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Antihypertensive effect of *Lepidium sativum* L. in spontaneously hypertensive rats

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Abstract

The antihypertensive and diuretic effects of the aqueous extract of *Lepidium sativum* L. (LS) were studied both in normotensive (WKY) and spontaneously hypertensive rats (SHR). Daily oral administration of the aqueous LS extract (20 mg/kg for 3 weeks) exhibited a significant decrease in blood pressure ($p < 0.01$) in SHR rats while in WKY rats, no significant change was noted during the period of treatment. The systolic blood pressure was decreased significantly from the 7th day ($p < 0.05$) to the end of treatment ($p < 0.01$) in SHR rats.

The aqueous LS extract enhanced significantly the water excretion in WKY rats ($p < 0.001$) but no statistically significant change was observed in SHR rats. Furthermore, oral administration of aqueous LS extract at a dose of 20 mg/kg produced a significant increase of urinary excretion of sodium ($p < 0.05$), potassium ($p < 0.01$) and chlorides ($p < 0.01$) in WKY rats. In spontaneously hypertensive rats, the aqueous LS extract administration induced a significant increase of urinary elimination of sodium ($p < 0.01$), potassium ($p < 0.001$) and chlorides ($p < 0.001$). Glomerular filtration rate showed a significant increase after oral administration of LS in normal rats ($p < 0.001$) while in SHR rats, no significant change was noted during the period of treatment. Furthermore, no significant changes were noted on heart rate after LS treatment in SHR as well as in WKY rats.

Our results suggest that daily oral administration of aqueous LS extract for 3 weeks exhibited antihypertensive and diuretic activities.

Keywords: Antihypertensive; Diuretic; Glomerular filtration rate; *Lepidium sativum*; Systolic blood pressure; SHR; WKY

1. Introduction

Herbal medication has been and remains commonly used instead of chemical drugs because of its minor side effects. *Lepidium sativum* L. (LS) Cresson "hab arachad" is a native shrub belonging to Brassicaceae family wildly grown in Morocco where LS is largely recommended by traditional herbal healers for hypertension, diabetes control, renal disease and phytotherapy (Jouad et al., 2001). The seeds are consumed wildly as salad and spice (Maier et al., 1998). Previous studies have demonstrated the protective action of LS against carcinogenic compounds (Kassie et al., 2003) and growth inhibition of *Pseudomonas aeruginosa*, a bacteria strain with a potent antibiotic resistance (Aburjai et al., 2001). According to our ethnobotanical survey in Fes-Boulmane region, LS seeds were wildly used in the management of hypertension (Jouad et al., 2001). As far as this pharmacological activity of LS seeds is concerned, there are no previous reports in the literature.

We have used Irbesartan as reference antihypertensive drug. This angiotensin (AT) II antagonist is considered as one of the widely used antihypertensive drugs in the treatment of hypertension with less adverse events compared to other

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antihypertensive medication (Moller-Nordhorn and Willich, 2003; Malmqvist et al., 2003).

In the context of our research on medicinal plants from the South–eastern region of Morocco, the present study reports the pharmacological activity of LS seeds on arterial blood pressure and renal function in normotensive and spontaneously hypertensive rats.

2. Material and methods

2.1. Plant material

The plant used in this study was collected from its natural habitat, i.e. from the Errachidia region (Morocco) in May–June 2001, and dried with hot air (40–60 °C). The plant was identified and authenticated as *Lepidium sativum* L. with assistance of Professor M. Rejdali (Veterinary and Agronomy Institute, Rabat, Morocco). Voucher specimen (EM 11) was deposited at the herbarium of the Faculty of Sciences and Techniques, Errachidia.

2.2. Preparation of the aqueous extract

The aqueous extract was prepared in a standardized manner by boiling 1 g of dried powdered seeds of LS in 100 ml of distilled water for 10 min and left for 15 min to infuse. Thereafter, the extract was cooled and filtered before use to remove particular matter. The filtrate was lyophilized and the desired dose (milligram of lyophilized aqueous LS extract per kilogram body weight) was then prepared and reconstituted in 10 ml of distilled water per kilogram body weight just before oral administration.

