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ECBALLIUM ELATERIUM; A POSSIBLE TOPICAL ANTI-INFLAMMATORY DRUG

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نظرا لأن قثاء الحمار نبات سام عند تناوله بالفم فإن الهدف من هذه الدراسة تحضير هيلامى من هذا النبات لعلاج موضعى للإلتهاب. ولقد درس هذا الهيلامى لقثاء الحمار مقارنة بعقار الدكلوفيناك صوديوم فى هيئة الهيلام وذلك بإستخدام طريقة الإلتهاب فى المخلب الأمامى فى ذكر فأر الألبينو بإستخدام حقن الكارجينان ولقد ثبت أن تأثير إختزال الورم فى المخلب الأمامى للفأر بإستخدام هيلام قثاء الحمار أقل من هيلام دكلوفيناك صوديوم. ولقد استخدمت ثلاث قواعد لتركيب الهيلام هم: صوديوم كاربوكسى ميثيل سليلوز ، بذور بلانتاجو وصوديوم الجينات. ولقد وجد أن أعلى معدل إختراق للعقار خلال غشاء السلوفان وجلد الفأر المحلوق وجد للهيلام المكون من 3% صوديوم كاربوكسى ميثيل سليلوز. ولقد وجد من الدراسة أن قثاء الحمار لها تأثير موضعى مضاد للإلتهاب فى الهيلام المحضر موضوع الدراسة مقارنة بالهيلام الخاص بالدكلوفيناك صوديوم المتواجد فى السوق الدوائى.

Owing to the toxicity of Ecballium elaterium (EB) when taken orally, the aim of this investigation was to prepare gel formulations of this drug for the treatment of inflammation. The anti-inflammatory activities of EB extract was studied in comparison with Diclofenac sodium market topical gel (Diclogesic gel®), using the carrageenan induced paw edema model in male albino rats. The pharmacological screening revealed that the percent reduction of edema produced by EB extract (85.32%) was lower than that of Diclogesic gel® (78.78%), i.e. it is more effective as anti-inflammatory agent than the market diclofenac sodium. Three types of gel formulations were prepared containing 1, 2 or 3% of EB in three gel bases, namely; Sodium carboxymethyl-cellulose (NaCMC), Plantago seed husks (PT), and Sodium alginate (NaAlg). EB 3% in NaCMC-gel was found to have comparatively the highest releasing and penetration power through the cellophane membrane and hairless mouse skin, respectively. NaCMC-gel showed similar diffusion coefficients (D_{app}) to PT- gel (1-1.5), while NaAlg preparations showed lower results (0.5). However, the permeability coefficient results revealed that NaCMC formulations have higher values followed by PT and NaAlg. It could be concluded that EB is a promising topical anti-inflammatory agent.

INTRODUCTION

The squirting cucumber, *Ecballium elaterium*, has been used as a medicinal plant for over 2,000 years. The plant is a very powerful purgative that causes evacuation of water from the bowels.[1] The juice of the fruit has antirheumatic, cardiac and purgative activities.[2-6] It is used internally in the treatment of oedema associated with kidney complaints, heart problems, rheumatism, paralysis and shingles.[1,5] Externally, it has been used to treat sinusitis and painful joints.[1] It should be used with great caution and only under the supervision of a qualified practitioner.[1,7] Excessive doses have caused gastroenteritis and even death. It should not be used by a pregnant woman since it can cause an abortion.[8] The active principle, cucurbitacin B, was isolated from the chloroform extract of the fruit juice of Ecballium elaterium, and showed a significant anti-inflammatory activity in mice.[9] Patients exposed orally or intranasally should be closely followed by upper airway obstruction. Patients exposed ocularly should have their eyes promptly irrigated to prevent corneal and conjunctival injury.[10] Satar *et al*.,[11] presented a life-threatening uvular angioedema caused by nasal aspiration of undiluted juice of squirting cucumber.

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Thus, the objective of this investigation was to prepare topical gel formulations containing 1%, 2% and 3% Ecballium extract, using Plantago, Sodium alginate (NaAlg) or sodium carboxymethylcellulose (NaCMC) as gel-bases. The release study of this extract from topical gel formulations through a cellophane membrane and its permeability through a hairless mouse skin was assessed. Also, the in vivo anti-inflammatory activity of Ecballium elaterium extract in rats

Materials

1- Drugs and chemicals

Ripe fruit of Ecballium elaterium. Sodium carboxymethylcellulose (NaCMC): S & C CHEMICALS, SUPPLICO. Sodium alginate (Na-Alg); S.D. Fine-Chem Ltd., Gujarat, India. Plantago ovata (PL) seed husks; Psyllium husks Sat Isabgol, MFRs, India. Carrageenan (sigma chemical co. Steinheim, Germany) it was used as 1% solution in normal saline. Normal saline (Batch: 2302, Dar AL Dawa, Amman, Jordan). 1%w/w Diclofenac® gel (Dar Al Dawa Na'ur, Jordan).

