e- ISSN 0975 – 9328
Print ISSN 2229 – 7472

International Journal of Phytopharmacology

Journal homepage: www.onlineijp.com

IJP

PHYTOCHEMICAL SCREENING AND ANTI-INFLAMMATORY PROPERTY OF ONE RECIPE FROM MOROCCAN TRADITIONAL MEDICINE

Latifa El Mansouri[1,2], Dalila Bousta[1*], Smahane Boukhira[1], Mounyr Balouiri[1], Abdessalam El Khanchoufi[1], Hassane Akdime[1], Bahia Bennani[3], Sanae Achour[4]

[1]LPAMSN, National Institute of Medicinal and Aromatic Plants, [2]LAMEC, Faculty of Sciences-Fez, [3]Faculty of Medicine of Fez, [4]CHU-Fez, Morocco.

ABSTRACT

The present study aims to investigate the inflammatory property of the recipe consisting of five plants from South of Morocco (Rissani -ER-rachidia region). Extract of plants is obtained by aqueous cold maceration and administered to Wistar rats orally. The extract has a significant anti-inflammatory effect compared to the positive control (indomethacin), 5 h after induction of inflammation by carrageenan at 0.5%. The aqueous extract is then formulated into syrup; its anti-inflammatory potency was also evaluated orally in Wistar rats. This syrup is a natural non-steroidal anti-inflammatory elaborated from plants used in Folk Moroccan Medicine and more powerful than indomethacin. In this context, this natural anti-inflammatory will help to improve the quality of life and well-being in the case of inflammation.

Key words: Natural Anti-inflammatory, Flavonoids, Phytochemical Screening, Traditional Medicine.

INTRODUCTION

Natural products have, until recently, been the primary source of commercial medicines and drug leads. A recent survey revealed that 61% of the 877 drugs introduced world-wide can be traced to or were inspired by natural products (Leland *et al*., 2006). Classic examples of herbs traditionally used to treat inflammation in Moroccan medicine are Fredolia aretioides (Coss. & Dur) Coss. & Moq.), *Citrullus colocynthis* (L.) Schard., *Euphorbia resinifera* Berg., *Ricinus communis* L. (Bellakhdar, 1997).

The five species studied were demonstrated active for many pharmacological or biological effects. Thus, the anti-arthritis effect of Phoenix dactylifera in rats has been demonstrated (Doha et al., 2004). The volatile part of the other species of the syrup has been tested primarily developed for other biological activities such as antifungal activity (Hmiri *et al*., 2011). The species Rosmarinus officinalis is known as an antioxidant and hepatoprotective in rats (Sotelo-Félix *et al*., 2002). In addition, the hydro-alcoholic extract of this species has an anti-ulcerogenic decreasing power index ulcerative lesion induced by indomethacin (Patrícia *et al*., 2000). Study on anti-inflammatory and antioxidant activity of pure compounds obtained from plants grown in vitro culture of R. officinalis were performed on isolated cells in vitro (Annette *et al*., 2006).

Other studies have shown that the methanol extract of R. officinalis is known for its antioxidant and antibacterial effect (Catalina *et al*., 2009). The essential oil of Artemisia herba-alba species was studied on the phytochemical and biological. It turned out that the essential oil of sagebrush is endowed with a variety of activities such as antioxidant, anti-venous anti-bacteriet al., anti-spasmodic and hypoglycemic activities, antifungal (Abou El-Hamd *et al*., 2010).

The essential oil of Mentha pulegium was also studied on the map phytochemical and chemical compounds were identified by GC-FID and GC-MS

Corresponding Author

Pr. Dalila Bousta
Email: boustadalila@gmail.com

(Derwich *et al*., 2012). It was also shown that the essential oil of Mentha suaveolens is inhibitory, more than 50% of acetylcholinesterase (Ferreira *et al*., 2010).

Inflammation is a dynamic process that is elicited in response to mechanical injuries, burns, microbial infections, and other noxious stimuli that may threaten the well-being of the host. This process involves changes in blood flow, increased vascular permeability, destruction of tissues via the activation and migration of leucocytes with synthesis of reactive oxygen derivatives (oxidative burst), and the synthesis of local inflammatory mediators, such as prostaglandins (PGs), leukotrienes, and platelet-activating factors induced by phospholipase A2, cyclooxygenases (COXs), and lipoxygenases (Christophe, 2006).

Anti-inflammatory conventional drug treatments are limited in their effectiveness in managing the incidence and outcome of many inflammatory diseases. They also present a significant number of side-effects in patients and recently, it has been shown that non-steroidal anti-inflammatory agents may even attenuate the healing process. Actually, it's there an urgent need to find safer and more effective medicines (Ayoola *et al*., 2009).

Where the object of our present study, whose purpose is to develop a non-steroidal anti-inflammatory nature et al. free of preservatives and other chemical addition that could be harmful to health. As we know, until now, no study has begun the synergistic effect of plants on the one hand, on the other hand, according to our surveys in the area of ER-Rachidia Rissani, most plants used in traditional medicine are used in combination. In this context, our goal is, in a first step, confirm or deny the use of a traditional recipe with anti-inflammatory effect in a second step, highlight the synergistic effect of different components of the mixture.

