Licorice: A Possible Anti-inflammatory and Anti-ulcer Drug

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ABSTRACT

The purpose of this investigation was to study the anti-inflammatory activities of both glycerrhitinic acid (GA) and the aqueous licorice extract (ALE) in comparison with diclofenac sodium (DS) (10 mg/kg), using the carrageenan-induced paw edema model in male albino rats. In addition, the anti-ulcer activities of ALE, famotidine (FT), and a combination of ALE and FT using indomethacin-induced ulceration technique in rat stomach were investigated. Conventional DS tablets containing GA, as well as DS chewable tablets containing either GA or ALE with different tastes were prepared. Also, rapidly disintegrating FT tablets were prepared using direct compression and camphor sublimation methods. ALE or GA produced significant anti-inflammatory activity similar to DS, and when taken concomitantly, there is no possible antagonism. The anti-ulcer activity of licorice was found to be similar to that of FT in indomethacin-induced ulceration technique in rat stomach. Combination therapy of both FT and licorice showed higher anti-ulcer activity than either of them alone. Generally, tablets containing the crosslinked sodium carboxymethyl cellulose (AcDisol) showed more rapidly disintegrating effect than those including Sodium starch glycolate (Primojel). The oral disintegration was very rapid for all the tested formulations. Also, the amount of FT absorbed from the oral cavity was nearly 9 from 10 mg theoretically present in each formula. It could be concluded that both GA and ALE have anti-inflammatory activity comparable with DS. It may be recommended to add ALE to either FT or diclofinac for more effective anti-inflammatory or anti-ulcer formulations, respectively.

KEYWORDS: glycerrhitinic acid, licorice extract, anti-inflammatory, anti-ulcer

INTRODUCTION

The intent of the present work was to study the possible anti-inflammatory activities of both glycerrhitinic acid (GA) and the aqueous licorice extract (ALE) in comparison with diclofenac sodium (DS), and to prepare conventional, as well as chewable tablets containing DS with either GA or ALE. In addition, this study was conducted to evaluate the anti-ulcer activity of ALE, famotidine (FT), and a combination of ALE and FT. Another goal of this study was to produce rapidly disintegrating tablets containing ALE with FT.

Licorice roots are straight pieces of wrinkled, fibrous wood, which are long and cylindrical and grow horizontally underground. Licorice belongs to the Leguminosae family.[1] The licorice extract can be used as a sugar substitute, where it has an antioxidant action in food and strengthens food aroma.[2] The sweet taste of licorice is entirely due to glycyrrhizin, which is 50 times sweeter than sugar.[3] In Japan, glycyrrhizin has been used for more than 20 years as a treatment for chronic hepatitis.[4] Licorice is known for its anti-inflammatory and anti-allergic effects. These actions are due to the effect of glycyrrhizin on the adrenal gland, which is responsible for producing cortisol, the body's own anti-inflammatory adrenal steroid hormone. It also has adaptogenic properties by stimulating cortisol production[5] and inhibiting the deactivation of glucocorticoid in the liver when there is not enough, and promoting the breakdown of cortisol when there is too much.[6]

Moreover, one of the better-known folk uses of licorice in Europe has been in the treatment of gastric ulcer. It had been shown that licorice-derived compounds can raise the concentration of prostaglandins in the digestive system that promote mucus secretion from the stomach; it was also reported that licorice prolongs the life span of surface cells in the stomach and has an antipepsin effect. The combined effect may lead to the healing of ulcers.[7] On hydrolysis, glycyrrhizin loses its sweet taste and is converted to glycerrhetinic acid and 2 molecules of glucuronic acid. The anti-ulcer drug carbenoxolone, a succinate derivative of glycerrhetinic acid, was developed in London in the early 1960s and has become the preferred form of licorice used to promote healing of ulcers.[8] Licorice has also been shown to help inhibit the growth of potentially harmful intestinal bacteria, such as *Helicobacter pylori,* through the flavonoids that it has.[9]

DS was not approved for use in the United States until 1988; however, it has been available elsewhere since 1974 and is perhaps the most widely used prescription nonsteroidal anti-inflammatory drug (NSAID) in Europe.[10,11] In addition to inhibiting prostaglandin synthesis secondary to

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the inhibition of cyclooxygenase enzyme, high doses of DS may reduce formation of lipoxygenase products by reducing the intracellular concentration of free arachidonate in leukocytes, perhaps by altering the release or uptake of fatty acid.[12,13]

Indomethacin is also an NSAID that is known to cause mucosal ulceration and increase the mucosal vascular permeability in the gastrointestinal tract. Indomethacin causes microvascular endothelium damage in the stomach and small intestine.

