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MODULATION OF THE ANTIDIABETIC EFFECT OF GLICLAZIDE BY ALOE AND SILYMARIN IN DIABETIC RATS

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ABSTRACT

Diabetes mellitus is one of the dangerous diseases affecting a lot of population all over the world. Natural products are nowadays a target of researches for adaptation of some antidiabetics with less or minimal side effects. Aloe; is one of the natural products that commonly used in folk medicine. Silymarin, is another natural substance which is the product of choice for treatment of liver diseases.

These natural products were tested for their hypoglycemic activity when given alone and in combination with gliclazide in diabetic rats. Gliclazide, aloe and silymarin when given alone significantly decreased blood glucose levels (-72%, -73% and -16%), glycated heamoglobin (-23.2%, -26.4% and -12.4%), malondialdehyde (MDA) (-44.55%, -39.6% and -54%), total cholesterol (TC) (-29%, -58% and -36%) and low density lipoprotein cholesterol (LDL) (-48%, -63% and -39%), while increased insulin (90.6%, 49.6% and 43.3%) and the high-density lipoprotein cholesterol (HDL)(99 %, 51% and %7.5%). The effects of these drugs were more pronounced after 30 days of treatment.

Both aloe and silymarin significantly increased the hypoglyemic effect of gliclazide. Aloe and gliclazide combination decreased blood glucose levels by 80%, TC levels by 56% and LDL levels 69% as compared with gliclazide alone which produced 72%, 29% and 48% respectively.

The combination of silymarin and gliclazide decreased the blood glucose levels by 76%, TC levels by 56% and LDL by levels 53% with respective values of 72%, 29% and 48% for gliclazide alone.

INTRODUCTION

According to the American Diabetes Association[1], diabetes mellitus is defined as a group of metabolic diseases charecterized by hyperglycemia resulting from defectes in insulin secretion, insulin action or both. Oxidation reaction may damage both glucose and lipids directly, generating reactive carbonyl containing compounds. These reactive fragments can then cause irreversible modification of proteins. The term glyoxidation is used to cover this type of protein modification if the reactive fragments are derived from carbohydrates, but if they are derived from lipids, the term lipoxidation is used[2].

Chronic hyperglycemia causes an increased production of reactive oxygen species (ROS) due to the autoxidation of monosaccharrides, accumulation of non-enzymaticaly glycosated products and osmotically active sugar alcohols such as sorbitol[3]. These all may lead to a long-term damage, dysfunction and failure of various organs, especially the eye[4], kidneys, nerves, heart and blood vessels[1].

Sulphonylureas have been used for several decades in the treatment of non-insulin dependent diabetes mellitus due to their ability to increase insulin secretion[5] and glucose utilization by various tissues through a post-insulin receptor mechanism, probably related to a normalization of glucose transporters (GLUT4)[6].

Natural products are nowadays a target of researches (especially that have the antioxidant properties) for adaptation of some antidiabetics with inducing fewer side effects. Aloe is one of the natural products that commonly used in folk medicine to inhibit infection, promote healing of minor burns[7] and treat a lot of diseases[8]. Silymarin is the natural product of choice that used for treatment of liver disease due to its free radical scavenging effect and membrane stabilizing activity[9]. Our investigation aimed at studying the hypoglycemic activity of gliclazide in combination with either aloe or silymarin in streptozotocin (STZ)-diabetic rats .

MATERIAL AND METHODS

Animals:

Adult male albino rats with initial body weight of 200 ±15 gm, have been used in our study. Animals were kept at 12 h dark /12 h light cycle and allowed free on access special diet and water *ad libitum*

Drugs and chemicals:

Streptozotocin (STZ) was purchased from Sigma Chemical Co. (St . Louis, MO), gliclazide was obtained from Servier Co. France. Silymarin was a gift from Acapi-Egypt and aloe was purchased from a Herbalist Shop.

Experimental procedures:

Adult male albino rats were divided into 7 groups, one group served as control (taken saline, n=10), the other 6 groups were treated daily by oral administration of aloe (500 mg/kg), silymarin (100 mg/kg), gliclazide (7.2 mg/kg) and the combination of each two interchangeably for 30 days after the induction of diabetes. Diabetes was induced according to the method of **Zhiyong and Helga**[10] using STZ (38 mg/kg) intraperitoneally. Blood samples were collected from sinus-orbital vein via glass capillaries after 0, 15 and 30 days of administration.

