Egypt. Acad. J. biolog. Sci., 2 (1): 153-162 (2009)
E.mail.egyptianacademic@yahoo.com ISSN: 1687–8809
Received: 11/2/2009 www.eajbs.eg.net

Effect of Ginger Extract Consumption on levels of blood Glucose, Lipid Profile and Kidney Functions in Alloxan Induced-Diabetic Rats.

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ABSTRACT

In recent years, ginger has become a subject of interest because of its beneficial effects on human health. The purpose of the present study was to investigate the effects of daily oral administration of ginger extract for 6 weeks on plasma glucose, lipid profile and kidney functions in alloxan-induced diabetic rats to show the ameliorating and partly curative effects in alloxan induced-diabetic rats (150 mg/kg i.p.(Intrapretonial). Rats (130-150gm) were divided into 4 groups; normal control rats, diabetic control rats, diabetic rats post-treated with ginger and diabetic rats pretreated with ginger. Ginger extract was administered orally for 6 weeks to post-treated and pre-treated rats, and they were compared with the normal and diabetic groups, respectively. Plasma glucose was reduced significantly in both post-treated and pre-treated groups. The post-treatment with ginger extract reduced plasma cholesterol, triglyceride and LDL-cholesterol, but during the pre-treatment with ginger extract produced insignificant change only in plasma triglyceride level. The plasma HDL-cholesterol was significantly increased in post-treated and pre-treated groups. The plasma creatinine, urea and uric acid levels were significantly reduced in post-treated group; also in pre-treated group, they were reduced but urea level statistically did not change. It is concluded that the consumption of ginger produced a significant hypoglycemic effect in diabetic rats. In addition, ginger is capable of improving hyperlipidemia and the impaired kidney functions in alloxan-induced diabetic rats.

Keyword: ginger, kidney function, glucose, plasma, lipids, alloxan, rats, diabetic.

INTRODUCTION

Diabetes mellitus is a common disorder associated with markedly increased morbidity and mortality rate. Diabetes mellitus can be defined as a group of metabolic disease characterized by chronic hyperglycemia resulting from defects in insulin secretion, insulin action or both resulting in impaired function in carbohydrate, lipid and protein metabolism (Zhang *et al.*, 2006).

Ginger is an underground rhizome of plant *Zingiber Officinale* belonging to the family *Zingibeaceae*, and now, it is considered a common constituent of diet worldwide (Stertie' *et al.*, 1991). Moreover, ginger is well known all over the world especially for its use in disorders of the gastrointestinal tract such as constipation, dyspepsia, nausea and vomiting (Tyler, 1993). It was reported that ginger has medicinal properties against digestive disorders, rheumatism, and diabetes (Afzal *et al*., 2001).Ginger extract possesses antioxidative characteristic, since it can scavenge superoxide anion and hydroxyl radicals (Krishnakantha and Lokesh, 1993). Akhani *et al*., (2004) reported that ginger pretreatment inhibited the induced hyperglycemia and hypoinsulinaemia. Other investigators (Sharma *et al*., 1996) have showed that the hypolipidemic effect of ginger. Furthermore, Ajith *et al*., (2007) studied the protective effect of ginger extract against the induced nephrotoxicity and renal failure. Ginger is

also recommended by the traditional herbalist in south Asia for using in cardiopathy, high blood pressure, (Duke, 2002). Its use was recorded in early Chinese text and documented in ancient Greek, Roman and Arabic medical literature (Bhandari *et al*., 2001).

In addition, phytochemical reports have shown that the main constituents of ginger are Gingerol, Shagaols, Zingerone and Paradol. It was reported that 6-gingerol and 6-shogaol are the major Gingerol and Shogaol present in the rhizome (Comell and McLachlan, 1972).

Sharma and Shukla, (1997) reported a significant blood glucose lowering effect of ginger juice in diabetic and non-diabetic animals. In addition, Ahmed and Sharma, (1997) reported a significant hypoglycemic activity in rats after administration of ginger extract. Akhani *et al*., (2004) reported that ginger pretreatment inhibited streptozotocin hyperglycemia and hypoinsulinaemia. Furthermore, Bhandari and Grover, (1998) reported the blood glucose and blood urea ware lowered after administration of ethanolic extract of ginger in diabetic rats.

