

ANTIARTHRITIC ACTIVITY OF GLYCYRRHIZA GLABRA LINN.

By

M. L. GUJRAL, K. N. SAREEN, K. K. TANGRI, A. K. ROY,

G. P. GUPTA AND M. K. P. AMMA

(Department of Pharmacology, K.G. Medical College, Lucknow University, Lucknow).

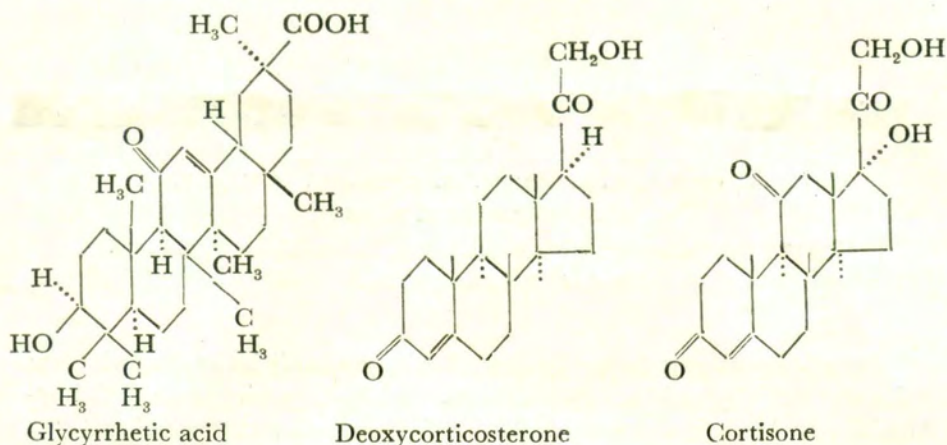
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Glycyrrhiza (liquorice or licorice; Indian-mulethi) is the dried rhizome and root of *Glycyrrhiza glabra* Linn. (family-Leguminosae, sub-family-Papilionaceae). It is a perennial plant which grows in the sub-Himalayan belt of India, Pakistan, China, Iran, Egypt, Southern Europe and Southern Russia. *Glycyrrhiza* (meaning sweet rhizome) is known as madhuyashti or yasthimadhu in Sanskrit, süssholz or süssholzwurzel in German, and bois doux or racine douce in French, all of which mean sweet wood or sweet root.

The use of liquorice in medicine has been known for several thousand years. The ancient Hindu texts like Atharava Veda, Rigveda, the Charaka and the Sushruta Samhitas (Charaka; Sushruta) and the various Nighantus (Dhanvantri) describe its use in affections of the eyes, throat, lungs, and stomach, in inflammations and as an aphrodisiac. In old Chinese medicine the prolonged use of the drug was reputed to have rejuvenating properties. Liquorice has been extensively used in all parts of the world as a demulcent and expectorant, as a mild laxative and as a sweetening agent to disguise the taste of unpleasant mixtures and in confectionery and tobacco industry.

Aqueous extracts of liquorice contain 5-10 per cent of a sweet, white, crystalline diglucuronide known as glycyrrhizin (madhuyashtin in the Hindu literature), which is calcium and potassium salts of glycyrrhizic or glycyrrhizinic acid, $C_{42}H_{82}O_{18}$, m.p. 205°. Glycyrrhizin, on acid or enzymatic hydrolysis, yields the triterpenoid "aglycone" glycyrrhetic or glycyrrhetic acid, $C_{30}H_{48}O_4$, m.p. 303 - 304° (287 - 93° of dimorphic form, [α]_D + 163°, λ max 248 m μ , log ϵ 4.1, methyl ester, m.p. 259°, acetate, m.p. 309-13°, methyl ester acetate, m.p. 300-301°) and two mols of glucuronic acid, $C_6H_{10}O_7$. Glycyrrhetic acid is 11-oxo-oleanol-30-carboxylic acid (β -amyrin series). The root also contains 5-10 per cent sugars (sucrose, dextrose), starch, an acid resin, a bitter principle asparagine, malic acid, and some proteinous, fatty and inorganic matters.

During recent years interest in liquorice has been greatly stimulated by the structural similarity of glycyrrhetic acid and corticosteroids.