Blood glucose level was measured directly by rapid glucose sensor system; GLUCOMEN, Mineria, Germany; glycoheamoglobin was measured by Stanbio Glycoheamoglobin Kit, according to Abraham et al.[11] method, procedure (No. 0350). Serum insulin was measured quantitatively according to Schliebs et al.,[12] by using immunoenzymatic assay kit supplied by Biosource Europe S.A (Catalog NO.001301). Area under glucose curve (AUC) was measured from the oral glucose tolerance test according to the method of Corteze et al.,[13]. MDA was measured in serum according to Draper and Hahdley[14]. Serum total cholesterol (TC), low-density lipoproteins (LDL) and high density lipoproteins (HDL) levels have been measured using kits according to Stein method[15]. All the previous parameters were measured at 0, 15 and 30 days (except insulin and glycohaemoglobin were measured at 0 and 30 days) after administration. Data were expressed as the mean ± SE for at least 6 independent experiments. Statistical comparisons were performed using unpaired Students t-test for differences between drug effects in different animals.

RESULTS

Oral administration of gliclazide, silymarin and aloe when given alone to adult male diabetic rats significantly reduced the blood glucose levels (Fig.1). Glycated hemoglobin (Fig. 2), malondialdhyde (MDA) (Fig.4), total cholesterol (TC) (Fig. 5) and low density lipoproteins cholesterol (LDL) (Fig. 6) were significantly reduced. On the other hand, insulin and the high density lipoproteins cholesterol (HDL) were significantly increased (Fig. 3) and (Fig.7) respectively. These effects were more pronounced after 30 days of treatment. On the other hand, aloe and gliclazide combination decreased the blood glucose levels by 80%, TC levels by 56% and LDL levels 69% as compared with gliclazide alone which produced 72%, 29% and 48% respectively.

Table (1): Effect of oral administration of gliclazide (7.2 mg/kg), silymarin (100 mg/kg), aloe (500 mg/kg) and their combinations for 30 days on area under the curve (AUC) (mg.min.dl^{-1}) in adult male albino diabetic rats.

Treatment	AUC mg.min.dl^{-1} X±SE	Compared with diabetic control	
		% Effect	% Change
Normal	26328±538.8	-	-
Diabetic	46041±1408 ^{A}	100	-
Silymarin	44100±2121.12	95.9	-4.08
Aloe	34800±1909.6^{B}	75.58	-24.41
Silymarin+aloe	36067.5±1482.6^{B}	78.33	-21.66
Gliclazide	34432.5±984.8^{B}	74.78	-25.21
Gliclazide+aloe	32340±1232.4^{B}	70.24	-29.7
Gliclazide+ silymarin	34661.2±1272.4^{B}	75.28	-24.7

A= significantly different from the normal rats at p<0.05.
B= significantly different from the diabetic rats at p<0.05.

The combination of silymarin and gliclazide decreased the blood glucose levels by 76%, TC levels by 56% and LDL by levels 53% with respective values of 72%, 29% and 48% for gliclazide alone.

The area under glucose curves of diabetic rats recorded a significant decrease after 30 days treatment with gliclazide, silymarin, aloe and their combinations compared with control values (Table 1, Fig. 8).

DISCUSSION

Sulfonylureas have been reported to stimulate insulin secretion as their predominant contribution towards their decreasing effects on blood glucose levels in diabetic patients[16]. They bind to a K^+ adenosine triphosphatase channel on at least one-sulfonylurea receptors[1] and block potassium efflux from the cell. Insulin secretion from beta cells is a function of blood glucose, or exposure to sulfonylurea and some to other substrates. Branched chain amino acids seem to activate beta cells through a pathway different from that of glucose stimulation[1,17].

Gliclazide, an oral hypoglycemic sulfonylurea, is widely used for treatment of non-insulin –dependent diabetes mellitus[18]. In addition to stimulating insulin secretion, gliclazide has a variety of bioactivities, e.g. scavenging a free radicals[19], inhibition of TNF-α production and prostanoid release in both animals and humans[20].

Data of this study showed that administration of aloe significantly decreased blood glucose level and increased the serum insulin. When given with gliclazide, aloe produced more pronounced effect on blood glucose and insulin. Similarly, aloe gel given orally may produce a mild reduction in mean glucose levels, but the mechanism has not been elucidated[21]. In animal study, aloe extract has been reported to improve glucose tolerance in normal and diabetic mice. It also decreased fasting blood level and decreased post-prandial elevation in blood glucose levels in rats[22].

