Biological activities of extracts from local medicinal plants: *Clematis flammula, Fraxinus angustifolia et Pistacia lentiscus*

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Abstract

Introduction
Oxidative stress is responsible for many inflammatory-related pathologies. The objective of this study was to examine the hepato-protective, anti-inflammatory, diuretic and hypo-uricemic activities of three medicinal plants: *P. lentiscus*, *Fraxinus angustifolia and Clematis flammula* used in traditional Algerian medicine.
Materials and methods
The hepato-protective effect of the plant extracts was estimated by the analysis of histological sections of mice livers, while the anti-inflammatory activity was evaluated by the carrageenan-induced paw edema model. The diuretic test was based on the quantification of cumulative urinary volume and the concentration of Na^+ and K^+ in extract-treated rats. The hypo-uricemic potential was evaluated by the quantification of serum uric acid and the inhibition of hepatic xanthine oxidoreductase activity.
Results and discussion
Histological analysis indicated that the hepato-protective effect was significant after treatment with *F. angustifolia* and *P. lentiscus* leaves extracts (50mg/kg). Ethanolic leaf extracts of *F. angustifolia* (200mg/Kg) and aqueous of chloroform of *C. flammula* (200mg/Kg) exhibited an interesting anti-inflammatory effect, comparable to that of diclofenac (50mg/Kg). The fruit extracts of *P. lentiscus* (200mg/Kg) induced a significant increase in urinary volume, similar to that of furosemide. Moreover, the extracts of *P. lentiscus* and *C. flammula* have demonstrated a considerable hypo-uricemic effect, with an appreciable inhibition of xanthine oxydoreductase activity.
Conclusion
Obtained results contribute to validate the therapeutic effects of tested medicinal plants. Identification of active molecules responsible for these effects is under way.
Key words: Anti-inflammatory, hypouricemic, diuretic, xanthine oxidoreductase.

1 Introduction

Clematis flammula (Ranunculaceae), *Pistacia lentiscus* (Anacardiaceae) and *Fraxinus angustifolia* (Oleaceae), are used in local traditional medicine against rheumatoid arthritis and artificial burns, for the treatment of throat infections, diarrhea and jaundice and used for rheumatism, inflammation and as a laxative, respectively. In addition, the plants are known for their diuretic properties (Beloued, 1998; Lahsissen et *al.*, 2009).
It has been proven that extracts derived from both plants are potent free radical scavengers, prevent lipid peroxidation *in vitro* (Atmani et *al.*, 2009) and strong inhibitors of xanthine oxidase (Berboucha et *al.*, 2010). This study was therefore undertaken to investigate the hepatoprotective, diuretic, anti-inflammatory and hypouricemic potentials of C.flammula, *P. lentiscus* and *F. angustifolia* ethanolic and aqueous extracts.

2 Experimental


Clematis flammula


Fraxinus angustifolia


Pistacia lentiscus

❑Anti-inflammatory activity (Winter et *al.*, 1962): Ethanolic extracts (200mg/Kg) or diclofenac (50mg/Kg) were administered orally one hour before carrageenan (1%) injection in the left hind paw. Measurements of the edema produced were effected one hour later and during 5 hours.

❑Hepatoprotective activity (Naskar et *al.*, 2010): Ethanolic extracts (50mg/Kg) or vitE (50mg/Kg) were administered orally for seven days and two hours before receiving CCl_4 that was given every 72hours. 24hrs after the last dose of CCl_4 mice were sacrified and their liver excised . Histologic sections were analysed to detect the presence or absence of liver damage.

❑Diuretic activity on rats (Kau et *al.*, 1984): Plant extracts (200mg/Kg) or the reference drug furosemide (20mg/kg) were administrated orally or injected by intraperitoneal way, respectively to rats and urine output was measured at several intervals of time after administration of a single dose. Na^+ and K^+ levels were determined by atomic absorption spectrophotometer.

❑Hypouricemic activity on mice (Zhu et *al.*, 2004):mice were injected intraperitoneally with potassium oxonate (250 mg/kg) 1 h before the final drug administration to increase the serum urate level, for three consecutive days. Serum uric acid levels and liver XOR activities were evaluated.

3 Results

Table I: Effect of *Fraxinus angustifolia* and *Pistacia lentiscus* extracts and diclofenac on carrageenan-induced paw edema in mice.