MATERIALS AND METHODS

Materials

Carrageenan (Sigma Chemical Co, St. Louis, MO, USA) was used as 1% solution in normal saline. DS, tutti frutti, chocolate, and aspartame were kindly supplied by the United Pharmaceutical Manufacturing Co (Amman, Jordan). Glycyrrhetinic acid, 18-beta-glycrrhetinic acid, 96% (Acros Organics, Morris Plains, NJ) was used as 100 mg/kg. Talc was obtained from Frutarom (Bellingham, Cleveland, TS23 1LQ, UK). Aerosil 200 was obtained from Degussa AG (Frankfurt, Germany). Tragacanth powder LR (Laboratory Rasayan) came from Laboratory Rasayan, SD Fine Chem Ltd, Boisar, India. Avicel was obtained from Janssen Chimica (Geel, Belgium). Menthol was obtained from (Medex, Naefby, Northants, UK). Normal saline was obtained from Dar Al Dawa (batch no. 2302, Naur, Jordan). Carboxy methyl cellulose was from HiMedia laboratories PVT (Bombay, India). Tween 80 was from Riedel de Haem (Seelze, Germany). Indomethacin, FT, aspartame, direct compression sugar (DCS), and AcDisol were kindly supplied by Dar Al Dawa Co (Amman, Jordan). Camphor was obtained from S&C Chemicals (Amman, Jordan). Primojel came from Avebe (Veendam, The Netherlands). Mannitol was obtained from Merck (Darmstadt, Germany). Polyethylene glycol 6000 (PEG_{6000}) was obtained from Janssen Chimica (Geel, Belgium).

Thirty-six 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 1 week to be acclimatized in the animal house before the experiment, and they were maintained on unrestricted supplies of food and water.

Methods

Aqueous Extraction of Licorice

One kilogram of powdered licorice was extracted 2 times with boiling water, then filtered, and the filtrate was evaporated until it became syrupy. For precipitation of glycosides (mainly glycyrrhizin), the aqueous extract was poured on excess acetone. The precipitate was decanted, dried, and finely crushed and passed through a 250-μm sieve (56 g).

Screening of the Anti-inflammatory Activity of Drugs Under Investigation

The anti-inflammatory activity of the agent under study was investigated by using the carrageenan-induced edema model.

Rats were divided into the following 6 groups, each composed of 6 rats:

(1) Negative control group received 0.2 mL of sesame oil.
(2) Positive control group was injected intramuscularly (IM) with DS (10 mg/kg).
(3) Treated group 1 was injected IM with GA (100 mg/kg).
(4) Treated group 2 was injected IM with ALE (250 mg/kg).
(5) Treated group 3 was injected IM with a combination of GA and DS (100 mg/kg and 10 mg/kg, respectively).
(6) Treated group 4 was injected IM with a combination of ALE and DS (250 mg/kg and 10 mg/kg, respectively).

The doses of 100 mg/kg of GA and 250 mg/kg of ALE that were administrated to rats were selected according to several trials, with 50, 75, and 100 mg of GA, as well as 200, 250, and 300 mg of ALE.

All drugs were administered 30 minutes before an intradermal injection of carrageenan (0.1 mL of a 1% solution in 0.9% saline) into the plantar surface of the right hind paw. The left paw was injected with 0.1 mL saline. Five hours after carrageenan injection, the right and the left paws were cut under ether anesthesia at the tibiotarsal articulation and weighed. The percentage increase in the weight of the right paw in comparison with the left one of each rat was calculated as an indication of the inflammation using the following equation:

$$\% \text{ Increase in Paw Weight} = \frac{R - L}{L} \times 100, \qquad (1)$$

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

The mean percentage reduction was measured from the difference in percentage swelling between the treated group and the negative control group using the following equation:

$$\% \text{ Reduction in Edema} = \frac{C - T}{C} \times 100, \qquad (2)$$

where C is the control group and T is the treated group.[14]

Table 1. Effect of Intramuscular Administration of Drugs Under Investigation on Carrageenan-induced Paw Edema in Rats*

Treatment (mg/kg)	Mean % Increase in Paw Weight ± SE	% Reduction of Edema
Sesame oil	46.2 ± 3.1	–
DS (10 mg/kg)	12.04 ± 1.1†	73.9
GA (100 mg/kg)	19.88 ± 1.7†	58.6
ALE (250 mg/kg)	15.31 ± 1.4†	66.8
DS/GA (10/100 mg/kg)	10.37 ± 1.1†	78.3
DS/ALE (10/250 mg/kg)	11.53 ± 1.1†	76.1

*DS indicates diclofenac sodium; GA, glycerrhitinic acid; and ALE, aqueous licorice extract.
†Significantly different from the control.

Results were presented as mean ± SE. Statistically significant differences between treatment groups were evaluated by Student *t* test ($P < .05$ represented significant difference) as shown in Table 1.