In this study, we aimed to (i) investigate some pharmacological activities such as the potential anti-inflammatory-like profile of aqueous extract of recipe in the rat, (ii) measure the percentage of some chemical components like flavonoids, total polyphenols, tannins and others compounds.

MATERIALS AND METHODS

Plant collection

Based on ethno-pharmacological surveys conducted in the region of Rissani Errachidia-(South of Morocco), we selected a recipe containing five plants; known for its anti-inflammatory traditional effect.

The five species are harvested in the region of Rissani-ER-Rachidia identified and deposited in the herbarium of the National Institute of Medicinal and Aromatic Plants (NIMAP) Taounate-Morocco [*Rosmarinus officinalis* (exsiccata N° INP216), *Phoenix dactylifera* (exsiccata N° INP215), *Artemisia herba-alba* (exsiccata N° INP218), *Mentha pulegium* (exsiccata N° INP221) and *Mentha suaveolens* (exsiccata N° INP220)].

Extract Preparation

The aerial parts of five plants were dried and crushed. They have been macerated in equal parts of distilled water for 3 hours with continuous agitation. Then the macerate was filtered through a Whatman N°. l. The filtrate obtained underwent a rotation evaporator (Rotavapor R-205 BÜCHI), and a dry residue was recovered with a yield of 31.92%.

Phytochemical screening

Phytochemical screening of dry extract was achieved through techniques described by (Bruneton, 1993; N'Guessan et et al., 2009; Dohou et et al., 2003; Diallo, 2005; Mun et et al., 2009; Karumi et et al., 2004]. These tests demonstrate the presence of a number of chemical groups of the dry extract of the species studied, which may be responsible for its anti-inflammatory effect.

Determination of total polyphenols

The total phenolic contents of the extract of recipe were determined with the Folin-Ciocalteu reagent, using gallic acid as standard (Singleton *et al*., 1965). The result was expressed as gallic acid equivalent (mg gallic acid/g of plant).

Determination of total tannins

The total tannins content was determined by spectrophotometer by using method described by (Sarneckis and *et al*., 2006).

Determination of flavonoids

Total flavonoids content was determined by spectrophotometer by using method described in the work of (Lamaison and *et al*., 1991).

Determination of catechins

Using the method proposed by (Swain and Hillis, 1959), based on the ability of catechins condensation with carbonyl compounds in acidic media. The result of the reaction is measured at 500 nm.

Syrup formulation

The syrup of the aqueous extract was made in our laboratory. We used sucrose as an excipient, with a percentage of 131.57%. For a bottle of 38 ml syrup, we dissolve 50 g of sugar in 27 ml of pure water. Then, the mixture is maintained at 50 °C in water bath until completely dissolved. The mixture is filtered under sterile conditions and mixed with the extract dissolved in 11 ml of pure water.

MICROBIOLOGICAL CONTROL SYRUP

Microorganisms used

The microorganisms employed in the current study were procured from the American type of cell culture collection (ATCC). Strains of gram-positive bacteria S. aureus (ATCC 29213), gram-negative bacteria E. coli (ATCC.25922), and fungal strain C. albicans (ATCC 10231) were employed.

MEDIA

Plate Count Agar (PCA) (BK144HK)

The medium is based on yeast extract, it is rich in tryptone, vitamin factors and glucose, energetic, sources promote the growth of bacteria count.

Malt Extract Agar (EMA) (BK045)

The medium is based on the malt extract, it is rich in carbohydrates, provides all the nutrients needed for the metabolism of yeasts and molds.

Microbial load

The microbial load of syrup was evaluated according to the following test (Geneviève *et al*., 2011). 100 µl of the syrup was taken in 900 µl of sterile distilled water. Then a series of dilution (10^{-2} to10^{-4}) were prepared. 100 µl of each dilution was spread on the surface of Petri dishes (9 cm) containing 25 ml of PCA environments for bacteria or EMA (20 g malt extract, 20 g agar) for fungi. The incubation was performed for 24 h at 37 °C for PCA and 7 days at 30 ° C for EMA. The results are provided by CFU per µl of syrup.

Animals

Male Wistar Rats (100-150g) were kept in well-ventilated environment and had a free access to water and food ad libitum and were housed in a quiet room under a "12-h light: 12-h dark" cycle for 2 weeks before experimentations.

Acute toxicity

Eighteen male rats are divided into 3 groups, each group containing 6 animals. All the groups were orally fed with aqueous extract in increasing doses of 200, 1000 and 5000 mg/kg b.w. The animals were observed for 2 h for any behavioral changes, neurological and autonomic profiles or cases of death after 24 h, 72 h and 5 days.