Groups	Paw thickness at different time intervals (in mm) % edema inhibition (max)						
	0H	1H	2H	3H	4H	5H	24H
Control	2.31±0.20	3.11±0.22	3.28±0.31	3.48±0.19	3.70±0.26	3.58± 0,16	3.56±0.09
C.Flammula (200mg/kg)	2.41±0.045	3,26±0.017	3,28±0.03	3,23±0.025	3,82±0.032	3,93±0.03	3,97±0.025
F. angustifolia (200mg/Kg)	2.37±0.19	2.99±0.32	3.00±0.39	3.02±0.41	3,06±0.38 47.81%[a]	3.19±0.29	3.21±0.36
P. lentiscus (200mg/Kg)	2.33±0.07	3.11±0.16	3.02±0.16 40.47%[a,b]	3.07±0.14	3.27±0.06	3.31±0.15	3.28±0.16
Diclofenac (50mg/Kg)	2.41±0.03	2.88±0.30	2.74±0.11 65.31%[a]	2.64±0.30	2.91±0.36	2.92±0.26	2.91±0.30

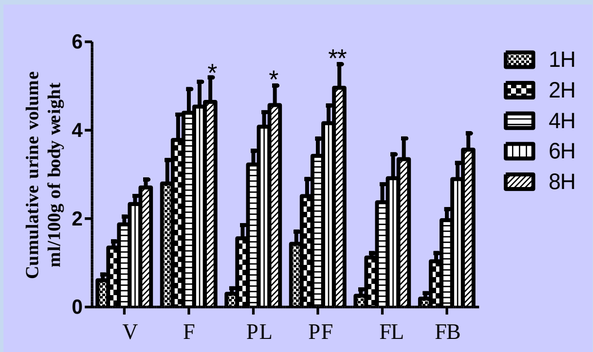


Figure 02: Effect of oral administration of Furosemide (10mg/kg) and ethanolic extracts of *Pistacia lentiscus* leaves (PL) and fruits (PF), *Fraxinus angustifloia* leaves (FL) and barks (FB) at 200mg/kg on cumulative urine volume per 100 g of body weight per rat. **Mean±S.E.M., n =6. *p < 0.05 vs. control, **p < 0.01 vs. control, ***p < 0.001 vs. control.**

Table II: Effect of oral administration of Furosemide (10mg/kg) and ethanolic extracts of *Pistacia lentiscus* leaves (PL) and fruits (PF), *Fraxinus angustifloia* leaves (FL) and barks (FB) at 200mg/kg on pH, conductivity and cumulative urinary excretion of sodium and potassium in rats.

	pH	Conductivity	Na^+	K^+
Vehicle	7,13±0,33	12,18±0,98	100,5±2,95	66,12±0,63
Furosemide	6,18±0,07	15,30±0,95	160,16±2,30***	150,498±0,42***
PL	7,90±0,44	13,9±2,25	126,06±3**	208,5±1,79***
PF	7,59±0,68	14,05±2,45	89,46±2,3	150,426±1,89***
FL	7,79±0,8	14,20±0,9	96,84±2,18	182,85±1,4***
FB	7,05±0,35	15,33±0,35	145,14±2,4***	155,77±1,86***

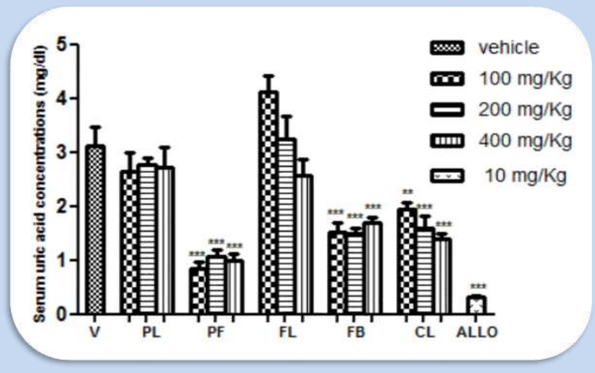


Figure 03: Effect of allopurinol (10mg/kg) and ethanolic extracts of *Pistacia lentiscus* leaves (PL) and fruits (PF), *Fraxinus angustifloia* leaves (FL) and barks (FB), and Clematis flammula leaves (CL) at 200mg/kg on serum urate level in normal mi**ce. Mean±S.E.M., n =6. *p < 0.05 vs. control, **p < 0.01 vs. control, ***p < 0.001 vs. control.**