Screening of the Anti-ulcer Activity of Agents Under Investigation

Rats were housed in groups of 6 rats per cage under controlled conditions at 21°C. Animals were given 12 hours of artificial light and 12 hours of darkness per day and fed on balanced rat chow and water ad libidum. Then, rats were fasted for 24 hours with free access to water. Four groups of 6 rats each were pretreated by intragastric administration of one of the following treatments:

(1) FT 1.14 mg/kg
(2) Licorice 100 mg/kg
(3) Combination of 100 mg/kg Licorice and 0.57 mg/kg FT
(4) Normal saline as control

After 30 minutes, gastric mucosal lesions were induced by the intragastric administration of 20 mg/kg indomethacin. Four hours after indomethacin administration, the rats were killed by an overdose of diethyl-ether.[15] The ventral body wall was cleaned with 70% alcohol. Using clean instruments, a longitudinal cut, extending from the perineum to the xiphoid process and 2 transverse cuts medially on either side were made, exposing the entire abdominal cavity. The stomach was freed from the peritoneum and removed. After emptying the food remnants from the stomach, it was cut open along the greater curvature and was washed several times in normal saline. The specimen was then transferred to a Petri dish containing normal saline, where it was mounted on white rubber. A uniform wire mesh of quadrangular units (1.7 mm^2/U) was superimposed on the stomach.

Measurement of Percentage of Ulceration

Under a dissecting microscope, the hemorrhagic lesions and erosions were similarly quantified fresh without fixation. The area of the glandular stomach and the area of the lesion were calculated by counting the mesh squares covering each of them. Lesion quantity was expressed as percentage lesion area relative to the glandular area of the stomach according to the following equation:

$$\% \text{ of Ulceration} = \frac{\text{Area of total lesions}}{\text{Glandular area of the stomach}} \times 100 \quad (3)$$

Statistical Analysis

Results were presented as mean ± SE. Statistical significant differences between treated groups were evaluated by Student *t* test ($P < .05$ represented significant difference) as shown in Table 2.

Preparation of Diclofenac Chewable Tablets

First, conventional DS tablets containing GA were prepared according to formula (A) presented in Table 3. The ingredients were thoroughly mixed in a cubic mixer (Erweka, Heusenstamm, Germany) for 10 minutes and

Table 2. Effect of Administration of Drugs Under Investigation in Rats*

Parameters Symbol	Drugs	Doses (mg/kg)	Mean Ulceration Area in mm^2 ± SE	% of Ulceration in the Glandular Area
A	Indomethacin	20	24.9 ± 0.37	3.59
B	FT	1.14	4.6 ± 0.08	0.72
C	Licorice	100	4.9 ± 0.26	0.74
D	FT and licorice	0.57 & 100	2.0 ± 0.06	0.32

*FT indicates famotidine. Intergroup comparisons show that there are significant differences between each pair of groups except between B and C.

Table 3. Excipients of 12.5-mg Diclofenac Sodium Chewable Tablet Formulations

Symbol	Conventional Tablets (GA)	Chewable Tablets				
Materials (mg)	A	B	C	D	E	F
GA	125	125	125	–	–	–
ALE	–	–	–	175	175	175
Avicel	49.5	44.5	44.5	44	44	44
Tragacanth	10	10	10	12.5	12.5	12.5
Aspartame	–	3	3	–	–	–
Tutti frutti	–	2	–	2.5	–	–
Chocolate	–	–	2	–	2.5	–
Menthol	–	–	–	–	–	2.5
Talc	2	2	2	2.5	2.5	2.5
Total tablet weight (mg)	200	200	200	250	250	250

*GA indicates glycerrhitinic acid and ALE, aqueous licorice extract. The formulations contain 1 mg Aerosil/tablet. A dash refers to the other tablet ingredients.

passed through sieve no. 355 μm. The mixture was compressed into 0.200-g flat tablets (8 mm in diameter) using a Korsch tableting machine (Korsch EK/O, Heusenstamm, Germany). Chewable tablets containing DS with GA (formula B and C) or ALE (formula D, E, and F) (Table 3) were prepared. Aspartame was essentially added to tablets containing GA to mask its bitter taste, whereas it is not needed for those of ALE due to its sweet flavor.