The animals were observed for obvious toxic symptoms and mortality in each group during 5 days by studying a single administration of four doses of aqueous extract: the general behavior of the animet al., the weight, the morphological appearance of organs (liver, spleen, stomach and kidneys), and the relative organ weights in comparison with the control group, calculated by the following formula: ROW = (organ weight / body weight) x1000 (Ramadan and *et al*., 2010).

Flow cytometer

The animals are anesthetized by "ip" with sodium pentobarbital at the dose of 30 mg/kg, 24 h, after treatment with extract of five plants and the syrup. The blood is collected in heparinized tubes, and then, subjected to analysis by FCM (flow cytometer Epics-XL MCL type). FCM was used to evaluate the proportion of leukocyte sub-populations. This is based on cell morphology, size and structure.

Aspartate Aminotransferarse (ASAT) and Alanine Aminotransferase (ALAT) level

The ALAT transfer the amino group of the alanine (500 mmol/L) on the 2-oxoglutarate (12 mmol/L) to form pyruvate and glutamate. The addition of pyridoxal phosphate (0.25 ml/0.1 mmol/L) in the reaction mixture gives the maximum level of ALAT catalytic activity. The reaction between pyruvate and NADH (0.20 mmol/L) is catalyzed by lactate dehydrogenase (LDH) (≥ 1.8 kUl/L) to form lactate and NAD+. The absorbance decrease due to consumption of NADH is measured at 340 nm and is directly proportional to the ALAT activity in the sample. Endogenous pyruvate disappears during the incubation period.

For Aspartate aminotransferase, it catalyzes the transamination of aspartate (240mmol/l) and 2-oxoglutarate (12mmol/l) to form L-glutamate and oxaloacetate. The addition of pyridoxal phosphate (0.1mmol/l) in the reaction mixture gives the maximum level of ASAT catalytic activity. Oxaloacetate reduced L-malate by malate dehydrogenase (≥0.6 kU/l); NADH (0.20 mmol/l) is converted to NAD simultaneously. The decrease in absorbance due to consumption of NADH is measured at 340 nm and is directly proportional to the activity of ASAT in the sample. Endogenous pyruvate disappears because of the LDH (≥0.9kU/l) reaction during the incubation period.

Anti-inflammatory activity

Acute inflammation in the rats was produced according to the method described by (Winter and *et al*., 1961). Five groups on rats each containing five animals per group were used for the study.

Group 1 concerned the control group receiving saline solution (0.9%) 10 ml/kg, animals in group 2 were given 10 mg/kg of indomethacin orally while groups 3, 4 and 5 received the plant extracts at doses of 200, 1000 and 5000 mg/kg b.w, respectively.

Animals were given a saline solution, indomethacin and the appropriate dose of the extract depending on the group, 1 hour before administration of an intradermal injection of carrageenan (0.1 ml of a 0.5 % solution in 0.9% saline solution) into the plantar region of the right hand paw. The paw size was measured before injection of carrageenan and each hour during 6 h.

Measurement of paw size was carried out by wrapping a piece of cotton thread round the paw and measuring the circumference with a meter rule. The average increase in paw size of each group was calculated and compared with the control (saline solution) and indomethacin groups. The percentage inhibition was then calculated (Ayoola *et al*., 2009)

$$\% \text{ inhibition} = \frac{(St\text{-}S0)\ \text{control} - (St\text{-}So)\ \text{treated}}{(St\text{-}S0)\ \text{control}} \times 100$$

St = the mean paw size for each group after carrageenan treatment

S0 = the mean paw size obtained for each group before carrageenan injection.

Statistical analysis

Data were expressed as the mean ± SEM. Comparisons of means were performed by using the t-test of Student. The level of statistical significance was set at $p < 0.05$.

RESULTS

Phytochemical screening

As shown in Table 1, phytochemical screening of extract of five plants studied revealed an abundance of flavonoids, tannins, mucilages, anthraquinones, coumarins, oses and holosides, compounds that known for their anti-inflammatory effects. However, saponosids were not detected (Table1).

Determination of total polyphenols

The total of polyphenols content was determined from regression calibrate curve (Y= 463.03x -4.7419; R2= 0.9994) and expressed in gallic acid equivalent. The result was calculated at 14.20 mg gallic acid/g of extract.

Determination of tannins

The total of tannins content was determined from regression calibrate curve (Y= [Ac280-Am280)+0.0065]/0.0029) and expressed in equivalent of gallic acid. The result was calculated at 1.35 mg gallic acid/g of extract.

Determination of flavonoids

The total flavonoids content was also determined from regression calibrate curve (Y= 0.0245x + 0.2602; R2= 0.9357) and expressed in quercetin equivalent (QE). The result was calculated at 16,934 mg /l QE.

Determination of catechins

The catechins content was determined from regression calibrate curve (Y= 340.72x -50.56; R2= 0.998) and expressed in D-catechin equivalent. The result was calculated at 1.22 mg D-catechin/g of extract.

