

EFFECTS OF *Rosmarinus officinalis* L. AQUEOUS EXTRACT ON ACUTE INFLAMMATION

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Résumé

Dans cette étude, l'extrait aqueux de *Rosmarinus officinalis* L. a été évalué pour l'activité anti-inflammatoire par l'utilisation, comme modèle, l'œdème de la patte du rat induit par le Carrageenan. Deux doses de l'extrait aqueux (1500 mg/Kg et 3000 mg/Kg) ont été administrées par gavage, puis le Carrageenan a été injecté dans la patte postérieure droite des rats. L'extrait, qui est riche en composés phénoliques ($196,63 \pm 3,09$ mg GAE/ g E) et en flavonoïdes ($2,22 \pm 0,09$ mg RE/ g E), a montré une bonne action inhibitrice (44,62 % à la troisième heure pour la grande dose. **: $p < 0,1$) et proche de celle de l'aspirine, qui a été utilisée comme un standard (31,87 % à la 3^e heure pour 200 mg/Kg d'aspirine. ***: $p < 0,05$). Le même effet a été observé macroscopiquement sur l'ulcère gastrique pour les deux substances. Cependant, les altérations tissulaires de l'estomac étaient différentes à l'étude histologique.

Mots clés : Réponse inflammatoire, agents anti-inflammatoires, *Rosmarinus officinalis* L.

Abstract

In this study, *Rosmarinus officinalis* L. aqueous extract was evaluated for anti-inflammatory activity by using the model of Carrageenan-induced rat paw oedema. Two doses of the aqueous extract (1500 mg/Kg and 3000 mg/Kg) were given orally by gavage technique, and then carrageenan was injected into the right hind paw of the rat. The extract, which was found rich in phenolic content (196.63 ± 3.09 mg GAE/ g E) and flavonoids (2.22 ± 0.09 mg RE/ g E), has shown a good inhibitory action (44.62 % in the third hour for the higher dose. *: $p < 0.1$) and close to that of aspirin that was used as a standard (31.87 % in the third hour for 200 mg/Kg aspirin. **: $p < 0.05$). The same effect was observed macroscopically on the gastric ulcer for both substances. However, the tissue alterations of the stomach were different at the histological study.

Keywords: Inflammation, anti-inflammatory activity, *Rosmarinus officinalis* L.

ملخص

في هذه الدراسة تم تقدير تأثير المستخلص المائي لنباتة *Rosmarinus officinalis* L. على التفاعل الالتهابي الحاد باستعمال تقنية وذمة رجل الجرذ عن طريق حقن مادة carrageenan. جرعتان من المستخلص المائي (1500 ملغ/ كغ و 3000 ملغ / كغ) استعملتا عن طريق تقنية الأنبيبة، ثم تم حقن المادة المسببة للوذمة في الرجل السفلية اليمنى للجرذ. المستخلص، الذي وجد غنيا بالمواد الفينولية (196.63 ± 3.09 ملغ/ح غ م) والفلافونويدات (2.22 ± 0.09 ملغ ر / غ م)، أظهر نشاطا تثبيطيا جيدا (44.62% في الساعة الثالثة للجرعة العالية $p < 0.1$ **) بالمقارنة مع ذلك المتحصل عليه من طرف الأسبرين المستعمل بهدف المقارنة (31.87% في الساعة الثالثة لجرعة 200 ملغ/ كغ $p < 0.05$ ***). نفس الأثر كان مرئيا للمادتين على تكوين القرحة المعدية، لكن تبين بعد الدراسة النسيجية أن التأثير على الأنسجة كان مختلفا.

الكلمات المفتاحية: التفاعل الالتهابي، مضادات الالتهاب، *Rosmarinus officinalis* L.

The inflammatory response is a critical mechanism for the body to ward off any harmful agent or cellular malfunction. This process is orchestrated by cells that are controlled by several types of chemicals in a delicate manner. However, if the balance between those chemicals was interrupted, this protective barrier would develop some serious damage that sometimes exceeds the one caused by the harmful agent itself. In that case, inflammation has to be stopped before any appearance of dramatic outcomes such as tissue damage (Lawrence & Gilroy, 2007).