2- Animals

Male albino rats (weighting 200-250 g) of a 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.

Methods

1- Extraction of *Ecballium elaterium*

One Kg of ripe fruit of Ecballium elaterium was extracted three times with absolute ethanol. After filtration, the filtrate was evaporated till syrupy. The extract was collected in small beaker, which produced about 13 g (used as powder extract without separation of any ingredients).

2- Screening of the anti-inflammatory activity of *Ecballium elaterium*

The anti-inflammatory activities of the agents under study were investigated by using the carrageenan-induced edema model.

Rats were divided into 3 groups, each comprised of 5 rats:

1- Control group: received 2 g of NaCMC gel base only.
2- Treated group (1): received 2 g of 3% of NaCMC-Ecballium elaterium gel.
3- Treated group (2): received 2 g of diclofenac sodium gel (Diclogesic 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 in to the treated paw. Three hours after the carrageenan injected the right and left paws were cut under ether anesthesia at the tibiotarsal articulation and weighed .[12]

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:

$$\% \text{ Increase in paw weight} = \frac{L-R}{L} X100$$

Where:
R: weight of right leg.
L: weight of left leg.[13]

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

$$\% \text{ Reduction of edema} = \frac{C-T}{C} X100$$

Where:
C: % swelling of control group.
T: % swelling of treated group.[14]

Statistical analysis

Results were presented as mean ± S.E. statistically significant differences between treated groups were evaluated by students *t*-test ($p<0.05$ were taken as representing significant differences) as shown in Table (II) Figs. (7,8).

Determination of ($k_{o/w}$)

A measurement of a drug's lipophilicity and an indication of its ability to cross cell membranes is the oil/water partition coefficient in system such as octanol/water *($k_{o/w}$)*.[15]

Fifty mg of Ecballium elaterium extract was added to 100 ml of octanol in a stoppered bottle in water bath at 37°, with continuous agitation using a shaker (Gesell Schaft Fur, GFL, 1083, Germany), at 400 rpm over night. Then distilled water (100 ml) was added to the bottle with shaking at the same temperature for 24 hours. The two layers were then separated using a separating funnel, and the absorbance of drug in the aqueous layer was determined of Ecbalium at λ_{max} 291 nm (which have been determined spectrophtometrically by scanning using CECiL CE-6602, UK) Then the concentration of drug in aqueous layer was determined from previuosly prepared calibration curve for the same drug. The concentration of 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.

3. Preparation of *Ecballium elaterium* topical gel

Three types of Ecballium elaterium gel were prepared according to the formula in Table (I), by dissolving the specified amount of Ecballium extract in the needed volume of water, which were then added to 5% of the base. The specified amount of glycerol and propyleneglycol were then added with continuous stirring at room temperature for 15 minutes using mechanical stirrer (Servodyne, mixer controller, Cole Parmer Instruments Co. Chicago, USA) at 500 rpm. The gels were then keep in a dark cool place overnight at 10-15°.

4. *In-vitro* release study through cellophane membrane

The release of Ecballium from each of the prepared gels was studied using a stainless steel diffusion cell, Fig. (1). Two grams sample of each formulation was accurately weighed and placed in the hollow bottom of the diffusion cell (donor part), the fisher 32/30 standard membrane was adjusted between the two joints and the two screws were fitted, and the cell was then placed in a beaker containing 500 ml phosphate buffer prepared according to USP/NF 1995 procedures by using SCHOTT pH-meter (G 840) at a pH of 7.4, which was then adjusted to a water bath at 37°, and agitated at 100 rpm. Samples of 5 ml were withdrawn at 5, 10, 15, 30, 45, 60, 75, 90 min intervals, and analyzed spectrophotometrically (by using UV-VIS Spectrophotometer model 7800 Jasco-Japan) at 291 nm for Ecballium content. The volume of diffusion was maintained constant by replacing the amount withdrawn with an equal volume of the dissolution medium at 37±0.5°. The concentration of Ecballium in each sample was determined using a previously constructed standard curve.