Microbiological control syrup

The counting of bacterial colonies on PCA medium was made for the 10^{-2} dilution is approximately 4.10^4 CFU / ml. On fungi count was made for the 10^{-1} dilution and gave 7.4 10^3 CFU / ml. The bacterial load was found relatively higher fungal burden. Comparing these results with the standards of the International Pharmacopoeia (total viable aerobic count. not more than 10^2 micro-organisms (aerobic bacteria and fungi) per gram, per milliliter or per patch (including the adhesive and backing layer) (WHO, 2012), the syrup is considered contaminated, hence the necessity of sterilization and the use of an effective preservative.

Acute toxicity

The results revealed that the doses of 200 and 1000 mg/kg, b.w. of aqueous extract did not produce any acute toxic effects or deaths in the two tested groups of rats (N=4). However for the ranging dose of 5000 mg/kg, b.w., animals manifested some side effects like tiredness, slight decrease in relative liver weight and the appearance of ulcerations in the gastric mucosa (Table 2).

Flow cytometer

Blood analysis of treated rats with syrup induces a decrease mainly in the percentage of lymphocytes compared to the control due to their migration on inflammation sites (Figure 1) (Ivan and *et al*., 2002; Anthony and *et al*., 2007) and this immunosuppressive action results also from the inhibition of the synthesis and release of many lymphokines, with inhibition of the activation of T lymphocytes as the general case for anti-inflammatory medicines. However, we don't note any significant modification on granulocytes subpopulation number (neutrophils), which are the most important cells involved in inflammatory process certainly due to the anti-inflammatory property of the extract. There is also a remarkable decrease of monocytes in the blood, due mainly to the migration into inflamed tissue through chemokine receptors and adhesion molecule.

ASAT and ALAT Transaminases level:

The effect of the administration of syrup on some biochemical parameters is presented in Figure 2. Biochemical analysis of blood showed a significant increase ($p<0.001$) in the levels of ASAT and ALAT in the Carrageenan and Indomethacin treated rats when compared to the control rats. We note also that the levels of ASAT and ALAT in the syrup treated groups increase significantly at 24h and 7 days.

Calculating the ASAT / ALAT ratio, syrup, which is <1 (0.93 at 24h and 0.75 at 7 days), we can deduce that the increase of two enzymes levels is harmless (Nicole and *et al*., 2009).

Anti-inflammatory activity

Injection of carrageenan into the hind paw induced a progressive edema reaching its maximum at 3 h. This increase was greater in the treated than in saline treated with the extract and indomethacin.

The extract administered orally at different doses (0.2, 1 and 5 g / kg b.w) has an important anti-inflammatory effect from 3h, These results are significant from 5h ($p < 0.05$). Thus, the extract at 1g/kg, b.w, has the best anti-inflammatory effect (82.98%) compared to that of indomethacin at 5h after induction of inflammation (Figure 3, Table 3).

Based on these results, we opted for a syrup formulation dosed at 1g/kg, b.w., and then it was administered to animals. Formulated syrup has a significant anti-inflammatory activity from 3h, compared to that of indomethacin or 84.04% at 5 h, the significance is important at 5h and 6h (Figure 4). No signs of acute toxicity were noted in animals treated with syrup (1g/kg, b.w) (Table 4).

Table 1. Phytochemical screening of the extract of five plants studied

chemical constituent	Extract of five plants
Flavonoids	+
Saponins	-
Tannins	-
Gallic	+
Catechin	+
Coumarins	+
Anthraquinones	+
Steroidal sapogenins	-
Cardiac glycosides	+
Free Quinones	+
Mucilages	+
Oses and holosides	+

Presence of chemical compounds is: (+) = presence; (-) = absent

Table 2. Effect of aqueous extract at a dose 5g/kg b.w. on the relative weight of the liver, spleen, stomach and kidneys. n= 4 for each group

Groups	**Dose (g/kg b.w.)**	**Body weight (g)**		**Relative organ weights (g)**	
		Liver	**Stomach**	**Kidney**	**Spleen**
Control	0	148±7.21	38.27±0.48	10.58±0.09	3.20±0.09
Treated	5	2.83±0.07	158±23.59	36.43±0.61	9.90±0.06
Significativity		3.73±0.09	2.70±0.10	-------	*(p<0.05)
		Ns	Ns	Ns	

