

Comparative study of *Inula Racemosa* and *Saussurea Lappa* on the glucose level in Albino rats

P. CHATURVEDI*, S.SHUKLA, P. TRIPATHI, S.CHAURASIA, S.K. SINGH* and Y.B. TRIPATHI *

Department of Medicinal Chemistry, Institute of Medical Sciences, Banaras Hindu University, Varanasi – 221 005

** Department of Medicine, Institute of Medical Sciences, Banaras Hindu University, Varanasi – 221 005.*

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ABSTRACT : *Inula racemosa* and *Saussurea lappa* have been used in ayurvedic system for the management of diabetes. The result of this communication concludes that *I. racemosa* reduces the blood glucose earlier as compared to *S. lappa*. Maximum response in case of *I. racemosa* is noted between 2 to 4 hours after drug administration while for *S. lappa* while for *S. lappa*, it is 4 to 8 hours. *S. lappa* can be used as substitute for *I. racemosa* for the management of diabetes, but it should not be taken for granted that this substitution should be applicable to all other systems, where *I. racemosa* has been recommended as a drug of choice.

INTRODUCTION

Management of diabetes without any side effect is still a challenge to the medical system. Several laboratories are involved in isolating or synthesizing new oral hypoglycemic agents. In this field of research medicinal plants of different systems of medicine such as Indian System, Chinese system and Tibetan system could prove to be of great importance.

In the Indian system of medicine, there are several plants which are used clinically for the management of diseases. Many plants have been reported as the substitute for other drugs in a specific ayurvedic formulation. *I. racemosa* Hook. F. (Compositae) and *S. lappa* Clarke (Compositae) are two examples (1,2). Both of them are clinically used as hypoglycemic agents (3).

In the Ayurvedic text, *I. racemosa* has been recommended for chest pain, cough and dyspnea (4). Water decoction of the root has been reported not only to lower the fasting blood glucose in normal rabbits, but also to protect the rabbit against glucose-included hyperglycemia (5,6). Its crude extract is clinically used for the management of angina pectoris (7). The petroleum ether extract of the root has been reported to lower plasma insulin and glucose level. This extract also showed negative inotropic and negative chronotropic effects on frog heart (8).

S. lappa root has been described as the substitute for *I. racemosa* root and is used for the cure of leucoderma, itching, scabies, epilepsy, headache and hysteria (9). Water decoction of *S.lappa* root lowers blood

glucose in glucose induced hyperglycemic rabbits (3). Its role in diabetic cardiovascular disorders has also been studied in patients (10).

In our previous reports the effect of these drugs on the glucose metabolism with special reference to their effect on other glands has been studied in detail (11) but the pharmacokinetics (acute response) of these extracts is yet to be investigated. The present report deals with the hourly effect of a single dose of these extracts on the glucose metabolism in rats. Its comparative data shows that *I. racemosa* possess quicker response in comparison to *S. lappa*. This study also supports the practice of substitution described in the ayurvedic text i.e. recommendation of more than one plants for making a specific ayurvedic preparation.

METHODS

Ratio-immuno-assay kit for insulin was purchased from Bhabha Atomic Research Centre, Bombay. Other chemicals of analytical grades were purchased from Qualigens Fine Chemicals, India. Pentobarbitone sodium and heparin sodium were procured from Sigma Chemical Company, St. Louis, USA.

Preparation of Drug

Roots of *I. racemosa* and *S. lappa* were purchased from the ayurvedic pharmacy, Institute of Medical Sciences, Banaras Hindu University. Their authenticity was confirmed by the direct comparison with the samples preserved in the Department and other pharmacognostical parameters as described earlier (8, 12). The drug samples were from the same lots which were used for preparing the effective ayurvedic medicines for clinical use in hospitals.

The dried root-samples were separately powdered and extracted with ethanol in a soxhlet apparatus. The extracts were distilled under reduced pressure in the Buchi type rotary evaporator. The solvent free extracts (residue) were kept in a vacuum desiccator until fixed weight was attained. The % yield of the residues were 6% for *I. racemosa* and 19% for *S. lappa*. The above solvent free extracts (residue after distillation) were used for the following biological experiments by dissolving in Tween-80-water mixture.

