THE EFFECT AQUEOUS EXTRACT OF THE LEAVES OF EUCALYPTUS GLOBULES ON CLINICAL LABORATORY PARAMETERS IN ALLOXAN – INDUCED DIABETIC RATS[+]

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Abstract

Eucalyptus globules leaves is considered extensively in the endogenous system of medicine as an antidiabetic agents in many countries . The current investigations focuses attention on the glucose and lipid lowering effect of the aqueous extract of E. globules leaves on experimentally induced diabetes in rats . The biochemical parameters studied were plasma glucose , insulin , total cholesterol , triglycerides , phospholipids , haemoglobin , and glycosylated haemoglobin . In addition body weight and renal glucose reabsorption were notified . Aqueous extract of E. globules were orally administered daily for 30 days in a dose of 150 mg / kg body weight to alloxan – diabetic rats , and a significant reduction in the parameters measured was investigated compared to diabetic rats , meanwhile , Glibinclamise was used as standard reference drug . In conclusion , E. globules leaves posses a hypoglycaemic with concurrent hypolipidemic effect in diabetic states , and may further suggests that E. globules may be useful in the therapy and managements of diabetic hyperlipidemia through reducing lipids levels .

المستخلص

تعتبر اوراق نبات اليوكاليبتوس من الاعشاب الطبية المستخدمة بالطب الشعبي في علاج داء السكري في بلدان
مختلفة من العالم ، ولذلك اجريت هذة الدراسة لتسليط الضوء على فعالية المستخلص المائي لاوراق نبات اليوكاليبتوس المزروع بالعراق فى تخفيض نسبة السكر والدهون بالدم فى الحيوانت المختبرية المخلقة بداء السكري . لقد تم قياس نسبة عدد من المعايير المختبرية السريرية التي تتغير عند الاصابة او تخليق داء السكري بالجرذان المختبرية ومنها تركيز الكلوكوز بالدم ونسبة هرمون النسولين وكذلك تركيز الهيموكلوبين والكلايكوسيلتد هيموغلوبين ، ومختلف انواع الدهون (الكوليسترول ، الترايكلسيرايد و الفوسفولبيدز)، بالاضافة الى ملاحضة اوزان اجسام الجرذان قبل وبعد الاصابة بالسكري وملاحظة وجود السكر بالبول .
ظهرت النتائج واثبتت قدرة المستخلص المائي لاوراق نبات اليوكاليبتوس عند استخدامة فمويا و بجرعة ١٥٠ ملغم / كغم وزن ولمدة ٣٠ يوم متواصلة قى تخفيض نسبة السكر بالدم بنسبة عالية وكذلك اثبتت الدراسة كفاءة النيات فى تقليل نسبة الدهون بانواعها وزيادة نسبة الانسولين و اعادة

[+]Received on 2008/4/2 , Accepted on 2008/12/23
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مستوى الهيموغلوبين بانواعة الى مستوياتة الطبيعية والحفاظ على وزن الجسم وزيادة طرح السكر مع البول وبالتالي يمكن اعتبار النبات كاحد المواد التى تمنع مضاعفات مرض السكر التي تتاتى من اختلال العمليات الايضية فى مراحل تقدم المرض .

Introduction

Approaches to the control and prevention of hyperglycaemia are central to the management of diabetes mellitus [1] . The development of new dietary adjuncts and novel antidiabetic agents , which reinstate a normal metabolic environment , thereby reducing the long - term complication associated with diabetes is required . Such agents would both ideally stimulate the secretion and improve the action of insulin [2] .Throughout the world , many traditional plant treatments for diabetes exist and there in lies a hidden wealth of potentially useful natural products for diabetes control [3 , 4 , 5] . Despite this , few traditional antidiabetic plants have received scientific or medical scrutiny , and the World Health Organization [6] recommended accordingly that this area warrants further evaluation . Diabetes mellitus is a syndrome resulting from variable interactions of heridatory and environmental factors , and characterized by depleted insulin secretion , hyperglycaemia , and altered metabolism of lipids , carbohydrates , and proteins , in addition to damage of B – cells of pancreas , with increased risk of complication of vascular disease [7] . A number of pharmacological and chemical agents act as diabetogenic and produce variety of diabetic complication . Alloxan induction of diabetes in experimental models widely used to study glycaemic and lipidemic changes in plasma . Many species of plants and herbs are known to act as anti – diabetic agents , but only few of them have been investigated [8] .

