avoid assuring these effects by extending treatment time (subchronic toxicity).

Then we investigated the effect on induced inflammation and psoriasis. Compared to the standard anti-inflammatory drug, Diclofenac, the extract has shown a percentage of inhibition of inducted oedema of 50, 62 and 58% after 180 min for the doses of 60, 80 and 100 mg/kg respectively. This effect is depending on its visnagin content may be due to the inhibition of transcription factors such as AP-1 and NF-beta (Lee *et al.*, 2010).

Psoriasis is a skin disease with massive skin cell proliferation (accelerated by releases of pro-inflammatory cytokines and growth factors) inflammatory cell infiltrates, generation of new blood vessels, modifications in lymphatic structure, and impaired differentiation of the epidermis. Then, growth of skin cells, accumulate and form thick red patches on the skin of diverse parts of the body. UV-B irradiation allows increasing the thickness of the skin, with creating ridges and in some cases, micro-abscesses like described for psoriasis. We have shown that treatment with retinoic acid has decreased the thickness of the skin. Similarly, treatment with the aqueous extract of the plant (300 and 600 mg/kg) effects comparable to that of retinoic acid or much more positive.

This effect was reported by Abdel-Fattah *et al.* (1983), where ten patients were treated orally with khellin (A.V.) and subsequently exposed to sunlight for four months, and 8 cases responded positively with variable degrees of clearance. No other studies were done on this plant since that. This effect is linked to its anti-inflammatory action, as showed before.

CONCLUSION

Based on the results obtained in this acute and subacute oral toxicity study in rats, it could be concluded that the use of *Ammi visnaga* L aqueous extract at a single dose can be considered non-toxic as long as the estimated LD50 exceeds 5000 mg/kg. Meanwhile, its repeated use for a long time should be used with caution, at least before further confirmatory studies, bearing in mind the signs of an observed adverse effect on haematological parameters and liver and kidney histology. The extract had also shown a high anti-inflammatory effect and an anti-psoriasis effect when they were induced experimentally. Further clinical studies will be conducted for an application in human health.

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Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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