A PRELIMINARY STUDY OF HYPOGLYCEMIC ACTIVITY OF *LYTHRUM SALICARIA*

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ABSTRACT.—A study of the hypoglycemic activity of several extracts of *Lythrum salicaria* was made in normoglycemic and hyperglycemic rabbits. Circulating insulin variations were studied as well. The results of this study prove a hypoglycemic activity of the plant.

Lythrum salicaria L. is a plant whose medicinal attributes have been known since Greek and Roman days. Dioscorides (1) recommended its use in dysentry and also as an antihemorrhagic, cicatrizant and for moderating the menstrual flow. Sagar (2) also recommended it for dysentry. Vincent and Segonzac (3) demonstrated that the antidiarrheal, antidysenteric action due to the antibiotic properties of the plant was active against *Staphylococcus* and against dysenteric *Bacillus* of His, Flexner and Shiga. M. Paris (4) demonstrated its antispamodic action on rabbit duodenum.

References to its hypoglycemic activity are very rare. Spyropulus (5) mentions that the salicaria can prevent phlorizinic-induced hyperglycemia in man. The plant is used in folk medicine as a hypoglycemic agent when administered in fluidextract form at a daily dose of 4–6 g.

In the present study, we have tried to discover that property of *Lythrum salicaria* which might justify its application as a hypoglycemic agent.

EXPERIMENTAL

PLANT MATERIAL.—The plant, in flower, was collected during July-September on the banks of the river Tambre in Sigueiro (La Coruña, Spain). A voucher sample is deposited in the herbarium of the Botany Department (Faculty of Pharmacy, Santiago de Compostela). The stems, leaves, flowers, and roots were separated and oven-dried at 40°. They were then triturated and stored in containers protected from light.

BIOLOGICAL ASSAYS.—To evaluate hypoglycemic activity, a study was carried out on the variations of blood glucose after the administration of several plant extracts to rabbits of both sexes, Australian strain (weighing 3.0±0.5 kg). The following technique was employed: the animals were fasted for 12 hours prior to testing but water was allowed *ad libitum*. The concentration of blood glucose was then determined, and noted as initial glycemia (Go). An extractive solution of the plant was administered orally, by means of an esophageal catheter, and blood glucose values were determined 1, 2 and 4 hours later. The percentage of plant extracts induced glycemia was calculated as a time function applying the formula:

$$\% \text{ induced glycemia} = \frac{Gx\text{-}Go}{Go} \cdot 100$$

Go and Gx are the values of initial glycemia (Go) and glycemia at 1, 2 and 4 hours (Gx_1, Gx_2, Gx_4), respectively. Animals concurrently dosed with distilled water served as controls. All doses indicated in the text were given as grams of dried plant per kilogram of body weight of the animal.

Blood glucose levels were determined with Dextrostix strips quantified in a Reflectance Meter (Ames Co).

The results were analyzed using STUDENT'S "t" test.

The following biological tests were carried out utilizing the indicated extraction procedures:

I.—HYPOGLYCEMIC ACTIVITY IN NORMOGLYCEMIC ANIMALS.

EXTRACTION WITH ETHANOL.—Powdered root, leaf, flower or stem (200 g) was extracted three times by heating with ethanol. The extractive solutions were evaporated *in vacuo* at

40°. The residue was extracted with distilled water and filtered; the aqueous solution was administered to the animals. The mean percentages of hypoglycemia induced by different parts of the plant are expressed in table 1.

TABLE 1. Hypoglycemic activity of the different parts of the plant.

Plant part	Dose g/kg	Mean % deviation from initial blood glucose conc.			N[a]
		Hours after administration			
		1	2	4	
Distilled water		− 0.5±1.5	− 1.4±3.8	− 2.6±2.6	15
Stem	1	−12.5±1.7**	− 9.8±1.5**	−16 ±2.7***	12
Leave	1	− 9.8±1.8**	−10.2±1.8**	−11.2±1.3**	12
Flower	1	—	−11.5±1.8**	−15.3±1.9***	12
Root	10	+0.75±4.7	+ 1.6±4.5	− 0.6±5.8	10

Mean ± standard deviation. Asterisks refer to significance between means **P<0.01 ***P<0.001.

Those without asterisks were not significantly from the control group.

[a]Number of animals tested.

FRACTIONATION WITH SOLVENTS.—The aqueous solution of the previous test (flower and stem) was treated successively in a separatory funnel with petroleum ether (bp-40–60), diethyl ether and ethyl acetate.