Our data and that of the others indicate that aloe may be able to decrease the glycogenolysis and gluconeogenesis processes. This mechanism is in agreement with that reported by Al-Awadi, et al.[22]. Moreover, aloe may increase glucose utilization by tissues. This was documented by the reduction in the total area under the curve (AUC) during the oral glucose tolerance test (OGTT). Increased glucose tissue utilization by aloe and its combinations with silymarin or gliclazide may be due to improving the liver status. Also, it is likely that improvement of glucose utilization allows the liver to be able to produce insulin-like growth factor I that facilitates glucose uptake in extrahepatic tissues or increased glucose transport receptors. However, this improvement may be due to the antioxidant properties of aloe, which may lead to increase of protein synthesis, increased levels of the reducing substances; glutathione, vitamins E and C in tissues.

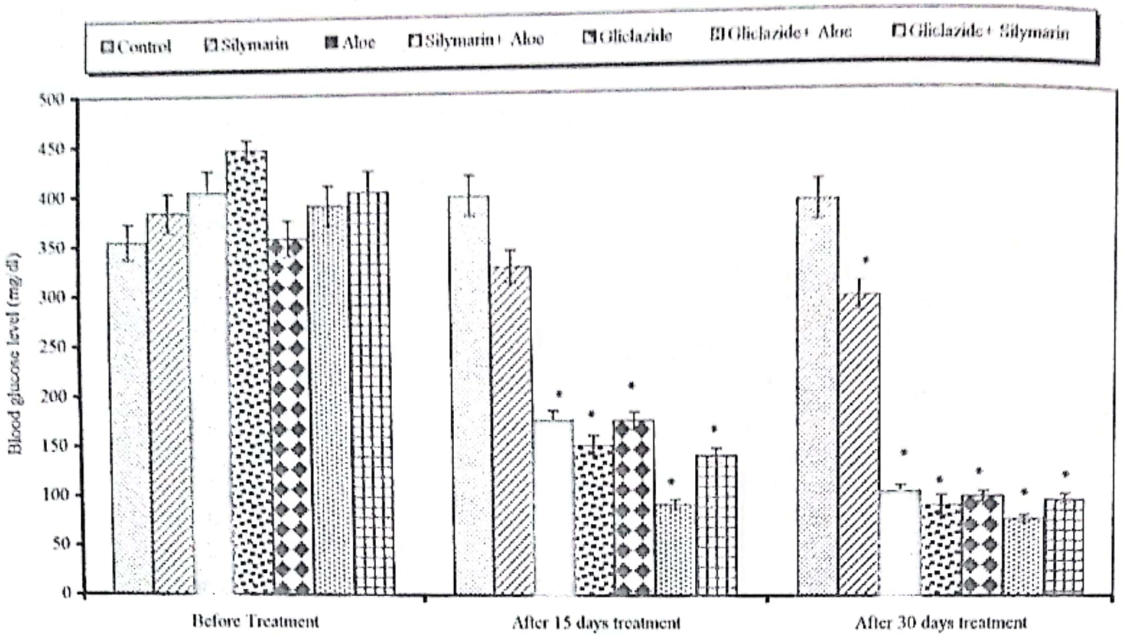

Fig. (1): Effect of oral gliclazide (7.2 mg/kg), silymarin (100 mg/kg), aloe (500 mg/kg) and their combinations on blood glucose level (mg/dl) of adult male diabetic rats.

