STUDY OF THE ANTI-INFLAMMATORY ACTIVITY OF SOME MEDICINAL EDIBLE PLANTS GROWING IN EGYPT

N. M. AMMAR*
S. Y. AL OKBI**
D. A. MOHAMED**

SUMMARY: Several edible plants are used in traditional medicine for the treatment of inflammatory conditions. The anti-inflammatory activity of some bioactive fractions isolated from the seeds of Trigonella foenum groecum, L., the roots of Glycyrrhiza glabra, L. and the fruits of Coriandrum sativum, L. were determined using the carragenan induced oedema method in comparison with two reference synthetic anti-inflammatory drugs. The petroleum ether and the aqueous methanolic extract of fenugreek and liquorice as well as the whole powdered fruit of coriander in a dose of 200 mg/kg exhibited a significant reduction in the volume of inflammation with variable degreeses. The same active dose from the three plants also inhibited prostaglandins E_2 levels in a range of 55-64%. Chromatographic fractionation of the bioactive components revealed the isolation of unsaturated fatty acids and flavonoids in the three plants, while saponins were only present in fenugreek and liquorice. The anti-inflammatory activity may be attributed to the presence of the forementioned bioactive compounds.

Key Words: Anti-inflammatory, medicinal, edible plants.

INTRODUCTION

The severe side effects of steroidal and nonsteroidal anti-inflammatory drugs evoked us to search for new anti-inflammatory agents from natural botanical sources which may have minimal drawbacks.

Our review of 'folk medicine' indicated that many plants possessing an anti-inflammatory activity were sometimes consumed by humans. These were the roots of *Glycyrrhiza glabra*, L., family Leguminosae (Liquorice), the seeds of *Trigonella foenum groecum*, L., family Leguminosae, (Fenugreek) and the fruits of *Coriandrum sativum* L., family Umbelliferae (Coriander).

The anti-inflammatory activity of liquorice has been studied and still current research is being carried out by some authors (1-2). The majority of literature deals only with the activity of the the aqueous extract and its main component, glycyrrhizin. The anti-inflammatory activity of fenugreek seeds have been reported (3), the authors noticed only the topical anti-inflammatory activity of the aqueous alcoholic extract on treating Aphthus ulcers and attributed its activity to the presence of triterpenoid saponins. Scanty literature concerning the anti-inflammatory effect of coriander fruits were avail-

*From Department of Pharmaceutical Sciences, National Research Center, Dokki, Cairo, Egypt.
**From Department of Nutrition, National Research Center Dokki, Cairo, Egypt.

able, only the topical anti-inflammatory activity of glycerol-ethanol extract of coriander fruits was studied and proved to be successful (4). So we decided to extend our study to include the systemic anti-inflammatory activity of several bioactive fractions isolated from the three aforementioned plants in comparison with two reference synthetic anti-inflammatory drugs. The aim of the present work is to evaluate their anti-inflammatory activity on the induced acute inflammation using carrageenan model in experimental rats, in addition, the measurement of PGE_2 will be carried out.

MATERIALS

1. Plant Materials

The plant materials used in this investigation consisted of: The seeds of *Trigonella foenum groecum*, L., family Leguminosae, (Fenugreek), the roots of *Glycyrrhiza glabra*, L., family Leguminosae (Liquorice), and the fruits of *Coriandrum sativum* L., family Umbelliferae (Coriander), which were collected from Egyptian markets. All samples were authenticated by Miss Badia Hassan Diwan, Consultant of Taxonomy Department of Systematic and Egyptian Flora, El-Orman Garden-Giza, Egypt.

2. Materials for Chromatography

1) Plates of silica gel 60 GF_{254} [0.25 mm. thickness, E. Merk, Dermastadt, Germany] for TLC.

2) Sheets of Whatmann filter paper No. 1 for Pc.

3) Sheets of Whatmann filter paper (3MM) for PPc.

3. Animals

White female albino rats of Sprague-Dawely strain of 150g average body weight were used. The animals were kept individually in wire bottomed cages at room temperature of 25±2°C and a relative humidity of about 55%.

4. Drugs (Synthetic anti-inflammatory agents)

Two reference anti-inflammatory drugs were used in our study.

1) Commercial name: Indomethacin, Chemical name: 1-(4. Chlorobenzoyl)-(-methoxy-2-methylindol-3-yl) acetic acid obtained from Pharco Pharmaceuticals Company.

2) Commercial name: Urbason retard, Chemical name Methyl Prednisolon-6 α- methyl predinosolone 11 β, 17 α, 21-Trihydroxy-6 α- methyl pregna-1,4-diene-3,20-dione, obtained from Hochest Orient Company.

5. Chemicals Used for Induction of Inflammation

λ-Carrageenan, type IV (Sigma, USA) Carrageenan is a polysaccharide isolated from two species *Girgartina aciculaire* and *G. pistillata*, which grow together in the sea. Carrageenan is prepared by dissolving 0.05 gm in 5ml distilled water.