The tablets were evaluated for weight, thickness, size, tensile strength (Ts), friability, and taste. In order to ensure uniformity of weight, 20 tablets taken randomly were weighed; the SD and coefficient of variation percentage (CV%) were calculated. The thickness of 20 tablets was measured individually by Erweka hardness tester, and the average and CV% were calculated. Size was evaluated by selecting 20 tablets at random; for each one the diameter was measured using Erweka hardness tester, and the average and CV% were calculated. The Ts was calculated from the following equation:

$$Ts = 2H/\pi TD \ldots (N/cm^2),$$

where T is the thickness, D is the diameter, and H is the hardness of the tablets in Newtons. The hardness was determined using Erweka TBH_{30} hardness tester; the average hardness of 10 tablets was taken randomly and the CV% (CV%= SD*100/Mean) can be also calculated. The percentage weight loss was determined after rotation of 20 preweighed tablets for 4 minutes at 25 rpm using Erweka friabilator TAR20. Finally, a test was made of the palatability. This test is made to evaluate the taste of each prepared formula. A random sample of 42 students from the College of Pharmacy in Al-Isra University chewed a tablet from each formula to evaluate the taste.

Preparation of Directly Compressed Rapidly Disintegrating Famotidine Tablets

FT tablets containing ALE were prepared using Primojel and AcDisol as super disintegrants according to formulations presented in Table 4, formulas $P_{1,2,3}$ and $A_{1,2,3}$, respectively, by direct compression technique. The ingredients were thoroughly mixed in a cubic mixer for 5 minutes. The mixture was compressed into 250-mg tablets using a Korsch tableting machine. Another batch of FT tablets containing ALE was prepared using camphor sublimation method. In this method mannitol and camphor (a sublimating substance) were used. The ingredients were thoroughly mixed in a plastic bottle for 2 minutes. Then the mixture was compressed into 250-mg tablets using the tableting machine. The resulting tablets were weighed and labeled. Next, the tablets were placed in an oven at 80°C for 30 minutes to eliminate camphor[16] (by sublimation); then these tablets were weighed to determine the lost weight. According to this method, 3 formulas were prepared: the first one contained mannitol and camphor; the second one contained mannitol, camphor, and Primojel; and

Table 4. Excipients of 10-mg Famotidine Tablets*

Formula	Primojel			AcDisol		
Materials (mg)	P_1	P_2	P_3	A_1	A_2	A_3
Mannitol	60	40	20	60	40	20
Primojel	20	40	60	–	–	–
AcDisol	–	–	–	20	40	60

*Each formula contains 50 mg aqueous licorice extract, 3.75 mg polyethylene glycol 6000, 60 mg direct compression sugar, and 46.25 mg aspartame. A dash refers to the other tablet ingredients.

Table 5. Excipients of 10-mg Famotidine Tablets Prepared by Camphor Sublimation Method*

Formula Materials (mg)	Camphor			Primojel With Camphor			AcDisol With Camphor		
	C_1	C_2	C_3	PC_1	PC_2	PC_3	AC_1	AC_2	AC_3
Mannitol	60	40	20	60	40	20	60	40	20
Primojel	–	–	–	10	20	30	–	–	–
AcDisol	–	–	–	–	–	–	10	20	30
Camphor	20	40	60	10	20	30	10	20	30

*Each formula contains 50 mg aqueous licorice extract, 3.75 mg polyethylene glycol 6000, 60 mg direct compression sugar, and 46.25 mg aspartame. A dash refers to the other tablet ingredients.

the third one contained mannitol, camphor, and AcDisol (Table 5, formulas $C_{1,2,3}$, $PC_{1,2,3}$, and $AC_{1,2,3}$, respectively).

The prepared tablets were evaluated in the same way as the DS tablets, and the following 2 tests were also performed: disintegration time in the oral cavity and in vivo oral absorption test.

The time required for complete disintegration of a tablet in the oral cavity, over the tongue, was collected from 10 healthy volunteers administered each formula at 24-hour intervals.[17] The end point is the exact time required for complete disintegration of the tested tablet.

In vivo tests, similar to that of Ishikawa et al,[18] were performed for studying the oral absorption effect of the chosen FT tablets by administering each formula to 10 healthy volunteers (21–25 years old). Volunteers kept the tablet in the oral cavity over the tongue until disintegration, and then rinsed their mouths with an aliquot of distilled water. This procedure was followed by quantitative assay (spectrophotometrically at 265 nm) to determine the amount of FT remaining in the oral cavity. The amount of drug absorbed through the oral mucosa could be calculated by subtracting the amount remaining from the initial concentration, 10 mg. A washing period of 3 days was taken in consideration to free the body from the drug before dosing.

A similar palatability test was made to evaluate the taste of the prepared tablets. A random sample of 20 students from the College of Pharmacy in Al-Isra University tested the taste of these tablets.

RESULTS AND DISCUSSION

In Vivo Anti-inflammatory Activities

The intraplantar injection of the hind paw by carrageenan induced a progressive edema; this model is useful to detect anti-inflammatory activity of different agents.[19] The results of this study showed an anti-inflammatory activity of all agents under investigation similar to that obtained by DS (10 mg/kg) (Table 1). The combination group of DS and GA acid produced the greater reduction of edema (78.3%). However, when compared with DS alone (73.9%), there was no significant difference.

