Biochemical Studies on the Influence of Certain Herbs in Streptozotocin Induced Diabetic Rats

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Abstract

The aim of this work was to investigate the effect of certain herbs on some bichemical parameters of experimentally induced diabetic rats. For this purpose one hundred and twenty male albino rats were used randomly and classified into: Control group: not received any thing. Diabetic group divided into: Subgroup (1): Control diabetic group injected with Streptozotcin not received any herbs. Subgroup (2): diabetic rats received Rosemary. Subgroup (3): diabetic rats received Cress. Subgroup (4): diabetic rats administrated Heliotropia. Subgroup (5): diabetic rats administrated Surangan. Subgroup (6): diabetic rats administrated a mixture of Rosemary +Cress+ Heliotropia + Surangan (M_4).subgroup (7): diabetic rats received a mixture of Rosemary + Cress + Hellotropia + surangan in addition to Sage + Artemisia + Termis + Fenugreek + Dandelion (M_9). All group were received the herbs for thirty days. Blood samples were collected from all animals groups at 10, 20 and 30 day from administration of herbs. The collected sera were subjected for determination of glucose, Lactate, insulin, glucagon, total cholesterol Triglycerides, HDL-Cholesterol and LDL-Cholesterol. The obtained results revealed that herbs especially M_4, M_9 and Rosemary ameliorate most of biochemical changes. Thus, Mixture of herbs M_4, M_9 and Rosemary are useful in diabetes.

Introduction

Diabetes has been a treatable but chronic condition, and the main risks to health are its characteristic long-term complications include cardiovascular disease, chronic renal failure and retinal damage **(10)**. It can be reversed by a variety of measures and medications that improve insulin sensitivity or reduce glucose production by the liver. So antidiabetic drugs are medicines that help control blood sugar levels in people with diabetes mellitus, but most drugs for diabetes have a dangerous side effect. In a striking example, on March 21, 2000, the U.S. Food and Drug Administration (FDA) removed one of the most widely prescribed diabetes drugs; Rezulin (troglitazone), from the market after it was linked to 90 cases of liver failure and 63 deaths **(17)**.

The first registered use of herbal extracts as anti-diabetic drugs was by Indians in the Amazon Basin for the treatment of type 2 diabetes, and today promoted as vegetable insulin but not formally an insulin analog **(30).**

Although research on rosemary and watercress is scant, some studies have focused on antioxidant effects of diterpenoids, especially carnosic acid and carnosol, isolated from rosemary leaves **(3).**

Accordingly this work was carried out to investigate the effect of some herbs such as Rosemary, cress, Heliotropium, Surangan, and a mixture of the four herbs, as well as a mixture consist of nine herbs (Rosemary, Cress, Heliotropium, Surangan, Sage, Artemisia, Termis, Fenugreek and Dandelion) on certain biochemical blood parameters as Glucose, Lactate, Insulin, Glucagon, Total cholesterol, Triacylglycerol and high-density lipoprotein Low-density lipoprotein, of experimentally induced diabetic rats.

Material and Methods

One hundred and twenty male albino rats (4-5 months old), weighing (200-220 gram) were used throughout these experiments. Kept at constant environmental and nutritional Conditions and left 14 days for acclimatization before the start of the experiment water were supplied ad-labium. The rats were randomly divided into two main groups:

I-Control group:- Comprise **15** rats maintained on basal diet and not received any thing.

II-Diabetic groups: - Comprise **105** rats intraperotineally injected with Streptozotcin for induction of diabetes, at a dose of **60** mg/kg.b.wt. Experimentally induced diabetic rats were divided into seven subgroups each comprise **15** rats.

Subgroup (1) Control diabetic group not received any herbs.

Subgroup (2) diabetic rats received **Rosemary (54** mg/kg.b.wt.) daily.

Subgroup (3) diabetic rats received **Cress (2.88** mg/kg.b.wt.) daily.

Subgroup (4) diabetic rats administrated **Heliotropia (0.024** mg/kg.b.wt.) daily.

Subgroup (5) diabetic rats administrated **Surangan** (27 mg/kg b.wt.) daily.