Ginger acts as a hypolipidaemic agent in cholesterol-fed rabbits (Bhandari *et al*., 1998). Akhani *et al*., (2004) reported that ginger treatment significantly decreased both serum cholesterol and triglycerides. In addition, Fuhrman *et al*., (2000) reported that ginger decreased LDL-cholesterol, VLDL-cholesterol and triglycerides levels in apolipoprotein-E deficient mice. Furthermore, Bhandari *et al*., (1998b) have reported that an ethanolic extract of ginger prevent hypercholesterolemia and development of atherosclerosis in cholesterol-fed rabbits. Bhandari *et al*., (2005) found that, the ethanolic extract of ginger significantly reduced serum total cholesterol and triglycerides and increased the HDL-cholesterol levels; also, the extract can protect tissues from lipid peroxidation and exhibit a significant lipid lowering activity in diabetic rats. The study of Gujaral *et al.*, (1978) revealed that serum and liver cholesterol decreased when ginger was administered to hypercholesterolemic rats.

In the diabetic rats, an increase in serum uric acid, urea and creatinine levels was observed (Ozsoy-Sacan, *et al*., 2006). The study of Ajith *et al*., (2007) was designed to evaluate the protective effect of ginger against cisplatin-induced oxidative stress and acute renal failure in kidneys of mice. In addition, this study observed the effect of pre-treatment with ginger on the levels of serum creatinine and urea, and concluded that the administration of ethanol extract of ginger before and after cisplatin injection significantly lowered the elevated levels of serum creatinine and urea.

MATERIALS AND METHODS

Ginger extract:

The fresh rhizomes of *Zingiber officinale* were obtained from local market and identified by the herbarium staff of the Botany Department, Assuit University, Egypt. Ginger juice was prepared using the method of Akhani *et al*., (2004), fresh rhizomes of ginger (1Kg) were collected and crushed, then squeezed in muslin cloth to obtain the juice, which was stored in the refrigerator at 2-8°C in a well-closed glass container.

Induction of diabetes:

Male Wistar rats (130-150gm.) were housed in standard condition and fed with normal diet and water ad libitum. Diabetes was induced in fasted rats (12hrs) by a single dose intraperitoneal injection of 150mg/kg body weight of alloxan. After the injection, they had free access of food and water; the rats were given 5% glucose solution after 6hrs of alloxan injection to drink overnight to counter hypoglycemic

shock. The diabetic state was assessed by measuring the non-fasting plasma glucose concentration 72hrs after alloxan treatment. The rats with a plasma glucose level above 250mg/dl were selected for the experiment and considered as diabetics (Zhang *et al*., 2006).

Experimental protocol:

The experimental animals were divided into 4 groups; each group contained ten animals: Control group G1 (normal without treatment), diabetic group G2 (injected with 150mg/kg b.w. of alloxan), diabetic rats post-treated with ginger for 6, weeks G3 (firstly, the rats were injected with alloxan then given ginger), normal rats pretreated with ginger before they were made diabetics G4 (firstly, the rats were given ginger for 6 weeks then injected with alloxan). Ginger extract was given orally to the rats through a gastric tube daily for 6 weeks at a dose of 4ml/kg body weight.

Collection of blood samples:

At the end of the 6 weeks of post-treatment, blood samples were collected by sacrificing the animals and the blood was collected in clean EDTA tubes, then plasma was separated by centrifugation and stored at-20°C for biochemical analysis. While the groups of rats pretreated with ginger were decapitated after 3 days from the injection of alloxan and blood samples were collected as mentioned above.

Chemicals:

Alloxan monohydrate was obtained from sigma chemicals company, Egypt. Kits of glucose, cholesterol, triglycerides, HDL, creatinine, urea and uric acid were purchased from Spinreact, S.A. Ctra. Santa Coloma, Spain. All other chemicals used were of analytical reagent grade.

Biochemical analysis:

Glucose determination was carried out according to the method of (Trinder, 1969), while Cholesterol was determined by the enzymatic method as described by (Richmomd,1973).Triglycerides were determined by the enzymatic colorimetric method as described by (Young, 2001). Low-density lipoproteins (LDL) and very low density lipoproteins (VLDL) in sample precipitate with phosphotungestate and magnesium ions. After centrifugation, the cholesterol concentration in the HDL fraction, which remains in the supernatant, is determined as described by Lopes-Virella, (1977).

Creatinine and urea were determined by enzymatic method according to the method (Patton, and Crouch, 1977).

