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Anti-inflammatory Activities of Colocynth Topical Gel

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Owing to the purgative effect of Colocynth extract when taken orally, the objective of this investigation was to prepare Sodium carboxymethyl cellulose (5%) topical gel formulations containing 3% of colocynth extract, hydrolyzed extract, or acetylated extract and to study their release through cellophane membrane and their permeability through hairless mouse skin. Also, to study the *in vivo* anti-inflammatory activity of the different types of colocynth extract using the carrageenan induced paw edema model in albino rats in comparison with the commercial Voltarin Emulgel®. The acetylated extract gel showed comparatively rapid permeability through hairless mouse skin, with low release rate through cellophane membrane. The pharmacological screening revealed that the percent reduction of edema produced by Colocynth extract was 45.39%, the hydrolyzed extract produced 54.11% inhibition and the acetylated extract produced 64.95%, while Voltarin Emulgel produced 63.35%. This means that acetylated colocynth extract can be used as an effective local anti-inflammatory agent.

Key words: Colocynth, gel, anti-inflammatory, permeability, carrageenan induced paw edema

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INTRODUCTION

The Colocynth, *Citrullus colocynthis* (Fam. Cucurbitaceae) was collected from the Maritime Plain between the mountains of Palestine and the Mediterranean. It is a powerful drastic hydragogue cathartic producing, when given in large doses, violent griping with, sometimes, bloody discharges and dangerous inflammation of the bowels (Galvez *et al*., 1996, Berrut *et al*., 1987). Recently, it could be proved to have antimutagenic (Farid, 1994) and anticancer (Jayaprakasam *et al*., 2003) effects.

The active principal, cucurbitacin B, was isolated from the chloroform extract of the fruit juice of Ecballium elaterium and showed a significant anti-inflammatory activity in mice (Yesilada *et al*., 1988). Also, in a previous study, topical Ecballium gel preparation showed systemic anti-inflammatory effect in rats (Aly and Mazen, 2003). Consequently, due to the presence of cucurbitacin B in Colocynth, it may have anti-inflammatory effects.

The objective of this investigation was to study the release of; colocynth extract, hydrolyzed extract and acetylated extract topical gel formulations (Sodium carboxymethyl cellulose; 5%) through cellophane membrane and its permeability through hairless mouse skin. Also, to study the *in vivo* anti-inflammatory activity of colocynth extract and its derivatives in rats.

MATERIALS AND METHODS

Drugs and chemicals: Sodium carboxymethyl cellulose (NaCMC) was from S and C Chemicals, (Amman, Jordan) Supplico. Chemicals. Carrageenan was supplied from Sigma Chemical Co. Steinheim, Germany, it was used as 1% solution in normal saline. Normal saline was from the market: Dar AL Dawa Na'ur, Amman, Jordan, (Batch No. 2302). Voltarin Emulgel ® was from the market: Novartis, Switzerland).

Animals: 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.

Extraction of colocynth: One kilogram of colocynth fruits was extracted by ethanol (70%) till exhaustion. The combined extracts were evaporated to dryness to give 40 g solid residue. Twenty grams were kept for further study and the other 20 g were directed to further hydrolysis and acetylation as follows:

Hydrolysis: In order to investigate the anti-inflammatory action of Cucurbitacins as aglycones in comparison with their glycosides, 10 g of extract were dissolved in 150 mL of ethanol-water mixture (1:2) and reflexed with hydrochloric acid (final solution 5% HCl) for about 3 h. After completion, the aglycones were extracted by shaking with ethyl acetate three times. The combined ethyl acetate extracts were evaporated till syrupy to give 5.5 g and kept for further investigation.

Acetylation: This procedure was done to compare the activity of glycosidal acetates in comparison with natural form. Ten gram of colocynth extract was acetylated by dissolving in the least amount of pyridine and then acetylated overnight at room temp by adding sufficient amount of acetic anhydride (20 mL). Water was added and the solution was shaken with ethyl acetate three times. The organic layer was evaporated to dryness to give 1-3 g and kept for further investigation.

Partition coefficient ($K_{o/w}$) determination: The partition coefficient is defined as the ratio of un-ionized drug distributed between the organic and aqueous phases at equilibrium (Leon, 1986).

$$K_{o/w} = \{C_{oil}/C_{water}\} \text{ equilibrium}$$

Ten milligram of each of the prepared extracts namely; colocynth extract, hydrolyzed extract, or acetylated extract, was separated, added to 50 mL of octanol in a stoppered bottle in a water both at 37°C with continuous shaking (Gesell schaft fur, GFL, 1083, Germany) over night. Then distilled water (50 mL) was added to the bottle with shaking at the same temperature for 24 h. The two layers were then separated using a separating funnel and the absorbance of drug in the aqueous layer was determined, at the specific λ_{max} (predetermined by scanning using CECIL CE-6602, UK.) for each formula of colocynth extract; colocynth extract 322 nm, after hydrolysis at 274 nm and the acetylated at 222 nm. Then the concentration of drug in the aqueous layer could be determined by preparing a calibration curve for each formula.