Subgroup (6) diabetic rats administrated a mixture of (Rosemary +Cress+ Heliotropia +Surangan) (M_4) daily.

Subgroup (7) diabetic rats received a mixture of (Rosemary +Cress +Heliotropia+ Surangan) in addition to (Sage **72** mg/kg.b.wt.+ Artemisia **150** mg/kg.b.wt.+ Termis **150** mg/kg. b. wt. + Fenugreek **18** mg/kg. b. wt. +Dandelion **144** mg/kg .b. wt.) (M_9) daily.

All group were received the herbs for thirty days from the onset of diabetes.

The herbal doses were calculated **according** to **(18).**

Blood samples for serum separation were collected from all animals group at **10, 20** and **30** day from the onset administration of herbs. All sera were subjected for determination of glucose (*27*), Lactate **(5),** insulin and glucagon **(6),** total cholesterol **(25)** Triglycerides **(7),** HDL-Cholesterol and LDL-Cholesterol **(21).**

Statistical analysis of the results was carried out according to **(8).**

Results and Discussion

The obtained data (Table, 1) showed high significant increase of serum glucose level after injection of single dose of streptozotcin, this results agreed with **(16)** who revealed that insulin biosynthesis and secretion are suppressed rapidly with one hour after exposure to STZ in vivo followed by necrosis of beta cells of pancreas. While, the mean value of serum glucose concentration decreased significantly after 10 and 20 days of Surangan and Heliotropium administrated to diabetic rats, this decrease became non significant after 30 days of treatment. Supplementation of M4 exhibits a highly significant decrease in serum glucose conc. after 10 days this decrease become significant at the last two periods of the experiment. Also

Rosemary and M_9 administration decreased glucose concentration significantly allover the period of the experiment. Similarly **(2)** found that, Rosemary leaf contains phenolic acids (2-3% Rosmarinic, chlorogenic, and caffeic), which had hypoglycemic effect. Thus it was indicated that, Rosmarinic acid might be beneficial for blood sugar reduction **(1)**. As it showed positive effects on both fasting and postprandial glucose in patients with type 2 diabetes that used a preparation of fresh leaf powder mixed in water for four weeks **(29).**

Moreover, Sage may also lower blood glucose levels, via two ways—by increasing the ability of liver cells to take up sugar and by decreasing the body's ability to convert fats and proteins from food into sugar. As well as, prolonged treatment with sage may increase the number of insulin-producing cells in the pancreas **(9).** Also dandelion was reported to decrease blood glucose levels in non-diabetic rabbits, as it is high in insulin; which is composed of fructose chains, it may act to buffer blood glucose levels, thus preventing sudden and severe fluctuations. Moreover, it increases the production of insulin; results are inconclusive for its actual effect on blood sugar **(13).**

Regarding, Fenugreek seeds, it was reported that, Fenugreek reduce blood glucose levels in type 1 and type 2 diabetes, as it contain a high proportion (40%) of a soluble fiber known as mucilage. This fiber forms a gelatinous structure, which may have effects on slowing the digestion and absorption of glucose from the intestine **(28)**. In addition, it increased erythrocyte insulin receptors, thus improve peripheral glucose utilization, which contributes to an improvement in glucose tolerance **(32).**

Concerning serum lactate concentration the obtained data (Table,1) revealed that the mean value of serum lactate concentration increased non significantly with Rosemary, Surangan, Cress, Heliotropium, M_4 and M_9 administration allover the period of experiment.

Lactate is a metabolic intermediary, originated in the lactic fermentation from glucose, which accumulates during high intensity exercise because of the associated increase in glycolytic activity. The formation of ATP is linked

to the generation of lactate and H^+. If fatigue develops, the increased levels of lactate correlate with the reduction of force **(5)**.