Plasma uric acid was determined by quantitative determination of uric acid (Young, 2001).

Statistical analysis:

Data were statistically analyzed by one-way analysis of variance followed by Duncant's test (PC-stat computer program). Finally, significant difference (L.S.D) was used to test the difference among treatments. Results were considered statistically significant when ($P < 0.05$).

RESULTS

Glucose:

The administration of ginger extract to the diabetic rats significantly reduced plasma glucose level when compared with diabetic group. This reduction was not enough to reach normal rats, but it was still significantly higher when compared with the normal group, as shown in Table 1 and illustrated in (Figs. 1& 2). On the other hand, from Table 1, it was clear that the level of plasma glucose was decreased in rats

pretreated with ginger extract and then induced-diabetes when compared with diabetic group, but it was still significantly higher than normal group.

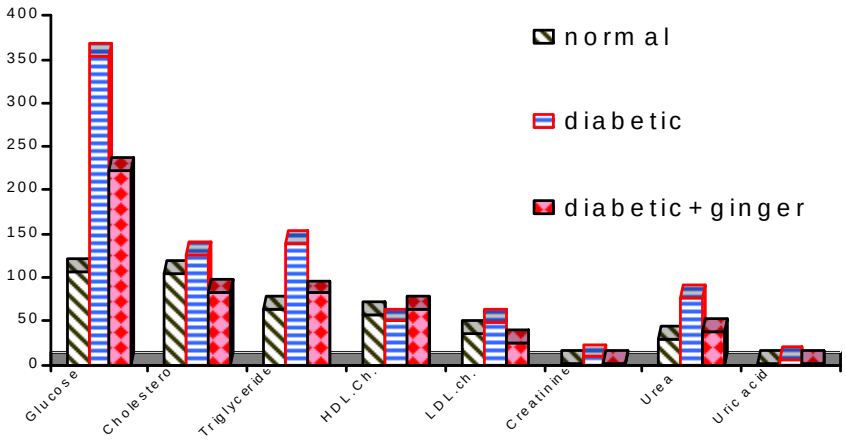

Fig. 1: Effect of post-treatment of ginger extract 4 ml/kg (body weight) on the levels of plasma glucose, lipid profile, Creatinine, urea and uric acid in alloxan-induced diabetic rats.

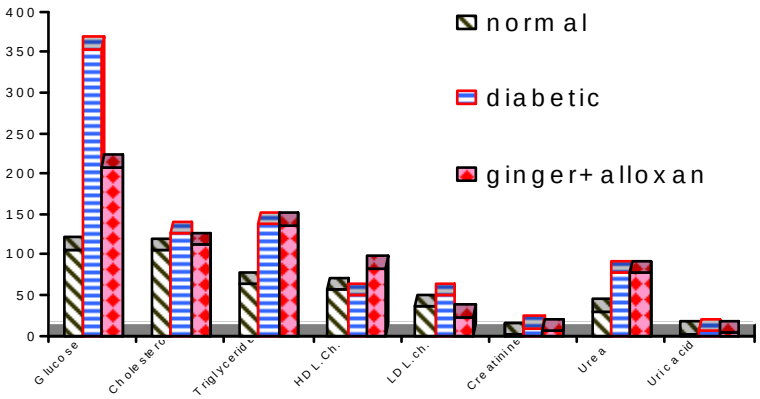

Fig. 2: Effect of pre-treatment with ginger extract 4 ml/kg (body weight) on the levels of plasma glucose, lipid profile, Creatinine, urea and uric acid in alloxan –induced diabetic rats.

Lipid Profile:

As shown in Table 1, alloxan produced significant hyperlipidemic action, where a significant increase was recorded in the levels of plasma cholesterol, triglycerides and LDL-cholesterol when compared with normal group. But, HDL-cholesterol level statistically without change when compared to normal group, while, post-treatment with ginger extract to diabetic rats produced significant reduction in the levels of plasma cholesterol, triglycerides and LDL-cholesterol when compared with diabetic group, but HDL-cholesterol recorded significant elevation when compared with diabetic group. It was clear from Table 1 that the reduction in the levels of cholesterol, triglycerides and LDL-cholesterol reach normal levels.