Each of the fractions was dehydrated with anhydrous Na_2SO_3 and concentrated to dryness. The residue was suspended in distilled water and tested for hypoglycemic activity. The aqueous solution remaining after extraction with these solvents was also tested. The results are shown in table 2.

TABLE 2. Hypoglycemic activity of the different extracts.

Fractions administered[b]	Mean % deviation from initial blood glucose conc.			N[a]
	Hours after administration			
	1	2	4	
Distilled water	− 0.5±1.5	− 1.4±3.8	− 2.6±2.6	15
Stem				
Ethanolic extract	−11.8±3.2**	—	−19.2±3.0***	14
Petroleum ether	− 3.2±1.9	—	− 1.6±3.8	6
Ethyl ether	−14.8±3.1**	−18.2±2.8	−21.2±3.9***	24
Ethyl acetate	−13.4±3.5**	−11.5±3.2	−10.4±2.8	11
Exhausted ethanolic extract	− 0.2±0.6	—	− 0.4±0.6	8
Flower				
Ethanolic extract	−15.9±3.1***	—	−20.9±4.2***	12
Petroleum ether	− 4.3±2.8	− 3.9±4.2	− 4.2±5.8	5
Ethyl ether	−18.8±1.6***	−18.8±5.4***	−21.2±4.2***	10
Ethyl acetate	−14 ±5.9**	−10.9±5.7	−16.2±5.2***	9
Exhausted ethanolic extract	− 3.2±3.0	− 2.2±3.6	− 1.5±2.4	6
Ethyl ether extract[c]	−12.4±5.2**	−16.4±4.6**	−21.0±3.4***	10
Ethanolic extract[d]	−12.6±2.4**	−19.8±1.3***	−10.8±1.9**	14

[a]Number of animals tested.

[b]All fractions were given at the dose of 10 g/kg.

[c]Ethyl ether extract obtained directly by Soxhlet.

[d]Ethanolic extract of powder exhausted with ether.

Mean ± standard deviation. Asterisks refer to significant differences as referred to in table 1.

DURATION OF ACTION.—The ethyl ether fraction obtained in the previous test was administered, and successive values of blood glucose concentration were determined until the initial values were reached. Thus, we were able to determine the duration of the hypoglycemic effect and the time taken to reach a peak response (table 3).

TABLE 3. Flower. Duration of hypoglycemic action.

Fraction	Dose g/kg	Mean % Deviation from initial blood glucose conc.					N[a]
		Hours after administration					
		1	2	4	6	8	
Distilled water	—	−0.5±1.5	−1.4±3.8	− 2.6±2.6	−0.8±1.2	−1.6±0.9	15
Ethyl ether	10	− 14±0.9*	− 17±1.6***	−22.2±1.2	− 14±3.2**	−7.5±6.2	6

Mean = standard deviation. Asterisks refer to significant differences as refered to in table 1.
[a]Number of animals tested.

DOSE RESPONSE RELATIONSHIP.—The dose-effect relationship of the ethyl ether fraction was represented graphically as follows: the decrease in blood glucose four hours after the administration of the ethyl ether fraction was expressed in ordinates as a mean percent deviation from initial blood-glucose concentration and plotted against the logarithm of the doses of ethyl ether fraction. A straight line was observed (fig. 1).

INTRAVENOUS ADMINISTRATION.—The ether fraction (flower) was dissolved in sterile physiological saline serum and injected into the marginal ear vein of the rabbit. Blood glucose values were determined every 15 min during a 75 min period. The results are given in table 4.

TABLE 4. Flower. Hypoglycemic activity by intravenous administration.

Fraction	Dose g/kg	Mean % Deviation from initial blood glucose conc.					N[a]
		Minutes after administration					
		15	30	45	60	75	
Ethyl ether	5	−6±2.2	−10±2	−21±4***	−8±2.8	−8.2±2.8	6

***P<0.001.
[a]Number of animals tested.

DIRECT EXTRACTION WITH DIETHYL ETHER.—Powder (flower) (200 g) was extracted in a Soxhlet with diethyl ether until completely extracted. The concentrated solution was extracted into a separatory funnel with distilled water and tested for hypoglycemic activity (table 2).

The powder already extracted with diethyl ether was extracted in a Soxhlet with ethanol. The ethanolic residue was concentrated and extracted with distilled water and filtered. The aqueous solution was then tested for hypoglycemic activity (table 2).