Drug release data was treated by *equation I* described by Higuchi :[16]

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

Or:

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

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.

5. Permeation through hairless mouse skin

Full-thickness skin was obtained from hairless male mice 25-30 g. The mice were sacrificed by 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 cm X 3 cm) were then stretched between the two joints over the orifice of the diffusion cell Fig. (1), with the stratum corneum side downwards (i.e. facing the gel, the donor side), and then proceeded as the above in-vitro study.

Permeation profiles were constructed by plotting the cumulative amount of drug permeated versus time. The permeability coefficient was calculated from the slope (B)

Table I: The contents of Ecballium-gel formulations.

Materials	Concentration (w/v)
Ecballium extract	1,2 or 3%
Gel base (Plantago, NaCMC, or NaAlg)	5%
Glycerol	10%
Propylene glycol	5%
Water up to	10 ml

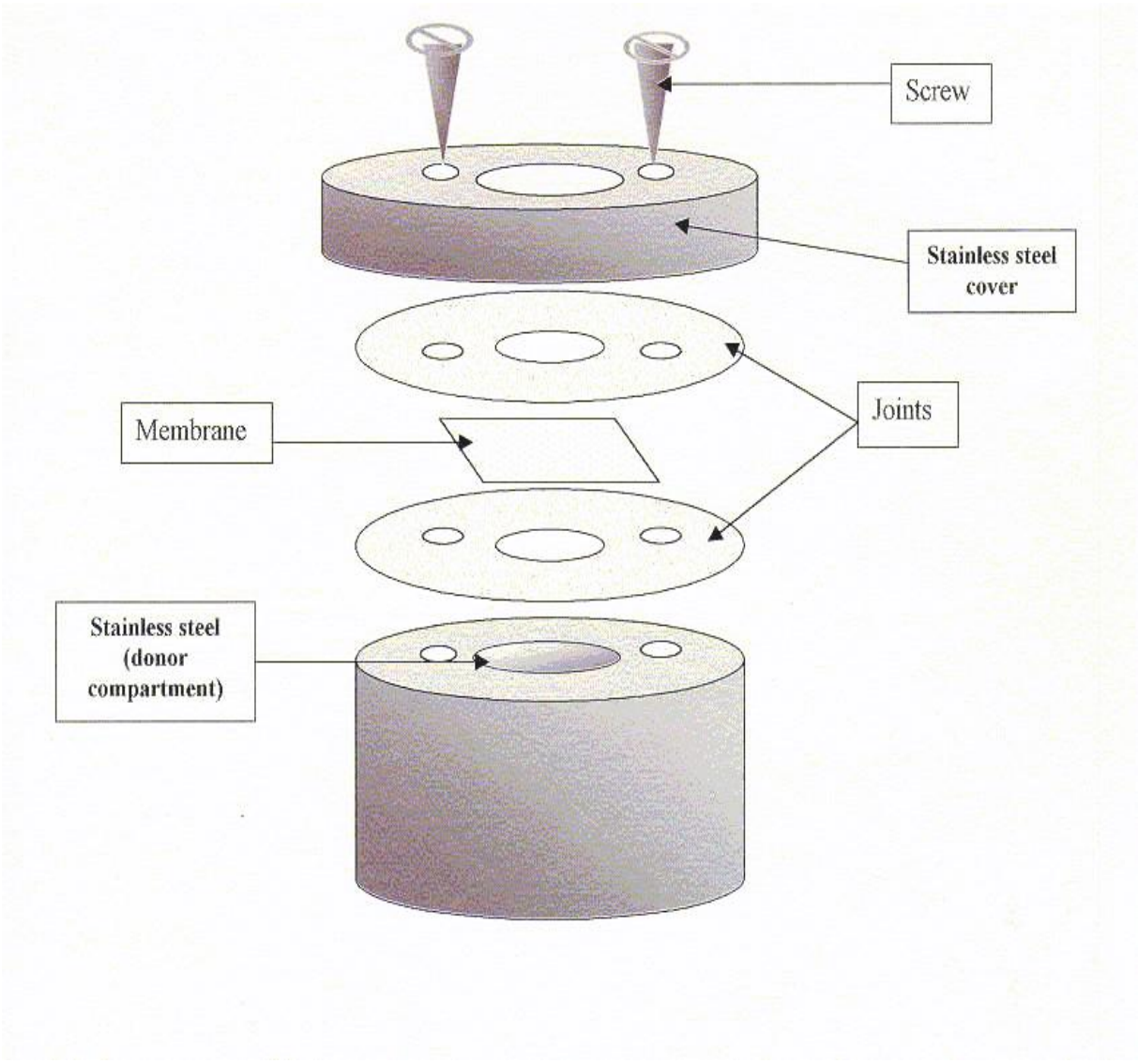

Fig. 1: Illustrative diagram of the diffusion cell used for both release and permeation tests.