METHODS

1. Preparation of Plants for Phytochemical Studies

The three plants were separately air dried, reduced to No. 36 powder and kept in tightly closed containers. 500 g of the dried powder of each plant under investigation were placed in a continuous extraction apparatus and subjected to successive extraction using petroleum ether (60°-80°C) then 50% aqueous methyl alcohol. For each organic solvent the extraction was continued till no residue was obtained when small aliquot colourless extract was evaporated to dryness in a small glass watch. For each extract the solvent was completely removed by distillation under reduced pressure at a temperature not exceeding 40°C and dried to a constant weight in a vacuum dessicator over anhydrous calcium chloride.

2. Phytochemical Study of the Biologically Active Extracts

The solvent free petroleum ether and alcoholic extracts of each of the three plants were subjected to phytochemical examination using chemical and chromatographic methods. The extracts of the three plants were separately screened for the following constituents, carbohydrates and (glycosides, tannins, flavonoids alkaloids and/or nitrogenous bases, saponins unsaturated sterols and/ triterpenes, coumarins and volatile oils (5).

3. Chromatographic Investigation

The bioactive extracts of the three plants were subjected to chromatographic identification using thin layer, paper, as well as preparative layer chromatography. Sterols and triterpenes (5) were detected using the bioactive petroleum ether extract of the three plants separately by spotting an aliquot of each sample on silica gel G plates using (benzene:ethanol) (19:1) as solvent system and detection was carried out by vanillin sulphuric acid reagent followed by heating at 100°C for 5 minutes. Saponins were isolated from the remaining aqueous concentrate of liquorice and fenugreek after evaporation of the bioactive hydromethanolic extract under vacuum at 60°C (6). The isolated saponin compounds from each plant were investigated by TLC on silica gel 'G' plates developed with (n-butanol:acetic acid:water) (4:1:1) or (chloroform:

methanol:water) (65:40:10). The spots were visualized by spraying with Modified Khagi Mishner reagent (7). Acid hydrolysis of each saponin was carried out with 2N sulphuric acid. The sapogenins isolated were detected by spotting on silical gel 'G' plates using [petroleum ether:benzene:ethyl acetate:acetic acid] [10:20:6:0.5] as solvent system and detected by spraying with Khagi Mishner reagent. Flavonoids were detected and isolated in the three plants by spotting an aliquot of the bioactive aqueous methanolic extract of each separately on thin layer chromatography using silica gel GF_{254} as adsorbant development was carried out using (methanol: chloroform) (8:2) or on paper chromatography using (n butanol-acetic acid-water) (4:1:5) as solvent system. The fluroescent spots were detected with aluminium chloride reagent under UV. The flavonoid glycosides were hydrolysed to their respective aglycones and sugars with 2N HCL. All the isolated compounds were identified by physical and spectral analysis as well as co-chromatography with authentic samples and comparison with reported literature. Sugars in all cases were detected by using Kieselguhr impregnated with Phosphate buffer (pH 5) as adsorbant and developed with (n-butanol-acetone-phosphate buffer (pH 5)) (40:50:10) the spots were visualized with naphthoresorcinol sulphuric acid reagent and heating at 105°C for 10 minutes (8).

4. Design of the Experimental Biological Work

1) Induction of Inflammation by Carrageenan and Measurement of Inflammation Volume.

Rats were maintained on laboratory stock diet and fasted for 16 hours before starting the experiment and divided into 18 groups each comprised of six rats.

The groups were:

Two control groups where rats did not recieve any medication.

Test groups:

Eight groups, where rats were given one oral dose of either 200 or 500 mg of either alcoholic or petroleum ether extracts of fenugreek, or liquorice/kg rat body weight. Two groups, where rats received one oral dose of 500 mg of either the alcoholic or petroleum ether extract of coriander fruits/kg rat body weight. Two groups where rats received one oral dose of either 200 or 500 mg of the whole powdered coriander fruits/kg body weight. Four groups, where rats received one oral dose of either 5 or 30 mg of Indomethacin or Urbason retard/kg rat body weight (9). After an hour of the oral medication, all rats were injected into the subplanter surface of the right hind paw with 1% λ-carrageenan suspension (0.05 ml/animal). Paw volumes were measured using differential volume meter immediately before the injection of carrageenan and after 30 minutes, 1, 1.5, 2, 3 and 4 hours of carrageenan injection. The volume of inflammation in the hind paw volume of rats given different anti-inflammatory agents was compared with that of the control inflammed rats.

2) *Determination of Prostaglandin E_2 in Plasma of Inflammed Rats:*

Plasma prostaglandin levels were determined nearly at the time of the best anti-inflammatory effect of the natural and synthetic agent proved to have an anti-inflammatory activity from the previous experiment.