In vivo, experiment were carried out on inbred Horts Men strain of albino rats. Overnight fasting animals were divided into two groups. The control group orally received the drug vehicle (distilled water : Tween – 80; 9:1) and the experimental group received the alcoholic extract of these drugs at a dose of 400 mg/kg body weight. Animals of all the three groups were sacrificed at 2h, 4h, 8h, 16h and 24 hours after drug / vehicle administration. Blood was collected for the estimation of glucose and insulin. The liver was harvested for the estimation of glycogen. Each point represents an average of seven observations. Results have been presented in the form of mean \pm SD. Student's 't' test was used to evaluate the level of significance.

Estimation of Plasma insulin

Method described in the kit was been followed. Radio immunoassay kit for insulin, contains insulin standard, anti insulin serum, insulin free serum, $^{125}_1$ – insulin, second antibody in Iyophylised form and polyethylene glycol (PEG) and buffer solution. At the time of assay the above samples were dissolved in buffer. Plasma samples were mixed with assay buffer, antiinsulin serum and kept at 4⁰C overnight. Next day 100 μ l of $^{125}_1$ – insulin was added

to each tube, mixed gently and further incubated at 4⁰ for 5 hours. Then the tubes were take out and 100 µl of second antibody was added and mixed. At the end 1 ml of PEG was added, mixed, incubated for 20 minutes at 1500 g. Supernatant free precipitate was counted in a well type counter. Values were estimated by the help of a standard curve and represented here as µU/ml.

Estimation of blood glucose

Blood was collected in a containing sodium fluoride and potassium oxalate (1:3) an anticoagulant. Modified Nelson Somogyi method was used (13). Zinc hydroxide – Barium sulfate was used for precipitating the protein and arsenomolybdate solution was used for estimating the reduced copper. The absorbance was recorded at 680nm. Concentration of unknown samples was calculated from a standard glucose curve.

Estimation of Liver glycogen

Method described by Hasside et al (14) was used. 1g liver was dissolved in 30% KOH by heating for 30 minutes on waterbath. Glycogen was precipitated by adding half volume of 95% ethanol. Isolated glycogen was hydrolyzed by adding 6 ml of 6N HCl for 2.5 hours on boiling waterbath. The solution was neutralized with 0.5N NaOH and glucose was estimated using 2% anthrone solution in conc. H₂SO₄. Absorbance was recorded at 620 nm.

RESULTS

Plasma Glucose

I. racemosa initiated its hypoglycemic response after two hours of its oral administration. Plasma glucose level (60.43 ± 9.43 mg%) decreased after 4 hours of drug

administration and returned to normal at 16 hour (86.76 ± 10.09 mg%) (Table-1). In the case of *S. lappa* treated rats the significant hypoglycemic response was observed at 8 hours of drug administration.

Liver Glycogen

In the *I. racemosa* treated group, a significant increase was noted only at 4 hours after extract administration (22.29 ± 2.29). In the *S. lappa* treated animals liver glycogen was increased only at 8 hours (Table-2).

Plasma Insulin

In the *I. racemosa* treated group of animals, a significant reduction in plasma insulin (23.86 ± 2.79) was observed (P<0.01) 4 hours after drug administration, which returned to normal value (28.93 ± 2.18) was observed at 8 hours, and remained low upto 16 hours (Table-3).

DISCUSSION

Glucose level in blood is a sensitive parameter and is regulated by hormonal, chemical and nervous factors. Insulin plays the major role in transporting blood glucose to the liver and other peripheral tissues. Catecholamines, glucagons, growth hormones etc. release the glucose from the stored glycogen (15). Besides the direct effect of these hormones on glucose metabolism, they also affect the function of each other. Thus the action of insulin is regulated at the level of its synthesis and action (16). The hypoglycemic drugs belong to different class of chemicals and have different mechanisms of action. The two plants discussed here also have the net hypoglycemic effect. The experiments described here reveal their acute response on blood glucose level.