The pharmacological screening was performed to determine the possible anti-inflammatory activity of licorice extract that contained ∽10% of GA, as well as GA alone, and their combination with DS. In addition, to find any possible interaction between DS and other agents under investigation, the development of edema in the paw of the rat has been described as a biphasic event. The initial phase is attributed to the release of histamine and serotonin. The second phase, which is the accelerating phase of swelling, is due to the release of a prostaglandin-like substance. It has been reported that the second phase of edema is sensitive to both clinically useful steroidal and nonsteroidal anti-inflammatory agents.[20-22] It has been reported that most anti-inflammatory agents might produce gastric irritation and even ulceration with prolonged use.[23]

It has been reported that licorice is one of the most commonly used herbal drugs in traditional Chinese prescriptions. It is mainly used as a demulcent and a sweetener.[1,2,7]

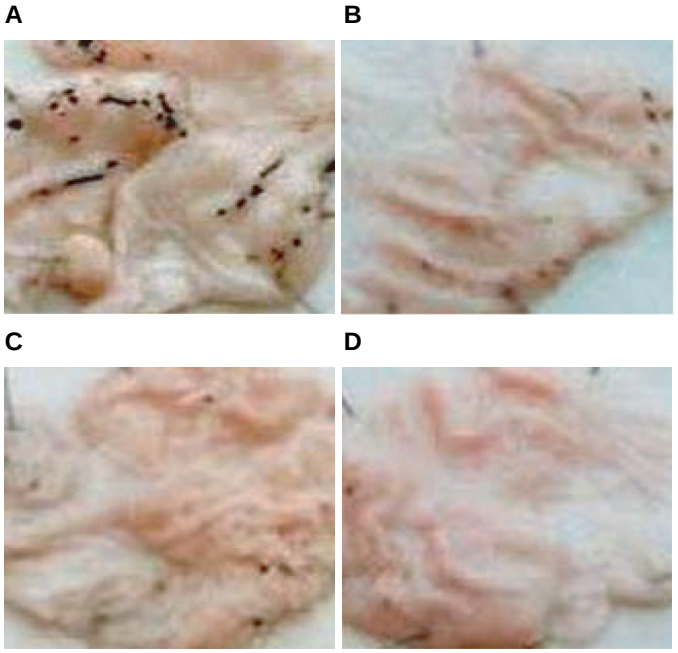

Figure 1. Photograph showing indomethacin-induced gastric ulcer: (A) untreated, (B) treated by FT, (C) treated by licorice, and (D) treated by combination of FT and licorice.

Table 6. The Physical Properties of the Prepared Diclofenac Sodium Chewable Tablets*

Formula	Properties		Uniformity of Weight (g)	Uniformity of Diameter (mm)	Uniformity of Thickness (mm)	Tensile Strength (N/cm^2)	Palatability Significance Test *P* Value
Glycerrhitinic acid	A (no additives)	Mean	0.2104	8.0035	4.365	126.90	Not tested
		CV%	5.586	0.2745	1.0927	3.3511	
	B (Chocolate)	Mean	0.227	8.0125	6.0250	43.28	.001
		CV%	5.0725	0.3433	0.7374	15.9005	
	C (Tutti-frutti)	Mean	0.2008	8.0180	4.4600	43.83	–
		CV%	4.3595	0.3337	0.4310	7.0986	
Aqueous licorice extract	D (Chocolate)	Mean	0.2364	8.0775	4.3025	23.46	.001
		CV%	3.4501	0.3080	0.4463	8.5582	
	E (Tutti-frutti)	Mean	0.2626	8.0825	5.0150	33.63	.05
		CV%	2.7057	0.2951	0.4569	6.2693	
	F (Menthol)	Mean	0.2371	8.047	5.0625	26.89	.01
		CV%	2.5873	0.5034	0.4277	10.4003	

*CV% indicates coefficient of variation percentage; dash indicates other tablet ingredients.

Several studies reported that licorice may possess anti-inflammatory activity, and that this may be owing to its effect on the adrenal glands, which are responsible for producing cortisol.[5,24] In addition, licorice-derived compounds can raise the concentration of prostaglandins in the digestive system, which promote mucus secretion from the stomach and therefore may lead to the healing of ulcers.[25] The aim of this part of the study is to assess the anti-inflammatory activity of licorice as well as the possible interaction that may develop when these plant extracts are taken concomitantly with a nonsteroidal anti-inflammatory agent such as DS. The results of this work demonstrate that licorice extract or GA produced significant anti-inflammatory activity resembling that produced by DS, and when taken concomitantly, there is no possible antagonism. From these observations, the combination may be beneficial owing to the anti-ulcer activity of licorice that counteracts ulcer development after NSAIDs.