II.—ACTION ON GLUCOSE-INDUCED HYPERGLYCEMIA.

Initial glycemia was determined in fasting rabbits, and the plant extract was then administered. Two hours later, the glycemia value was again determined and a dose of 2 g/kg of glucose was then administered orally. Glucose values were determined to be 0.5, 1 and 2 h later. At the same time, a control test was carried out using only glucose. The mean percentages of induced hyperglycemia are tabulated in table 5.

III.—INSULIN VARIATIONS IN BLOOD.

The ether fraction (flower) was administered (10 g/kg) and successive extractions of blood

TABLE 5. Flower. Action on glucose-induced hyperglycemia.

Fraction	Dose g/kg	Mean % Deviation from initial glucose conc.			N[a]
		Hours after administration			
		½	1	2	
Glucose	2	76 ±5	66±9.4	34.4±8.7	11
Glucose + Ethanolic extract	2 5	43.5±6.1***	26±5.1***	−5.2±2.3***	16

Mean ± standard deviation. Asterisks refer to significant differences as referred to in table 1.

[a]Number of animals tested.

were carried out 2 and 4 h later. The values of glucose and insulin were then determined. Insulin was determined by radioimmunoassay according to Hales and Randle (7). The mean values are shown in fig. 2.

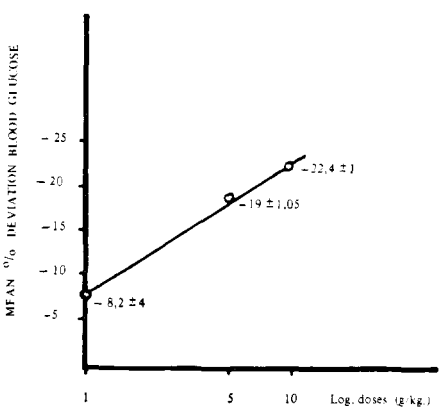

FIGURE 1

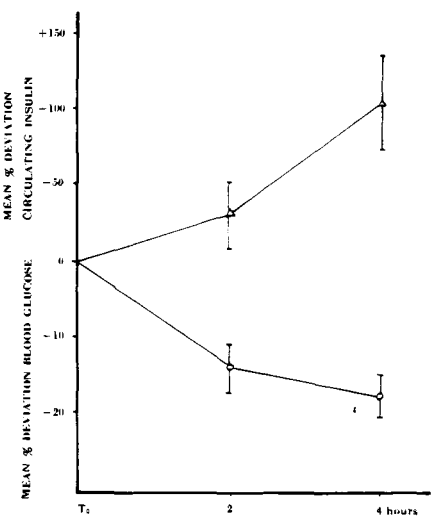

FIGURE 2

FIG. 1. Dose-response relationship of the ethyl ether fraction, 4 hours after administration.
FIG. 2. Blood insulin and glucose variations under oral administration of ethyl ether extract from flowers of *L. salicaria*.
T_0=Initial time.

DISCUSSION

From the test carried out, it could be observed that the flower and stem of *Lythrum salicaria* possessed hypoglycemic activity and, to a lesser extent, the leaf; the root was inactive. In normoglycemic animals, maximum hypoglycemia was observed after 4 h; recovery to initial values occurs in 8 h (table 3).

When fractionation of the ethanol extract was carried out with solvents, maximum activity was observed with the ethyl ether fraction (table 2). It is

interesting to note that, once the aqueous solution was exhausted with all the solvents, (exhausted ethanolic extract), it lost its hypoglycemic activity. Direct extraction of the flower powder with ethyl ether in a Soxhlet gave results identical to the ether fraction already mentioned.

The ether-exhausted powder, extracted with ethanol, continued to present activity even though the maximum decrease was reached in 2 h and recovery was very rapid. This leads us to believe that there might be two types of hypoglycemic substances.

The test carried out on glucose-induced hyperglycemia also confirmed the hypoglycemic activity of the plant, as there was a statistically significant difference between treated and untreated results.

Finally, it was observed that the decrease of glucose in the blood was accompanied by a notable increase in circulating insulin. This increase reached 100% of initial value four hours after the administration of the extract and coincided with the maximum decrease of glucose in blood. This would suggest that the active principles of the plant may act by provoking the liberation of insulin.

All the results obtained in this study confirm that the flower and the stem of *Lythrum salicaria* possess hypoglycemic activity.

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