Regarding serum Insulin level the obtained data (Table, 1) revealed that, the mean value of serum insulin level was significantly increased allover the periods of the experiment after Rosemary administration. Moreover, supplementation of M_4 and M9 caused significant increases after 20 and 30 days of the experiment. These results came in accordance with the recorded data of **(9)** who found that, Sage may help to lower blood sugar levels for individuals with diabetes. They added that, prolonged treatment with Sage might increase the number of insulin-producing cells in the pancreas. As sage may boost insulin activity two- to five fold or more in patients with Type II diabetes **(23)**. Moreover, dandelion extracts improve the health of the pancreas which releases insulin to help each cell absorb glucose to burn as energy **(22)**, as well as, Fenugreek fibers improving peripheral insulin sensitivity, via increasing the number of insulin receptor sites occurs and the cells become more sensitive to the action of insulin **(4)**, in addition Rosmarinic acid may improve pancreatic beta cell function and thus enhance insulin secretion **(29)**.

Concerning serum glucagons level, the obtained data (Table, 1) revealed that, the mean value of glucagon concentration increased non significantly with Rosemary, Surangan, Cress, Heliotropium , M_4 and M_9 treatment allover the period of experiment. These results were nearly similar to that reported by **(14)** who showed that, dandelion tend to normalize pancreatic secretions, raising glucagon levels, thus helping to moderate swings in blood sugar levels.as its extracts benefit the spleen and improve the health of the pancreas **(22)**.

Regarding Lipid Profiles, The obtained data (Table, 2) revealed that, the mean value of total cholesterol, Triacylglecerols and LDLC concentration decreased significantly. However, HDL-cholesterol concentration increased non significantly after 10, 20 and 30days of M_9 administration .This came in accordance with the recorded data of **(11)**, who reported that, Dandelion might lower cholesterol, improve lipid profiles (mainly, lower total cholesterol, Triacylglecerol,while increasing HDL ["good"] cholesterol) in

diabetic micem, as it has a lipotropic effect that can help reduce cholesterol,and increase the "good" cholesterol, high-density lipoproteins (HDL) **(12)**. Also turmis seed inhibited diet-induced hypercholesterolemia,via reduced liver esterified and total cholesterol,and decreasing plasma LDLc and VLDL through stimulation of LDL receptors by a well-defined protein component of the lupin seeds **(15)**.

Moreover, Artemisia proved superior to Green Tea in terms of reducing total blood cholesterol. As it reduced blood cholesterol by 17%. **(31)**, in addition, fenugreek seed lower serum cholesterol, Triacylglycerol, LDLC and VLDL, as it contains about 54% fiber that frequently inhibits the absorption of cholesterol and facilitates its movement through the system and about 5% steroidal saponins, inhibit cholesterol production by the liver **(23)**. The lipid-lowering effect of fenugreek might also be attributed to its estrogenic constituent, indirectly increasing thyroid hormone, as well as, Fecal bile acid and cholesterol excretion are increased by fenugreek administration **(24)**. Another hypothesis attributes the cholesterol-lowering activities to the fiber-rich gum portion of the seed that reduces the rate of hepatic synthesis of cholesterol. It is likely that both mechanisms contribute to the overall effect **(19)**. Moreover Fenugreek seed contains the alkaloid Trigonella, nicotinic acid and Coumarins, that has the ability to reduce total cholesterol, and triglycerides while raising HDL and pancreatic function **(20)**.

It could be concluded that, a mixture of herbs M_4, M_9 and Rosemary are considered the best available herbs in serum glucose level control. .Thus, we recommended that a mixture of herbs especially M_4, M_9 and are useful in diabetes.

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Table (1): Mean values of serum glucose, lactate, insulin and glucagon concentration after herbs (R, S, Cr and H) adminsitration and their combination (M_4&M_9) in diabetic rats.

