

INFLUENCE OF LIQUORICE ON THE BLOOD GLUCOSE, POTASSIUM AND SODIUM LEVELS AND ITS INTERACTION WITH INSULIN IN NORMAL, DIABETIC AND ADRENALECTOMIZED RATS

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الإسراف في تناول العرقسوس كمشروب شعبي قد يؤدي إلى مخاطر صحية خاصة في بعض المرضى ومن هذه المخاطر زيادة نسبة الجلوكوز في الدم ولذلك هدفت هذه الدراسة إلى إلقاء الضوء والتأكد من هذه المخاطر وذلك بدراسة تأثير العرقسوس على نسبة الجلوكوز في الدم سواء في حيوانات طبيعية (فئران بيضاء) أو فئران مصابة بمرض السكر التجريبي ودراسة تأثيره على مفعول الأنسولين المنخفض لنسبة السكر في الدم ودور الغدة فوق الكلوية في إحداث تأثيرات العرقسوس وذلك بإعطاء العرقسوس لفئران أزيلت منهم هذه الغدة جراحياً. وكذلك أجريت دراسة لتوضيح التأثير المنفرد للعرقسوس وتأثيره في وجود الأنسولين على مستوى الصوديوم والبوتاسيوم في بلازما هذه الحيوانات.

أوضحت هذه الدراسة أن إعطاء العرقسوس بجرعة مقدارها 5.2 جرام لكل كيلو جرام من وزن الحيوانات السليمة والمصابة تجريبياً بمرض السكر يؤدي إلى زيادة في معدلات سكر الدم (الجلوكوز) في هذه الحيوانات ما عدا الحيوانات التي أستأصلت منها الغدة فوق الكلوية فلم يحدث فيها أي تغيير في نسبة الجلوكوز في الدم. أظهرت النتائج أيضاً أن تناول المشترك للأنسولين و العرقسوس بواسطة الحيوانات السليمة والمصابة تجريبياً بمرض السكر ينتج عنه إضعاف أو تقليل لتأثير الأنسولين المنخفض لسكر الدم. بينما لا يؤثر تناول العرقسوس في تأثير الأنسولين المنخفض للسكر في الدم في الحيوانات المستأصلة منها الغدة فوق الكلوية.

أوضحت الدراسة أن تناول العرقسوس بنفس الجرعة يؤدي إلى زيادة نسبة الصوديوم وإنخفاض نسبة البوتاسيوم في الدم في جميع الحيوانات ما عدا الحيوانات المستأصلة منها الغدة فوق الكلوية فلم يحدث فيها أي تغيير في نسبة الصوديوم والبوتاسيوم.

أثبتت الدراسة أن تناول المشترك للعرقسوس والأنسولين (نصف وحدة لكل كيلو جرام من وزن الحيوانات) يؤدي إلى زيادة في التأثير المنخفض للبوتاسيوم الذي ينتج عن تناول الأنسولين فقط في الحيوانات السليمة والمصابة ولم يحدث هذا التأثير في الحيوانات المستأصلة منها الغدة فوق الكلوية.

نتائج هذه الدراسة تدعم ما سبق من أبحاث ونتائج نتجت من الإسراف في تناول العرقسوس وأهمها مخاطر زيادة نسبة السكر في الدم وإنخفاض نسبة البوتاسيوم وزيادة نسبة الصوديوم والإقلال من تأثير الأنسولين المنخفض للسكر في الدم.

Excessive ingestion of the popular herb, liquorice, may lead to various health problems, among of which its reported hyperglycemic effect. The aim of the present study was to evaluate this possible effect in both normal and diabetic rats. The mechanism of this hyperglycemic effect of liquorice was also investigated by studying the role of the adrenal glands after giving liquorice to adrenalectomized rats. The effect of liquorice on the hypoglycemic effect of insulin as well as its effect on the plasma electrolyte (Na^+ & K^+) levels were also studied.

Results of this study revealed that liquorice (5.2 g/kg, orally) produces a significant ($P < 0.05$) increase in the blood glucose level (B.G.L) in both normal and diabetic rats but not in

adrenalectomized rats. Concurrent administration of insulin (0.5 u/kg) and liquorice produced an inhibition of the insulin-induced hypoglycemia in both normal and diabetic animals. However, liquorice ingestion didn't affect the insulin hypoglycemic effect in adrenalectomized rats. Oral administration of liquorice (5.2 g/kg) produced a significant ($P < 0.05$) reduction in plasma potassium and elevation in plasma sodium levels in both normal and diabetic animals. However, in adrenalectomized animals, liquorice failed to produce a significant change in plasma electrolyte (Na^+ & K^+) levels.