and the drug concentration (C_o) according to Chow and Kakai[17] equation:

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

RESULTS AND DISCUSSION

Partition coefficient

The partition coefficient of Ecballium could be calculated as follows;

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

$$K_{o/w} = \frac{0.283}{0.231} = 1.225$$

So it may be expected for Ecballium extract to penetrate the skin membrane because it's partition coefficient is more than 1.

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 Ecballium extract. The results of the study showed an anti-inflammatory activity of 3% Ecballium gel under investigation which was better than that obtained by; Diclogesic gel®, which is commercially available. The percent reduction of edema produced by EB extract (85.32%) was lower than that of Diclogesic gel® (78.78%), i.e. it is an effective anti-inflammatory agent similar to diclofenac sodium (Table II and Fig. 2). Thus it could be concluded that carrageenan-induced paw edema in rats can be considered as a acceptable method for screening the anti-inflammatory activity of Ecballium elaterium.

Release study

Owing to the high toxicity of internal administration of EB, it may be recommended to formulate EB for topical application. Three types of gel formulations were prepared containing 1, 2 or 3% of EB (powder extract) in three gel bases, namely; sodium carboxymethylcellulose (NaCMC), Plantago (PT) and sodium alginate (NaAlg). The prepared gel formulations were found to have acceptable rheological properties. The release of EB from each formula was studied through a cellophane membrane using the described stainless steel diffusion cell. The results are represented in Table (III) and Figs. 3-5. After 90 min. it could be observed that EB release was very high from all the tested gel formulations reaching 50 mg from NaCMC gel containing 3% EB, 43 and 28 from PT and NaAlg, respectively (Fig. 6). Also, by increasing EB concentration, a pronounced increase in the release rate could be produced for the three tested formulations (Table III and Figs. 3-5). Such figures revealed also, that an initial rapid release through the first 30 min followed by comparatively a slower release within the following 60 min. The linear correlations obtained upon plotting the square root of time versus the amount of EB released / unit area (Figs. 7-9), i.e Higuchi equation,[18], ($r \approx 0.99$ in all cases) confirms these results.

Permeability study

The permeability study of EB through hairless mouse skin, using the same diffusion cell, revealed that the amount of EB permeated increased by increasing its percentage in the formula for all the tested gel-bases (Figs. 10-12). Formulations containing 3% EB, as shown in Fig. 13, permeated the largest amount of drug from each of the tested base, especially that containing 3% of NaCMC where 42 mg EB were permeated through 90min. followed by PT (28.8 mg) and NaAlg (21.6 mg), i.e. the same releasing sequence.

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 IV). The release of EB through cellophane membrane revealed that NaCMC and NaAlg gel preparations release EB according to Higuchi equation rather than zero-order (r values is nearly 0.99 in all formulations), whereas, PT gel preparations release EB in accordance with zero-order ($r \approx 0.99$) with lower r values on applying Higuchi equation in all EB concentrations. On the other hand, the mechanism of permeation of EB gel formulations through hairless mouse skin (Table V and Figs. 14-16) could be explained by Higuchi rather than the zero-order in all the tested preparations, i.e. the release rate

decrease by time (all the tested formulations showed r values of nearly 0.99).

Table III represents the release and permeability characteristic of the tested EB gels. Nearly similar results of diffusion coefficient (D_{app}) for NaCMC and PT gels (1-1.5) could be observed. While NaAlg preparations showed lower results (0.5), whereas, the permeability coefficient results revealed that NaCMC formulations have higher values followed by PT and NaAlg.

Table II: Effect of topical administration of Ecballium gel or Diclogesic gel® on Carrageenan-induced paw edema in rats.

Treatment mg/kg	Mean % increase in paw weight ± S.E.	% Reduction of edema	Significance
Gel base pure (control)	41.17 ± 2.430	---	---
Diclogesic gel®	8.734 ± 0.803	78.78	0.002*
Ecballium gel	6.042 ± 0.454	85.32	0.001*

*Significant difference

Table III: Release and permeability characteristics of the prepared Ecballium gel formulations.