2.3. Animals used

Experiments were performed in WKY and spontaneously hypertensive rats (SHR) male rats (GENEST Saint ISLE-France) weighing between 210 and 260 g, housing at 23 ± 1 °C with 12-h light/12-h dark photoperiod, with food (standard rat diet) and water ad libitum. Animals were assigned to three different groups of five rats each ($n = 5$) and treated as follows: the first group received distilled water and served as control group, the second received the aqueous LS extract at a dose of 20 mg/kg and the third was treated with Irbesartan (20 mg/kg). The rats were given the drug solutions (LS or Irbesartan) or vehicle (distilled water) by oral gavage at a volume of 10 ml/kg once a day during 3 weeks. The influence of circadian rhythms was avoided by starting all experiments at 10:00 a.m. immediately after gavage, the rats were individually housed in metabolic cages and allowed an ad libitum access to water and food throughout the period of treatment. The animals were maintained on a 12-h light/12-h dark cycle during this study.

Urine was collected in graduated cylinders at D0, D7, D14 and D21 after daily oral administration and its volume and electrolytes content were measured. Arterial blood pressure and heart rate of the conscious animals were measured using a blood pressure recorder (Ugo basile biological research apparatus APELEX Comerio-(va)-Italy).

The systolic blood pressure and heart rate were measured before starting the experiments (D0), 1 week (D7), 2 weeks (D14) and 3 weeks (D21) after oral administration. The mean bloodless pressure recording was avoided at the same time in order to discard the influence of the circadian rhythm.

Blood samples for plasma electrolytes, creatinine and urea determinations were obtained from all animals under anaesthesia (ether inhalation) from the retroorbital sinus. Blood samples were centrifuged at 4000 rpm for 10 min at 4 °C and then the plasma was recuperated.

2.4. Determinations of parameters

Sodium, potassium, chloride, creatinine and urea levels were determined in plasma and urine samples using an autoanalyser (AU 400 Olympus France); pH and potassium content of the aqueous LS extract were also determined.

2.5. Statistical analysis

All data were recorded on standardized forms and were expressed as means ± S.E.M. for five determinations. Mean values were considered significantly different if $p < 0.05$. The experimental design allowed us to apply a factorial ANOVA (time, treatment and interaction) for comparison.

3. Results and discussion

The aim of this study was to investigate the antihypertensive effect of aqueous extract of *Lepidium sativum* seeds in normotensive and spontaneously hypertensive rats. According to our previous ethnopharmacological survey carried out in the north centre region of Morocco, seeds of *Lepidium sativum* were largely used for the treatment of hypertension and renal disease (Jouad et al., 2001), but no previous pharmacological or clinical study was carried out to test the antihypertensive property of this plant.

After 3 weeks oral administration, the aqueous LS extract showed an antihypertensive response. Although, LS elicited significant systolic blood pressure decreases in SHR rats with the screening dose used (20 mg/kg) (Fig. 1, Panel b). Additionally, the reduction of blood pressure was greater in SHR than in WKY rats. In hypertensive rats, LS caused a significant fall in systolic blood pressure (SBP) from the 1st ($p < 0.05$), 2nd ($p < 0.01$) to the 3rd week of treatment ($p < 0.01$) (Fig. 1, Panel b) while, in WKY rats, daily oral administration of aqueous LS extract did not cause any significant change of SBP (Fig. 1, Panel a). Thus, the effect of aqueous LS extract seems to exhibit more antihypertensive than hypotensive activity. In addition, the systolic blood pressure was markedly reduced after daily oral administration of