Revers (1946 and 1948) and Mulhuysen et al. (1950) found liquorice extract to be effective in the treatment of peptic ulcer. This use was previously known in India and Dr. Geo S. Keith (quoted by Chopra, 1933) reported that "for relieving pain, discomfort and other symptoms caused by acrid matter in the stomach, it is wonderful. It seems to remove the irritating effects of acids in a better way than alkalis". Ito and coworkers (1955 a, b & c) and Adamson and Tillmann (1955) reported beneficial effects of liquorice extracts and glycyrrhetic acid in a variety of dermatoses. Although Warin and Evans (1956), Donaldson and Duthie (1956) and Tillmann et al. (1957) reported adverse results, Evans (1956), Annan (1957), Chakravorti (1957), Sommerville (1957) and Colin-Joans (1957 a & b) confirmed the anti-inflammatory properties of glycyrrhetic acid in dermatoses.

Cornforth and Long (1954) showed certain fractions of liquorice, like hydrocortisone, to suppress the tuberculin reaction in BCG-sensitised guinea pigs. Gujral and Saxena (1956) were the first to report on the antiarthritic effect of aqueous extract of glycyrrhiza in formaldehyde-induced arthritis in rats. Later significant antiarthritic activity of glycyrrhetic acid, by the same test, was also reported by Gujral and coworkers (1957 a & b; 1958 a & b). Somers (1957), D'Archy and Kellett (1957) and Logemann et al. (1957) found glycyrrhetic acid active in the cotton pellet test. Finney and Somers (1958) determined the anti-inflammatory activity of glycyrrhetic acid and some of its derivatives using four methods including the rat foot test. In a later paper Finny et al. (1958) discussed the pharmacology of glycyrrhetic acid.

The present paper includes the work on glycyrrhiza glabra and its constituents carried out in this department since November 1955, when the first paper was communicated.

METHODS AND MATERIALS

Preparation of glycyrrhizin. Coarsely powdered glycyrrhiza root was macerated and then exhausted with hot water in a percolator. Crude glycyrrhizin was precipitated from the dark brown percolate with dilute H_2SO_4 (10 per cent), filtered, washed, dissolved in NH_4OH and then made free from excess ammonia by boiling. After dilution glycyrrhizin was precipitated with lead acetate. The washed lead salt was suspended in water and the lead removed with H_2S . The precipitate of glycyrrhizin and PbS was washed, dried and extracted with alcohol in a soxhlet. The extract, after concentration and then keeping in a refrigerator, gave light brown glycyrrhizin, m.p. $180-85^\circ$. Repetition of the lead precipitation and several crystallisations from alcohol yielded a white crystalline sample, m.p. $203-5^\circ$ (Vogel, 1843; Tschirch and Cederberg, 1907).

Preparation of glycyrrhetic acid. Pure glycyrrhizin was hydrolysed by heating with 100 parts of 3 per cent H_2SO_4 for 20-24 hours in a water bath. The cooled liquid was filtered, washed and repeatedly crystallised from methanol to give white crystals of glycyrrhetic acid, m.p. $297-300^\circ$ (Karrer et al. 1921; Ruzicka and Coworkers, 1936 and 1937).

Testing. The anti-inflammatory activity of glycyrrhizin and glycyrrhetic acid was studied in formaldehyde-induced arthritis in rats produced by method of Selye (1949) as modified by Brownlee (1950). Adult male albino nonadrenalectomised rats of the same strain weighing between 100 and 110 gm. and maintained under uniform conditions were selected and divided into groups of six rats each. One group functioned as untreated control while the remaining groups were administered hydrocortisone and butazolidine as reference standards and the test drugs. The animals were so selected that the mean linear cross section immediately below the ankle joints in each group was within ± 0.1 mm. from that of the control group. 0.1 ml. of a 2 per cent formaldehyde solution was injected in each hind limb of the rat just beneath the plantar aponeurosis. The injection was repeated on the third day. The dose of hydrocortisone, butazolidine and the drugs to be tested was suspended in 1 cc. of 3 per cent solution of gum acacia and introduced into the stomach by means of a gastric cannula daily for ten days from the commencement of the experiment. Day to day changes in size were recorded for each group by measuring the linear cross section immediately below the ankle by means of a micrometer screw gauge. The mean measurement to the nearest half mm. of both feet in each group of six rats was made also for a period of ten days. The mean value for all the observations with their standard error derived from the group means and t and P values were calculated.