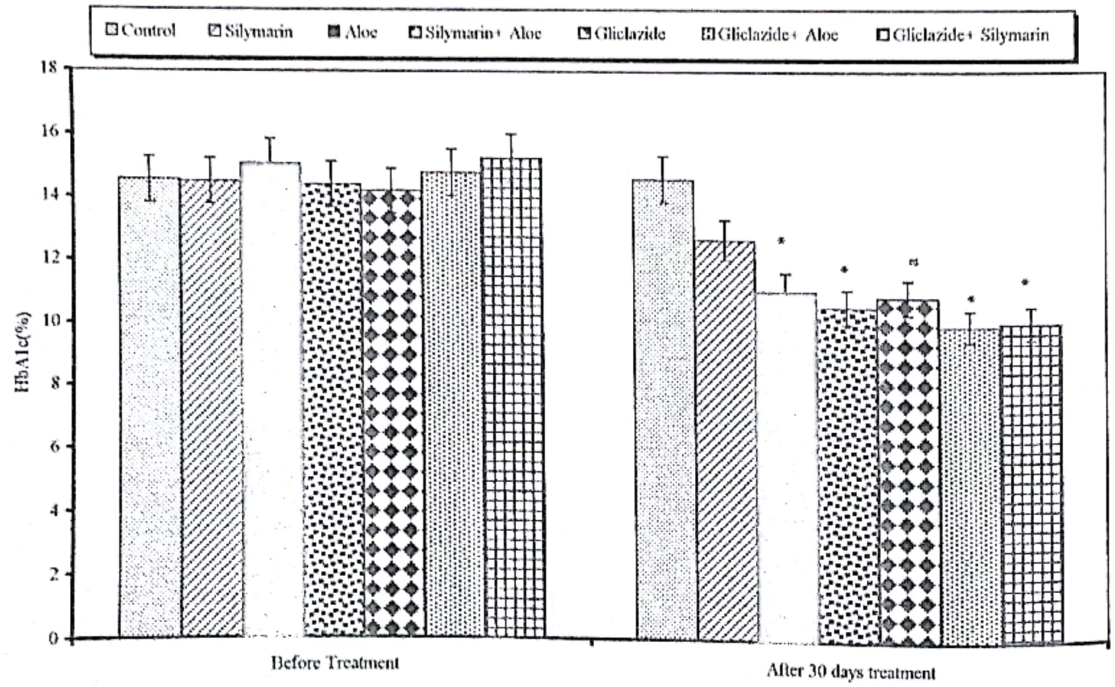

Fig. (2): Effect of oral gliclazide (7.2mg/kg), silymarin (100mg/kg), aloe (500mg/kg) and their combinations on glycated heamoglobin(HbA_{1c}) level (%) of adult male diabetic rats.

Data represents the mean ± SE of 6 rats / group.
*: significantly different from the mean value of control rats at $p<0.05$.

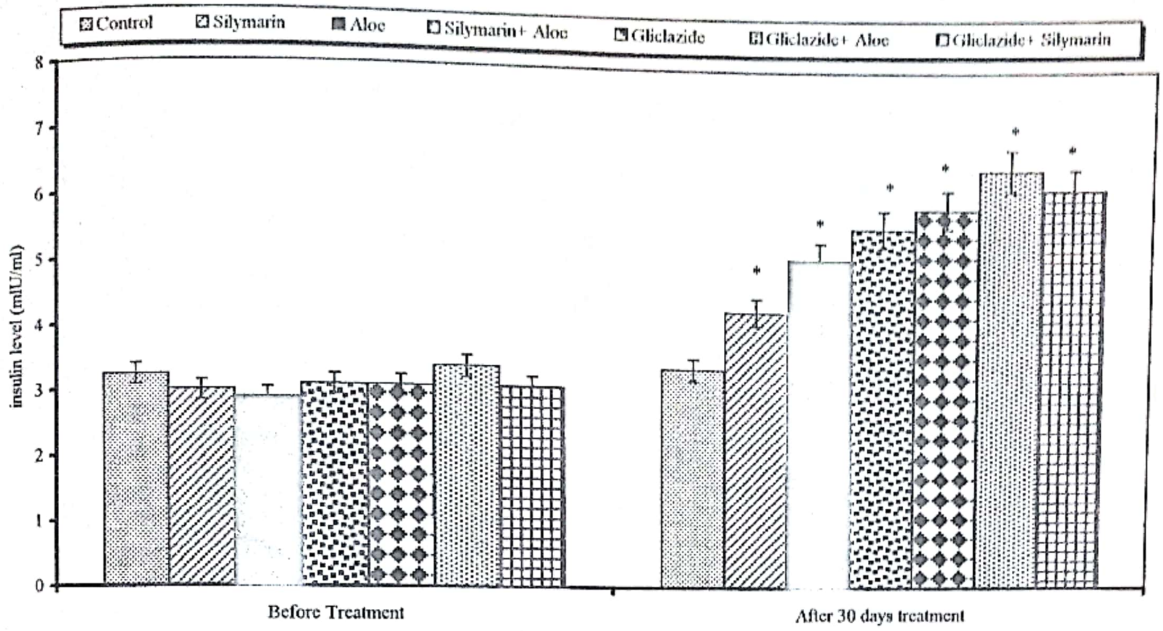

Fig. (3): Effect of oral gliclazide (7.2mg/kg), silymarin (100mg/kg), aloe (500mg/kg) and their combinations on serum insulin level (mU/ml) of diabetic adult male rats.