The same experiment of induction of inflammation was repeated using another rats of the same sex and body weight which were given only the low dose of the plant extract (200mg/kg). In this experiment the control inflammed groups contain twelve rats. A normal control group of 6 rats was added (where no injection of carrageenan or oral medication was given). Concerning reference drugs; only two groups of rats were run where rats received one oral dose of 5 mg of either Indomethacin or Urbason retard/kg rat body weight. After one and half an hour of carrageenan injection rats which were given oral doses of the alcoholic or the petroleum ether extract of liquorice or fenugreek (200 mg/kg rat body weight), 6 rats from the control inflammed group and the normal control rats were anaesthesized and blood samples were drawn and collected on EDTA. After 3 hours of carrageenan injection, blood samples of all the residual groups were also collected on EDTA. The plasma was separated by centrifugation at 3000 r.p.m. for 15 minutes for the determination of prostaglandin E_2 by a radioimmunoassay (10).

RESULTS AND DISCUSSION

Carrageenan induced oedema of rat foot is used widely as a working model of inflammation in the search for new anti-inflammatory agents (11) and appeared to be the basis for the discovery of Indomethacin, the anti-inflammatory drug (12). The oedema which develops in rat paw after carrageenan injection is a biphasic event (13). The initial phase is attributed to the release of histamine and serotonin, the oedema maintained between the first and second phase to kinin, and the second phase to prostaglandin (14). All the mediators appear to be dependent upon an intact complement system for their activation and release (15). It has been shown (16) that, in the early

Table 1: Mean hind paw volume at different time intervals of carrageenan injection after administration of natural or synthetic anti-inflammatory agents.

Groups		Time (hours)						
		0	0.5	1	1.5	2	3	4
Control	Mean	1.340	1.488	1.618	1.734	1.806	1.890	1.863
	± S.E.	0.037	0.036	0.031	0.039	0.063	0.035	0.045
Alcoholic ext. of liquorice 200 mg/kg	Mean	0.979	1.017	1.017	1.050	1.154	1.146	1.188
	± S.E.	0.033	0.039	0.039	0.046	0.067	0.059	0.050
Alcoholic ext. of liquorice 500 mg/kg	Mean	0.917	0.954	0.992	1.038	1.103	1.108	1.154
	± S.E.	0.026	0.028	0.033	0.050	0.075	0.057	0.053
PE. ext liquorice 200 mg/kg	Mean	0.817	0.867	0.838	1.000	1.179	1.188	1.183
	± S.E.	0.023	0.032	0.020	0.025	0.032	0.053	0.042
PE. ext liquorice 500 mg/kg	Mean	0.821	0.879	0.946	1.075	1.225	1.250	1.230
	± S.E.	0.044	0.034	0.037	0.049	0.047	0.049	0.056
Alcoholic ext. of fenugreek 200 mg/kg	Mean	1.354	1.483	1.547	1.683	1.717	1.784	1.788
	± S.E.	0.033	0.020	0.029	0.058	0.042	0.053	0.051
Alcoholic ext. of fenugreek 500 mg/kg	Mean	1.369	1.478	1.640	1.652	1.730	1.794	1.818
	± S.E.	0.017	0.009	0.017	0.028	0.029	0.031	0.047
PE. ext. of fenugreek 200 mg/kg	Mean	0.804	0.904	0.900	0.971	1.129	1.204	1.229
	± S.E.	0.024	0.019	0.013	0.034	0.027	0.057	0.053
PE. ext. of fenugreek 500 mg/kg	Mean	0.904	0.967	0.954	1.073	1.271	1.313	1.367
	± S.E.	0.016	0.028	0.019	0.085	0.058	0.071	0.076
Indomethacin 30 mg/kg	Mean	0.808	0.817	0.908	0.958	0.913	1.017	1.067
	± S.E.	0.030	0.041	0.063	0.066	0.055	0.057	0.035
Urbason retard 30 mg/kg	Mean	0.796	0.896	0.908	0.946	0.896	1.033	1.046
	± S.E.	0.022	0.016	0.039	0.022	0.028	0.030	0.019
Control	Mean	0.758	0.871	0.938	1.063	1.163	1.371	1.439
	± S.E.	0.026	0.027	0.029	0.032	0.056	0.051	0.091
Coriander fruits powder 200 mg/kg	Mean	1.075	1.125	1.117	1.196	1.354	1.311	1.354
	± S.E.	0.022	0.016	0.017	0.023	0.031	0.043	0.051
Coriander fruits powder 500 mg/kg	Mean	0.804	0.988	0.958	1.088	1.113	1.192	1.227
	± S.E.	0.024	0.017	0.020	0.032	0.063	0.057	0.064
Alcoholic ext. of coriander 500 mg/kg	Mean	0.767	0.879	0.950	1.038	1.158	1.406	1.463
	± S.E.	0.019	0.024	0.025	0.049	0.084	0.079	0.071
PE. ext. coriander 500 mg/kg	Mean	0.688	0.808	0.854	0.971	1.071	1.395	1.365
	± S.E.	0.024	0.033	0.023	0.032	0.065	0.049	0.069
Indomethacin 5 mg/kg	Mean	0.842	0.971	0.946	0.979	1.050	1.121	1.125
	± S.E.	0.036	0.019	0.025	0.019	0.022	0.034	0.040
Urbason retard 5 mg/kg	Mean	0.858	0.933	0.954	0.950	0.983	1.004	1.004
	± S.E.	0.031	0.015	0.012	0.012	0.018	0.018	0.018