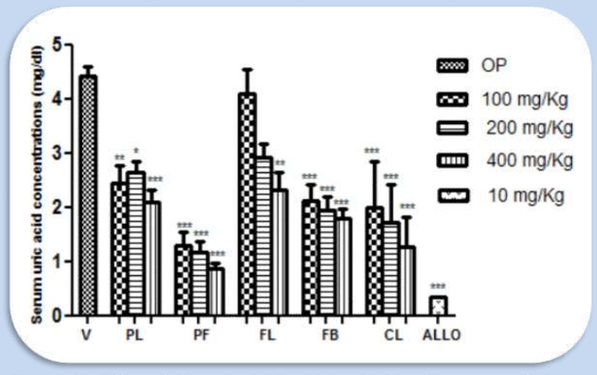
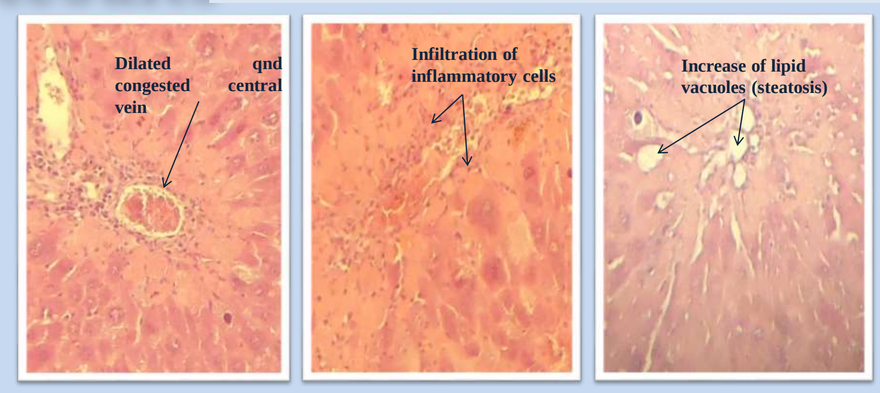
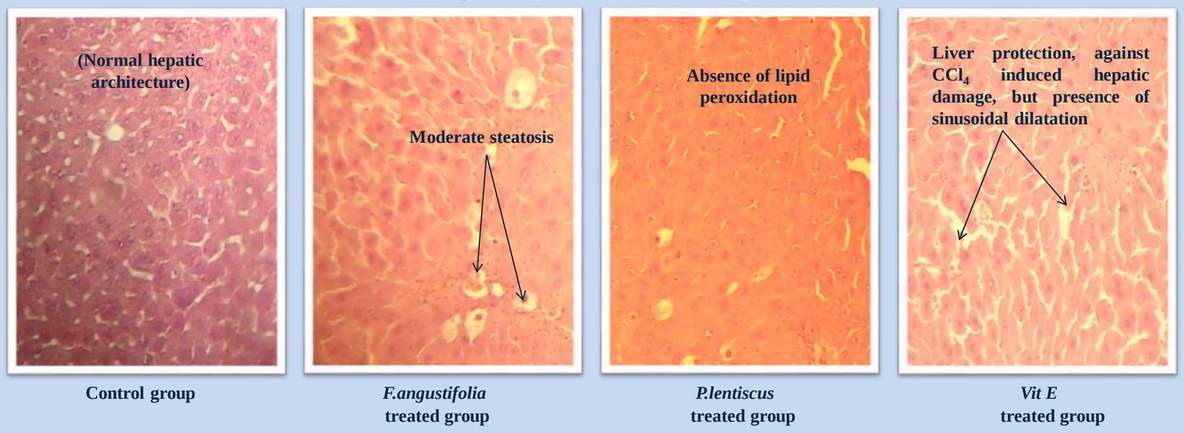


Figure 04: Effect of allopurinol (10mg/kg) and ethanolic extracts of Pistacia lentiscus leaves (PL) and fruits (PF), *Fraxinus angustifloia* leaves (FL) and barks (FB) and *Clematis flammula* leaves (CL) at 200mg/kg on serum urate level in hyperuricemic mice. **Mean±S.E.M., n =6. *p < 0.05 vs. control, **p < 0.01 vs. control, ***p < 0.001 vs. control.**