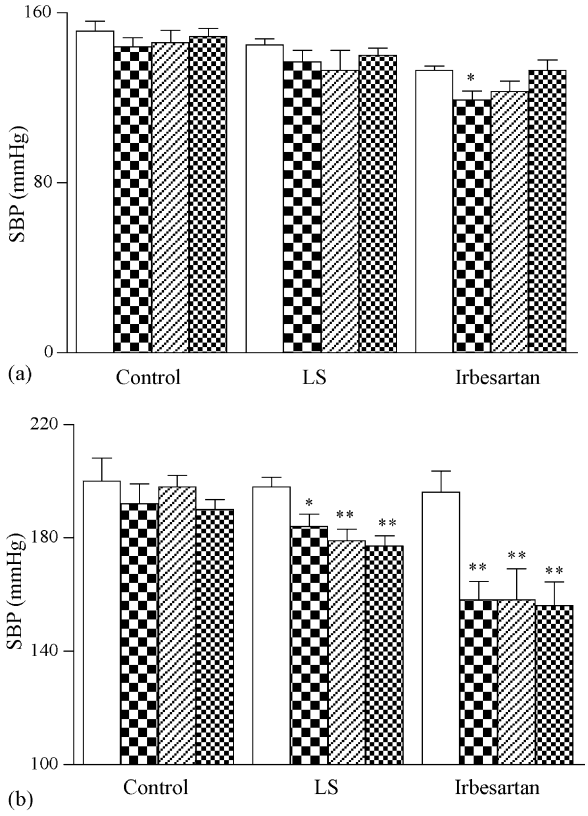

Fig. 1. Effect of oral administration of the aqueous LS extract (20 mg/kg) on arterial blood pressure (SBP) (mmHg) in both WKY (Panel a) and SHR (Panel b) rats. Data are expressed as means ± S.E.M. for five determinations. $^{*}p < 0.05$; $^{**}p < 0.01$ when compared to baseline values (the start of treatment); (□) day 0, (▩) day 7, (▨) day 14 and (▩) day 21 of treatment.

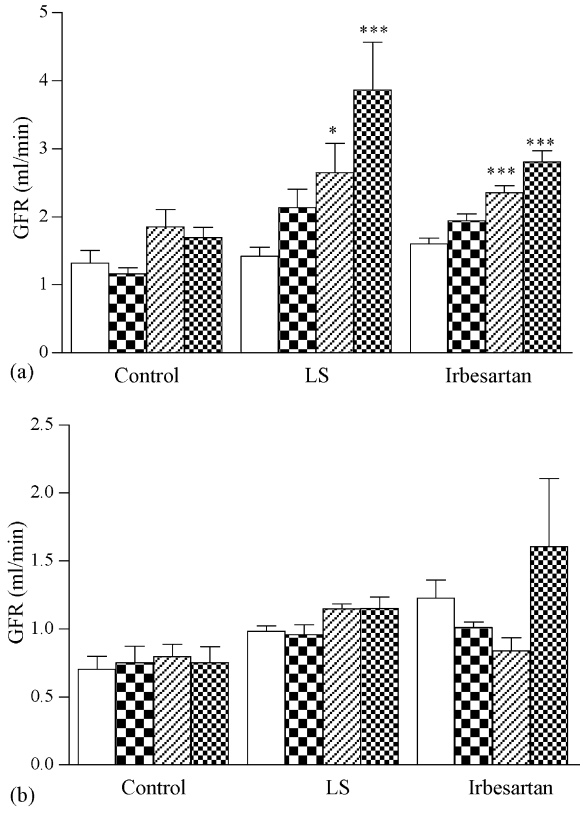

Fig. 2. Effect of oral administration of aqueous LS extract on glomerular filtration rate (GFR) in both WKY (Panel a) and SHR (Panel b) rats. Data are expressed as means ± S.E.M. for five determinations. $^{*}p < 0.05$; $^{***}p < 0.001$ when compared to baseline values (the start of treatment); (□) day 0, (▩) day 7, (▨) day 14 and (▩) day 21 of treatment.

Irbesartan (20 mg/kg) in both WKY ($p < 0.05$) (Fig. 1, Panel a) and SHR ($p < 0.01$) (Fig. 1, Panel b) rats. In WKY, the effect of Irbesartan on SBP was reversible while in SHR, Irbesartan (20 mg/kg) showed a sustained effect during all period of treatment.