Table 2: The volume of inflammation of the hind paw (c.c) at different time intervals of carrageenan injection after administration of natural or synthetic anti-inflammatory agents in comparison to control inflammed rats.

Groups		Time (hours)					
		0.5	1	1.5	2	3	4
Control	Mean	0.148	0.278	0.394	0.466	0.550	0.523
	± S.E.	0.023	0.034	0.028	0.041	0.035	0.043
Alcoholic ext. of liquorice 200 mg/kg	Mean	0.038****	0.038*****	0.071*****	0.175****	0.167*****	0.208*****
	± S.E.	0.009	0.009	0.023	0.066	0.054	0.048
	% Inhibition	74	86	82	64	70	60
Alcoholic ext. of liquorice 500 mg/kg	Mean	0.038****	0.075*****	0.121*****	0.186****	0.192*****	0.238****
	± S.E.	0.015	0.013	0.028	0.057	0.044	0.046
	% Inhibition	74	73	69	60	65	54
PE. ext of liquorice 200 mg/kg	Mean	0.050***	0.125*****	0.183****	0.363	0.408***	0.367**
	± S.E.	0.020	0.027	0.017	0.027	0.037	0.031
	% Inhibition	66	55	54	22	26	30
PE. ext of liquorice 500 mg/kg	Mean	0.058**	0.125***	0.254****	0.413	0.408**	0.413
	± S.E.	0.020	0.027	0.019	0.009	0.035	0.026
	% Inhibition	61	55	36	11	26	21
Alcholic ext. of fenugreek 200 mg/kg	Mean	0.129	0.193	0.296	0.363	0.430*	0.433
	± S.E.	0.019	0.018	0.039	0.031	0.037	0.048
	% Inhibition	13	31	25	11	22	17
Alcholic ext. of fenugreek 500 mg/kg	Mean	0.108	0.386	0.283***	0.462	0.425	0.448
	± S.E.	0.013	0.106	0.026	0.093	0.038	0.049
	% Inhibition	27	-	28	1	23	14
PE. ext. of fenugreek 200 mg/kg	Mean	0.100	0.096****	0.167****	0.325*	0.400	0.425
	± S.E.	0.021	0.027	0.043	0.045	0.069	0.054
	% Inhibition	32	65	58	30	27	23
PE. ext. of fenugreek 500 mg/kg	Mean	0.063**	0.050*****	0.169**	0.367	0.408	0.463
	± S.E.	0.022	0.019	0.076	0.049	0.061	0.068
	% Inhibition	57	82	57	21	26	11
Indomethacin 30 mg/kg	Mean	0.100	0.100*	0.150***	0.104*****	0.208*****	0.258*****
	± S.E.	0.048	0.068	0.069	0.051	0.037	0.005
	% Inhibition	32	64	62	78	62	51
Urbason retard 30 mg/kg	Mean	0.100	0.113****	0.15*****	0.092*****	0.238*****	0.25*****
	± S.E.	0.011	0.020	0.013	0.023	0.019	0.014
	% Inhibition	32	59	62	80	57	52

Values significantly differ from the control:

*:P<0.05, **:P<0.025, ***:P<0.010, ****:P<0.005, *****:P<0.001

Table 2: Continue

Groups		Time (hours)					
		0.5	1	1.5	2	3	4
Control	Mean	0.113	0.211	0.296	0.404	0.613	0.681
	± S.E.	0.020	0.046	0.052	0.073	0.055	0.087
Coriander fruits powder 200 mg/kg	Mean	0.050**	0.042****	0.100****	0.279	0.235*****	0.279****
	± S.E.	0.009	0.011	0.009	0.045	0.058	0.063
	% Inhibition	56	80	66	31	62	59
Coriander fruits powder 500 mg/kg	Mean	0.179	0.150	0.279	0.304	0.388*	0.423
	± S.E.	0.025	0.032	0.034	0.076	0.077	0.079
	% Inhibition	-	29	6	25	37	38
Alcholic ext. of coriander 500 mg/kg	Mean	0.113	0.183	0.271	0.392	0.639	0.697
	± S.E.	0.014	0.026	0.044	0.081	0.082	0.075
	% Inhibition	0	13	8	3	-	2
PE. ext. of coriander 500 mg/kg	Mean	0.121	0.167	0.267	0.383	0.708	0.678
	± S.E.	0.012	0.008	0.031	0.057	0.033	0.051
	% Inhibition	-	21	10	5	-	-
Indomethacin 5 mg/kg	Mean	0.129	0.104	0.138**	0.208*	0.279*****	0.283****
	± S.E.	0.022	0.018	0.026	0.029	0.041	0.038
	% Inhibition	-	51	53	49	54	58
Urbason retard 5 mg/kg	Mean	0.075	0.096	0.092***	0.125****	0.146*****	0.146*****
	± S.E.	0.032	0.027	0.029	0.021	0.037	0.052
	% Inhibition	34	55	69	69	76	79