Gel Formula		Amount of drug Released through 90 min (mg)	Cumulative amount of drug permeated through 90 min (mg)	Diffusion Coefficient (D_{app}) (10^4 cm^2/min)	Permeability Coefficient (10^6 cm/min)
NaCMC	1%	13.48	9.17	2.2824	4.6874
	2%	30.73	24.98	1.5490	5.7779
	3%	50.37	42.23	1.8232	5.5636
Plantago	1%	12.04	8.21	2.1702	4.1184
	2%	28.33	16.83	2.7069	4.1791
	3%	43.19	28.81	2.1443	4.5834
Na Alginate	1%	9.17	6.77	0.9970	3.4358
	2%	18.75	13.96	0.9265	3.4695
	3%	28.33	21.62	0.9779	3.4695

Table IV: Mechanism of Ecballium release from NaCMC, Plantago or Na Alginate gel formulations through a cellophane membrane:

Mechanism / Formula		Higuchi			Zero order		
		A	B	r	A	B	r
NaCMC	1%	-1.7106	1.0784	0.9866	0.9838	0.0894	0.9695
	2%	2.2936	1.7769	0.9933	6.6303	0.1498	0.9926
	3%	3.9858	2.8916	0.9907	11.157	0.2411	0.9788
Plantago	1%	-2.3300	1.0515	0.9925	0.1639	0.0899	0.9958
	2%	-4.9851	2.3489	0.9929	0.7009	0.1992	0.9978
	3%	-3.8410	3.1359	0.9826	3.6706	0.2679	0.9947
Na Alginate	1%	-0.5229	0.6871	0.9927	0.8857	0.0618	0.9625
	2%	-0.8416	1.4117	0.9925	2.0050	0.1275	0.9570
	3%	-0.3066	2.0119	0.9970	3.4615	0.1868	0.9596

Table V: Mechanism of drug permeation NaCMC, Plantago or Na Alginate gel formulations through hairless mouse skin:

Formula \ Mechanism		Higuchi			Zero order		
		A	B	r	A	B	r
NaCMC	1%	-1.6506	1.1291	0.9860	1.16504	0.0938	0.9850
	2%	0.1545	2.8989	0.9638	7.7790	0.23118	0.9543
	3%	5.0712	4.0716	0.9929	15.4000	0.33388	0.9822
Plantago	1%	-1.6986	0.9755	0.9866	0.0824	0.0824	0.9873
	2%	-2.2208	2.0149	0.9886	2.8095	0.16714	0.9720
	3%	-3.4842	3.2784	0.9917	4.5287	0.27504	0.9883
Na Alginate	1%	-1.1084	0.8251	0.9936	0.9404	0.06874	0.9808
	2%	-1.8462	1.6684	0.9961	2.3041	0.1388	0.9820
	3%	-2.2376	2.4832	0.9947	3.8729	0.2082	0.9882

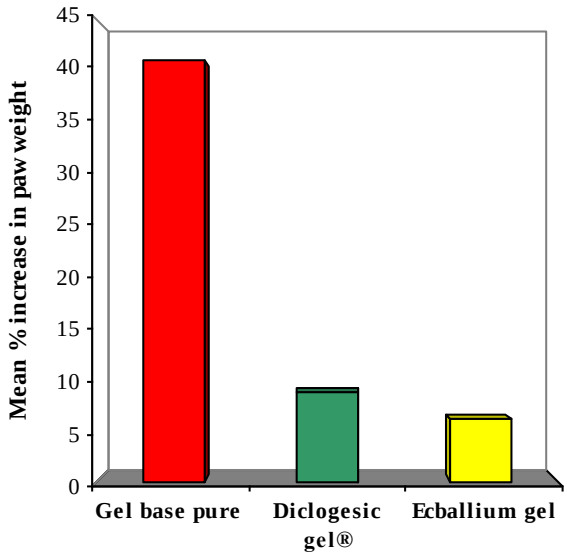

Fig. 2: Mean % increase in paw weight for each of the tested topical gel formulations.