The heart rate did not show any significant change after LS administration in both normal and spontaneously hypertensive rats (data not shown). Nevertheless, the investigation of the glomerular filtration rate (GFR) (Fig. 2) will be interesting to determine the part of renal glomerular and tubular effects of the aqueous LS extract. Then, oral administration of the aqueous LS extract did not produce any significant change of glomerular filtration rate similar to Irbesartan in SHR rats (Fig. 2, Panel b) while, the effect was more important in both LS ($p < 0.001$) and Irbesartan ($p < 0.001$) treated WKY groups (Fig. 2, Panel a). LS administration increased GFR similar to Irbesartan, possibly by promoting dilatation in the afferent arteriole of renal vasculature, since Irbesartan is known by its action on ATII receptor of angiotensin (Sealy and Laragh, 1995). In this view, GFR is known to be increased consequently to the elevation of renal blood flow (Melis, 1992). In addition, administration of LS produced an increase on urine output which was apparent at the 7th day ($p < 0.05$) of treatment and became more potent from the 14th day onwards ($p < 0.001$) (Fig. 3, Panel a) in WKY rats. This diuretic effect was markedly accompanied with an increase on the urinary elimination of sodium ($p < 0.05$) (Fig. 4, Panel a), potassium ($p < 0.01$) (Fig. 4, Panel b) and chlorides ($p < 0.01$) (Fig. 4, Panel c) after 3 weeks of LS daily oral administration. In SHR, administration of LS reduced blood pressure without affecting the excretion of water (Fig. 3, Panel b), but induced a significant increase of sodium from the 2nd to the 3rd week of treatment ($p < 0.01$) (Fig. 5, Panel a), potassium which was apparent at the 14th day ($p < 0.05$) and became more potent at the end of treatment ($p < 0.001$) (Fig. 5, Panel b) and chlorides ($p < 0.001$) (Fig. 5, Panel c).

On the other hand, Irbesartan at a dose used produced a significant and an important diuretic effect in both SHR and WKY rats. The peak of water excretion was observed at the fourteenth day ($p < 0.001$) (Fig. 3, Panel a) in WKY and after 3 weeks of treatment ($p < 0.01$) (Fig. 3, Panel b) in SHR rats. Furthermore, in WKY rats, Irbesartan caused a significant increase on the urinary excretion of sodium ($p < 0.05$) (Fig. 4, Panel a), potassium ($p < 0.05$) (Fig. 4, Panel b) and chlorides ($p < 0.01$) (Fig. 4, Panel c). In hypertensive rats, daily oral administration of Irbesartan increased significantly

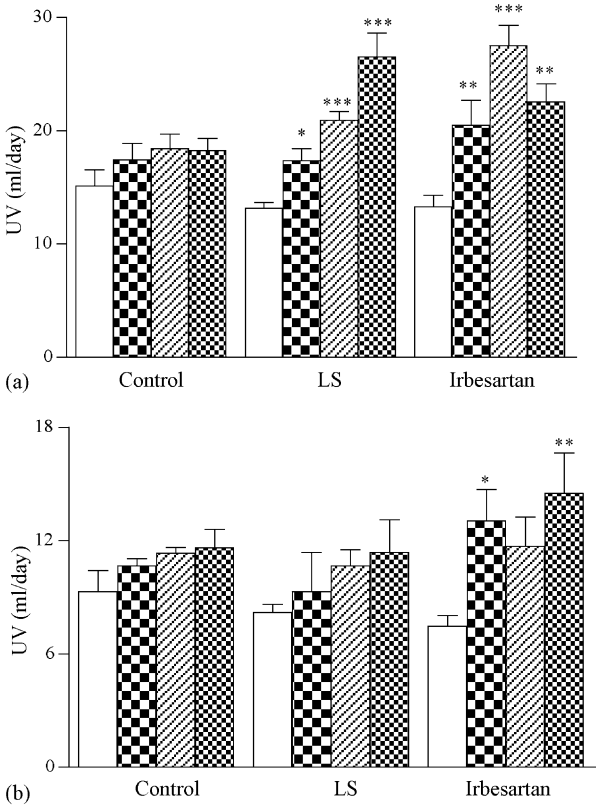

Fig. 3. Average urinary volume excretion (UV) in urine samples of normal (Panel a) and hypertensive (Panel b) rats treated by oral administration of aqueous LS extract. Urine samples were pooled for 24 h. Data are expressed as means ± S.E.M. for five determinations. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ when compared to baseline values (the start of treatment); (□) day 0, (▩) day 7, (▨) day 14 and (▩) day 21 of treatment.

the excretion of sodium ($p < 0.01$) (Fig. 5, Panel a), chlorides ($p < 0.05$) (Fig. 5, Panel c) and potassium ($p < 0.01$) (Fig. 5, Panel b); these effects were observed only at the end of treatment. The basal plasma levels of sodium, chloride and urea did not differ significantly in both LS and Irbesartan-treated