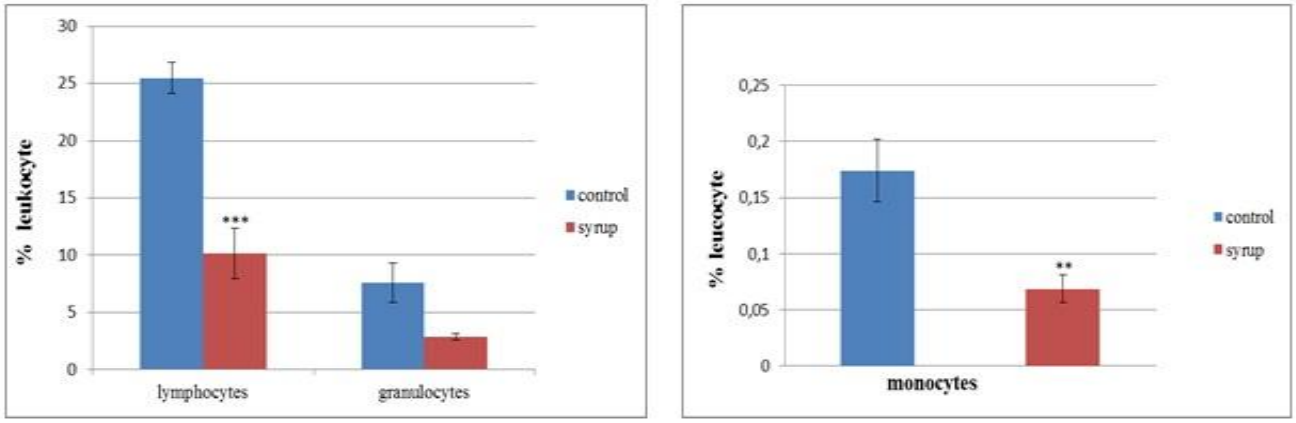
Table 3. Anti-inflammatory effect of extract of five plants studied, n= 9.

Treatment	**Dose (mg/kg)**	**Inhibition of edema (%).**			
		3h	4h	5h	6h
Indomethacin recipe	10	2.34 ± 0.081	2.34 ± 0.099	2.28 ± 0.093	2.28 ± 0.093
	200	2.56 ± 0.107	2.44 ± 0.081	2.4 ± 0.104	2.52 ± 0.177
	1000	2.72 ± 0.203	2.58 ± 0.124	2.48 ±0.101	2.54 ± 0.169
Significativity	5000	2.48 ± 0.124	2.4 ± 0.130	2.38 ± 0.115	2.36 ± 0.102
		ns	ns	ns	p<0.05

Table 4. Effect of syrup on the relative weight of the liver, spleen, stomach and kidney n= 6

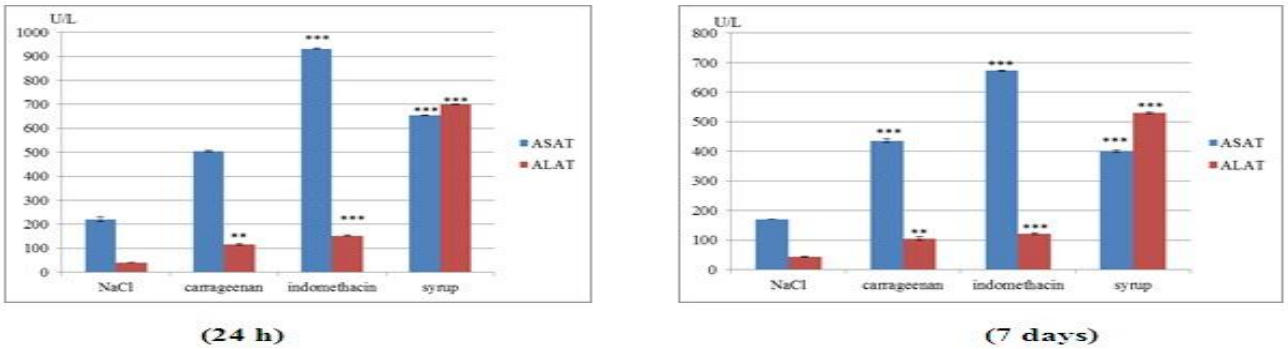
Groups	**Dose (g/kg b.w.)**	**Body weight (g)**		**Relative organ weights (g)**	
		Liver	**Stomach**	**Kidney**	**Spleen**
Control	0	148±7.21	38.27±0.48	10.58±0.09	3.20±0.09
Treated	1	2.83±0.07	111.33±16.16	38.69±0.51	12.43±0.22
Significativity		3.40±0.01	3.02±0.03	-------	--------
		ns	ns	ns	ns

Fig 1. Effect of syrup in lymphocytes, granulocytes, and monocytes sub-populations of rats during 7 days of treatment.



Values are expressed as Mean ±SEM; n = 10 for each group. *** p < 0.001; **: p<0.01.

Fig 2. Effect of syrup in transaminases levels after 24 hours and 7 days of treatment



Values are expressed Mean ±SEM; n = 3 for each groups. *** p < 0.001, p<0.01.