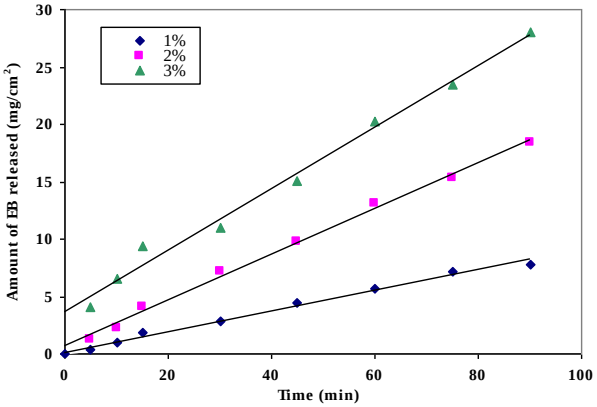

Fig. 4: Release profiles of the amount of EB released /unit area versus time for topical gel containing different Plantago percents.

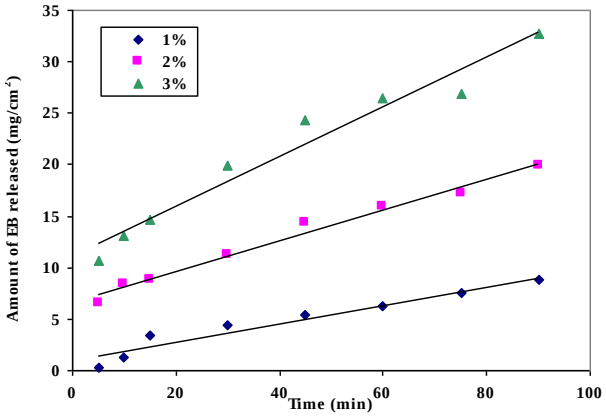

Fig. 3: Release profiles of the amount of EB released /unit area versus time for topical gel containing different NaCMA percents.

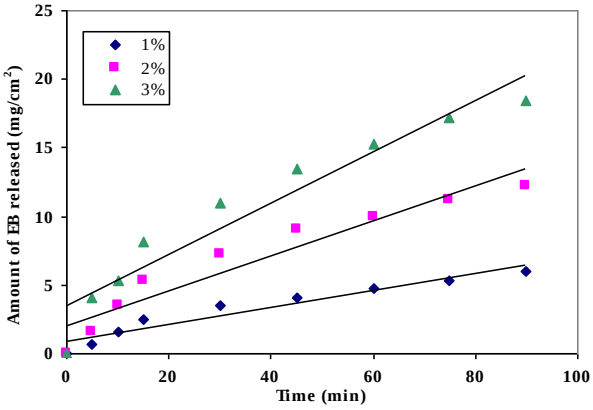

Fig. 5: Release profiles of the amount of EB released /unit area versus time for topical gel containing different Na Alginate percents.

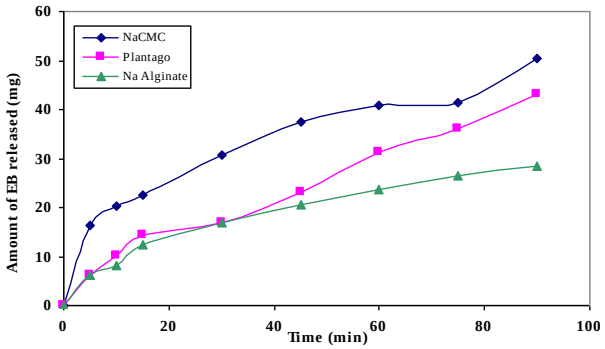

Fig. 6: Release profiles of EB from 3% concentrations of different gel formulations through cellophane membrane.

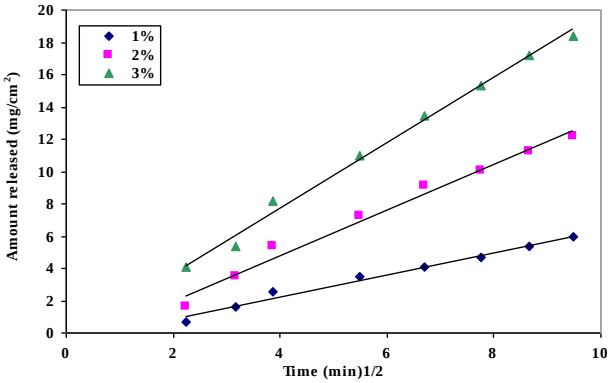

Fig. 9: Square root of time versus the amount of EB released / unit area for topical gel containing different Na Alginate percents.