Table 1
Effect of oral administration of aqueous extract of *Lepidium sativum* (LS) at dose of 20 mg/kg on plasma sodium, potassium, chlorides and urea concentrations (mmol/l) after 3 weeks of treatment

Treatment	Sodium	Chlorides	Potassium	Urea
Control				
Day 0	166.2 ± 1.2	118.6 ± 0.87	6.00 ± 0.08	7.0 ± 0.4
Day 21	166.0 ± 0.7	119.0 ± 0.70	5.22 ± 0.12***	8.4 ± 0.3*
Lepidium sativum				
Day 0	166.2 ± 1.16	118.6 ± 0.87	6.00 ± 0.08	7.26 ± 0.38
Day 21	163.0 ± 1.48	116.0 ± 1.00	4.46 ± 0.2***	9.00 ± 0.54**
Irbesartan				
Day 0	166.2 ± 1.2	118.6 ± 0.87	6.00 ± 0.08	7.3 ± 0.4
Day 21	162.8 ± 1.2*	117.4 ± 0.40	5.22 ± 0.18***	7.8 ± 0.2

Values are expressed as mean ± S.E.M. for five determinations.
* $p<0.05$.
** $p<0.01$.
*** $p<0.001$ when compared to baseline values (start of treatment).

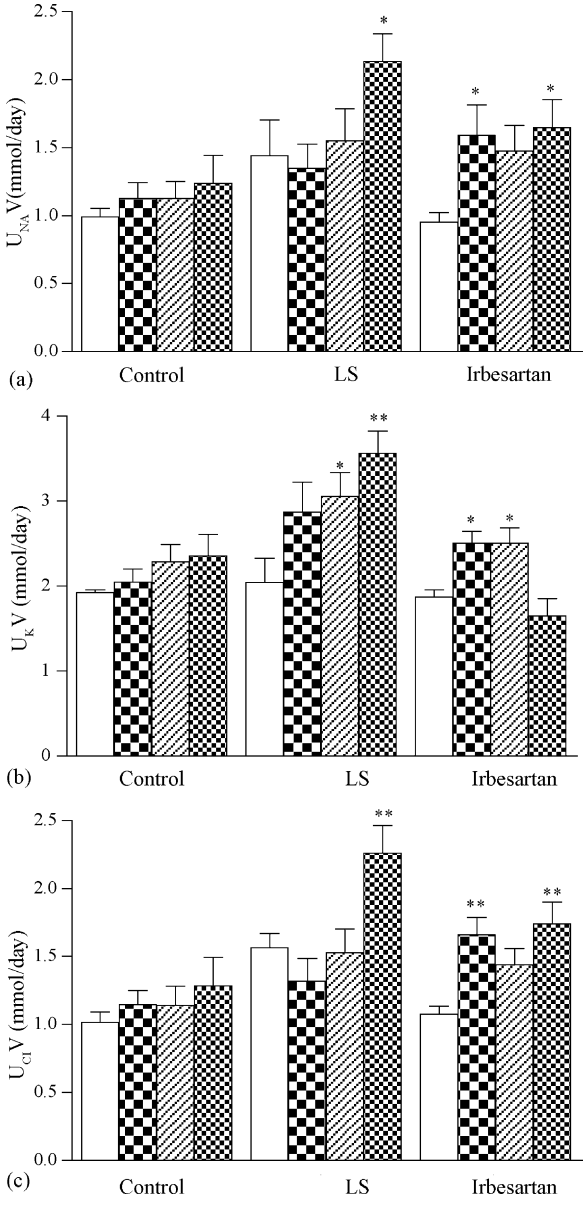

Fig. 4. Effect of oral administration of aqueous LS extract (20 mg/kg) on urinary excretion of sodium ($U_{Na}V$) (Panel a), chlorides ($U_{Cl}V$) (Panel b) and potassium ($U_K V$) (Panel c) in WKY rats. Data are expressed as means ± S.E.M. for five determinations. $^{*}p < 0.05$; $^{**}p < 0.01$ when compared to baseline values (the start of treatment); (□) day 0, (▩) day 7, (▨) day 14 and (▩) day 21 of treatment.

SHR groups (Table 1) while, plasma potassium levels showed a significant decrease for all SHR groups. In WKY rats, no changes were noted in plasma electrolytes and urea (data not shown).