Values significantly differ from the control:

*:P<0.05, **:P<0.025, ***:P<0.010, ****:P<0.005, *****:P<0.001

phase of the oedema, the dominant cells are polymorphonuclears whereas in advanced stages mononuclears predominate. The effect of administration of the different natural agents and the reference drugs on carrageenan oedema are present in Tables 1 and 2. In our study Urbason retard (steroidal) and indomethacin (non-steroidal) anti-inflammatory drugs were tested on carrageenan oedema. It was noticed that Indomethacin and Urbason retard on oral administration of 30 and 5 mg/kg respectively (Table 2) showed a significant inhibitory effect starting after 1 and 1.5 hours following carregeenan injection. The activity profile of both Urbason retard and Indomethacin is similar. At high dose (30 mg/kg), the anti-inflammatory activity of both drugs increased gradualy till it reached its maximum 2 hours after carrageenan injection (80 and 78% for Urbason retard and Indomethacin respectively) then starts to decline till 4 hours but the effect was still significant. At low dose (5mg/kg), the anti-inflammatory activity progressed with time till it reached its maximum 4 hours after carrageenan injection (79 and 58% for Urbason retard and Indomethacin respectively). It was reported (1) that administration of Prednisolon at 5 and 30 mg/kg doses produced 40% and 60% inhibition of carrageenan oedema in mice respectively after 4 hours. The activity profile of low dose of Indomethacin in our

Table 3: Plasma levels of PGE_2 (pg/ml) of inflammed rats pretreated orally with the different natural (200 mg/kg) or synthetic (5 mg/kg) anti-inflammatory agents.

A. After 1.5 hour of carrageenan injection.

Groups	Normal control	Inflammed control	PE. ext. of fenugreek	Alcoholic ext. of liquorice	PE. ext. of liquorice
Mean	197.320	516.242	186.495*****	245.133*****	202.921*****
± S.E.	19.192	43.786	15.432	31.067	23.490
% Change	-	-	64	53	61

B. After 3 hours of carrageenan injection.

Groups	Normal control	Inflammed control	Alcholic ext. of fenugreek	Coriander powder	Indomethacin	Urbason retard
Mean	197.320	617.037	254.241*****	279.458*****	199.531*****	199.978*****
± S.E.	19.192	41.953	9.210	14.794	2.895	26.100
% Change	-	213	59	55	68	68

Values significantly differ from the inflammed control: ***** P<0.001

study agreed with that of Ozaki (17). Examination of the nonsteroidal anti-inflammatory drugs on carrageenan model has shown that they suppressed mainly the last phase of the response, namely the 'prostaglandin phase'. Their ability to suppress this phase correlates directly with their ability to suppress mononuclear leucocyte migration into the inflammed tissues (14). The present study indicated that the petroleum ether and the alcoholic extracts of liquorice and fenugreek and the whole powdered coriander fruits exhibited anti-inflammatory effects on carrageenan induced oedema in rat paw at both 200 and 500 mg/kg doses. It is worthy to mention the administration of both petroleum ether or alcoholic extract of liquorice as well as the whole powdered coriander fruits in a low dose (200 mg/kg) is more potent than the high dose (500 mg/kg). This low dose of any of the mentioned natural agents was more significant than Indomethacin used in a dose of 5 mg/kg. The activities of those natural agents differ from that of Indomethacin and Urbason retard since the significant inhibitory effect of them started 0.5 hour following the injection of the phlogistic compound and continued during the 4 hours. This means that the anti-inflammatory mechanism of action of Liquorice extracts and the whole powdered Coriander fruits may include an inhibitory effect on all the mediators released before the second phase as well as on prostaglandin; the mediator of the second phase. In our study the least anti-inflammatory activity on this model was due to the petroleum ether and the alcoholic extracts of fenugreek. The significant anti-inflammatory effect of both doses (200 and 500 mg/kg) of the petroleum ether extract of fenugreek was only restricted between 0.5 and 2 hours after carrageenan injection. The administration of the alcoholic extract of fenugreek in a dose of 200 and 500 mg/kg produced a significant anti-inflammatory effect 3 hours and 1.5 hours after carrageenan injection respectively. So this extract may act either through inhibition of prostaglandin or one of the transmitters released before the second phase of carrageenan oedema or both collectively. The petroleum ether and the alcoholic extracts of coriander were devoid of any anti-inflammatory activity when tested separately. However the whole coriander powder fruits

produced a potent anti-inflammatory activity. This suggests that a synergism may be induced from the combination of both extracts in the mother coriander fruit powder. Plasma levels of PGE_2 of different experimental groups are present in Table 3. It is obvious that the prostaglandin levels in control inflammed rats were significantly higher than control normal rats where $p<0.001$. Our study showed that the plasma levels of prostaglandin E_2 were inhibited significantly after the administration of any of the anti-inflammatory natural agents. This means that the mechanism of action of all the tested anti-inflammatory agents included prostaglandin inhibiting activity.