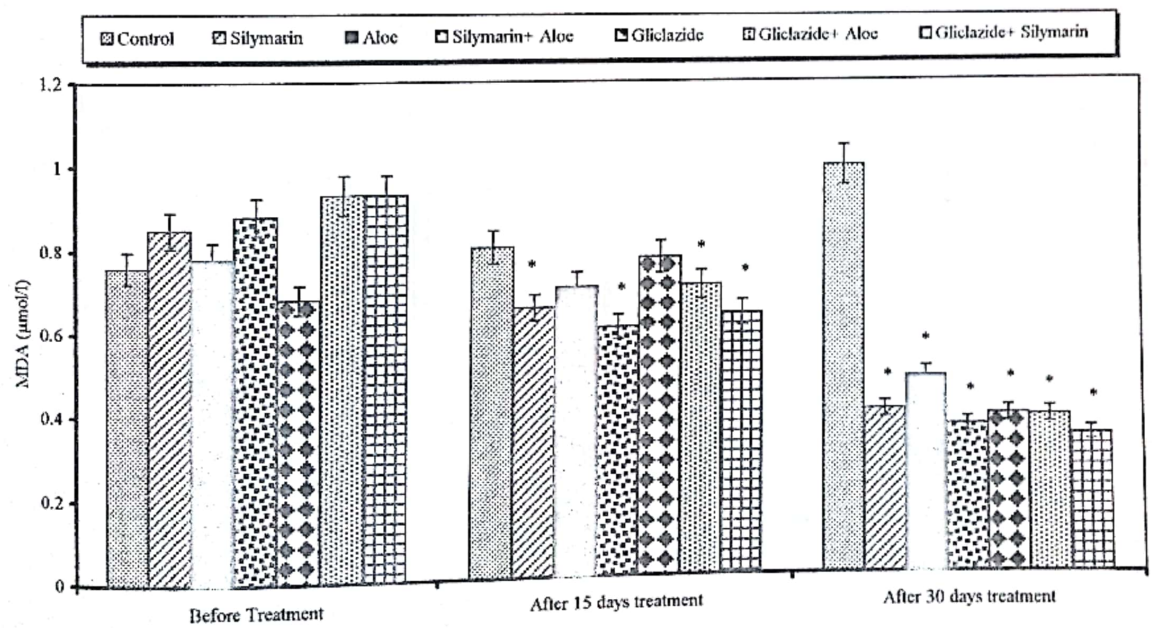

Fig. (4): Effect of oral gliclazide (7.2mg/kg), silymarin (100mg/kg), aloe (500mg/kg) and their combinations on serum malondialdhyde (MDA) level (µmol/l) of adult male diabetic rats.

Data represents the mean ± SE of 6rats / group.
*: significantly different from the mean value of control rats at $p<0.05$.