In Vivo Anti-ulcer Activities

The results obtained showed that the stomach of rats treated with intragastric indomethacin (20 mg/kg) developed gastric ulceration after 4 hours of administration. Rats

Table 7. The Physical Properties of the Prepared Famotidine Tablets by Direct Compression

Formula	Properties	Uniformity of Weight (mg)	Uniformity of Thickness (mm)	Uniformity of Diameter (mm)	Hardness (Newton)	Disintegration Time (minutes)
Primojel	20%	254.9 (11.60)	1.55 (0.041)	12.98 (0.025)	52.50 (7.500)	3.65 (0.631)
	40%	267.6 (11.92)	1.40 (0.0401)	13.02 (0.044)	55.17 (6.568)	3.18 (0.389)
	60%	259.4 (11.90)	1.52 (0.062)	13.00 (0.029)	52.00 (5.066)	2.01 (0.294)
AcDisol	20%	260.8 (9.38)	1.52 (0.024)	13.04 (0.17)	32.17 (4.140)	2.33 (0.576)
	40%	264.7 (10.77)	1.65 (0.007)	12.65 (0.448)	19.67 (2.357)	1.57 (0.312)
	60%	259.4 (8.83)	1.61 (0.018)	13.10 (0.017)	23.83 (2.544)	1.03 (0.272)
Camphor	20%	240.1 (10.56)	1.54 (0.087)	13.00 (0.018)	24.67 (1.795)	7.00 (0.315)
	40%	214.2 (11.70)	1.64 (0.021)	12.99 (0.036)	29.00 (4.435)	2.16 (0.851)
	60%	195.8 (9.11)	1.65 (0.008)	12.60 (0.216)	20.50 (1.258)	0.87 (0.298)
Camphor and Primojel (1:1)	20%	239.5 (11.93)	1.57 (0.024)	12.96 (0.034)	26.00 (6.298)	0.80 (0.291)
	40%	232.7 (9.42)	1.60 (0.041)	12.99 (0.011)	30.33 (4.190)	0.80 (0.177)
	60%	228.4 (9.03)	1.65 (0.007)	11.95 (0.039)	17.33 (1.106)	0.76 (0.434)
Camphor and AcDisol (1:1)	20%	246.4 (11.88)	1.63 (0.020)	12.97 (0.027)	35.33 (3.350)	1.79 (0.281)
	40%	239.6 (11.19)	1.63 (0.023)	12.90 (0.045)	25.67 (5.249)	0.78 (0.258)
	20%	254.9 (11.60)	1.55 (0.041)	12.98 (0.025)	52.50 (7.500)	3.65 (0.631)

The values between parenthesis are the coefficient of variation percentage (CV%).

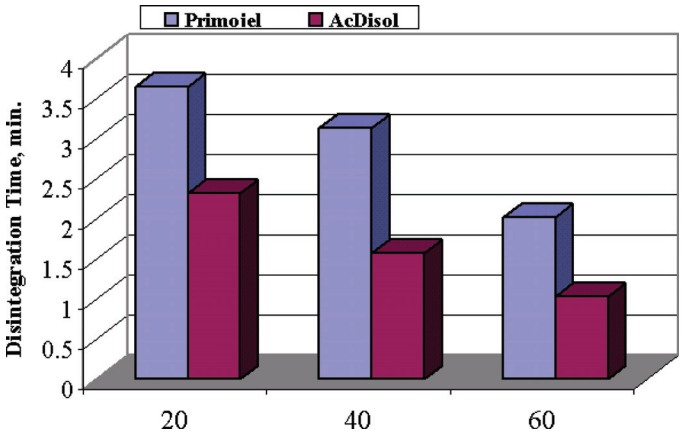

Figure 2 . Disintegration time of the prepared tablets containing Primojel or AcDisol.

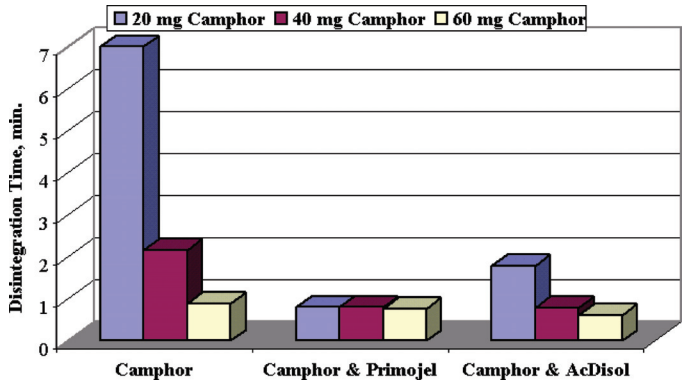

Figure 3 . Disintegration time for the tested tablets containing camphor alone and with Primojel or AcDisol.

treated with indomethacin alone (Group A) developed an ulceration area of 24.9 mm^2 that represents 3.59% of the glandular area. Stomachs of rats that were pretreated with FT (Group B) developed an ulceration area of 4.6 mm^2, representing 0.72% of the glandular area. However, stomachs of rats that were pretreated with licorice (Group C) developed an ulceration area of 4.9 mm^2, representing 0.74% of the glandular area. Rats pretreated with combination of FT and licorice (Group D) developed an ulceration area of 2 mm^2, which represents 0.32% of the glandular area (Table 2 and Figure 1).