Parameters	Animal Groups / Duration	R ($\overline{X}$ ±S.E)	S ($\overline{X}$ ±S.E)	Cr ($\overline{X}$ ±S.E)	H ($\overline{X}$ ±S.E)	M_4 ($\overline{X}$ ±S.E)	M_9 ($\overline{X}$ ±S.E)	C_D ($\overline{X}$ ±S.E)	C ($\overline{X}$ ±S.E)
Glucose mg/dl	10 day	138.29*±11.79	140.14*±10.38	140.29±15.97	139*±11.54	134.14**±5.16	135*±8.97	213.14±11.63	108.86±10.54
	20 day	136*±10.16	139.43±16.22	142±15.89	137.43±14.16	134.29*±10.98	133*±10.96	223.24±12.87	101.29±10.68
	30 day	135.71*±14.62	143.57±17.23	144.43±16.62	141.14±14.78	135.43*±14.84	132.43*±14.48	239.86±13.80	99.14±10.03
Lactate mg/dl	10 day	37.000±2.75	32.86±2.60	32.86±2.59	32.29±2.08	38.14±2.99	37.286±2.87	27.143±1.16	43.29±3.89
	20 day	38.14±2.80	35.86±2.60	33.14±1.46	34.000±1.59	34.57±2.31	33.43±1.64	29.29±1.24	41.429±0.782
	30 day	33.29±0.246	31.86±0.001	37.29±2.35	37.71±2.68	33.86±0. 732	39.43±2.93	31.71±0.685	42.286±0.680
Insulin pg/ml	10 day	11.516*±0.578	9.86±1.093	9.522±1.249	10.43±3.262	10.592±1.499	12.582*±1.249	7.465±0.431	24.396±1.816
	20 day	12.55*±1.304	9.56±0.896	9.87±1.006	9.49±1.175	13.43*±1.219	12.380±1.8.4	6.78±0.587	20.524±0.347
	30 day	10.97*±0.539	8.40±0.654	9.496±0.821	9.846±0.976	12.258*±0.876	12.356*±0.891	6.552±0.657	21.526±0.491
Glucagon pg/ml	10 day	86.19±1.044	84.77±1.115	85.164±0.6	83.138±0.025	83.374±0.216	84.13±0.622	82.62±0.184	120.9±30.488
	20 day	85.74±2.428	79.47±0.500	86.33±2.93	78.24±0.156	79.28±0.567	81.35±1.093	77.81±0.05	115.14±4.47

Table (2) **Mean values of Serum total cholesterol, triacylglycerols, HDL-cholesterol and LDL-cholesterol concentration after herbs (R, S, Cr, H) adminstration and their combination (M4&M9) in diabetic rats.**

Parameters	Animal Groups / Duration	R ($\overline{X}$ ±S.E)	S ($\overline{X}$ ±S.E)	Cr ($\overline{X}$ ±S.E)	H ($\overline{X}$ ±S.E)	M_4 ($\overline{X}$ ±S.E)	M_9 ($\overline{X}$ ±S.E)	C_D ($\overline{X}$ ±S.E)	C ($\overline{X}$ ±S.E)
Total cholesterol mg/dl	10 day	123.43±4.45	129.86±4.47	128.71±3.73	121.29±6.96	121.14±6.55	119.71*±3.46	141±2.27	110.71±9.97
	20 day	125.86±7.44	116.43±8.20	115.57±9.59	117.43±8.23	112.43±9.30	110.14*±4.20	150±3.78	108.71±2.81
	30 day	127.57±9.03	125.00±9.97	126.71±9.85	125.57±9.32	121.43±9.27	116.71*±7.99	156.71±1.85	113.71±2.50
Tri-acylglycerol mg/dl	10 day	128.43±0.51	123.71±6.16	120.57±8.30	124.29±9.29	120.00±6.51	112.00*±7.93	148.29±6.26	108.29±6.02
	20 day	139.29±6.72	126.57±6.92	130.57±8.20	141.14±5.38	125.86±11.02	136.14*±3.26	159.43±3.14	112.14±1.71
	30 day	141.43±12.20	132.86±9.97	124.00±12.92	126.71±12.81	120.86±13.85	112.29*±7.59	166.57±1.67	118.29±2.45
HDL-cholesterol mg/dl	10 day	25.14±2.060	27.000±2.540	23.429±1.026	25.143±2.156	25.286±2.451	27.286*±1.098	19.714±0.818	30.714±1.451
	20 day	28.571±1.935	25.429±0.097	26.143±1.156	27.571±1.261	28.000±1.948	30.429*±0.935	22.71±1.29	31.571±0.649
	30 day	32.571±2.397	30.000±0.585	30.286±0.482	29.000±0.099	31.43±1.234	33.86*±1.370	27.857±0.553	35.571±0.751
LDL-cholesterol mg/dl	10 day	70.86±3.867	72.00±2.390	65.71±4.96	68.14±4.527	66.86±4.731	60.00*±3.051	83.71±3.306	59.00±6.51
	20 day	58.43±4.80	58.00±4.06	55.00±4.876	53.71±4.94	56.00±5.615	52.714*±1.78	69.71±2.36	55.57±1.69
	30 day	63.71±5.21	64.43±3.94	69.14±0.71	68.00±1.85	62.96±.1.50	62.00*±2.64	77.71±3.50	54±1.60