Treatment of rats with Indomethacin and Urbason retard in our study produced a significant reduction of plasma prostaglandin E_2 by 68% where $p<0.001$. Our results agreed with that of Jaffee *et al.* (10) who reported that treatment of rats with Indomethacin in some inflammatory conditions produced a significant reduction of plasma prostaglandin E_2 levels by 67%.

The results in our study revealed that the inhibition of prostaglandin E_2 levels in serum after the administration of the different anti-inflammatory agents is not proportional to the inhibitory effect of oedema volume. The natural anti-inflammatory agents and reference drugs can be arranged according to their potency in inhibiting serum prostaglandin E_2 in the following order: Urbason retard and Indometha-cin> petroleum ether extract of fenugreek>petroleum ether extract of liquorice>alcoholic extract of fenugreek> whole powdered of coriander>alcoholic extract of liquorice.

The mechanism of action of steroidal anti-inflammatory agent is the blocking of all the known pathways of eicosanoid metabolism possibly by stimulating the synthesis of several proteins collectively called lipocortin. Lipocortins inhibit phospholipase A_2 activity preventing the release of arachidonic acid (18). It was reported (19) that the anti-inflammatory effects of steroidal drugs results from their ability to inhibit both cellular and fluid egress from the vascular space to inflammatory sites and their ability to inhibit the cellular function involved in the inflammation process. The inhibition of immune function also occurs to some degree. Dale and Foneman (20) reported that the steroidal anti-inflammatory drugs reduce the vasodilation which occurs during inflammation. The non-steroidal anti-inflammatory drugs block prostaglandin and thromboxane formation by inhibiting cyclooxygenase activity. Indomethacin, in addition, inhibits phospholipase by increasing intracellular cyclic adenosine monophosphate (18).

Phytochemical study of the bioactive extracts revealed the presence of unsaturated fatty acids and flavonoids in the petroleum ether and aqueous alcholic extracts in the three plants under investigation. While saponins were present in Fenugreek seed and Liquorice, the anti-inflammatory activity of the aqueous alcoholic extract of liqourice root may be due to a great extent to the presence of the triterpenoidal saponins (glycyrrhizin) and the flavonoids liquiritigenin and liquiritin. Aleskinskaya (21) demonstrated that glycyrrhetinic acid salts (the aglycone of glycyrrhizin) were similar to cortisone in effectiveness against inflammation. Nikita (22) has emphasized the anti-inflammatory properties of the parent saponin whilst Ikram and Zirvi (23) concluded that the flavonoids liquiritin, liquiritigenin are amongst the active principles. The origin of the anti-inflammatory activities of saponins compounds is considered to be their structural similarity to adrenal corticoid hormones. Capaso *et al.* (24) failed to observe any effects of the aglycone on the synthesis or liberation of prostaglandin. Inoue *et al.* (25) showed that, these compounds slightly inhibited the activity of enzymes responsible for prostaglandin synthesis, in comparison, glycyrrhizin was devoid of activity. *In vitro* study showed that glycyrrhizin inhibited prostaglandin E_2 production in rats, also it inhibited the release of arachidonic acid (26). In agreement with our results, Nasyrov (27) reported that glycyrrhizic acid showed pronounced anti-inflammatory action, inhibited the development of histamine-serotonin and bradykinin induced oedema and decreased vascular permeability. Concerning the onset of liquorice extract, the results of Inoue *et al.* (28) agreed with ours since they reported that glycyrrhetinic acid is capable of inhibiting inflam-

mation in an earlier time than corticosteroids. It has also shown by the same authors that glycyrrhetinic acid produced significant inhibition of PGE_2. Akamatsu *et al.* (2) reported that glycyrrhizin exerts its anti-inflammatory action by inhibiting the generation of reactive oxygen species by neutrophils, the most potent inflammatory meditor at the site of inflammation. Mitscher *et al.* (29) found O-acetylsalicylic acid in liquorice. Its presence might contribute to the therapeutic properties of the aqeous extract.