RESULTS

In the first series of experiments glycyrrhizin 10 mg./100 gm. body weight was used as a test drug. The results of these experiments are given in Figure 1 and Table 1. A perusal of these will make it clear that glycyrrhizin shows a significant anti-arthritic activity. The probability that the difference observed between the means for the control group and the glycyrrhizin treated group is real, is expressed by a figure for $t=3.4936$ which corresponds to a probability of 1 in 100 that this difference might arise by chance. Hydrocortisone at 0.5 mg./100 gm. and butazolidine at 10 mg./100 gm. body weight showed a greater activity ($P=0.001$) than glycyrrhizin.

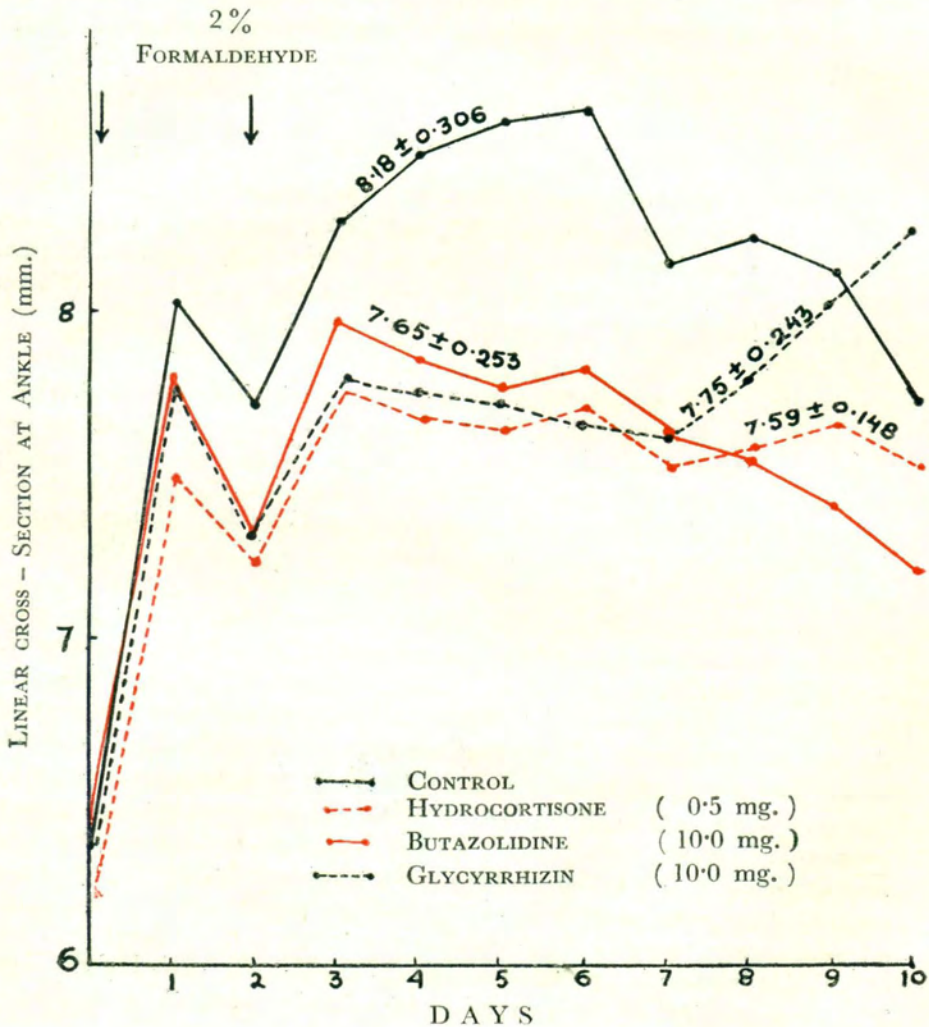
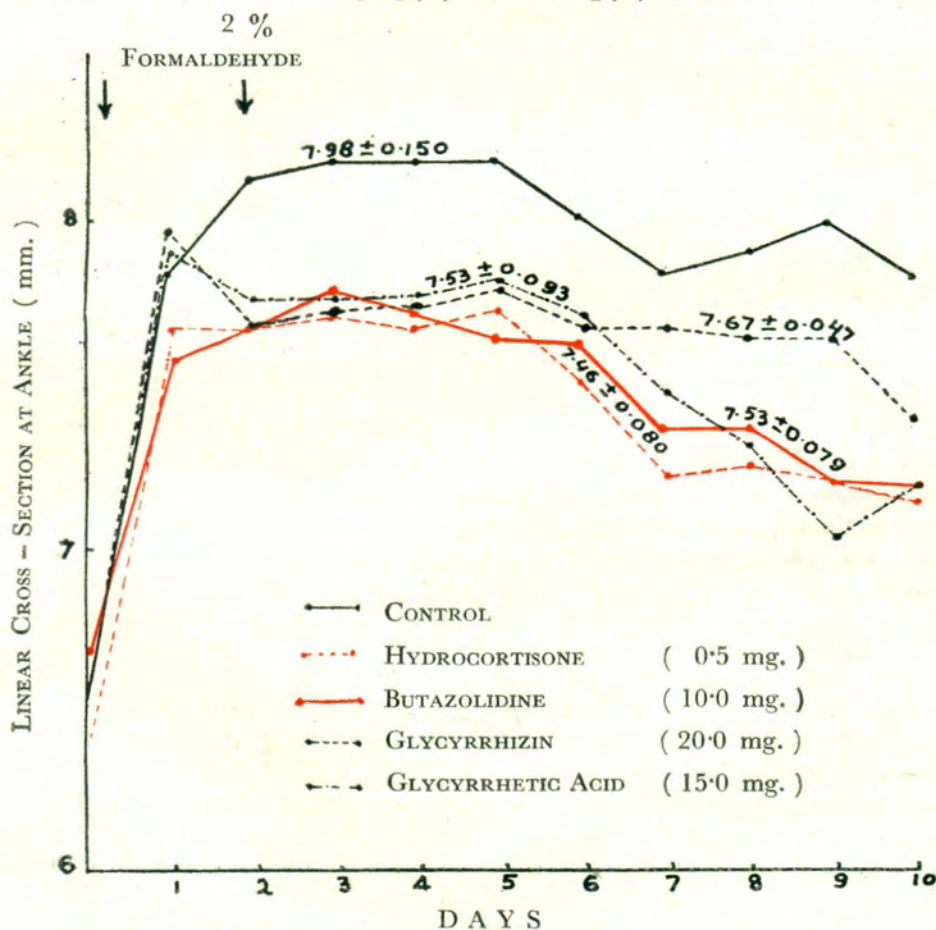