Eucalyptus globules (eucalyptus , blue gum tree) is traditionally used to treat diabetes in South America , Africa ,and Asia [9] , the medicinal part of the plant the leaves from which tea is made [10] The use of Eucalyptus globules leaves in the treatment of diabetes mellitus was first advocated by Faulds [11]. Recent studies in streptozotocin diabetic mice confirm the hypoglycaemic efficacy of Eucalyptus [12]. Also Vallasensor & Lamadrid [13 , 14] showed that Eucalyptus tereticonis exhibit anti-hyperglycaemic activity upon using oral glucose tolerance test in a dose of 5 mg / 20 gm mice .Leaves of eucalyptus are reported to contain eucalplol (cineol) together with rutin, terpineol , sesquiterpene , aliphatic aldehydes , isoamylalcohol , terpenes and tannins [15]. However , no scientific studies are so far carried out to investigate the efficacy of E. globulus in the management of hyperlipidemia , and other biochemical changes associated with diabetic state . The present study was undertaken to investigate the effect of aqueous extract of E. globulus . on blood glucose , serum insulin , lipid profile , haemoglobin , glycosylated haemoglobin , body weight and glucose reabsorption in alloxan induced diabetes in rats .

Materials and Methods

Dried eucalyptus leaves were obtained from commercial source and a voucher specimen of the plant was identified at the National Herbarium of Iraq Botany Direvtorate in Abu-Ghraib . The leaves were shed dried at 25 C and homogenized to a fine powder and stored at room temperature 25 C until use . Aqueous extract of

eucalyptus were prepared by maceration process of powdered material at 250 g / L [16]. In brief a suspension of 25 g of the Eucalyptus leaves powder in 100 ml of distilled water was stirred magnetically overnight (16 hours) at room temperature 25 C , and this was repeated three consecutive times . The residue was removed by filtration and extract was evaporated to dryness at a low temperature (40 C) under reduced pressure in a rotator evaporator .The residual extract were dissolved in normal saline whenever used in the experiments and administered orally to the experimental animals in a dose of 150 mg /kg body weight / day for 30 days [17] .All the experiments were carried out using Wister male rats (180- 200 g) obtained from the animal house of College of Pharmacy in University of Baghdad , Iraq . The animals were housed in a polypropylene cages , and provided with water and standard pellets diet ad libitum .Diabetes was induced in rats by intraperitoneal injection of 100 mg / kg body weight of alloxan monohydrate (5 % w/v) , freshly dissolved in physiological
saline immediately before use [18] . The diabetic state was noticed 48 h after alloxan injection as body weight loss , glucosurea [19] , and hyperglycaemia, and the animals which presented blood glucose level above 200 mg / 100 ml , as well as with the clinical signs of polydypsia , polyuria , and polyphagia were selected for the experiments [20] . Animals were devided in to following four groups 6 in each group as follows :Group I : normal rats received only physiological saline .Group II : control diabetic rats received physiological saline .Group III : diabetic rats received aqueous extract of E. globules (150 mg / kg body weight) / orally / daily for 30 days .

Group IV : diabetic rats received glibinclamide 600 microgram / kg body weight / orally / daily for 30 days .

At the end of the 30 days , the animals were deprived of food overnight and sacrificed by by decapitation . Blood was collected in two differents tubes (i.e.) one with anticoagulant (potassium oxalate and sodium floride) for plasma , and another without anticoagulant for serum which is separated by centrifugation .

Fasting blood glucose level was estimated by O- toluidine method [21]. Plasma insulin level was assayed by Enzymetic Linked Immunosorbant Assay
(ELISA) kit using human insulin as standarad . Haemoglobin was estimated according to [22] , and glycosylated haemoglobin according to [23] Total cholesterol and triglyceride were estimated according to [24]and [25] respectively. Phospholipids were analyzed accorording to [26] .The results were expressed as mean +- SEM statistically significance of the differences in parameters before and after treatment was calculated using Students -
paired –t – test .

Results and Discussion

Table 1 – shows the levels of blood glucose , plasma insulin , total haemoglobin , and glycosylated haemoglobin . There was s significant elevation in blood glucose and glycosylated haemoglobin levels ($P<0.0005$) , while the plasma insulin and total haemoglobin levels decreased significantly ($P<0.0005$) in alloxan induced diabetic rats when compared with normal rats . Administration of E.. globules and glibiclamide tends to bring the parameters significantly towards the normal values ($P<0.0005$, $P<0.005$) .

In diabetic rats , there is significant changes in body weight ($P<0.0005$) compared to normal rats , and the urine sugar since urine containing sugar were

noticed (+++) , but upon treatment with 150 mg / kg body weight of aqueous extract of E . globules and glibinclamide reduced uine sugar take place and maitanance body weight were recorded , these effects were shown in , Table - 2 .

Table -3 shows the level of cholesterol , triglycerides , and phospholipids in the plasma of control and experimental rats , Diabetic rats showed significantly increase levels of cholesterol , triglycerides , and phospholipids ($P<0.0005$) when compared with normal rats . In rats treated with aqueous extract of E. globules and glibinclamide , there was a significant decrease in the levels of cholesterol ($P<0.005$, $P<0.05$ respectively) , triglycerides ($P<0.01$) , and phospholipids ($P<0.0005$, $P<0,005$ respectively) when compared with diabetic control rats .