The anti-inflammatory drugs are used practically in a daily basis these years, especially the non-steroidal anti-inflammatory drugs (Charles *et al.*, 2010), as well as glucocorticoids (Perretti & D'Acquisto, 2009); they are taken with and without a prescription. These drugs have an instant anti-inflammatory action: after a couple of hours the signs of inflammation fade significantly.

On the other side, these drugs have some bad side effects that must be taken in consideration (Langman *et al.*, 1994; Rhen & Cidlowski, 2005). Peptic ulcer, for example, is one of the serious side effects that was, and still, associated with NSAIDs; and for that, second thoughts should be taken in count, especially when these drugs are prescribed for long lasting treatments. That is why people use the steroidal anti-inflammatory drugs, their effect is stronger and their mechanism implies the release of body hormones that interfere with the inflammatory cascade. Nevertheless, their mechanism of action is unstable and can weaken the immune response; plus, the side effects are clearly significant. So the research for natural anti-inflammatory molecules has become an interesting field of study. Many medicinal plants have been cited for their anti-inflammatory action (Calixto *et al.*, 2003), especially those in Lamiaceae family because of their low toxicity and high availability.

Rosmarinus officinalis L. is a Labiatae of Mediterranean origin that is known from the ancient times and appreciated for its taste and aroma. This plant has a wide range of medicinal, pharmaceutical and non pharmaceutical applications (González *et al.*, 2007). In this study, some effects of *Rosmarinus officinalis* L. on acute inflammation are evaluated in a way that allows us to test its effectiveness as herbal tea like it is used in folk medicine.

MATERIAL AND METHODS

Plant material

Rosmarinus officinalis L. was brought from Adrar (south west of Algeria) where it was cultivated, harvested, collected and left for a total air-dry method for a week. It was ground with a wooden mortar and pestle (Vekoo®).

Animals

Female *Wistar* rats were purchased from Pasteur institute (Algiers) and put in a suitable place for adaptation with a normal temperature, a regular day / night light system and free access to food and water in a 28 days period. Then, they were divided into four groups according to weight closeness.

Chemicals

Folin–Ciocalteu reagent, Sodium Carbonate, Gallic acid, Quercetin, Rutin, Aluminum Trichloride, Tannic acid, Acetylsalicylic acid and Carrageenan were purchased from Sigma-Aldrich® as well as Methanol and Chloroform. Formalin and sodium chloride were bought from Prolabo®. NaCl 0.9 % (sterile) was purchased from Sidal®. Fresh cattle blood was brought from a local butchery.

Phytochemical screening

-Aqueous extract preparation: The protocol of extraction was carried out as described by Mbiantcha *et al.* (2010).

-Phytochemical screening: Phenolic content was determined by the Folin-Ciocalteu method (Boudries *et al.*, 2012). Total flavonoid content of the crude extract was assessed by aluminumtrichloride colorimetric method (Baharun *et al.*, 1996). The tannin content was evaluated by the method described by Ferreira *et al.* (2003).

Carrageenan-induced rat paw oedema

Carrageenan-induced paw oedema was carried out as described by Mbiantcha *et al.* (2010): The rats' paw volumes were measured plethysmographically (UGO BASILE®-7140). The oedema was expressed as an increase in the volume of paw ($\Delta V = V_t - V_0$), and the percentage of inhibition (*I* %) for each treatment was obtained by pursuing the equation 1:

$$I (\%) = [(\Delta V_c - \Delta V_{tr}) / \Delta V_c] \times 100 \quad (1)$$

Where ΔV_{tr} = right hind paw average increased volume in treated group and ΔV_c = right hind paw average increase in control groups.

Gastric ulcer evaluation

The macroscopic examination was carried out as described by Shuai *et al.* (2011) with the use of a digital camera (Canon® IXUS): The photos were transferred to computer and the area of ulcers was assessed by assuming the surface proportion. Ulcerated surface (US, %), was computed as in equation 2.

$$US = (A/B) \times 100 \quad (2)$$

Where A is the total area covered by ulcers and B is the total corpus mucosal surface.