Results of the present work support the previous reports concerning the various hazards of liquorice frequent administration to normal and diabetic individuals. According to our data, the most important of these hazards are hyperglycemia, inhibition of insulin induced hypoglycemia, hypokalemia and hypernatremia. Our result may also support the assumption of the possible direct or indirect glucomineralocorticoid activity of liquorice.

INTRODUCTION & AIM OF THE WORK

Liquorice is among the most popular and commonly used herbs in several countries. It has been also taken medicinally for thousands of years.¹ However, excessive use of liquorice or its derivatives may cause hypertension, hypokalemia and low aldosterone excretion² and may also produce serious risks for diabetic patients under treatment with different agents.³ It has been reported that excessive ingestion of liquorice by diabetic patients treated with tolbutamide, decreases the hypoglycemic effect of the latter drug. This action was attributed to the severe hypokalemic effect of liquorice which inhibits the release of insulin.

Carbenoxolone, which is a semisynthetic derivative of glycyrrhetic acid (one of the main constituents of liquorice used in treatment of gastric ulcer⁴ produced significant decrease of the glucose uptake and the incorporation of glucose into triglycerides and CO_2 in rat epididymal fat pads. The effect produced by insulin on these metabolic pathways was reduced when adipose tissue was incubated with insulin in presence of carbenoxolone.

The present study was designed to evaluate the effect of excessive ingestion of liquorice on the blood glucose level and to assess the modification of the antidiabetic effect of insulin by liquorice in normal, diabetic and adrenalectomized rats. In addition, the changes in the plasma electrolyte (Na^+ & K^+) levels was evaluated following the administration of

liquorice and its concurrent administration with insulin.

MATERIALS AND METHODS

Experimental protocol

Albino rats (150-250 g), were used and divided into three main groups; normal (control), diabetic and adrenalectomized rats. Each group was subdivided into 4 subgroups as follows:

- The first, (control), received only an alcoholic saline solution (5.2 ml/kg/orally)
- The second, received only insulin (0.5 U/kg s.c.).
- The third, received, 5.2 g/kg of liquorice orally (as an alcoholic extract).
- The fourth, received both insulin 0.5 U/kg s.c and 5.2 g/kg alcoholic extract of liquorice.

Blood samples were collected as above described from all groups after 5, 15, 30, 60 minutes and 2, 4, 6, 12 and 24 hours following drug administration for determination of the plasma glucose and the electrolyte (Na^+ & K^+) levels.

Induction of diabetes

Diabetes was induced experimentally by injecting alloxan monohydrate (100 mg/kg) I.P.⁵ Two days later, B.G.L was determined and those rats which showed B.G.L higher than 250 mg/100 ml were considered diabetic.

Procedure of Adrenalectomy in rats

Rats were surgically adrenalectomized bilaterally according to the method of Exton and his associates⁶ and Lindsay *et al.*⁷. This method was subjected to some modification as follows; each rat was anaesthetized by ether and fixed on the board in its supine position with its back upwards. The dorsal hair was shaved around the loin. The shaved area was sterilized by topical application of 70% ethyl alcohol.

A skin incision was made on both sides of vertebral column just above the anatomical site of the kidneys. The adrenal glands were exposed, dissected from the kidneys and removed. After adrenalectomy, the incised muscles were stitched with catgut and the incised skin was stitched with silk. Each adrenalectomized animal received 5 ml of normal saline by a stomach tube and was then provided with saline solution instead of drinking water for the next 24 hours to avoid severe hyponatremia; after which, rats were used in the study.

Collection of blood samples

Blood samples were collected from the retro orbital plexus of rats^{7,8} using a capillary tube previously moistened with heparin solution (25 u/ml normal saline). About 0.5 ml of blood was taken into the heparinized tube and centrifuged for 10 minutes at 4500 r.p.m. 10 μ l of clear plasma were withdrawn by a micropipette and transferred into a stoppered glass tube. Another stoppered glass tube containing 10 μ l of the standard glucose solution (1 mg/1 ml) was concurrently used as a standard.

Determination of the blood glucose level

The enzymatic "glucose Kits" colorimetric method^{7,9} was used.