Diabetes mellitus is one of the most common chronic disease associated with hyperlipidemia and co-morbidities such as obesity , hypertension . Hyperlipidemia is a metabolic complications of both clinical and experimental diabetes . Alloxan , a B cytotoxin induces " chemical diabetes " (alloxan diabetes) in a wide variety of animals species by damaging the insulin secreting pancreatic B cells resulting in a decrease in endogenous insulin release which paves the ways for the reduction in utilization of glucose by the tissues [27]. In this study , aqueous extract of E. globulus decreases blood blood glucose level in alloxan – diabetic rats . The possible mechanism of the extract could be correlated with the reminiscent effect ot hypoglycaemic sulphonylureas that promotes insulin secretion by closure of K+ ATPase channels , membrane depolarization and stimulation of Ca +2 influx , an initial key step in insulin secretion . In the study , a decrease in total haemoglobin during diabetes was notified , and this may be due to the formation of glycosylated haemoglobin . Increase in the levels of haemoglobins in animals given aqueous extract of E. globulus may be due to the decreased level of blood glucose and glycosylated haemoglobin .

E. globulus administration to alloxan – induced diabetic animals reversed the weight loss , this ability of E. globulus to recover the body weight loss seems to be due to its hypoglycaemic effect . Excess of fatty acids in serum produced by alloxan – induced diabetes promote conversion of excess fatty acids into phospholipids and cholesterol in the liver .

These two substances along with excess triglycerides formed at the same time in the liver may be discharged into the blood in the forms of lipoproteins [28] . The abnormal high concentration of serum lipids in the diabetic subject is due , mainly to the increase in the metabolism of free fatty acids from the peripheral fatty depots , since insulin inhibits the hormone sensitive lipase . Hypercholesteremia and hypertriglyceridemia have been reported to occurs in alloxan diabetic rats [29] , and a significant increases observed in our experiment was in accordance of these studies . The marked hyperlipdemia that characterize the diabetic state may therefore , be regarded as a consequence of the uninhibited actions of lipolytic hormones on fat depots [30] . Activation of enzymes suggests that enhanced lipid metabolism during diabetes is shifted towards carbohydrates metabolism , and it enhances the utilization of glucose at peripheral sites .

One of the possible action of E. globulus may be due to its inhibition of endogenous synthesis of lipids .Metabolic aberration in alloxan induced diabetes in rats suggests a high turnover of triglycerides and phospholipids . E. globules was antagonizing the metabolic aberration , and thereby restore the normal metabolism by tilting the balance from high lipids to high carbohydrate metabolism in alloxan diabetic rats.

These results suggest that E. globules exhibits hypoglycemic and hypolipidemic effects in also prevents the fatty acid changes produced during diabetes. The effect of 150 mg / kg body weight was similar to that obtained by glibenclamide .

: Table – 1: Changes in blood glucose , plasma insulin , haemoglobin and glycosylated haemoglobin levels of control and experimental animals .

Groups	Blood glucose level mg/dl	Plasma insulin milliunit /ml	haemoglobin mg / dl	Glycosylated Haemoglobin mg/g haemoglobin
Group I	96.5 +- 7.04	15.83+-1.02	12.65+- 0.7	0.24+- 0.01
Group II	241 +- 13.8 +++	4.65 +-0.96 +++	5.31 +- 0.42 +++	0.84 +-0.07 +++
Group III	199 +-14.9 **	13.95 +- 0.48 ***	11.32 +-0.93 ***	0.39 +-0.03 ***
Group IV	101+- 13.4 ***	10.31+-1.03 ***	10.31+-1.03 ***	0.42+-0.04 ***

+++ represents P< 0.0005 compared to control
*** represents P< 0.0005 compared to control diabetic ** represents P< 0.005 compared to control diabetic

Table-2 : changes in the body weight , and glucose renal reabsorption in control and experimental animals .

Groups	Body weight Gm		Urine sugar
	initial	Final	
Group I	194 +- 0.84	210 +- 8.9	Nil
Group II	208 +-12.2	148 +- 13.6 +++	+ + +
Group III	191 +- 14.4	186 +- 13.3 **	+
Group IV	184 +- 10.9	201 +- 13.14 ***	trace

+++ represnts P < 0.0005 compared to control
*** represents P < 0.0005 compared to control diabetic
** represents P < 0.005 compared to control diabetic

Table -3 : Change in serum cholesterol , triglyceride , phospholipids in control and Experimental animals .

Groups	Cholesterol mg / 100 ml	Triglycerides mg / 100 ml	phospholipids mg / 100 ml
Group I	75.1 +- 1.49	46.13 +- 3.76	81.15 +- 1.39
Group II	99.93 +- 4.83+++	61.23 +- 1.8 +++	97.05 +-2.68 +++
Group III	89.8 92.82 +- 2.68 *	57.53 +- 3.9 *	85.5 +- 2.66 ***
Group IV	86.13 +- 1.56 **	56.16 +- 1.79 *	89.71 +- 2.52 **

+++ represnts P < 0.0005 compared to control
*** represents P < 0.0005 compared to control diabetic
** represents P < 0.005 compared to control diabetic
* represents P < 0.01 compared to control diabetic

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