The microscopic examination was proceeded as described by Gwaram *et al.* (2012): The stomach sections were stained with haematoxylin and eosin and analyzed under light microscope at x10, 40 and 100 magnification.

RESULTS

Phytochemical screening

In this work, 300 g of dry matter of *Rosmarinus officinalis* L. were used to get the aqueous extract, which has a dark brown colour and a strong aroma that did not get weak.

The yield of the process was evaluated: an amount of 36.32 ± 1.67 g. of aqueous extract was recovered from the dry matter which means 12.11 % (± 0.55) of the total weight.

For the total phenolic content, the aqueous extract of *Rosmarinus officinalis* L. has 196.63 ± 3.09 mg Gallic acid equivalent of total phenols in 1 g. of the crude extract. For total flavonoid content, an amount of 1.37 ± 0.06 mg. Quercetin equivalent in 1g extract and 2.22 ± 0.09 mg Rutin equivalent in 1 g. extract was assumed. The tannin content was 2.09 ± 0.02 mg Tannic acid equivalent of tannins in 1 g. of extract.

Carrageenan –induced rat paw oedema

- Control: The results are present in figure 1 and tables 1 and 2. The injection of 0.1 mL Carrageenan (1%) in the right hind paw of the rat causes a visible inflammation after one hour. Then, the oedema volume increases till it gets to the maximum after 4 hours before it starts to decrease till the 8th hour but with no disappearance.

- Aspirin: The results are shown in the same figure and tables. The administration of Acetylsalicylic acid (as a standard anti-inflammatory agent) has reduced the oedema progressively. The maximum effect of inflammation, observed at the fourth hour, was reduced (23.36 %).

The effect of aspirin is present since the first hour after the shot and maintained all the way to the 8th hour. These results make the curve of aspirin a good standard to evaluate the anti-inflammatory effect of the extract.

-*Rosmarinus officinalis* aqueous extract: The results are present in figure 1, and tables 1, and 2. The aqueous extract of *Rosmarinus officinalis* has reduced the inflammation progressively after the first hour for dose two and after the second hour for dose one. The maximum effect of inflammation, observed in hour 4, was reduced as well: it reached 27.37 % for dose one and 40.15 % for dose two.

The maximum inhibitory effect was seen at the last hour of the experiment.

Macro and microscopic observation of gastric ulcer

- The macroscopic aspect : Stomach ulcerated surface was measured in order to evaluate the effect of the standard anti-inflammatory substance and *Rosmarinus officinalis* aqueous extract on gastric mucosa after 8 hours of treatment. The amount of injured area in all groups was different significantly from that in the untreated group. However, there was not a difference among the treated groups (between each other). The higher percentage was observed in Aspirin group (10.55 %, * $p < 0.05$) and the higher dose of the extract (8.75 %, ** $p < 0.01$) while the lower percentage was found in the untreated group (1.69%) and the lower dose of the extract (6.05%, * $p < 0.05$).

Table1: The influence of Aspirin and *Rosmarinus officinalis* aqueous extract on oedema volume

Groups	Oedema volume (% of the initial volume V0 \pm SEM)								
	0:30	1:00	2:00	3:00	4:00	5:00	6:00	7:00	8:00
Control Vehicle (Distilled water)	12.9 ± 5.05	25.3 ± 6.50	36.1 ± 7.50	40.8 ± 7.97	44.1 ± 7.70	41.8 ± 9.05	35.9 ± 7.87	33.4 ± 8.22	29.5 ± 7.42
Standard: Aspirin (200 mg/Kg)	07.8 ± 4.17	12.9 ± 4.64	15.9 ± 6.16	25.0 ± 6.35	30.4 ± 5.29	29.9 ± 4.95	27.2 ± 4.49	21.6 ± 4.54	17.7 ± 4.31
Aq. Ex (1500 mg/Kg)	12.0 ± 3.34	16.9 ± 4.05	22.4 ± 3.86	25.9 ± 4.49	31.7 ± 5.95	26.8 ± 6.15	24.6 ± 5.52	19.3 ± 5.48	14.4 ± 5.08
Aq Ex (3000 mg/Kg)	05.4 ± 2.15	09.3 ± 2.83	14.8 ± 4.73	20.0 ± 5.51	23.3 ± 5.49	17.8 ± 5.70	12.2 ± 4.21	07.3 ± 2.70	05.0 ± 1.52