Procedure of determination of plasma sodium and potassium levels

Alliquots of plasma were taken from control and treated animals as previously described. Plasma supernatant samples were diluted with double distilled water and their electrolyte contents were determined by photoelectric flame photometry for Na⁺ and K⁺.^{10,11}

Electrolyte levels were also determined in a control group of animals. Standard calibration curves for Na⁺ and K⁺ ions were constructed by preparing standard solutions of NaCl and KCl using Analar sources of these salts. The standard calibration curves were used for the determination of plasma contents of Na⁺ and K⁺ separately.

Chemicals and drugs

- Liquorice extract (B.P, 88, Pembroek company, Holland).
- Insulin (LED Nordisk Insulin Laboratorium Compenhagen).
- Glucoxy Kits (Bio Merieux, Paris, France).
- Standard glucose solution (Bio Merieux, Paris, France).
- Glucose Analar (BDH Biochemicals, England).
- Alloxan Monohydrate (BDH, Biochemicals, England).
- Pentobarbital sodium (May and Baker LTD, England).
- Saline (Nile Co, for Pharmaceutical and Chemical Industries, Cairo ARE).
- Heparin (Evans, Medical LTD Speke, Liverpool, England).
- Ethyl alcohol 70 % (El Nassr Pharmaceutical Chemical Co, Cairo, ARE).

Statistical analysis

Data are expressed as the mean \pm standard error of the mean. Significance of the difference between data was determined using the student's (t) test. The difference was regarded as significant when P < 0.05.

RESULTS

1- Control experiments

No significant changes were obtained in the blood glucose levels or the plasma Na⁺ and K⁺ levels after pentobarbital or alcoholic saline solution treatment in all groups.

2- Effect of liquorice (5.2 g/kg, orally), insulin (0.5 u/kg, S.C.) and their combination on the blood glucose level

a- Effect on normal rats

As shown in Figure 1, liquorice produced

a significant ($P < 0.05$) elevation in the blood glucose level after 4 hours (90.1 ± 3.2 Vs 70.2 ± 4.1 mg/100 ml), 6 hours (93.3 ± 2.1 Vs 75.4 ± 2.5 mg/100 ml) and 12 hours (84 ± 2.9 Vs 72.7 ± 3.2 mg/100 ml). Following the administration of liquorice, insulin injection produced maximum reduction in the normal blood glucose level after 1 hour (37.2 ± 1.4 Vs 71.4 ± 3.3 mg/100 ml). The B.G.L returned to its normal level after 3 hours. Combined administration of insulin (0.5 u/kg S.C.) and liquorice (5.2 g /kg orally) resulted in a significant ($P < 0.05$) reduction in the hyperglycemic effect of liquorice alone on the normal B.G.L (76.2 ± 2.3 Vs 90.1 ± 3.2 & 74.3 ± 3.1 Vs 93.3 ± 2.1 mg/100 ml after 4 and 6 hours respectively).

b- Effect on alloxan diabetic rats

Liquorice intake significantly increased ($P < 0.05$) the blood glucose level of the alloxan diabetic rats (167.4 ± 2.1 Vs 151.6 ± 5.1 and 172.4 ± 3.6 Vs 149.3 ± 6.2 mg/100 ml) after 4 and 6 hours respectively. Administration of liquorice (5.2 g/kg) orally into alloxan diabetic rats interferes significantly ($P < 0.05$) with the hypoglycemic effect of insulin. (110.5 ± 5.3 Vs 81.3 ± 4.3 mg/100 ml), as compared with the control non treated level (148.3 mg/100 ml ± 5.1), after two hours following the combined administration of liquorice and insulin (Fig. 2).

c- Effect on adrenalectomized rats

The oral administration of liquorice (5.2 g/kg) into adrenalectomized rats led to a non significant change in the blood glucose level at all tested time intervals. Insulin administration produced marked reduction of the blood glucose level of the adrenalectomized rats (37.4 ± 4.1 Vs 76.9 ± 3.1 , 30.8 ± 3.5 Vs 76.4 ± 2.6 and 35.7 ± 3.5 Vs 74.2 ± 3.5 mg/100 ml) 30 minutes as well as, 1 and 2 hours respectively after insulin S.C. injection. ($P < 0.01$). The concurrent administration of liquorice with insulin didn't produce any significant change in the hypoglycemic effect of insulin when given alone (Fig. 3).