These groups were evaluated statistically applying the Student *t* test (at $P < .05$). The results revealed that there was significant difference between group A and groups B, C, and D, while comparison of Group B with C showed no significant difference. The percentage of ulceration in rats treated with indomethacin alone (ie, group A) was 3.59%. However, significant difference was observed when groups B and C were compared with group D.

From the above results, it may be concluded that licorice has similar anti-ulcer activity to FT. The combination of FT with licorice showed superior anti-ulcer effect to either of them alone.

Diclofenac Sodium Chewable Tablet Properties

Table 6 represents the physical properties of the prepared DS tablet formulations. All the prepared tablet formulations (A to E) showed acceptable physical properties for the uniformity of weight, diameter, and friability results according to *United States Pharmacopeia/National Formulary (USP/NF)*2002. The Ts (127 N/cm^2) and thickness values are also suitable. Neither the friability nor the hardness values has any *USP* requirements for chewable tablets.

The comparison statistical study for the palatability of the prepared chewable tablets (Table 6) revealed that formula A has a comparatively bad taste. Upon applying the Chi-square test for the obtained results of taste on 42 students,

Table 8. Tablets Weight (mg) Before and After Camphor Sublimation and the Amount of Camphor Lost

Formula	Camphor Concentration (mg)	Weight Before Camphor Sublimation (mg)	Weight After Camphor Sublimation (mg)	Loss in Weight (mg)
Camphor alone	20	260.4 (10.26)	240.1 (10.56)	20.3 (0.98)
	40	254.5 (11.69)	214.2 (11.70)	40.3 (0.71)
	60	255.4 (9.54)	195.8 (10.92)	59.6 (0.88)
Camphor and Primojel (1:1)	20	252.9 (11.75)	241.5 (11.93)	11.4 (0.55)
	40	254.7 (8.56)	232.7 (9.42)	22.0 (0.65)
	60	259.2 (10.22)	228.4 (9.03)	30.8 (0.72)
Camphor and AcDisol (1:1)	20	258.0 (11.76)	246.4 (12.88)	11.6 (0.48)
	40	260.7 (11.89)	239.6 (11.19)	21.1 (0.74)
	60	258.6 (10.60)	227.8 (11.20)	30.8 (0.82)

The values between parenthesis are the coefficient of variation percentage (CV%).

Table 9. In Vitro Disintegration Time, Oral Disintegration Time, and the Amount of Famotidine Absorbed for the Chosen Formulas (Which Contain 60 mg)*

Properties Formula	In Vitro Disintegration Time (minutes)	Oral Disintegration Time (minutes)	Amount of FT Absorbed (mg)
Primojel	2.013 (0.294)	1.683 (0.322)	9.06 (0.09)
AcDisol	1.030 (0.272)	1.470 (0.285)	7.82 (0.53)
Camphor	0.870 (0.298)	0.717 (0.401)	9.58 (0.02)
Camphor and Primojel (1:1)	0.760 (0.434)	0.433 (0.292)	8.57 (0.45)
Camphor and AcDisol (1:1)	0.620 (0.280)	0.840 (0.282)	9.34 (0.13)

*FT indicates Famotidine.
The values between parentheses are the coefficient of variation percentage (CV%).

using formula C as standard formula, the results obtained (Table 6) revealed that by comparing group C to group F, a significant difference could be observed with *P* less than .01, whereas when comparing this group C with groups B and D, *P* was less than .001, which indicates a highly significant difference. Also, when this group (group C) was compared with group E, a significant difference was obtained with *P* less than .05. Thus, it could be concluded that the formula containing tutti-frutti with GA (ie, formula C) showed the most palatable formula followed by those containing ALE with chocolate (ie, formula E), then formula F, which contained ALE with menthol. Whereas the 2 formulas containing GA with chocolate (ie, formula B) and ALE with tutti-frutti (ie, formula D) have the lowest acceptability results.

