## NATURAL DRUGS

# ANTI-INFLAMMATORY ACTIVITY OF ACHILLEA AND RUSCUS TOPICAL GEL ON CARRAGEENAN-INDUCED PAW EDEMA IN RATS

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**Abstract:** The anti-inflammatory activity of Achillea and Ruscus extracts was studied in comparison with diclofenac sodium topical gel (diclosal Emulgel), using the carrageenan induced paw edema model in Albino rats. Gel formulation was prepared containing 6% of each extract in gel base, namely sodium carboxymethylcellulose (NaCMC). The kinetics of drug release from the prepared formulation was studied separately in each case. Results showed that the release follows the Higuchi square root equation. The pharmacological screening revealed that the percent reduction of edema for Achillea extract and Ruscus extract were 48.1% and 18.8%, respectively, while diclosal Emulgel produced 47% reduction of edema.

**Keywords:** Achillea, Ruscus, carrageenan-induced paw edema

Achillea fragrantissima (*Asteraceae*) is a common plant in the Mediterranean region and easily found growing in fields and on roadsides. It contains high percentage of flavonoids, tannins, volatile oils, sterols and triterpenes. Also, it contains unsaturated amides, and sesquiterpene lactones (1). Achillea was highly valued as a medicinal plant for its antiseptic properties. It was used to cover cuts and sores and hasten scar tissue formation, but till now no clinical uses for Achillea fragrantissima is described (2).

Ruscus aculeatus (*Liliaceae*) is growing wildly in the forests. It contains steroidal saponins derived from ruscogenin and neoroscogenin in addition to essential oils, flavonoids, resin and minerals. Ruscus is used in supportive therapy for venous insufficiencies such as circulatory disorders, edema, thrombophlebits, swelling and also used as diuretic. Since it may cause vascular contraction, caution should be used in individuals with hypertension (3). In very rare cases it may cause gastrointestinal upset.

This work was suggested on the basis of presence of flavonoidal and ruscosides constituent in the two investigated parts of plants, respectively, namely Achillea fragrantissima Linne, *Asteraceae* (dried flowers) and Ruscus aculeatus, *Liliaceae* (leaves). The aim of this study was to study their possible anti-inflammatory effect by formulation of the two extracts in a suitable gel formulation for topical administration and comparison of the prepared gels with a standard gel in the market by using the carrageenan-induced edema model.

The partition coefficient was determined for the two extracts as a measurement of a drug's lipophilicity. The *in vitro* release study through cellophane membrane of each prepared gel was studied using a stainless steel diffusion cell.

## MATERIALS AND METHODS

Sodium carboxymethylcellulose (Na-CMC), normal saline and diclofenac sodium emulgel (diclosal Emulgel) were obtained from Dar Al Dawa, Na'ur, Jordan. Carageenan (0.1% solution in normal saline) was obtained from Sigma Chemical Co. Steinheim, Germany. Ruscus (leaves) and Achillea (flowers) were obtained from the Jordanian market in March 2004. Organic solvents i.e. chloroform, methanol, acetone and octanol were of spectroscopic grade.

Male Albino rats (weighing 200-250 g) of local strain were used for the anti-inflammatory study by carrageenan induced rat paw edema method. The animals were kept for one week in the animal house before the experiment to be acclimatized, and they were maintained on unrestricted supplies of food and water.

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## Extraction

Achillea flowers (500 g) were extracted by 70% ethanol till exhaustion. The combined extracts were evaporated to dryness to give 51 g of solid residue.

Ruscus leaves (100 g) were extracted by 70% ethanol till exhaustion. The combined extracts were evaporated to dryness to give 9 g of solid residue.

## Determination of partition coefficient ($K_{o/w}$)

10 mg of each extract was added to 50 mL of distilled water in a stoppered bottle and shaked at $37^{\circ}C$ in water bath shaker (GFL 1083, Germany) overnight. Octanol (50 mL) was added to the bottle with shaking at the same temperature for 24 h. The two layers were then separated using a separatory funnel, and the absorbance of drug in the aqueous layer was determined, at 338.6 nm for Achillea extract and at 236 nm for Ruscus extract. The drug concentration in aqueous layer was determined by a calibration curve for each extract. The concentration of drug in octanol was determined by difference. The partition coefficient of drug between octanol and water ($K_{O/W}$) was calculated from the equation:

$$K_{o/w} = C_o / C_w$$

where: $K_{o/w}$ is the partition coefficient, $C_o$ is the concentration of drug in octanol and $C_w$ is the concentration of drug in water.