Chromatographic investigation of the aqueous alcoholic extract of fenugreek proved the presence of steroidal sapogenins as diosgenin, tiogenin, yamogenin and gitogenin, to which, the anti-inflammatory activity may be attributed. A previous clinical trial for the investigation of the topical anti-inflammatory effect of these steroidal compounds as well as the alcoholic extract of fenugreek proved to be successful (3). The anti-inflammatory activity of the petroleum ether extract of liquorice and fenugreek may be due to the presence of different unsaturated fatty acids of linoleic and linolenic series detected chromatographically and noticed by Frattini *et al.* (30). It was reported previously (31) that the essential fatty acids may have an anti-inflammatory therapeutic activity. Certain botanical lipids such as gamma-linolenic acid (18: 3n-6) can be converted rapidly to dihomo-gamma-linolenic acid (20:3 n-6), which has known anti-inflammatory and immunoregulating properties (32). Dihoma-gamma-linolenic acid competes with arachidonate for oxidative enzymes, thereby reducing production of cyclooxygenase products derived from arachidonate. In addition this fatty acid (dihomo-gamma-linolenic acid (DGLA)) can not be converted to inflammatory leukotriens by 5-lipoxygenase. Instead, it is converted to 15-hydroxy-DGLA, which can inhibit 5-lipoxygenase activity (33). Gamma linolenic acid enrichment of diet suppresses acute and chronic inflammation in several experimental models (34). Fatty acids also have a direct effects on cell function which are independent of products formed through the actions of the cyclooxygenase, and lipoxygnase.

The whole powdered Coriander fruit proved to have a potent anti-inflammatory activity. We suggest that its activity is due to the presence of unsaturated fatty acids and quercetin which may together produce a synergestic effect. These compounds are detected by a chromatographic investigation. The fruit is rich in palmitoleic, linoleic, linolenic acids as well having as high percentage of unsaturated fatty acids of oleic series. Quercetin which is also detected, is a flavonoid which proved to possess an anti-inflammatory activity through inhibition of lipoxygenase and consequently blocking the production of leukotrienes. Quercetin also has an anti-allergic action by blocking the histamine release (35).

CONCLUSION

The alcoholic and petroleum ether extracts of liquorice roots, fenugreek seeds and the whole powder of Coriander fruits can be used safely (within the extremes of our study) as anti-inflammatory agents. The potency of the samples under investigation in acute inflammation can be arranged in the following order: alcoholic extract of liquorice > Urbason retard > whole powder of Coriander > petroleum ether extract of liquorice and Indomethacin > petroleum ether extract of fenugreek > alcoholic extract of fenugreek.

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REFERENCES

1. Amagaya S, E Sugishita, Y Ogihara, S Ogawa, K Okada and T Aizawa : Comparative studies of the anti-inflammatory activities of the stereoisomers of glycrrhetinic acid. J Pharmacobio-Dyn, 7, 923-8, 1984.

2. Akamatsu H, J Komura, Y Asada and Y Niwa : Mechanism of anti-inflammatory action of glycyrrhizin: effect on neutrophil functions including reactive oxygen species generation. Planta Med, 57, 199-221, 1991.

3. Ammar N, S Gaafar and R Khalil : Anti-inflammatory effect of natural steroidal sapogenins on oral aphthus ulcers. Egyptian Dental Journal 38:89-98, 1992.

4. Yapeev Asylgaraj S, I Volfenzon Irina, G Galina Wlantseva, B Lidiya Sherman and V Popova Margarita : Caries prophylactis and anti-inflammatory toothpaste. Izobreteniya, 8, 204, 1993.

5. Harborne JB : Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis. Second edition, published in USA by Chapman and Hall, p 46, 1984.

6. Abdel Wahab SM, GM Wassel, EA Aboutabl, NM Ammar, NM El Fiki and MS Afifi : The saponin of Acacia nilotica, L. Bull Fac Pharm, Cairo Univ, vol 28, no 1, p 87-90, 1990.

7. Stahl E : Thin layer chromatography. Springer Verlag, Berlin, Heidelberg, New York, p 856, 1969.

8. Karawya MS, GM Wassel, HH Baghdadi and NM Ammar : Planta Med., 38, 73, 1980.

9. Lanhers CM, J Fleurentin, F Mortier, A Vinche and C Younos : Anti-inflammatory and analgesic effects of an aqueous extract of Harpagophytum procumbens. Planta Med 58, 117-123, 1992.

10. Jaffee B, HR Behrman and CW Parker : Radioimmunoassay measurement of prostaglandins E, A, and F in human plasma. J Clin Invest, 52:398-405, 1973.

11. Valencia Emir Feria Manueli, G Jesus Diaz, Antonio Gonzalez, and Jaime Bermejo : Antinociceptive, Anti-inflammatory and antipyretic effects of Lapidin, a bicyclic sesqiterpene. Planta Med, 60, 395-399, 1994.

12. Winter CA, EA Risley and GW Nuss : Anti-inflammatory and anti-pyretic activities of indomethacin, 1- (p-Chlorobenzyl) -5-methoxy-2-Methylindole 3-acetic acid. J Pharmac Exp Ther, 141, 369, 1963.