V0: initial volume; Aq. Ex: Aqueous Extract. Number of rats per group N = 6

Table 2: The proportion of inhibition (I %) of the rats paw oedema by Aspirin and Aqueous extract (Aq Ex) of *Rosmarinus officinalis* all along the experience.

Groups	Percentage of inhibition (I %) for each hour (compared to the positive control volume)									
	00:30	01:00	02:00	03:00	04:00	05:00	06:00	07:00	08:00	
Aspirin (200 mg/Kg)	39,74	42,76	52,49**	31,87**	23,36	19,92	15,00	26,73	32,58*	
Aq. Ex. (1500 mg/Kg)	3,85	30,26	36,20*	35,46*	27,37	35,16*	30,91	41,58 *	51,12*	
Aq. Ex. (3000 mg/Kg)	52,56	57,89**	53,85**	44,62*	40,15*	51,17**	61,36**	75,25**	80,34**	

Significance: *: $p < 0,1$. **: $p < 0,05$; N=6

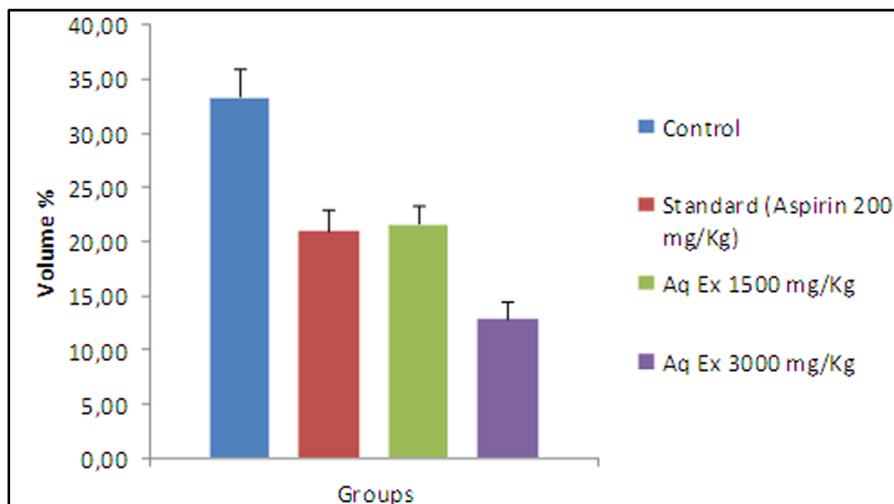


Figure 1: Total percentage of oedema volume for each group. (Aq Ex: *Rosmarinus officinalis* aqueous extract).