## Preparation of topical extract gel

Achillea and Ruscus gels were separately prepared according to the formula in Table 1, by dissolving weighed amount of extract in the needed volume of water, and then added to 5% of the base. The specified amounts of glycerol and propylene glycol were added with continuous stirring at room temperature for 15 min using mechanical stirrer. Each gel was kept separately in dark cool place overnight ($10\text{-}15^{\circ}C$).

## In vitro release study through cellophane membrane

The release of extract from its prepared gel was studied using a stainless steel diffusion cell. More specific, 2 g sample of each formulation was accurately weighed and placed in the hollow bottom of the diffusion cell (donor part), the Fisher 27/30 standard membrane was adjusted between the two joints and the tow screw was fitted, and the cell was then placed in a beaker containing 600 mL of phosphate buffer (pH = 7.4), which was adjusted to the water bath of the dissolution apparatus (ERWEKA GmbH, Heusentamm, Germany) operating at $37^{\circ}C$ and 100 rpm.

5 mL samples were withdrawn at predetermined time intervals and immediately replaced with equal volumes of phosphate buffer, then analyzed spectrophotometrically at mentioned above $\lambda_{max}$, using UV/Vis apparatus (model 7800 Jasco, Japan).

The kinetics of the release process was studied by analyzing the release data using two kinetic

Table 1. The content of gels formulations.

| Material | Concentration (w/w) % |
|---|---|
| Extract | 6 |
| Sodium carboxymethylcellulose | 5 |
| Glycerol | 10 |
| Propylene glycol | 5 |
| Water up to | 100 |

Table 2. Rats groups used in the carrageenan-induced edema model (each comprised of 4 rats) with the type of extract composition and dose received for each group.

| Group | Extract composition and dose received |
|---|---|
| 1- Control group | Received 2 g of sodium carboxymethylcellulose (gel base only). |
| 2- Treated group (1) | Received 2 g of commercial diclofenac gel (Diclosal Emulgel). |
| 3- Treated group (2) | Received 2 g of 6% of NaCMC-Achillea extract gel. |
| 4- Treated group (3) | Received 2 g of 6% of NaCMC-Ruscus extract gel. |

Table 3. Partition coefficient, release rate constant and $r^2$ values for Achillea and Ruscus extracts.

| Formula | $K_{o/w}$ | Zero-order rate constant ($K_0$) | Higuchi square root rate constant ($K_1$) |
|---|---|---|---|
| Achillea extract | 0.828 | 0.085 ($R^2 = 0.86$) | 1.184 ($R^2 = 0.95$) |
| Ruscus extract | 0.638 | 0.122 ($R^2 = 0.94$) | 1.627 ($R^2 = 0.96$) |

Table 4. Effect of topical administration of extract gel and diclofenac gel on carrageenan-induced paws edema in rats.

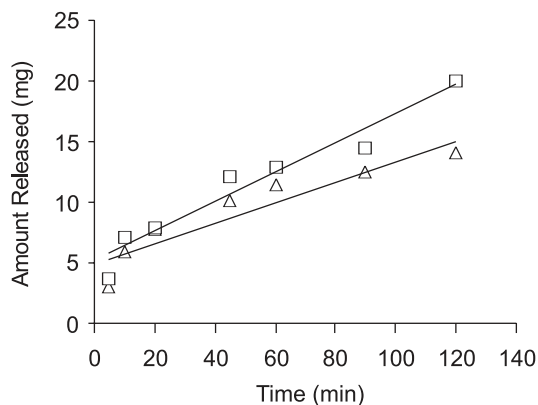| Treatment | Mean % increase in paw weight | % reduction of edema |
|---|---|---|
| Gel bases pure (control) | 34.5 ± 1.1 | 0.0 |
| Diclosal Emulgel (diclofenac sodium) | 18.3 ± 1.5 | 47.0 ± 0.8 |
| Extract of Achillea | 17.9 ± 1.3 | 48.1 ± 1.1 |
| Extract of Ruscus | 28.0 ± 1.8 | 18.8 ± 1.6 |