Fig 3. Anti-inflammatory effect of extract compared to indomethacin n=9, * : p<0.05 at 6h

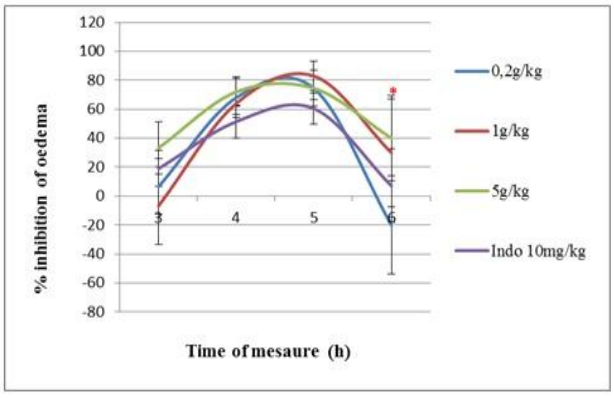
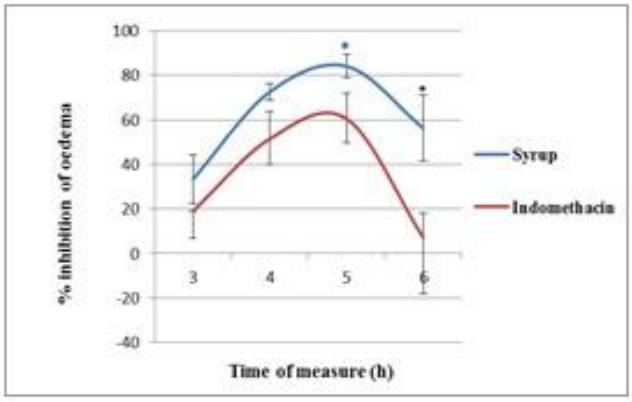


Fig 4. Anti-inflammatory profile of syrup, n=9, *: p<0.05



DISCUSSION AND CONCLUSION

The recipe evaluated in this study is recommended by traditional practitioners in the region southeast of Morocco to treat inflammatory diseases. In this study, we confirm in the laboratory the anti-inflammatory activity of this recipe by the model of acute edema of the rat paw induced by carrageenan described by (Winter and *et al*., 1961). We also demonstrated an increase of ALAT level which may be the result of liver damage after treatment with carrageenan and indomethacin (Nicole and *et al*., 2009). Indeed, the levels of transaminases in treated animals decreased at 7 days after a single administration compared to 24 h; this

decrease is due mainly to the metabolism of product in the body.

Generally an increase in ASAT / ALAT rate is considered intermediate (5-15 X the upper limit in normal conditions), which may be the result of lower limbs edema, fever, jaundice or other (Nicole and *et al*., 2009). Thus, if the ratio ASAT / ALAT> 1; we must mention hepatitis cytolysis or infringement of centrilobular regions by vascular mechanisms. This is not the case for the syrup, where the ASAT / ALAT ratio is less than 1.

The evolution of the volume of edema following injection of carrageenan is time-dependent and is divided into two phases: The first phase from 0 to 1 hour and a second phase from 1 to 5 hours. Indeed, the injection of the carrageenan-induced increase of mRNA synthesis of cyclooxygenase 2 (COX-2) where the increase in the concentration of this enzyme has a peak at 1 hour. This increase is accompanied by an increase in the synthesis of prostaglandins (PGs), mainly prostaglandin E2 (PGE2) (peak at 2 hours) involved in the process of pain and inflammation (Posadas and *et al*., 2004; Nantel and *et al*., 1999).

The anti-inflammatory activity of syrup, formulated was significant at the later phase, 5h after carrageenan injection. This explains the delayed effect of non-steroidal anti-inflammatory drugs (NSAIDs) such as acetyl salicylic acid, which has no effect at 1h. This is in relation to their mode of action involves the inhibition of PGs by the stimulation of both COX-1 and COX-2 (in Chatter and *et al*., 2011).

The anti-inflammatory activity of recipe is due to their richness in total polyphenols, flavonoids, tannins and mucilages. The anti-inflammatory of flavonoids and mucilages has been proven by several studies (González-Gallego and *et al*., 2007; García-Lafuente and *et al*., 2009; Sindhu and *et al*., 2012). These molecules inhibit cyclooxygenase, phospholipase A2 and lipoxygenase. arachidonic acid (Hyun and *et al*., 2004). The anti-inflammatory and antioxidant activity of tannins has been shown by study of Giovannelli and *et al*., (2000) (in Mavar and *et al*., 2004). Where they showed that they might both have a protective and a therapeutic potential in oxidative damage related to pathologies. In addition, the work of Perchellet *et al*., (1996) concluded also that some foliage tannins have potent antioxidant and anti-inflammatory activities.

The significant anti-inflammatory effect of our syrup observed after 5h and 6h is prolonged and its bioavailability is higher compared to indomethacin. Thus, this effect is due mainly to the synergistic effect of plants mixture that favored certainly the anti-inflammatory activity.

ACKNOWLEDGEMENTS

We are grateful to Professor ENNABILI Abdeslam for botanical identification of these plants and the Center Emirates Wildlife Propagation (ECWP) "Region, Missouri (Morocco) for the donation of animals. This work is supported by FP-7 INCO- MAP2ERA and EMAP.

REFERENCES

Abou El-Hamd H. Mohamed. Chemical Constituents and Biological Activities of Artemisia herba-alba. *Rec. Nat. Prod*, 4(1), 2010, 1-25.

Annette K & Claudia R. Phenolic Antioxidant Compounds Produced by in Vitro Cultures of Rosemary (Rosmarinus officinalis) and Their Anti-inflammatory Effect on Lipopolysaccharide-Activated Microglia. *Pharmaceutical Biology*, 44(6), 2006, 401–410.