On the other hand, as shown in Table 1 the pre-treatment of rats with ginger extract followed by the induction of diabetes caused a significant reduction in the plasma levels of cholesterol and LDL-cholesterol when compared with diabetic rats, but HDL-cholesterol level recorded significant elevation when compared with

diabetic rats, while plasma triglycerides statistically did not change when compared with diabetic rats, but there was a significant increase when compared with normal group. Furthermore, the levels of plasma cholesterol and HDL-cholesterol in rats pretreated with ginger extract recorded a significant increase when compared with normal group, in contrast, LDL-cholesterol recorded a significant decrease when compared with control group as shown in Table 1.

Kidney Functions:

As shown in Table 1, the alloxan produced significant increase in the levels of plasma creatinine, urea and uric acid when compared with normal group, while, post-administration of ginger extract to the diabetic rats significantly reduced the levels of plasma creatinine, urea and uric acid when compared with the diabetic group, but no significant changes were observed when compared with the normal rats. This indicates that, post-treatment with ginger extract normalized the plasma creatinine, urea and uric acid.

Table 1: Effect of post-treatment and pre-treatment with ginger extract 4 ml/kg (body weight) on the levels of plasma glucose, lipids profile, Creatinine, urea and uric acid in alloxan-induced diabetic rats.

parameters mg /dl	normal group G1M±S.E	Diabetic control group G2 M±S.E	post-treated group Diabetic + ginger G3 M±S.E	pre-treated group ginger + alloxan G4 M±S.E
Glucose	105.8±6.0	353.8±6.1*	223.2±6.7$^{*\#}$	208.3±5.5$^{*\#}$
Cholesterol	104.8±1.7	126.2±4.0*	83.7±2.6$^{*\#}$	112.5±2.4$^{*\#}$
Triglyceride	63.6±4.4	137.9±4.7*	81.7±3.1$^{*\#}$	136.7±3.2*
HDL.Ch.	56.5±3.6	49.6±2.0	62.7±2.4$^{\#}$	83.4±2.6$^{*\#}$
LDL.Ch.	35.6±3.3	49.1±3.2*	24.7±0.6$^{*\#}$	23.1±0.2$^{*\#}$
Creatinine	0.47±0.04	8.8±0.4*	4.0±0.01$^{\#}$	5.0±0.2$^{*\#}$
Urea	29.8±1.7	76.5±3.2*	38.3±1.9$^{\#}$	77.2±3.0*
Uric acid	1.6±0.1	4.8±0.3*	1.7±0.1$^{\#}$	2.8±0.1$^{*\#}$

Values are expressed as mean ± S.E of 10 animals.
Diabetic control is compared with normal.
Treated groups are compared with diabetic control.
* Values are statistically significant at P^{*} <0.05 when compared with normal.
#Values are statistically significant at $P^{\#}$ <0.05 when compared with diabetic control.

On the other hand, the pre-treatment followed by induction of diabetes decreased significantly the levels of plasma creatinine and uric acids were decreased significantly when compared with the diabetic group. Furthermore, they are still significantly higher than normal rats. In contrast, non-significant increase was observed in plasma urea when compared with diabetic group, but it was still significantly higher than normal rats as shown in Table 1.

DISCUSSION

In this work we have studied the effect of post-treatment with ginger extracts on blood glucose, lipid profile and kidney functions in alloxan-induced diabetic rats through 6 weeks of treatment. To examine these effects the levels of plasma glucose, cholesterol, triglycerides, HDL-cholesterol, LDL-cholesterol, creatinine, urea and uric acid were determined.

In the present study we found that, post-treatment and pre-treatment with ginger, extract to the diabetic rats reduced the plasma glucose, and the pre-treatment with ginger caused reduction of glucose in diabetic rats. These results are in agreement with the study of Akhani *et al*., (2004), who found that post-treatment and pre-treatment of streptozotocin-induced diabetic rats with ginger extract significantly decreased the blood glucose level and increased the insulin level. Kar *et al*., (1999) reported that, the inorganic part of a medicinal plant contains mainly mineral

elements, which are responsible for the hypoglycemic activity. In support of this view, a number of essential minerals (Ca, Zn, K, Mn and Cr), are known to be associated with the mechanisms of insulin release and its activity in different animals and in human beings (Castro, 1998).