Fig. 1

TABLE I
Comparative effects of Hydrocortisone, Butazolidine and
Glycyrrhizin in rat foot arthritis.

Drug	Dose per 100 gm.	Mean diameter (mm).	S. D.	S. E. of mean	t	P
Control	—	8.1823	0.306	0.0974	—	—
Hydrocortisone	0.5 mg.	7.5912	0.148	0.0471	5.5657	0.001
Butazolidine	10.0 mg.	7.6452	0.2525	0.0804	4.2626	0.001
Glycyrrhizin	10.0 mg.	7.7456	0.243	0.0773	3.4936	0.01

The results of the second series of experiments are embodied in Figure II and Table II. The test drugs, glycyrrhizin and glycyrrhetic acid, both showed



significant activity. Glycyrrhizin at a dose level of 20 mg./100 gm., glycyrrhetic acid at 15 mg./100 gm., hydrocortisone at 0.5 mg./100 gm. and butazolidine at 10 mg./100 gm. body weight were all found active at a level of $P=0.001$, showing thereby that the test drugs in the doses mentioned were equally effective with the reference standards.

TABLE II

Comparative effects of Hydrocortisone, Butazolidine, Glycyrrhizin and Glycyrrhetic acid in rat foot arthritis.

Drug	Dose per 100 gm.	Mean diameter (mm).	S. D.	S. E. of mean.	t	P
Control	—	7.9825	0.15	0.0477	—	—
Hydrocortisone	0.5 mg.	7.4608	0.08	0.0254	9.6611	<0.001
Butazolidine	10.0 mg.	7.5266	0.0787	0.025	8.4425	<0.001
Glycyrrhizin	20.0 mg.	7.6667	0.047	0.0149	6.316	<0.001
Glycyrrhetic acid	15.0 mg.	7.5291	0.093	0.0296	8.0964	<0.001

DISCUSSION

Ito et al. (1955 c), in studies on the hæmatological findings and skin functions in rabbits, reported activity in glycyrrhizin and a mixture of glycyrrhetic acid plus glucuronic acid (equivalent to reconstituted glycyrrhizin) but not in glycyrrhetic acid or glucuronic acid alone. In our work both glycyrrhizin and glycyrrhetic acid were found to be active. Our studies were, however, made in rat arthritis and not in hæmatological findings and skin functions in rabbits.

Finney and Somers (1958) did not test glycyrrhizin but reported anti-inflammatory effect from the use of glycyrrhetic acid and some of its derivatives. It may be pointed out that the method used by us differs greatly from the one employed by them. Whereas these authors administered the drug for six days previous to a single injection of formaldehyde in plantar aponeurosis of one foot only and did not follow it by further treatment with glycyrrhetic acid, we injected both the feet on the first and the third days and fed the drug for ten days starting with the day of the first injection. The effect investigated by these authors appears to be on the acute inflammatory

reaction during the first 24 hours; the effect reported in this paper is suppression of the "rheumatoid reaction" (Brownlee, 1950). The work of Finney and Somers (1958) with glycyrrhetic acid is in conformity with our earlier work (1956) in rat arthritis where we employed an aqueous extract of glycyrrhiza glabra. It is also in conformity with the work reported in the present study with this difference that whereas Finney and Somers used only glycyrrhetic acid and found it active, we employed both glycyrrhizin and glycyrrhetic acid and found both the drugs active. It is well known that some drugs are rendered inactive after their introduction into the body by their conversion to glucuronides. By the same logic one would consider that glycyrrhizin which is glycyrrhetic acid diglucuronide would be inactive. It is possible that this is the reason why glycyrrhizin itself has not been tested by a number of workers.

The precise mechanism of action of glycyrrhizin or glycyrrhetic acid is at the present time obscure. It is not even known if all the activity of glycyrrhizin is due to glycyrrhetic acid alone. It is possible that glucuronic acid may have a role of its own to play. In this regard the Japanese work (reported in this paper) and that of Peterman (1947) and of Hodas (1949) are pertinent. Ito et al. (1955 c), in experimental animals and human beings, have reported a strong cortisone-like activity in glycyrrhizin and a mixture of glycyrrhetic acid plus glucuronic acid. Louis and Conn (1956), on the other hand, in clinical studies, report a mineralocorticoid activity from the use of glycyrrhizin (ammonium salt). According to these workers glycyrrhizin has no demonstrable effects upon organic metabolism. Atherden (1958) considers that glycyrrhetic acid *in vitro* is a powerful inhibitor of the metabolism of both 11-deoxycorticosterone and progesterone and that its 11-oxo group is essential for the inhibition. Finney, Somers and Wilkinson (1958) also failed to get any glucocorticoid effect from the use of glycyrrhetic acid in experimental animals. These workers state that "much remains to be discovered about the mode of action of glycyrrhetic acid, but it offers a new approach to the treatment of inflammatory conditions free from the disadvantages of corticoids which have claimed so much attention and disproves the concept that an anti-inflammatory agent must of necessity have a concomitant corticoid-like action."

The experimental work in progress in this department and the clinical studies which are being carried out in the Gandhi Memorial Hospital, lead us to think glycyrrhiza glabra and its constituents may prove valuable agents in the treatment of rheumatic diseases, inflammatory dermatoses and certain other conditions.

SUMMARY

1. The antiarthritic effect of watery extract of Glycyrrhiza glabra previously reported by Gujral and Saxena (1956) is confirmed here and is ascribed to the presence in it of glycyrrhetic acid through glycyrrhizin.

2. The effect in the doses mentioned is comparable to that of hydrocortisone and butazolidine and is significant at a level of $P=0.001$.

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