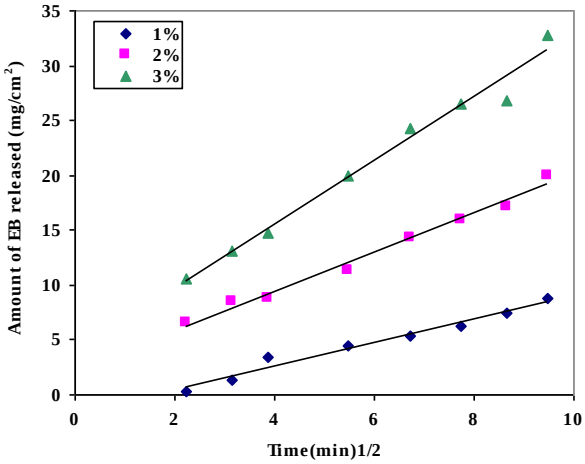

Fig. 7: Square root of time versus the amount of EB released / unit area for topical gel containing different NaCMC percents.

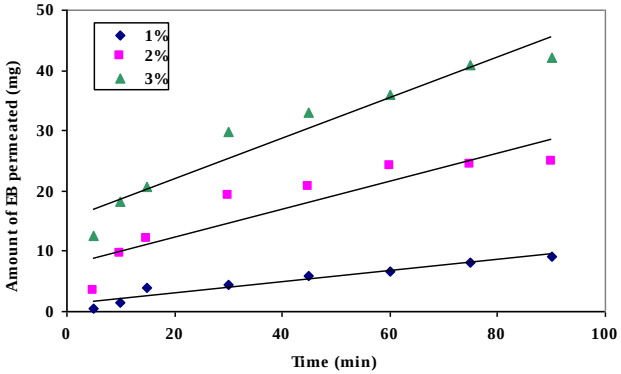

Fig. 10: Permeation profiles of EB from topical gels containing different NaCMC concentrations through hairless mouse skin.

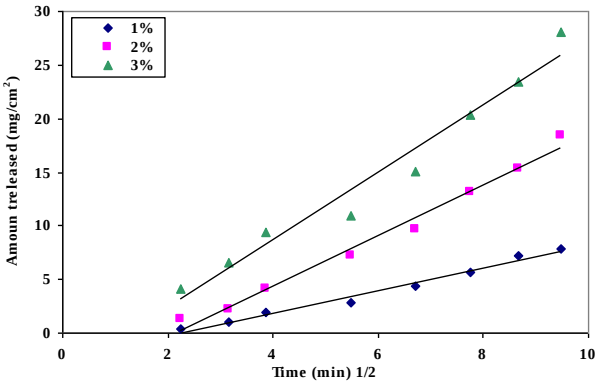

Fig. 8: Square root of time versus the amount of EB released / unit area for topical gel containing different Plantago percents.

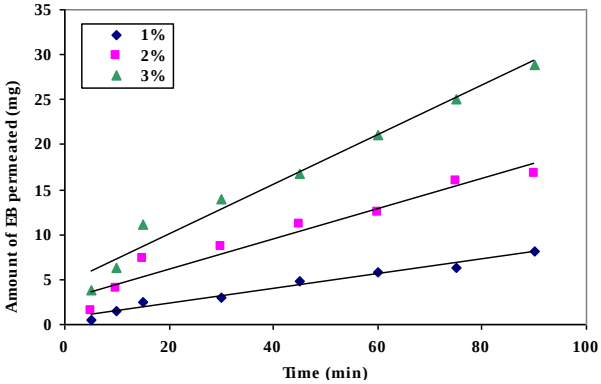

Fig. 11: Permeation profiles of EB from topical gels containing different Plantago concentrations through hairless mouse skin.

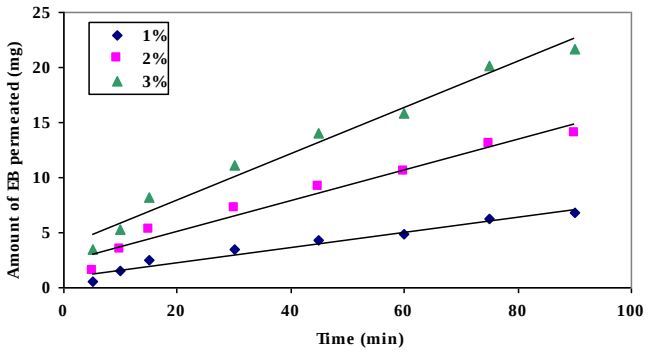

Fig. 12: Permeation profiles of EB from topical gels containing different Na Alginate concentrations through hairless mouse skin.