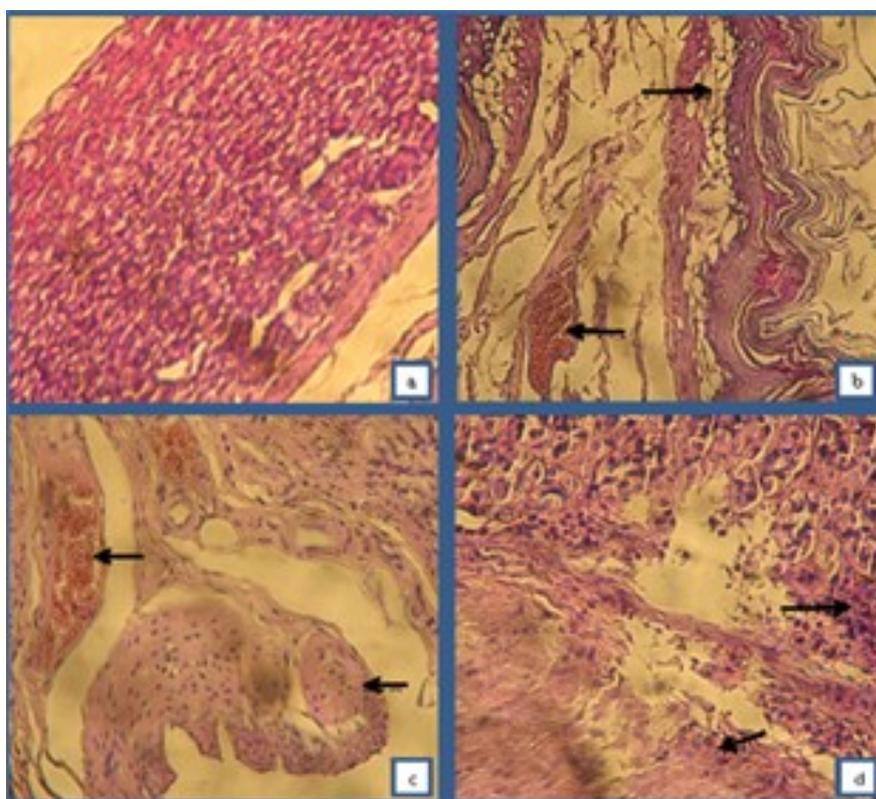


Figure 2: Histological examination of stomach of control and experimental group of rats (magnification $\times 100$, Haematoxylin and Eosin staining), a: untreated group, b: aspirin group, c: Aq. Ex of *Rosmarinus officinalis* L. 1500 mg/Kg, d: Aq. Ex of *Rosmarinus officinalis* L. 3000 mg/Kg.

- The microscopic aspect: The gastric walls taken out of the untreated rats were slightly inflamed with the presence of some lymphocytes, plasmocytes and polymorphonuclear neutrophils (Figure 2 a.). The blood vessels were congestive with the sign of an oedema in the site.

For the Aspirin-treated group, the gastric walls were edematous with an important presence of autolytic remains. The blood vessels were congestive (Figure 2 b.).

For the extract-treated rats with the lower dose, the main character of the gastric wall was the vascular congestion (Figure 2 c.). The same feature was observed in the group that had the higher dose (Figure 2 d.). However, the cellular content was different: the gastroesophageal wall was inflamed with the obvious presence of polymorphonuclear eosinophils and lymphocytes.

DISCUSSION

The phytochemical aspect of *Rosmarinus officinalis* aqueous extract

The content properties of *Rosmarinus officinalis* L. has been the subject of an intense study where the plant dry material was examined to evaluate its richness of active biomolecules. Al Sereiti *et al.* (1999) have cited that *Rosmarinus officinalis* aqueous extract has a variety of substances with so many biological activities.

The choice of distilled water as a solvent was taken because of the very low toxicity of *Rosmarinus officinalis* aqueous extract as it was investigated by Lemonica *et al.* (1996). The overall yield of the maceration process was 12.55 %; which was close to the results of Genena *et al.* (2008)(8.76 %) but with the choice of hexane as a solvent, and very low in comparison with ethanol extract of the same reference (30.25 %).

The phenolic content of *Rosmarinus officinalis* was evaluated deeply in literature. Al Sereitiet *et al.* (1999) have cited that when the aqueous extract was analyzed, the results were in favour of the following active principles: Rosmarinic acid (RA), Caffeic acid (CA), Chlorogenic acid, Carnosolic acid, Rosmanol, Carnosol and different diterpenes. In this study, the total phenolic content was very rich (19.66 %) and a bit higher than 14.20 % found by Genena *et al.* (2008) in ethanol extract. The flavonoid content, also, was acceptable as it reached 1.37 %. These results confirm the importance of *Rosmarinus officinalis* as a medicinal herb.