3- Effect of liquorice (5.2 g/kg, orally), insulin (0.5 u/kg s.c.) and their combination on the plasma potassium level

a- Effect on normal rats

A significant ($P < 0.05$) reduction in plasma potassium level was recorded, 4 hours after liquorice administration (3.8 ± 0.1 Vs 4.2 ± 0.2 mmol/l). The same hypokalemic effect was observed after insulin administration (3.4 ± 0.2 Vs 4.2 ± 0.1 , 3.3 ± 0.2 Vs 4.3 ± 0.2 and 3.7 ± 0.1 Vs 4.3 ± 0.1 mmol/l) 30 minutes as well as, 1 and 2 hours after insulin administration respectively. Liquorice intake prolonged the duration of the hypokalemic effect of insulin when used concurrently without a significant change as regards to the hypokalemic effect of either liquorice or insulin alone as shown in Figure (4).

b- Effect on alloxan diabetic rats

A significant ($P < 0.05$) hypokalemic effect was observed after 15 minutes with insulin (3.8 ± 0.1 Vs 4.2 ± 0.2 mmol/l) and after 4 hours with liquorice (4.0 ± 0.1 Vs 4.3 ± 0.2 mmol/l). The concurrent administration of both liquorice and insulin produced prolongation of the duration of hypokalemia. A significant ($P < 0.05$) potentiation of the hypokalemic effect was observed after 4 hours (3.7 mmol/l ± 0.1) as regards to the hypokalemic effect of either liquorice (4.0 mmol/l ± 0.1) or insulin (3.9 mmol/l ± 0.1) compared with the control plasma potassium level (4.3 mmol/l ± 0.2) (Fig. 5).

c- Effect on adrenalectomized rats

Liquorice administration did not exert any significant change in plasma potassium level. However a significant ($P < 0.05$), decrease in the potassium level was observed 60 minutes after S.C. administration of insulin (6.7 ± 0.1 Vs 7.2 ± 0.1 mmol/l). The same hypokalemic effect was observed also after the concurrent administration of insulin and liquorice (6.7 ± 0.1 Vs 7.2 ± 0.1 mmol/l), 60 minutes after their administration (Fig. 6).

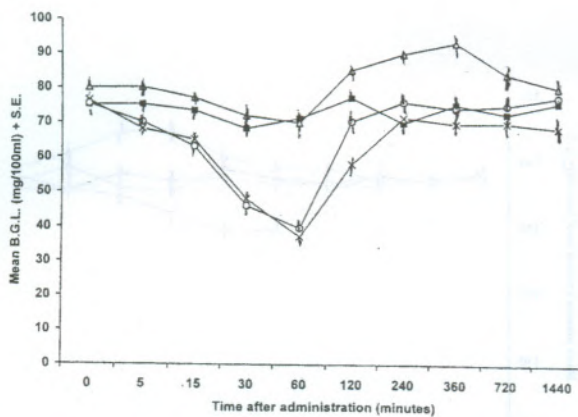


Fig. 1: Effect of liquorice (∇), insulin (x) and their combination (○), on the blood glucose level of normal rats (■).

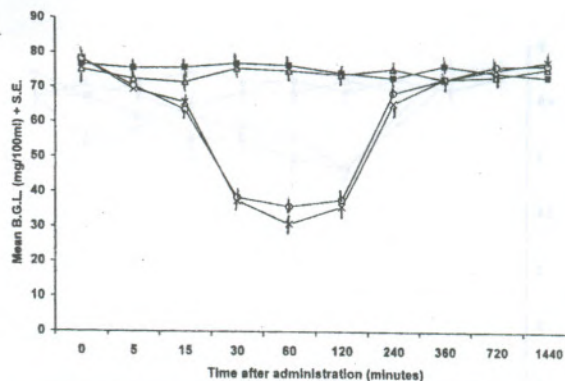


Fig. 3: Effect of liquorice (∇), insulin (x) and their combination (○) on the blood glucose level of the adrenalectomized rats (■).