In the present study, the post-treatment of the induced-diabetic rats with ginger extract caused reduction in the levels of plasma cholesterol, triglycerides and LDL-cholesterol, but plasma HDL-cholesterol level statistically increased. On the other hand, the pre-treatment with ginger extract followed by induction of diabetes caused reduction in the plasma levels of cholesterol and LDL-cholesterol, and increased the level of plasma HDL-cholesterol, but plasma triglycerides statistically did not change. These findings are in agreement with previous studies; Bhandari *et al*., (2005) revealed that ethanolic extract of ginger produced significant decrease in serum total cholesterol and triglycerides levels and increased HDL-cholesterol level as compared to diabetic rats, and the extract exhibit a significant lipid lowering activity and protect the tissues from lipid peroxidation. Furthermore, Fuhrman *et al*., (2000) reported that ethanolic extract of ginger reduced plasma cholesterol and inhibited LDL oxidation in atherosclerotic, apolipoprotein E-deficient mice. It was concluded that (E)-8 beta, 17-epoxyllabed-12-ene-15, 16-dial, a compound isolated from ginger, interfered with cholesterol biosynthesis in liver homogenates of hypercholesterolaemic mice causing its reduction (Tanabe *et al*., 1993).

In the present study, injection of alloxan caused an increase of plasma lipid profile. These results are similar to the study of Martinez-conde *et al*., (1984), who reported that streptozotocin have lipolytic action on adipocytes, increasing plasma levels of free fatty acids. On the other hand, Akhani *et al.* (2004) revealed that treatment with ginger juice significantly decreased triglycerides and cholesterol levels in diabetic rats, and he suggested that the reduction in serum lipid levels with ginger might be due to its antagonistic action on streptozotocin receptors, thereby increasing insulin levels. Srinivasan and Sambaiah, (1991) reported that feeding rats with ginger significantly elevated the activity of hepatic cholesterol 7-alpha-hydroxylase which is a rate-limiting enzyme in the biosynthesis of the bile acids and stimulates the conversion of cholesterol to bile acids leading to the excretion of cholesterol from the body. In support of this view, the study of (Bhandari *et al*., 1998 b) revealed that post-treatment with ginger extract to the cholesterol-fed rabbits for 70 days resulted in less marked hyperlipidaemic when compared to the pathogenic rats. Moreover, the marked hyperlipidemia that characterizes the diabetic state may therefore be regarded because of the uninhibited actions of lipolytic hormones on the fat depots due to the absence of insulin (Goodman and Gilman, 1985). Hypolipidaemic and anti-atherosclerotic effects of ginger extract were also demonstrated in cholesterol-fed rabbits (Bhandari, and Grover, 1998 A). It was concluded that the hypocholesterolaemic effect of ginger could have possibly resulted from the inhibition of cellular cholesterol biosynthesis after the consumption of the extract (Fuhrman *et al*., 2000). Furthermore, Neess *et al*., (1996) reported that the reduction of cellular cholesterol biosynthesis is associated with increased activity of the LDL receptor, which in turn leads to enhanced removal of LDL from plasma, resulting in reduced plasma cholesterol concentration.

Hypertriglyceridaemia is a common finding in diabetic patients and is responsible for vascular complications (Kudchodkar *et al*., 1988). In the study of Bruan and Severson, (1992) it was concluded that deficiency of lipoprotein lipase activity may contribute significantly to the elevation of triglycerides in diabetes. Furthermore, Lopes-Virella *et al*., 1983) reported that treatment of diabetes with insulin served to lower plasma triglycerides levels by returning lipoprotein lipase

levels to normal. In the present study the decreasing levels of plasma triglycerides following the treatment with ginger extract may due to the stimulating effect ginger extract on insulin. The diabetic hyperglycemia induces the elevation of the plasma urea and creatinine in diabetic rats, which are considered a significant marker of renal dysfunction (Almdal and Vilstriup, 1988). In the present study the effect of ginger on the kidney functions was assessed by the determination the levels of plasma creatinine, urea and uric acid, and the study revealed that post-administration of ginger extract to the diabetic rats reduced and normalized the levels of plasma creatinine, urea and uric acid. On the other hand, the pre-treatment with ginger before the induction of diabetes inhibited the higher increase of plasma creatinine and uric acid resulted from the induced-hyperglycemia by alloxan but they did not normalized. Moreover, the study of Ajith *et al*., (2007) demonstrated that ethanol extract of ginger rendered significant protection against induced nephrotoxicity, which was evident from the lowered serum urea, and creatinine levels in the mice that pre-treated with ginger extract, and this study concluded that ginger extract significantly protected the elevation of serum creatinine and urea levels. Furthermore, the treatment of ginger extract could significantly prevent the depletion of antioxidant concentration and antioxidant enzymes activities in the kidneys. In addition, Ajith *et al*., (2007) reported that the presence of polyphenols and flavonoids in ginger extract might be responsible for the antioxidant nephroprotective activities and the reduction of serum urea and creatinine levels.