Figure 1. A linear plot of amount released versus time for the release data in accordance with the zero-order equation. Achillea extract (-□-) and Ruscus extracts (-Δ-).
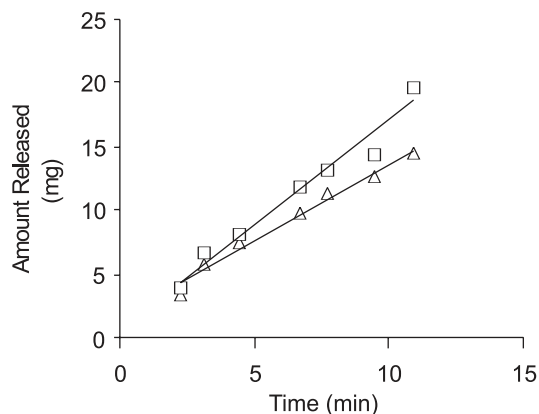


Figure 2. A linear plot of amount released versus square root of time for the release data in accordance with the Higuchi square root equation. Achillea extract (-□-) and Ruscus extracts (-Δ-).

equations, the zero-order equation and the Higuchi square root equation.

## Screening of anti-inflammatory activity of extracts gel

The anti-inflammatory activities of the agent under study were evaluated by using the carrageenan-induced edema model. More specific, rats were divided into 4 groups, each comprised of four rats as shown in Table 2.

The gel was applied to the planter surface of the left hind paw by gently rubbing 50 times with the index finger. Three hours after the dose, 0.1 mL of 1% carrageenan solution in normal saline was injected subplantarly into the treated paw. Three hours after the carrageenan injection, the right and the left paw were cut at the tibiotarsal articulation under chloroform anesthesia and weighed (4-6).

The percentage increase in the weight of the left paw in comparison with the right one of each rat, as an indication of the inflammation produced, was calculated by the following equation:

% increase in paw weight = (L – R / L) x 100

where: R is the weight of right leg and L: is the weight of left leg.

The mean percentage of inflammation reduction was measured from the difference in % swelling between treated groups and the control group by the following equation:

% reduction of edema = (C – T / C) x 100

where: C = % swelling of control group (untreated) and T = % swelling of treated group.

## RESULTS AND DISCUSSION

Achillea contains volatile oils, mainly azulenes which are anti-inflammatory and could be lost during evaporation. However, it contains also several known anti-inflammatory compounds as flavonoids. Also Ruscus contains ruscosides which are known as anti-inflammatory. In the UV spectrum of these extract, fortunately, the only very clear peaks were found at 338.6 nm for flavonoids in Achillea and 236 nm for ruscosides in Ruscus. No other significant peaks were found.

The partition coefficient of Achillea extract and Ruscus extract were 0.828 and 0.638, respectively, indicating that both extracts can be applied topically depending on their lipid solubility.

The prepared gel formulations were found to have acceptable rheological properties. Figure 1 shows that the Ruscus gel release was about 20 mg within 120 min, whereas only 14 mg was released from Achillea extract at the same time. This may be attributed to the lower aqueous solubility of Achillea extract.

In order to describe the kinetics of the release process of drug through cellophane membrane in the two gel formulations, two equations were used, namely, the zero-order rate equation and the Higuchi square root equation (7, 8). The release data obtained from the two formulations were plotted in accordance with the zero-order equation i. e. amount released as a function of time (Figure 1).

The release data obtained from the two gel formulations were plotted in accordance with the Higuchi square root equation, i.e. the amount released as a function of the square root of time (Figure 2). A linear relationship was obtained with $r^2$ value close to unity as shown in Table 3, indicating that the release kinetics follows the Higuchi square root equation and that the release process is diffusion-controlled (9, 10).

The pharmacological screening was carried out by using the carrageenan-induced edema model to evaluate the possible anti-inflammatory activity of the two extracts. As shown in Table 4, the reduction of edema was 48% for Achillea extract, nearly equally effective to that produced by the standard (Diclosal Emulgel) 47%, indicating an anti-inflammatory effect. This good result was not achieved by using Ruscus extracts, the reduction of edema was only 18%, which indicates that the anti-inflammatory effect of Ruscus extract was very low compared to that obtained for Achillea extract as well as that obtained from the standard.

From this study it can be concluded that NaCMC can be successfully used as gel base and that the kinetic of drug release from this formulation can be described by Higuchi square root equation. Also it can be concluded that both extracts have an anti-inflammatory effect, with Achillea extract to be nearly equally effective as anti-inflammatory agent at the market – diclofenac sodium.

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