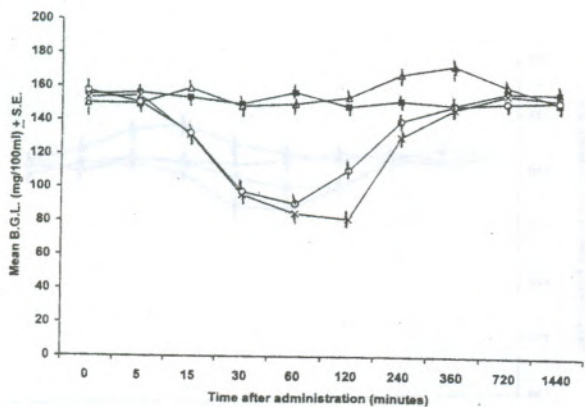


Fig. 2: Effect of liquorice (∇), insulin (x) and their combination (○) on the blood glucose level of the alloxan diabetic rats (■).

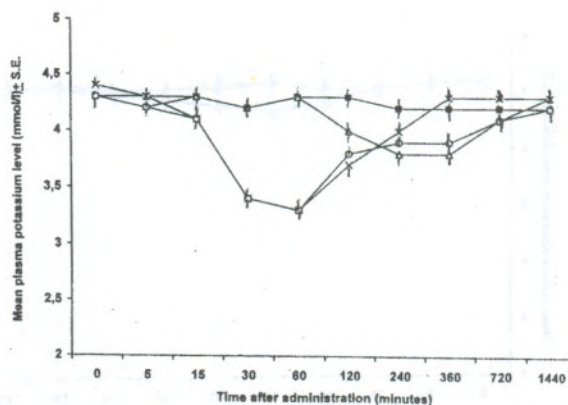


Fig. 4: Effect of liquorice (∇), insulin (x) and their combination (○) on the plasma potassium level of normal rats (■).

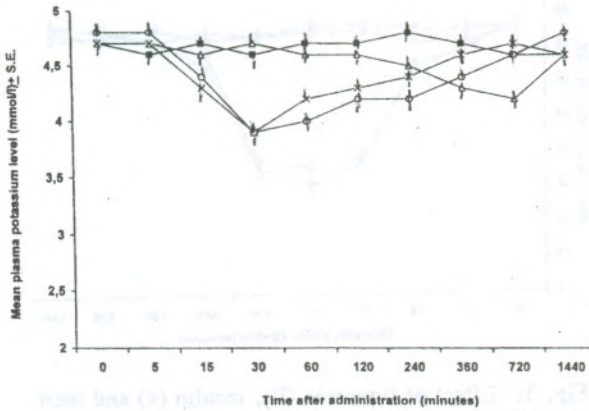


Fig. 5: Effect of liquorice (∇), insulin (x) and their combination (○) on the plasma potassium level of the alloxan diabetic rats (■).

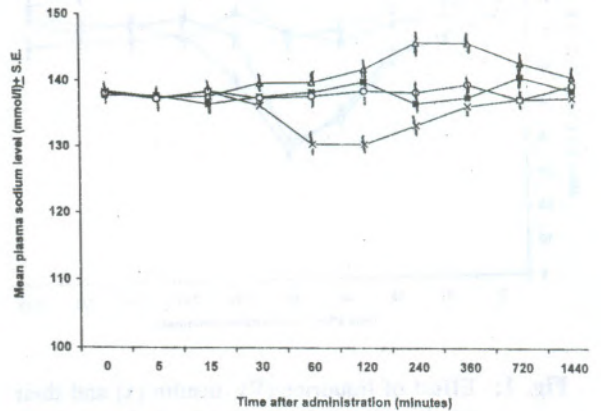


Fig. 7: Effect of liquorice (∇), insulin (x) and their combination (○) on the plasma sodium level of normal rats (■).

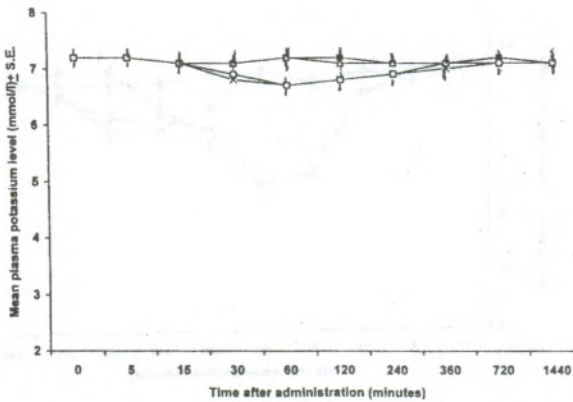


Fig. 6: Effect of liquorice (∇), insulin (x) and their combination (○) on the plasma potassium level of the adrenalectomized rats (■).