Anthony LD, Miranda R, Richard ML. Immunité, La réponse immunitaire dans les maladies infectieuses et inflammatoires. Editions New science press, 2007.

Ayoola GA, Akpanika GA, Awobajo FO, Sofidiya MO, Osunkalu VO, Coker HAB & Odugbemi TO. Anti-Iinflammatory Properties of the Allanblanckia floribunda (Guttiferae). *Bot. Res. Intl*., 2(1), 2009, 21-26.

Bellakhdar J. la pharmacopée marocaine traditionnelle, Médecine arabe ancienne et savoirs populaires. Ibis Press, Paris, 1997.

Bruneton J. Pharmacognosie, Phytochimie, Plantes médicinales. In: Technique et Documentation, 2nd éd., Lavoisier (ed). Paris, 1993.

Catalina SR, Karina A, Victoria R, Adrián AV, Silvia M. Synergistic antioxidant and antibacterial activity of rosemary plus butylated derivatives. *Food Chemistry*, 115, 2009, 456–461.

Chatter RR, Tarhouni S, Kharrat R. Criblage de l'effet anti-inflammatoire et analgésique des algues marines de la mer méditerranée. *Archs. Inst. Pasteur Tunis*, 88, 2011, 1–4.

Christophe W. Ethno-pharmacology of Medicinal Plants, Asia and Pacific. Edit. C. Wiart © Humana Press Inc., Totowa, NJ, 2006.

Dalmas E, Clement K, Guerre-Millo M. Defining macrophage phenotype and function in adipose tissue. *Trends Immunol*, 32, 2011, 307-314.

Derwich E, Benziane Z & Taouil R. GC/MS Analysis of Volatile Compounds of the Essential Oil of the Leaves of Mentha pulegium growing in Morocco. *Chem. Bull. "POLITEHNICA" Univ. (Timisoara)*, 55(69), 2010, 103-106.

Diallo A. étude de la phytochimie et des activités biologiques de syzygium guineense willd. (MYRTACEAE), *thèse de pharmacie*, 2005, 43.

Doha MA, Sahar YA. *In vivo* evaluation of antioxidant and anti-inflammatory activity of different extracts of date fruits in adjuvant arthritis. *Pol. J. Food Nutr. Sci*, 54(4), 2004, 397–402.

Dohou N, Yamni K, Tahrouch S, Idrissi Hassani LM, Badoc A, Gmira N. Screening phytochimique d'une endémique Ibéro-Marocaine, *Thymelaea lythroides*. *Bull. Soc. Pharm. Bordeaux*, 142, 2003, 61–78.

Ferreira A, Proença C, Serralheiro MLM & Araújo MEM. The *in vitro* screening for acetylcholinesterase inhibition and antioxidant activity of medicinal plants from Portug *et al*., *Journal of Ethnopharmacology*, 108, 2006, 31–37.

García-Lafuente A, Eva Guillamón, Ana VMA, Rostagno, José Alfredo M. Flavonoids as anti-inflammatory agents: implications in cancer and cardiovascular disease. *Inflamm. Res*., 58, 2009, 537–552.

Geneviève M, Marie-Claude B, Jacques L, Marie L. Méthode de laboratoire, dénombrement des bactéries et moisissures cultivables de l'air prélevées sur filtre de polycarbonate. *Publié par IRSST*, 2011.

Giovannelli L, Testa G, De Filippo C, Cheynier V, Clifford MN, Dolara P. Effect of complex polyphenols and tannins from red wine on DNA oxidative damage of rat colon mucosa *in vivo*. *European Journal of Nutrition*, 39, 2000, 207–212.

González-Gallego J, Sánchez-Campos S, Tuñón MJ. Anti-inflammatory properties of dietary flavonoids. *Nutr Hosp*, 22(3), 2007, 287-93.

Hmiri SH, Rahouti M, Habib Z, Satrani B, Ghanmi M & El Ajjouri M. Evaluation du potentiel antifongique des huiles essentielles de Mentha pulegium et d'Eucalyptus camaldulensis dans la lutte biologique contre les champignons responsables de la détérioration des pommes en conservation. *Bulletin de la Société Royale des Sciences de Liège*, 80, 2011, 824 – 836.

Hyun PK, Kun HS, Hyeun WC, Sam SK. Anti-inflammatory Plant Flavonoids and Cellular Action Mechanisms. *J Pharmacol Sci*., 99, 2004, 229-245.

Ivan MR, Jonathan B, David M. Immunologie, 3rd ed, De Boeck & Larcier. 2002.

Karumi Y, Onyeyili PA, Ogugbuaja VO. Identification of active principles of *M. balsamina* (Balsam Apple) leaf extract. *J. Med. Sci*., 4, 2004, 179-182.