Single oral dose of *I. racemosa* produces the maximum response at 4 hours, and at 8 hours these changes return to the normal value. These data refer to the conclusion that *I. racemosa* needs 2 to 4 hours for its absorption and assimilation through the gut. In another 4 hours, it becomes ineffective as its hypoglycemic response reduces to normal. As reported earlier, lowering of blood glucose at the same time indicates the role of insulin action but there is no increase in plasma insulin concentration. It is likely to be due to the enhancement in the peripheral sensitivity of insulin action. Reduction in plasma insulin might be a secondary response to low blood glucose.

On the other hand *S. lappa* shows its delayed response in comparison to *I. racemosa* because the maximum response has been noted from 4 to 8 hours of drug administration and at 16 hours the effect is not completely abolished. The above observations indicate the duration of effect of these plant extracts which might be helpful in deciding the daily doses. It also supports the use of these drugs as a substitute for the management of diabetes in

an ayurvedic formulation. This study suggests that in the case of non availability of *I. racemosa* the other plant may be used for preparing the antidiabetic preparations but it should not be taken for granted that this substitution would be applicable to all other systems where *I. racemosa* has been recommended as a drug of choice. However, it appears that *I. racemosa* possess comparatively quicker and short duration response than *S. lappa* and at the same dose former drug shows greater hypoglycemic response than the latter. Ayurvedic preparation from these medicinal plants are already in market and have proved to be a good hypoglycemic agent without any side.

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Table -1
EFFECT OF *I.RACEMOSA* AND *S.LAPPA* ALCOHOLIC EXTRACTS ON BLOOD GLUCOSE IN RATS

Group	N	Blood Glucose Control	Mg% (mean ± SD) Treated (I.R.)	Treated (S.L.)
2 hours	7	87.57 ± 5.39	85.71 + 9.41	87.4 + 6.68
4 hours	7	84.29 ± 9.34	60.40 + 9.43 **	86.71 + 3.54
8 hours	7	86.57 ± 8.72	78.43 + 4.80 *	73.79 + 4.06 **
16 hours	7	89.34 ± 6.47	86.76 + 10.09	79.91 + 10.80 *
24 hours	7	85.94 ± 7.12	85.31 + 8.32	88.43 + 10.63

Level of significance **p<.001, *p<.05

I.R = *I. racemosa*

S.L = *S. lappa*

Table – 2
EFFECT OF *I. RACEMOSA* AND *S. LAPPA* ALCOHOLIC EXTRACTS ON LIVER GLYCOGEN IN RATS

Group	N	Liver glycogen		Treated (S L)
		Control	Treated (I R)	
2 hours	7	17.68 + 2.46	19.29 + 1.98	16.35 + 2.08
4 hours	7	18.57 + 1.81	22.29 + 2.29 **	15.71 + 2.46
8 hours	7	17.14 + 2.21	17.71 + 3.35	20.29 + 3.50 *
16 hours	7	18.19 + 3.67	18.43 + 3.69	17.07 + 2.21
24 hours	7	19.04 + 2.68	18.21 + 2.67	17.71 + 2.21

Level of significance **p<.01, *p<.05

Table – 3
EFFECT OF *I. RACEMOSA* AND *S. LAPPA* ALCOHOLIC EXTRACTS ON PLASMA INSULIN IN RATS

Group	N	Plasma insulin μ U/ml (mean + SD)		Treated (S L)
		Control	Treated (I R)	
2 hours	7	30.21 + 3.66	27.43 + 2.57	29.43 + 2.00
4 hours	7	29.29 + 3.26	23.86 + 2.79 ***	28.07 + 1.17
8 hours	7	31.08 + 2.16	28.93 + 3.14	21.22 + 2.18 ***
16 hours	7	29.67 + 4.64	29.71 + 3.45	24.79 + 1.07 **
24 hours	7	30.19 + 2.37	29.12 + 2.96	29.14 + 2.34

Level of significance ***p<.001, **p<.01

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