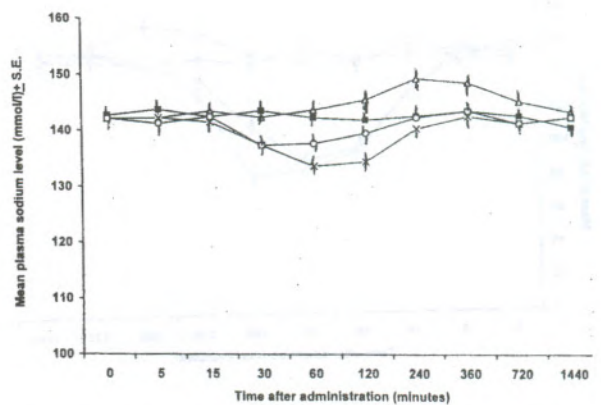


Fig. 8: Effect of liquorice (∇), insulin (x) and their combination (○) on the plasma sodium level of the alloxan diabetic rats (■).

4- Effect of liquorice, insulin and their combination on the plasma sodium level

a- Effect on normal rats

Liquorice exerted a significant elevation in plasma sodium level (145.7 ± 0.9 Vs 136.5 ± 1.9 and 145.6 ± 1.3 Vs 137 ± 1.1 mmol/l) 4 and 6 hours respectively after the oral administration of liquorice. Insulin produced a significant decrease in plasma sodium level (130.3 ± 1.1 Vs 138.2 ± 0.8 & 130.3 ± 0.9 Vs 139.8 ± 0.8 mmol/l) 1 and 2 hours respectively after administration. On the other hand, concurrent administration of liquorice and insulin produced a non significant change in plasma sodium level (Fig. 7).

b- Effect on alloxan diabetic rats

Liquorice administration produced the same but less marked hypernatremia compared with the normal rats (149.2 ± 1.4 Vs 142.5 ± 0.9 mmol/l) 4 hours after administration. Insulin produced a significant ($P < 0.05$) reduction in plasma sodium level (133.6 ± 1.9 Vs 142 ± 1.8 mmol/l) 1 hour after administration. Combined administration of liquorice and insulin produced a less marked decrease in plasma sodium level 30 minutes after administration (137.3 ± 1.3 Vs 143.5 ± 1.3 mmol/l) $P < 0.05$ (Fig. 8).

c- Effect on adrenalectomized rats

Neither liquorice nor insulin produced any significant change in the plasma sodium level (Fig. 9).

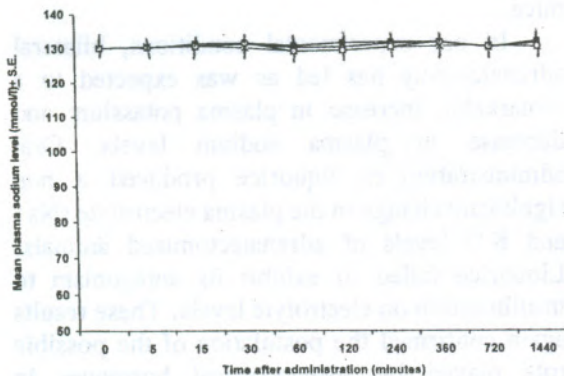


Fig. 9: Effect of liquorice (▽), insulin (x) and their combination (○) on the plasma sodium level of the adrenalectomized rats (■).

Excessive ingestion of liquorice can cause various health problems e.g. hypertension sodium retention and hypokalemia.² In addition, symptoms related to hyperglycemia and myopathy secondary to liquorice-induced hypokalemia and an inverse relations of fasting serum glucose and serum potassium were reported.¹² Liquorice has also a glucomineralocorticoid - like action as it is a potent inhibitor of 11 β -hydroxysteroid dehydrogenase enzyme which converts cortisol to cortisone in man and corticosterone to 11-deoxy-corticosterone in rats.^{13,14} These facts have tempted us to carry out the present work in order to evaluate the effect of liquorice on the blood glucose level and its possible interactions with the classic antidiabetic agent "insulin" as well as investigation of the role of suprarenal gland in these possible changes in B.G.L. and the mechanisms underlying these effects. The changes in the plasma electrolytes (Na^+ & K^+) were also evaluated. Three groups of animals were used; normal, alloxan-diabetic and adrenalectomized rats. Groups of animals received either, alcoholic extract of liquorice alone (5.2 g/kg, orally), insulin alone (0.5 u/kg, s.c.) or both liquorice and insulin. Control animals received only the alcoholic saline solution (20% ethyl alcohol) the vehicle of the liquorice extract. The effect of this vehicle on the B.G.L and plasma Na^+ and K^+ levels was studied.