The concentration of each drug in octanol could be determined by the difference. The partition coefficient of drug between octanol and water
($K_{o/w}$) could be 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.

Table 1: The content of Colocynth-gel formulation

Material	Concentration
Colocynth extract	3%
Base (NaCMC)	5%
Glycerol	10%
Propylene glycol	5%
Water up to	100 mL

Preparation of colocynth extracts topical gel: Three types of the prementioned colocynth extract gel were prepared according to the formula in Table 1, by dissolving weighed amount of each extract in the needed volume of water, (one milliliter of ethanol was needed to aid in solubilization) which were then added to 5% of the gel base (NaCMC). The specified amount of glycerol and propylene glycol were then added with continuous stirring at room temperature for 15 min using mechanical stirrer (Servodyne, Mixer Controller. Cole Parmer Instruments Co. Chicago, USA) and the prepared gels were kept at dark cool place overnights (10-15°C).

***In vitro* release study through cellophane membrane:** The release of colocynth from each of the prepared gels was studied using a stainless steel diffusion cell. A two gm 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 two screw were fitted and the cell was then placed in a beaker containing 600 mL phosphate buffer prepared according to USP/NF_{2000} procedures by using SCHOTT pH-meter (G 840) at a pH of 7.4, which was then adjusted to the water bath of the dissolution apparatus (ERWEKA GmbH Heusentamm Germany), at 37°C and 100 rpm (rounds per minute). Sample 5 ml were withdrawn at 5, 10, 20, 45, 60, 90, 120 min intervals and analyzed spectrophotometrically (by using UV-VIS spectrophotometer model 7800 Jasco-Japan) at the λ_{max}, as previously mentioned. The volume of diffusion was maintained constant by replacing the amount withdrawn with an equal volume of the dissolution medium at 37±0.5°C.

Drug release data was treated by equation I described by Higuchi (Higuchi, 1962):

$$Q = 2C_o(D_{app}.t/\pi)^{1/2} \quad (1)$$

or

$$D_{app} = (B/2C_o)^2.\pi \quad (2)$$

Where, Q = the amount of drug released to the sink per unit area at time t; D_{app} = apparent diffusion coefficient of the drug in the vehicle; C_o = the initial drug concentration in the vehicle.

Thus, a plot of Q vs. $t^{1/2}$ should produce a straight line, (B is the slope of this line) the gradient of which is related to the release of the drug out of the gel and can be used to calculate the apparent diffusion coefficient D_{app}. Each result stated was the mean of three determinations.

Permeation through hairless mouse skin: Full-thickness skin was obtained from hairless male mice 25-30 g. The mice were sacrificed snapping the spinal cord at the neck. The dermal side of the skin was carefully cleared of adhering blood vessels, fats, or subcutaneous tissues and washed with warm water. The prepared skin samples (3×3 cm) were then stretched between the two joints over the orifice of the diffusion cell, with the stratum corneum side downwards (i.e., facing the gel, the donor side) and then proceed as above *in vitro* study.

Permeation profiles were constructed by plotting the cumulative amount of drug permeated vs time. The permeability coefficient was calculated from the slope (B) and the drug concentration (C_o) according to Chow *et al.* (1984) equation:

$$\text{Permeability coefficient} = B/C_o$$

Screening of the anti-inflammatory activity of the prepared gels: The anti-inflammatory activities of the agents under investigation were studied using the carrageenan-induced edema model as discussed by Sang-cheol and Won (1999).

Rats were divided into 4 groups, each comprised of 5 rats:

- Control group: received 2 g of NaCMC gel base only.
- Treated group (1): received 2 g of commercial diclofenac gel (Voltarin Emulgel®).
- Treated group (2): received 2 g of 3% of NaCMC-Colocynth Extract gel.
- Treated group (3): received 2 g of 3% of NaCMC-Acetylated Colocynth Extract gel.
- Treated group (4): received 2 g of 3% of NaCMC-Hydrolyzed Colocynth Extract gel.
- The gels were applied to the plantar 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 left paws were cut under ether anesthesia at the tibiotarsal articulation and weighed (Sang-cheol and Won, 1999).

The percentage increases in the weight of the left paw in comparison with the right one of each rat was

calculated, as an indication of the inflammation produced by the following equation:

$$\% \text{ Increase in paw weight} = \frac{L-R}{L} \quad 100$$

Where, R: weight of right leg; L: weight of left leg

The mean percentage reduction was measured from the difference in% swelling between treated groups and the control group by the following equation (Winter *et al*., 1962; Di Rosa *et al*., 1971):

$$\% \text{ Reduction of edema} = \frac{C-T}{C} \quad 100$$

Where, C: % swelling of control group; T: % swelling of treated group (Di Rosa *et al*., 1971).