Carrageenan-induced rat paw oedema

The carrageenan-induced rat paw oedema is a good procedure to study the effect of *Rosmarinus officinalis* aqueous extract on acute inflammation. In this model, the early phase of swelling (0-1 h), which is not inhibited by aspirin, causes the release of histamine and bradykinin. In contrast, the second accelerating phase of oedema (1-6 h),

has been associated with the elevated production of prostaglandins and inducible cyclooxygenase (COX-2) in the hind paw. Neutrophil Leukocytes also contribute to this inflammatory response by producing oxygen-derived free radicals (Salvemini *et al.*, 1996).

Several researches have been assessed to evaluate the anti-inflammatory effect of *Rosmarinus officinalis*. Juhás *et al.* (2009) have investigated the anti-inflammatory effects of dietary administration of *Rosmarinus officinalis* essential oil in mice using carrageenan-induced mouse paw oedema. Their results show that *Rosmarinus officinalis* essential oil at high concentration (5 000 ppm) in the diet was able to attenuate the inflammation.

At a high dose, *Rosmarinus officinalis* aqueous extract has reduced the volume of carrageenan-induced rat paw oedema significantly. This is during the second hour (36.2 % per 1500 mg/Kg, 53.85 % per 3000 mg/Kg), the third hour (35.46 %, 44.62 %) and the last couple of hours (51.12%, 80.34 % for 1500 and 3000 mg / kg respectively) which defines the dose-related effect of the final outcome.

Juhás *et al.* (2009) gave the credit of the anti-inflammatory effect of *Rosmarinus officinalis* extract to the terpene components, and to the synergy of biologically active compounds of essential oil, in particular their antimicrobial, anti-oxidative effects.

In another research, Scheckel *et al.* (2008) investigated the effect of Rosemarinic acid on reducing cyclooxygenase-2 synthesis. The Rosemarinic acid, as cited in that article, was one of the important anti-inflammatory molecules that have been previously studied.

Last but not least, the effect was reached via oral administration of the extract, which confirms the benefit of the home-made herbal tea.

The effect of aspirin and *Rosmarinus officinalis* L. aqueous extract on gastric wall

The actions of aspirin on gastric epithelium may involve several mechanisms such as the direct killing of epithelial cells, inducing osmotic lysis, or reducing mucus and bicarbonate secretion. This damage is visible macroscopically (Wallace, 2008).

The same macroscopic aspect was determined for *Rosmarinus officinalis* extract with both doses. However, the microscopic aspect was different. For the group of lower dose the vascular congestion was the same as the one in non-treated group. As for the higher dose, the eosinophil leukocytes presence may be referable to a chemotactic system or a triggering system that was lunched because of the long time of the treatment. Further investigation needs to be proceeded.

Finally, Juhás *et al.* (2009) considered the vascular congestion and the cellular aspect was due to the high

concentration of some components in *Rosmarinus officinalis* (eucalyptol, α -pinene and borneol) that inhibited acetylcholinesterase and produced vascular relaxation and plasma leakage.

CONCLUSION

Rosmarinus officinalis L. is a very common herb among people. It is widely used as a spice, a tisane or herbal tea, in cosmetic industry and other important fields. Such diverse activities has generated great interest in its' extracts, and some of its isolated constituents have been subjected for many pharmacological investigations.

In this study, the anti-inflammatory effect of *Rosmarinus officinalis* L. was evaluated by the technique of rats paw oedema; and the aqueous extract was chosen for being the closest to the herbal tea most people use. In the experiment, the inhibition of oedema's volume by the extract was fast at the beginning of the effect, significant and reasonably similar to the one caused by aspirin in the maximal hour and even better in the last hours. Moreover, the effect of the extract on stomach mucosa was different from that of aspirin when the histological examination was assessed. Furthermore, the biochemical tests that were run at the very first time of the study claim the richness of the aqueous extract with natural antioxidants and other beneficial active biomolecules. These results claim the importance of *Rosmarinus officinalis* L. in the medical field.

A further investigation should be attempted in order to see the effect of this plant on chronic inflammation and the diseases related to that type of response such as rheumatoid arthritis and atherosclerosis. Also, the study of the effect of *Rosmarinus officinalis* L. aqueous extract on eosinophil leukocytes seems to be a pretty interesting investigation theme.

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