13. Vinegar R, W Schreiber and R Hugo : Biphasic development of carrageenan oedema in rats. J Pharmac Exp Ther, 166, 96, 1969.

14. DiRosa M and DA Willoughby : Screens for anti-Inflammatory drugs. J Pharm Pharmac, 23:297-298, 1971.

15. Giroud JP and DA Willoughby : The interrelations of complement and a prostaglandin-like substance in acute inflammation. J Path, 101, 241, 1970.

16. Perper BM, M Sanda, G Chinea and AL Oronsky : Leukocyte chemotaxis in vivo. 1 Description of a model of cell accumulation using adoptively transfered 51Cr- labeled cells. J Lab Clin Med, 84:378-383, 1974.

17. Ozaki Y, N Mamoru, K Hiroyuki and H Masatoshi : Studies on concentration of glycyrrhizin in plasma and its absorbtion after oral administration of licorice extract and glycyrrhizin. Yakugaku Zasshi, 110:77-81, 1990.

18. Katzung GB : Basic and Clinical Pharmacology. 5th edition Appleton and Lange, A publishing division of prentice Hall, pp 263-277, 1992.

19. Spiegel TM : Practical rheumatology. Printed by A Wiley, Medical Publication. John Wiley and Sons, USA, pp 161-183, 1983.

20. Dale MM and JC Foneman : Text book of immunopharmacology. First edition, printed in Great Britain, Butler and Tanner Ltd, Frome, Somerset, USA, pp 285-290, 1984.

21. Aleshinskaya E : Eksperimentalnoje issliedo wanie I nowyje aspekty kliniczeskowo primienienia glicrizinowoj I gliccirretinowoj kistot wydielennych iz Glycyrrhiza glabra. Herba Pol. 4:437-440, 1970.

22. Nikitina SS : Niekatoryje dannyje O mechanizmie protifwospalite-Inogo effekta glicirizinovoji glicciretinovoji glicciretinovoj kislot vydellennychiz soloki goloj. Farmacol Toksiol. 1:67-70, 1966.

23. Ikram M and AK Zirvi : Chemistry and pharmacology of licorice. Herba Pol, 3-4:312-320, 1976.

24. Capaso F, N Mascolo, G Autore and MR Durracio : Glycyrrhetinic acid, leucocytes and prostaglandins. J Pharm Pharmacol, 35:332-335, 1983.

25. Inoue H, H Saito, Y Koshihara and S Murata : Inhibitory effects of glycyrrhetinic acid derivatives on lipoxygenase and prostaglandin synthetase. Chem Pharm, Bull, 2:847-901, 1986.

26. Ohuchi K, Y Kamada, L Levine and S Tsurufuji : Glycyrrhizin inhibitis prostaglandin E2 production by activated peritoneal macrophages from rats. Prostaglandins Med, 7:457-63, 1981.

27. Nasyrov KhM and DN Lazareva : Study of the anti-inflammatory activity of glycrrhizic acid derivatives. Farmakol Toksikol (Moscow), 43:399-404, 1980.

28. Inoue H, T Mori, S Ashibata and Y Koshihara : Inhibitory effect of glycrrhetinic acid derivatives on arachidonic acid-induced mouse ear oedema. J Pharm Pharmacolo, 40:272-277, 1988.

29. Mitscher LA, YH Park, D Clark and JL Beal : Anti-microbial agents from higher plants. Anti-microbial isoflavonoids and related substances from Glycyrrhiza glabra L. var. typica. J Nat Product, 43:259-69, 1980.

30. Frattini C, C Bicchi, C Barettini and GM Nano : Volatile flavor components of licorice. J Agric Food Chem, 25:1238-1241, 1977.

31. Zurier RB : Fatty acids, Inflammation and Immune responses. Prostaglandins Leukotrienes and Essential Fatty Acids. 48:57-62, 1993.

32. Fantone JC, SL Kunkel, PA Ward and RB Zurier : Suppression by prostaglandin E1 of vascular permerability induced by vasoactive inflammatory mediators. J Immunol, 125:2591-600, 1980.

33. Ziboh VA and RS Chapikin : Biologic significance of polyunsaturated fatty acids in the skin. Arch Dermatol, 123:1686-1690, 1987.

34. Tate G, BF Madell, and RA Karmal : Suppressions of monosodium urate crystal-induced acute inflammation by diets enriched with gamma-linolenic acid and eicosapentaenoic acid. Arthritis Rheum. 31:1543-1551, 1988.

35. Metz SA : Anti-inflammatory agents as inhibitors of prostaglandin synthesis in man. Med Clin N Am, 65, (Symposium Issue), 713-757, 1981.

Correspondence:
N. M. Ammar
Department of Pharmaceutical Sciences,
National Research Center,
Dokki, Cairo,
EGYPT.