Directly Compressed Famotidine Tablets

The physical properties results of the tablets prepared by direct compression using either Primojel or AcDisol are represented in Table 7. All tablet formulations fulfill the *USP/NF* 2002 requirements for the uniformity of weight and diameter. The hardness values are also suitable for this type of tablets. The in vitro disintegration time of the prepared tablets, generally, was decreased by increasing the amount of disintegrant, reaching 1 minute in formula containing 24% AcDisol. Also, tablets containing AcDisol showed more rapid disintegrating effect than those of Primojel (Figure 2). On the other hand, tablets prepared by camphor sublimation method showed pronounced decreasing of in vitro disintegration time by increasing camphor concentration (Table 7 and Figure 3). However, the inclusion of Primojel with camphor markedly improved the disintegration time in the 3 tested formulations ($\backsim$0.8 minute). While formulations containing AcDisol showed 0.62-, 0.78-, and 1.79-minute disintegration time in tablets containing 30, 20, and 10 mg AcDisol, respectively (Table 7).

Table 8 shows tablet weight before and after camphor sublimation. For 20 tablets prepared using a mannitol/camphor compounding ratio of 1:3, 1:1, and 3:1, the mean loss in weight was 21.3, 39.6, and 59.7 mg, respectively, corresponding to the weight of camphor present in each formula (20, 40, and 60%, respectively). The other 6 formulas (containing Primojel or AcDisol) revealed similar results (Table 8). Those results indicate that there is no camphor remaining through the sublimation process.

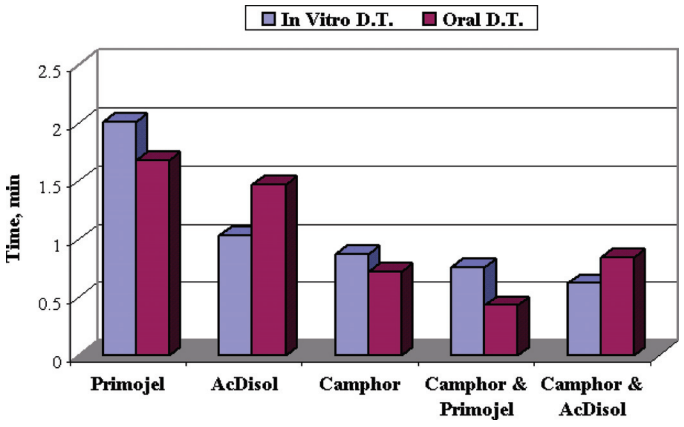

Figure 4. In vitro and oral disintegration time (DT) for the chosen tablet formulations.

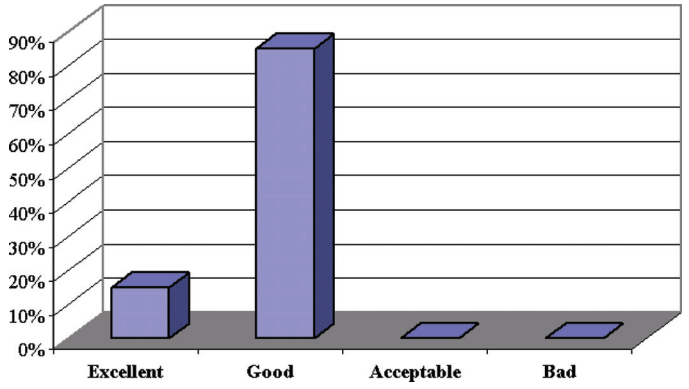

Figure 5. Palatability test for the prepared rapidly disintegrating FT tablets.

The most rapid in vitro disintegrating formulas (containing 60 mg) were chosen for the in vivo oral disintegration test and for the determination of the amount of FT absorbed after disintegration (Table 9 and Figure 4). The FT concentration was determined spectrophotometrically at 265 nm.

The oral disintegration values were very rapid for all the tested formulations (Table 9). This may be attributed to the comparatively large amount of disintegrant in the first 2 formulas and camphor in the third one. Also, the combination of disintegrant and camphor in the last 2 formulas showed the most rapid effect.

The amount of FT absorbed from the oral cavity was nearly 9 from 10 mg theoretically present, in each formula (Table 9). This indicates very rapid disintegration followed by rapid onset of action of drug when administered over the tongue.

The results of the palatability test revealed that 15% of students stated that the taste of the prepared FT tablets was excellent, while 85% stated that it was good, as seen in Figure 5.

CONCLUSION

It could be concluded from the results that the ALE or GA produced significant anti-inflammatory activity resembling that produced by DS, and when taken concomitantly, there is no possible antagonism. Combination therapy of both FT and licorice showed higher anti-ulcer activity than either of them alone. The oral disintegration of FT was very rapid for all the chosen tested formulations. Also, the amount absorbed from the oral cavity was nearly 9 from 10 mg theoretically present in each formula. It may be recommended to add ALE to either FT or DS for the production of more effective anti-inflammatory or anti-ulcer formulations, respectively.

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