CCl_4-treated groups: signs of liver damage



Control group | ***F.angustifolia* treated group** | ***P.lentiscus* treated group** | ***Vit E* treated group**

Figure 01 : Photomicrographs of liver sections from mice stained with eosin hematoxylin

Effect of ethanolic extracts of *Pistacia lentiscus* leaves (PL) and fruits (PF), *Fraxinus angustifloia* leaves (FL) and barks (FB) and *Clematis flammula* leaves (CL) on liver xanthine oxidoreductase activities in normal and oxonate pretreated mice, after oral three time administration. **Mean±S.E.M., n =6. *p < 0.05 vs. control, **p < 0.01 vs. control, ***p < 0.001 vs. control.**

Treatments	Dose (mg/kg)	XO (nmole/min/ mg of [proteins)	XDH (nmole/min/ mg of proteins)	% Inhibition XO	% Inhibition XDH
Vehicle	_	2.56±0.07	2.48±0.06	---	---
PL EtOH	100	2.36±0.10	2.37±0.03	8.13	4.75
PL EtOH	200	2.36±0.17	2.18±0.05	8.09	---
PL EtOH	400	1.84±0.08	2.09±0.05	28.28	21.04
PF EtOH	100	2.54±0.13	2.85±0.12	---	---
PF EtOH	200	2.70±0.17	3.19±0.26	---	---
PF EtOH	400	2.67±0.30	3.33±0.14	---	---
FL EtOH	100	1.84±0.26	1.87±0.22	25.80	21.75
FL EtOH	200	1.70±0.16	1.96±0.22	31.45	17.99
FL EtOH	400	1.51±0.16	1.30±0.09	39.11	45.60
FB EtOH	100	2.84±0.25	2.44±0.20	---	---
FB EtOH	200	2.48±0.08	2.52±0.41	---	---
FB EtOH	400	2.39±0.25	2.63±0.11	---	---
CL AqEA	100	0.87±0.16	0.55±0.05	68.84	76.74
CL AqEA	200	0.92±0.16	0.87±0.11	66.95	63.09
CL AqEA	400	1.10±0.12	1.12±0.12	60.69	52.72
Allopurinol	10	0.66±0.10	0.66±0.10	73.38	72.38

Treatments	Dose (mg/kg)	XO (nmole/min/ mg of proteins)	XDH (nmole/min/ mg of proteins)	% Inhibition XO	% Inhibition XDH
PO	250	2.59±0.16	2.83±0.17	---	---
PO+PL EtOH	100	2.41±0.08	2.54±0.12	7.21	10.09
PO+PL EtOH	200	2.18±0.05	2.40±0.07	15.95	15.29
PO+PL EtOH	400	2.09±0.10	2.30±0.08	19.46	18.79
PO+PF EtOH	100	2.56±0.04	3.15±0.13	---	---
PO+PF EtOH	200	2.97±0.17	3.28±0.23	---	---
PO+PF EtOH	400	2.88±0.21	2.82±0.16	---	---
PO+FL EtOH	100	1.44±0.16	1.53±0.09	33.94	40.92
PO+FL EtOH	200	1.37±0.07	1.31±0.10	37.15	49.42
PO+FL EtOH	400	1.56±0.25	1.44±0.31	28.44	44.40
PO+FB EtOH	100	2.33±0.03	3.55±0.61	---	---
PO+FB EtOH	200	2.39±0.02	3.75±0.25	---	---
PO+FB EtOH	400	2.40±0.21	2.72±0.14	---	---
PO+CL AqEA	100	1.03±0.14	1.59±0.26	61.15	47.46
PO+CL AqEA	200	0.78±0.07	0.84±0.09	70.31	71.95
PO+CL AqEA	400	1.00±0.04	1.15±0.06	62.07	61.71
PO+Allopurinol	10	0.62±0.11	0.60±0.10	71.55	76.83

4 Conclusion

Obtained results indicate that *Clematis flammula*, *Fraxinus angustifolia* and *Pistacia lentiscus* extracts exhibited promissing hypouricemic and anti-inflammatory potential and protected mice from liver damage, thereby validating their traditional use as antioxidants, anti-inflammatory and anti-hyperuricemic in North Africa.

6 Acknowledgements

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