Statistical analysis: Results were presented as mean±SE statistically significant differences between treated groups were evaluated by students t-test $p<0.05$ were taken as representing significant differences).

RESULTS AND DISCUSSION

Partition coefficient: The partition coefficient of Colocynth could be calculated according to the equation;

$$K_{o/w} = Co/C_W$$

Thus; for Colocynth extract; $K_{o/w}$ = 0.5226, for hydrolyzed Colocynth extract = 3.487, while for acetylated Colocynth extract = 2.90.

So it may be expected for Colocynth extract to show higher water solubility than lipid solubility (2:1) i.e., low penetration power to the skin membrane. In contrast, both the acetylated and hydrolyzed extract may reveal higher penetration power due to their higher lipid solubility; nearly three folds of their aqueous solubility.

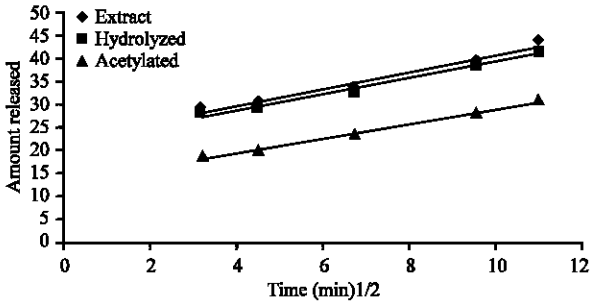

Fig. 1: Square root of time versus the amount of colocynth released for different gel formulations

Table 2: The amount of colocynth released through cellophane membrane from the prepared gel formulations

	Amount of colocynth released		
Time (min)	Extract	Hydrolyzed	Acetylated
5	0	0	0
10	29.36	28.31	19.16
20	30.47	29.18	19.62
45	33.72	32.78	23.46
60	34.86	33.67	25.25
90	39.44	38.99	28.3
120	44.05	42.03	31.2

Table 3: The amount of colocynth permeated through hairless mouse skin from the prepared gel formulations

	Amount of colocynth permeated		
Time (min)	Extract	Hydrolyzed	Acetylated
5	0	0	0
10	0	11.19	7.44
20	0	11.49	8.18
45	0.43	12.21	8.39
60	0.86	12.52	8.5
90	1.73	13.88	9.74
120	2.6	14.62	9.97

Table 4: Mechanism of colocynth release from NaCMC-gel formulations through cellophane membrane

	Higuchi			Zero order		
Mechanism formula	A^1	B^2	r^3	A	B	r
Colocynth extract	22.287	1.8382	0.9742	27.704	0.1324	0.99679
hydrolyzed extract	21.426	1.7965	0.9795	26.704	0.1285	0.99569
Acetylated extract	13.16	1.5996	0.9915	17.996	0.1131	0.99594

Where: A^1 is the intercept, B^2 is the slope and r^3 is the correlation coefficient

Table 5: Mechanism of colocynth permeation NaCMC-gel formulations through hairless mouse skin

	Higuchi			Zero order		
Mechanism formula	A^1	B^2	r^3	A	B	r
Colocynth extract	-1.4411	0.3354	0.9512	-0.4734	0.0245	0.988
hydrolyze extract	9.4905	0.446	0.9764	10.812	0.032	0.9951
Acetylated extract	6.473	0.3146	0.9588	7.4215	0.0223	0.9651

Where: A^1 is the intercept, B^2 is the slope and r^3 is the correlation coefficient

Release study: The three gel formulations containing 3% of; powder extract, acetylated extract or hydrolyzed extract in sodium carboxymethyl cellulose (NaCMC) gel base were found to have acceptable rheological properties. The release of compounds from each formula through cellophane membrane was studied using the described stainless steel diffusion cell and the results are represented in Table 2 and Fig. 1. It could be observed that Colocynth release was very high from all the tested gel formulations reaching 44 mg (about 66.74%) from Colocynth extract-NaCMC gel formula. However, the acetylated extract gel formula showed lower release value

Table 6: Release and permeability characteristics of the prepared NaCMC-Colocynth gel formulations

Gel formula	Amount of drug released (mg) through 120 min	Cumulative amount of drug permeated (mg) through 120 min	Apparent Diffusion Coefficient (D_{app}) ($10^{-4}cm^2\ min^{-1}$)	Permeability Coefficient ($10^{-4}cm\ min^{-1}$)
Colocynth Extract	44.05	2.6	3.11	4.08
Hydrolyzed Extract	42.03	14.62	2.97	5.33
Acetylated Extract	31.2	9.97	2.36	3.72

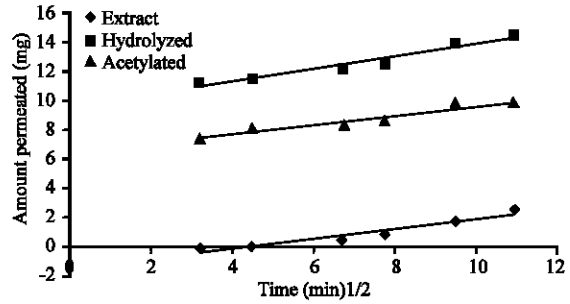

Fig. 2: Square root of time versus the amount of colocynth permeated for different gel formulation through hairless mouse skin

(31.2 mg), which may be attributed to its lower aqueous solubility ($K_{o/w} \approx 3$).