Data are presented as means ±S.E. * Significantly different at P<0.05

S.E.: stander error ** Highly significant differenet at p<0.01

الدراسات الكيميائية الحيوية علي تأثير بعض الأعشاب في الفئران المحدث فيها مرض البول السكرى بالأستربتوزوسين

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يعتبر مرض السكر من اكثر الامراض المزمنه شيوعا و تكمن خطورته في انه لا يعتمد علي تناول المريض العقار فحسب وانما علي سلوك المريض الغذائي. لذا فاتجاه المرضى للطبيعة واستخدامهم للأعشاب بدلا من العقاقير يدعوهم الي التفاؤل والأمل في انهم سيتفادوا الأعراض الجانبية التي قد تصاحب تناول العقاقير . لذلك استهدفت هذه الدراسه تاثير بعض الاعشاب الطبيعيه في الفئران لمعرفة التغيرات البيوكيميائية التى تحدث بالدم بعد استخدام المريميه والفسنتين وسورنجان و حشيشه العقرب واكليل الجبل و رشاد والترمس والحلبه و داندليون علي ذكور الفئران البيضاءالمحدث لها مرض السكر تجريبيا. وقد اجريت الدراسة في مدة ٣٠ يوم علي عدد مائه وعشرون فأر تم تقسيمهم إلى مجموعتين أساسيتين كلأتى: المجموعه الضابطه :فئران سليمة لم يتم اعطائهم اي اعشاب ، المجموعة الثانية: (المحدث بها مرض البول السكرى تجريبياً) وتم تقسيمها إلي سبع مجموعات متساوية اشتملت كلا منها علي ١٥ فأر:

المجموعه الاولي : (مجموعه ضابطه) محدث بها سكر ولم تتناول اي عشب .
المجموعه الثانيه : محدث بها سكرو تم اعطائها اكليل الجبل(الروزماري).
المجموعه الثالثه : محدث بها سكر تم اعطائها سورنجان (سورنجان).
المجموعه الرابعه : محدث بها سكر تم اعطائها رشاد (كرس).
المجموعه الخامسه : محدث بها سكر تم اعطائها حشيشه العقرب (هليوتروبيم).
المجموعه السادسه: محدث بها سكر تم إعطائها خليط من الاربعه اعشاب السابق ذكرها.
المجموعه السابعه : محدث بها سكر تم اعطائها خليط من الاربعه اعشاب السابقه ويزيد عليه الترمس والحلبه (الفينوجريك) و داندليون والمريميه (الساج) والافسنتين (الارتمسية). ثم تم ذبح الفئران علي فترات ١٠ و٢٠ و٣٠ يوم من بداية اعطاء الاعشاب وتم اخذ عينات الدم و فصل المصل لتعيين مستوي بعض المكونات البيوكيميائية مثل: الجلوكوز ، اللاكتات ، الانسولين، الجلوكاجون، الكوليسترول الكلى، الدهون الثلاثيه، الكوليسترول عالي الكثافه ،الكوليسترول منخفض الكثافه نستخلص من هذه الدراسه ان استعمال الاعشاب الطبيعيه مثل: اكليل الجبل/رشاد/حشيشة العقرب/سورنجان/مريميه/داندليون/ارتميسيه/ترمس/حلبه احدثت تغير في معدل السكر بالدم واللاكتات والانسولين و الجلوكاجون والكوليسترول الاجمالي والدهون الثلاثيه والكوليسترول عالي الكثافه و منخفض الكثافه لذا ينصح باستخدام هذه الأعشاب الطبيعيه كعلاج تكميلي في مرضى البول السكرى لما لها من دور فى ضبط معدل السكر فى الدم.