From the data obtained, it is concluded that post-treatment and pre-treatment with ginger extract produced a significant anti-hyperglycemic effect. Furthermore, ginger is capable of improving hyperlipidemia and the impaired kidney functions in alloxan induced- diabetic rats.

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ARABIC SUMMARY

تاثير تعاطى مستخلص الزنجبيل على مستويات الجلوكوز والدهون ووظائف الكلى فى دم الفئران المصابه بالسكرى المستحدث بواسطه الالوكزان

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لقد ظهر فى الاونه الاخيره استخدام الاعشاب والنباتات الطبيه للعلاج وتحسين بعض الحلات المرضيه.

تهدف هذه الدراسه لمعرفه تاثير تعاطى مستخلص من الزنجبيل فميا بجرعه مقدارها 4 ملى/ كجم من وزن الجسم كاجرعه علاجيه لمده 6 اسابيع وذلك بعد استحداث السكرى بواسطه الالوكزان , كما اعطيت نفس جرعه الزنجبيل (4ملى / كجم) امجموعه اخرى من الفئران لمده 6 اسابيع وذلك قبل استحداث السكرى لمعرفه الدور التحسينى والوقائى للزنجبيل.

قسمت الفئران الى 4 مجموعات ، المجموعه الاولى وهى العاديه ، المجموعه الثانيه مصابه بالسكرى المستحدث بواسطه الالوكزان (150 ملى جرام / كجم) ، المجموعه الثالثه وهى معالجه الفئران المصابه بالزنجبيل اما المجموعه الرابعه فقد اعطيت الزنجبيل اولا لمدة 6 اسابيع ثم استحدث السكرى بواسطه الالوكزان فى نهايه المده.

زبحت الفئران وجمعت عينات الدم وتم وفصل البلازما لتقدير مستويات الجلوكوز، والكوليسترول ، وثلاثى الجليسريدات ، البروتينات الدهنيه منخفضه الكثافة والبرتينات الدهنيه عاليه الكثافه وكذلك مستويات الكرياتنين , البولينا وحمض البوليك.

وقد تمت مقارنه المجموعات المعالجه بالزنجبيل مع كلا من المجموعه العاديه والمجموعه الضابطه المصابه بالسكرى المستحدث بواسطه الالوكزان.

اسفرت النتائج عن انخفاض مستوى الجلوكوز فى البلازما فى كلا المجموعتان المعالجه بالزنجبيل (المجموعه الثالثه والرابعه).كذلك لوحظ انخفاض مستوى الكوليسترول ، ثلاثى الجليسريدات ،والدهون البروتينيه منخفضه الكثافه وارتفاع مستوى الدهون البروتينيه عاليه الكثافه فى المجموعه الثالثه (المصابه السكرى) . اظهرت النتائج ايضا انخفاض مستوى الكوليسترول والبروتنيات الدهنيه منخفضه الكثافه وعدم تغير مستوى ثلاثى الجليسريدات عند المقارنه بالمجموعه المصابه بالسكرى ، بينما ارتفع مستوى البروتينات الدهنيه عاليه الكثافه وذلك فى المجموعه الرابعه (المعالجه بالزنجبيل سابقا).

اظهرت النتائج انخفاض فى مستويات الكرياتنين ، البولينا وحمض البوليك فى المجموعه الثالثه عند المقارنه بالمجموعه المصابه بالسكرى اما مستوى البولينا فى المجموعه الرابعه المعالجه بالزنجبيل سابقا لم يطرق عليها ايه تغيير على الرغم من انخفاض مستوى حمض البوليك والكرياتنين.

نستنتج من هذه الدراسه ان تعاطى مستخلص الزنجبيل لمده طويله يلعب دورا تحسينيا وجزئيا فى خفض مستوى السكرى فى الفئران المصابه بالسكرى المستحدث بواسطه الالوكزان ، وكذلك تحسين مستويات الدهون والضعف فى وظائف الكلى الناتج عن السكرى المستحدث بوسطه الالوكزان.