Permeability study: The permeability study of Colocynth through hairless mouse skin (Table 3 and Fig. 2), using the same previously described diffusion cell, revealed that the hydrolyzed extract of Colocynth showed the highest permeation rate and also the amount permeated, compared to the other two formulations, followed by the acetylated extract, while the original extract of colocynth showed very low permeability. This may be due to its low lipid solubility ($K_{o/w} \approx 0.5$) compared to the other two formulations ($K_{o/w} \approx 3$). The permeability results of Colocynth through hairless mouse skin were found to be in contrast to its release through cellophane membrane. The acetylated extract, which showed very rapid permeability, has very low release rate, which may be attributed to the higher lipid permeability of hairless mouse skin.

Release and permeability kinetics: Upon studying the release kinetics of the prepared gel formulations either through cellophane membrane or hairless mouse skin by applying the zero-order and Higuchi equations for each of the prepared formulations (Table 4 and 5). The release of Colocynth through cellophane membrane as well as its permeation through hairless mouse skin was according to zero-order rather than Higuchi equation ('r' values is nearly 0.99 in all formulations), which means that the release and permeation rate of Colocynth was constant all over the process. The constant rate of release and permeation (zero-order kinetics) my be due to the initial rapid lipid solubility of colocynth in contrast to the previous results of Ecbalium (Aly and Mazen, 2003) where the release and permeation showed comparatively slower initial release and permeation i.e., revealed Higuchian rather than zero-order kinetics.

Table 7: Effect of topical administration of Colocynth gel or Voltarin Emulgel® on Carrageenan-induced paws edema in rats

Treatment ($mg\ kg^{-1}$)	Mean%increase in paw weight±SE	%Reduction of edema	Significance
Gel base pure (control)	51.09±0.8520	0	0
Voltarin®	18.87±2.2284	63.35	0.005
Extract	28.12±1.8547	45.39	0.001
Acetylated	18.05±1.4758	64.95	0.000
Hydrolyzed	23.63±2.4787	54.11	0.002

Table 6 represents the release characteristics of the tested Colocynth gels. Nearly similar results of diffusion coefficient (D_{app}) for Colocynth-gel (≈ 3) could be observed. While the permeability results showed comparatively slightly higher permeability coefficient (5.3×10^{-4}) for formula containing the hydrolyzed extract gel, also, due to its higher lipid solubility.

***In vivo* anti-inflammatory activities:** The intraplantar injection of the hind paw by carrageenan induced a progressive edema and this model is useful to detect anti-inflammatory activity of different agents.

The pharmacological screening was carried out to determine the possible anti-inflammatory activity of the three types of the prepared Colocynth gel. The results of the study showed anti-inflammatory activity of the three tested Colocynth-NaCMC gel formulations. The reduction of edema values ($\approx$18-28) for all the tested formulations (Table 7) were found to be very low compared to the control value ($\approx$51), indicating their anti-inflammatory effect. The statistical t-test results revealed that $p<0.05$ for all the tested formulations when compared to the control base formula, as shown in Table 4, confirming their anti-inflammatory activity. Moreover, their effectiveness was comparable with Voltarin Emulgel®. The acetylated extract showed slightly higher percent increase in paw weight effect than the commercial Voltarin Emulgel®, Table 7. Similar results have been obtained before with Ecbalium which showed more reduction of edema than Voltarin (Aly and Mozen, 2003). Thus it could be concluded that carrageenan-induced paw edema in rats can be considered as an acceptable method for screening the anti-inflammatory activity of Colocynth extract.

CONCLUSIONS

From the result obtained it could be concluded that:

- The partition coefficient of colocynth extract (0.5), hydrolyzed colocynth extract (3.5) and acetylated colocynth extract (2.9).
- Carrageenan-induced paw edema in rat can be applied as a good method for screening and assessing of anti-inflammatory activity of Colocynth extract. Percent reduction of edema produced by acetylated Colocynth extract was 64.95%, while Voltarin Emulgel produced 63.35%.
- The acetylated extract gel, which showed very rapid permeability through hairless mouse skin, with very low release rate through cellophane membrane, can be used topically as anti-inflammatory.

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