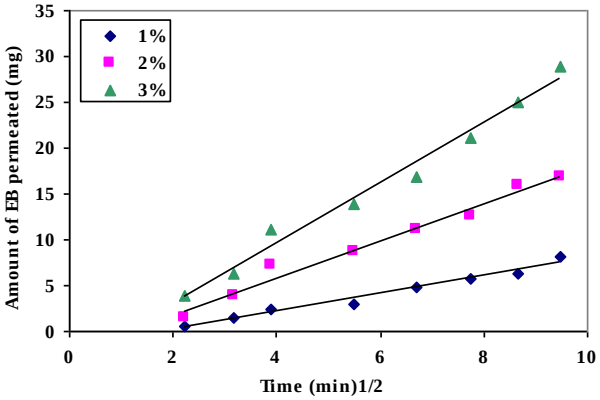

Fig. 15: Square root of time versus amount of EB permeated from topical gels containing different Plantago concentrations through hairless mouse skin.

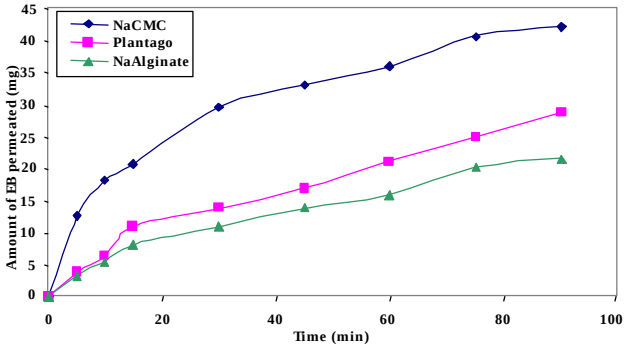

Fig. 13: Permeation profiles of EB from 3% concentrations of different gel formulations through hairless mouse skin.

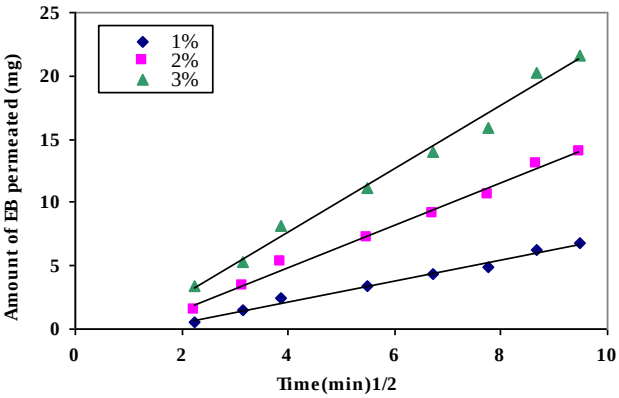

Fig. 16: Square root of time versus amount of EB permeated from topical gels containing different Na Alginate concentrations through hairless mouse skin.

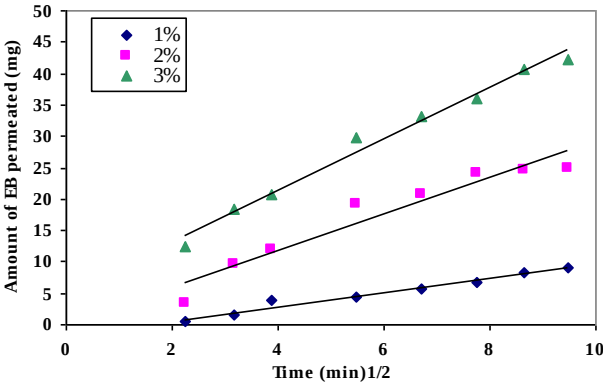

Fig. 14: Square root of time versus amount of EB permeated from topical gels containing different NaCMC concentrations through hairless mouse skin.

CONCLUSIONS

From the result obtained it could be concluded that:

1- The partition Coefficient ($k_{o/w}$) result of Ecballium (1.225) revealed that it can be applied topically depending on its lipid and aqueous solubility.

2- The pharmacological screening revealed that the percent reduction of edema produced by EB extract (85.32%) was lower than that of Diclogesic gel® (78.78%), i.e. it is more effective as anti-inflammatory agent than the market diclofenac sodium.

3- NaCMC can be used for preparing successful topical gel with high releasing and penetration power through cellophane membrane and hairless mouse skin. Plantago can also, be used but with less effectiveness.
4- Cellophane membrane can be used for studying the effectiveness of Ecballium penetration through its topical gel formulations as an alternative to the biological hairless mouse skin with nearly the same effect.
5- It could be concluded that EB is a promising topical anti-inflammatory agent.

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