Results revealed that oral administration of alcoholic saline solution has no effect on the BGL and plasma electrolytes of all animal groups. Oral administration of liquorice to normal rats led to significant hyperglycemia, four hours after the administration. This action persisted for 12 hours after which the BGL returned to its normal levels. These results are in agreement with the previously reported studies showing that excessive ingestion of liquorice leads to hyperglycemia in rats¹⁵ and in humans.¹² In the next set of experiments, a disease state which simulates diabetes mellitus in man was induced in experimental animals by the use of alloxan monohydrate.¹⁶

Liquorice was administered into insulin-treated and untreated-diabetic animals. Liquorice produced a significant increase in the blood glucose level of the diabetic untreated animals 4 hours after administration; reaching its maximum effect after 6 hours. This hyperglycemic effect of liquorice was less intense than that observed in normal rats.

Administration of liquorice to insulin-treated rats led to inhibition of insulin induced hypoglycemia. These results are in accordance with the previous study which reported that diabetic patients treated concurrently with insulin and liquorice developed a reduction in insulin activity, hypokalemia and sodium retention.³ In another study by Conn and his associates,¹⁷ liquorice remarkably reduced tolbutamide-induced hypoglycemia in diabetic patients. This effect was again attributed to the hypokalemic effect of this herb.

The results of our study show that liquorice ingestion produced a rise of sodium and decrease of potassium levels in plasma of the normal and alloxan diabetic rats. These results are in agreement with the observation of Farse¹⁸ and Murakami¹⁹ who reported that liquorice causes a significant decrease of the hyperkalemia associated with non-insulin-dependent diabetes mellitus due to hyporeninemia. Furthermore, liquorice is sometimes used to treat hyperkalemia due to selective hypoaldosteronism in diabetic patients.¹⁹ Jamil,¹² revealed that symptoms related to hyperglycemia and myopathy following liquorice administration are secondary to the hypokalemia it produces.

Hypokalemia and myoglobinuria were reported even after the ingestion of small amounts of liquorice. These effects were associated with sodium retention and suppression of plasma levels of both renin and aldosterone confirming a possible mineralocorticoid-like action of liquorice.¹ Our results revealed also that in its combined administration with insulin, liquorice reduces the hypokalemia produced by insulin in normal and diabetic rats.

These results may confirm the assumption that liquorice antagonizes insulin - induced hypoglycemia via its hypokalemic effect which

may also lead to inhibition of insulin secretion. Certain reports have also pointed to the adrenocortical hormones as possible targets for liquorice-induced inhibition of insulin hypoglycemic effect.^{7,20} Moreover the previously reported studies of Seelen *et al.*¹⁵ and Kageyama *et al.*²¹ stated that glucocorticoid-like action of liquorice may also decrease insulin sensitivity to its receptors and consequently inhibit its hypoglycemic effect. To clarify this point, another set of experiments was designed in order to evaluate the effect of single and combined administration of liquorice on B.G.L. in the absence of adrenal gland hormones. To achieve this goal, adrenalectomized animals were used. Our results showed that liquorice failed to produce a significant change in BGL of adrenalectomized rats. Furthermore, combined administration of liquorice with insulin to adrenalectomized rats revealed that liquorice ability to inhibit insulin induced hypoglycemia was very much reduced in the absence of adrenal gland hormones. This again may support the assumption that the adrenal gland hormones may play an important role in mediating both liquorice - induced hyperglycemia as well as its inhibition of insulin hypoglycemia. Bailey and his co-workers²² stated that adrenal gland hormones play an important role in the development of glucose intolerance. These authors also found that bilateral adrenalectomy leads to improvement in glucose tolerance in mice.

In our experimental conditions, bilateral adrenalectomy has led as was expected to a remarkable increase in plasma potassium and decrease in plasma sodium levels. Oral administration of liquorice produced a non significant change in the plasma electrolyte (Na^+ and K^+) levels of adrenalectomized animals. Liquorice failed to exhibit its antagonism to insulin action on electrolyte levels. These results again confirmed the postulation of the possible role played by adrenocortical hormones in mediating liquorice pharmacological actions.

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