Lamaison JL and Carnat A. Teneurs en principaux flavonoïdes des fleurs et des feuilles de *Crataegus monogyna* Jacq. et de *Crataegus laevigata* (Poiret) DC., en fonction de la période de végétation. *Plantes Med. Phytother*., 25(1), 1991, 12-16.

Leland JC, Ara K, Peter BKSL, James AD, Harry LB. Natural products from plants, 2nd ed., Taylor & Francis Group. LLC, 2006.

Mavar MH, Brkic D, Marie DEP, Quetin-Leclercq J. *In vivo* anti-inflammatory activity of Alchornea cordifolia (Schumach. & Thonn.) Müll. Arg. (Euphorbiaceae). *Journal of Ethnopharmacology*, 92, 2004, 209–214.

Mun FY, Lee FA, Omar ZA, Ibrahim MS, Hesham AA, Mohd ZA. Anti-inflammatory and Analgesic Effects of Elephantopus tomentosus Ethanolic Extract. *J Acupunct Meridian Stud*, 2(4), 2009, 280−287.

N'guessan K., Beugré K., Guédé N. Z., Dossahoua T. & Laurent A. A., Screening phytochimique de quelques plantes médicinales ivoiriennes utilisées en pays Krobou Agboville, Côte-d'Ivoire, Sciences & Nature, 6 (1), 2009, 1 – 15.

Nantel F, Denis D, Gordon R, Northey A, Cirino M, Metters KM and Chan CC. Distribution and regulation of cyclooxy-genase-2 in carrageenan-induced inflammation. *Br. J. Pharmacol*., 128, 1999, 853-859.

Nicole JS, Olivier P, Jacques C. Patient avec des tests hépatiques perturbés : que faire ? *Rev. Med. Suisse*, 5, 2009, 2410-2414.

Norman RF, Olayiwola A, Audrey SB, Djaja DS, Zhengang G. Place des plantes médicinales dans la thérapeutique. *Bulletin de l'organisation mondiale de santé*, 64(2), 1986, 159-175.

Patrícia CD, Mary AF, Ana P & João EC. Antiulcerogenic activity of crude hydroalcoholic extract of *Rosmarinus officinalis* L. *Journal of Ethnopharmacology*, 69, 2000, 57–62.

Perchellet EM, Gali HU, Makkar HPS, Perchellet JP. Ability of tannins extracted from the leaves of various trees and shrubs to inhibit the biomarkers of tumor promotion in mouse skin *in vivo*. *International Journal of Oncology*, 9, 1996, 801–809.

Posadas I, Bucci M, Roviezzo F, Rossi A, Parente L, Sautebin L, Cirino G. Carrageenan-induced mouse paw oedeme is biphasic, age-weight dependant and displays differential nitric oxide cyclooxygenase-2 expression. *British journal of pharmacology*, 142, 2004, 331-338.

Ramadan A, Soliman G, Sawsan SM, Salwa MN, Rehab F. Abdel-Rahman. Evaluation of the safety and antioxidant activities of Crocus sativus and Propolis ethanolic extracts. *Journal of Saudi Chemical Society*, 16, 2012, 13-21.

Sarneckis C, Dambergs RG, Jones P, Mercurio M, Herderich MJ, Smith P. Quantification of condensed tannins by precipitation with methyl cellulose: development and validation of an optimised tool for grape and wine analysis. *Australian Journal of Grape and Wine Research*, 12(1), 2006, 39-49.

Sindhu G, Ratheesh M, Shyni GL, Nambisan B, Helen A. Anti-inflammatory and antioxidative effects of mucilage of Trigonella foenum graecum (Fenugreek) on adjuvant induced arthritic rats. *International Immunopharmacology*, 12, 2012, 205–211.

Singleton VL and Rossi JA, Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. *Am J Enol Vitic*, 16, 1965, 144–158.

Sotelo-Félix JI, Martinez-Fong D, Muriel P, Santillán RL, Castillo D & Yahuaca P. Evaluation of the effectiveness of Rosmarinus officinalis (Lamiaceae) in the alleviation of carbon tetrachloride induced acute hepatotoxicity in the rat. *Journal of Ethnopharmacology*, 81, 2002, 145-154.

Swain T and Hillis WE. The phenolic constituents of prunus domestica L. The quantitative analysis of phenolic constituents. *Journal of the science of food and and agriculture*, 10, 1959, 63-68.

Vinegar R, Schreiber W, Hugo R. Biphasic development of carrageenan oedema in rats. *J. Pharmacol. Exp. Ther*., 166, 1969, 96-103.

Winter CA, Risley EA and Nuss CW. Carageenan-induced oedema in hind paws of the rat as an assay for anti-inflammatory drugs. *Proc. Soc. Expt. Biol. Med*, 111, 1962, 544-547.

World Health Organization, supplementary information, S.3.7 microbiological quality of non-sterile Products: recommended acceptance criteria for pharmaceutical preparations: Final text for revision of The International Pharmacopoeia, 2012.

Xu H, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, Sole J, Nichols A, Ross JS, Tartaglia LA, Chen H., Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *J Clin Invest*, 112, 2003, 1821-1830.