These results show that the reduction of blood pressure was markedly accompanied by an increase in urine output in addition to solute renal excretion revealing an eventual reduction of tubular renal reabsorption of water and accompanying anions (Lu et al., 1994) in both LS and Irbesartan-treated groups. Similarly, it has been reported that intrarenal or intra-

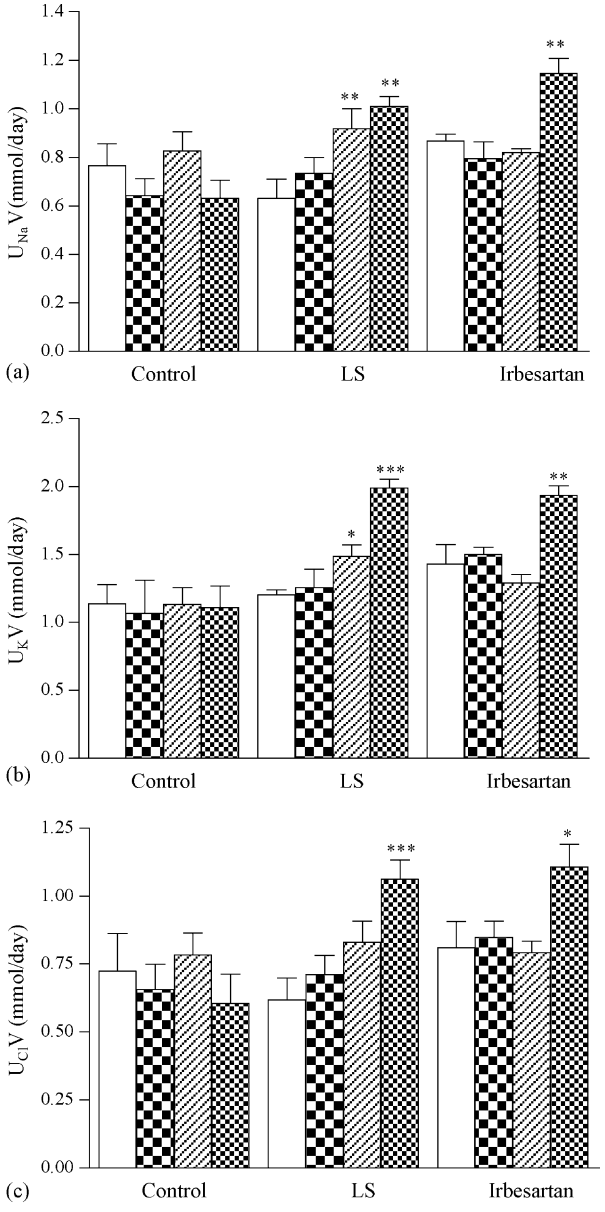

Fig. 5. Effect of oral administration of aqueous LS extract (20 mg/kg) on urinary excretion of sodium ($U_{Na}V$) (Panel a), chlorides ($U_{Cl}V$) (Panel b) and potassium (U_KV) (Panel c) in SHR rats. Data are expressed as means ± S.E.M. for five determinations. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ when compared to baseline values (the start of treatment); (□) day 0, (▩) day 7, (▨) day 14 and (▩) day 21 of treatment.

venous administration of vasodilators such as bradykinin and acetylcholin was associated with an increase on urinary Na^+ excretion (Earley and Freidler, 1966). Additionally, the elevation of GFR could involve an impairment of an autoregulatory renal mechanism by increasing the Na^+ filtered load (Melis, 1992).

The underlying mechanism of the antihypertensive activity observed in SHR rats, seems to be independent of the diuretic and natriuretic activities. LS had no influence on blood pressure in WKY rats.

4. Conclusion

We conclude that aqueous extract of *Lepidium sativum* L. seeds was effective in decreasing blood pressure and increasing water and electrolytes excretion. These results suggest that LS can improve hypertension with no influence on normotensive situation or on cardiac rate. Therefore, these findings support the use of *Lepidium sativum* L. decoction by the Moroccan population for the treatment of hypertension, cardiac and renal diseases.

Finally, the molecular mechanism(s) and site(s) of these activities and the active constituent(s) of *Lepidium sativum* L. involved are still to be determined.

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