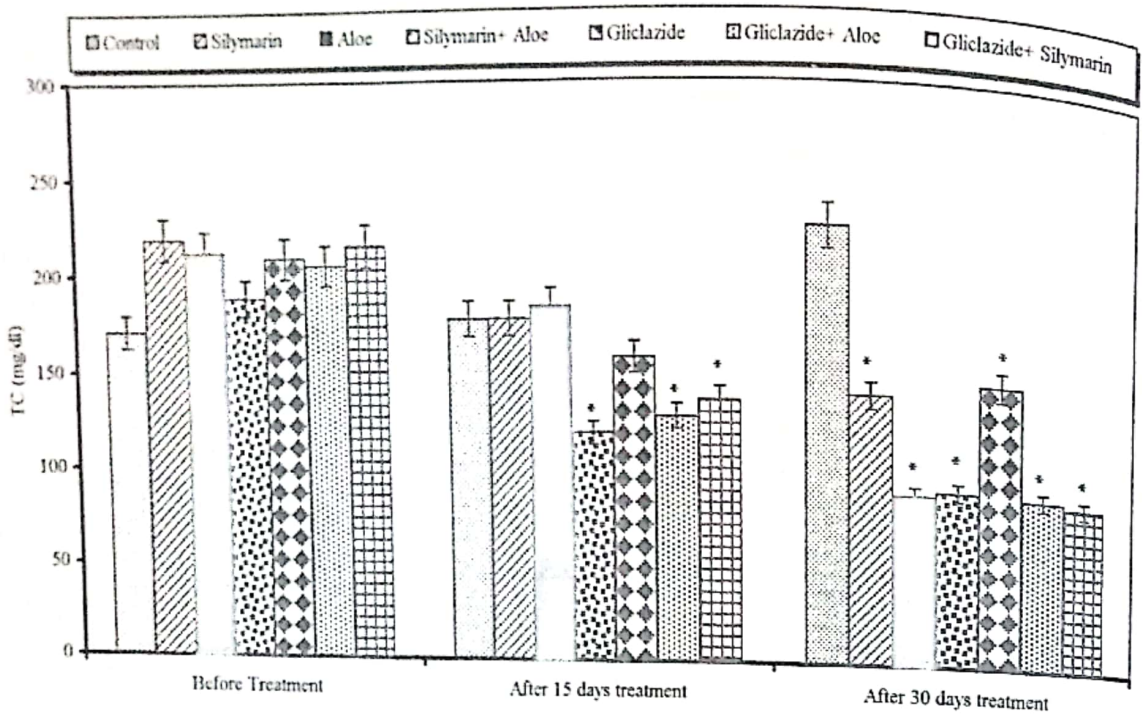

Fig. (5): Effect of oral gliclazide (7.2mg/kg), silymarin (100mg/kg), aloe (500mg/kg) and their combinations on serum total serum (TC) level (mg/dl) of adult male diabetic rats.

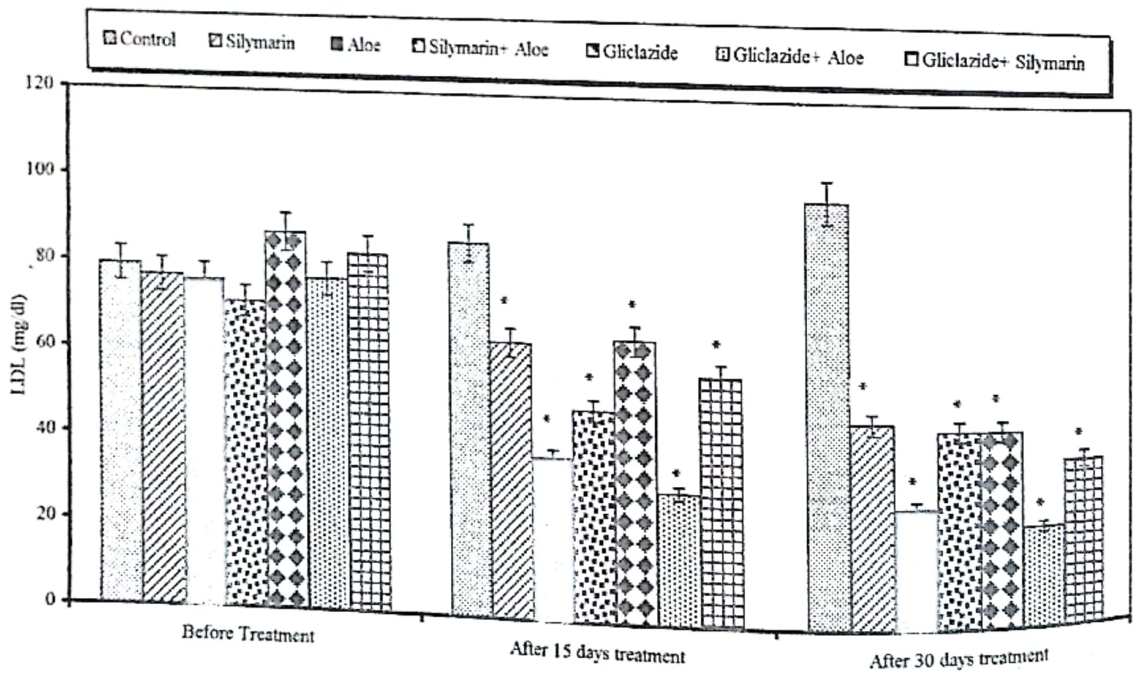

Fig. (6): Effect of oral administration of gliclazide (7.2mg/kg), silymarin (100mg/kg), aloe (500mg/kg) and their combinations on serum low denesity lipoprotien cholesterol (LDL) level (mg/dl) of adult male diabetic rats.

Data represents the mean + SE of 6rats / group.
*: significantly different from the mean value of control rats at $p<0.05$.

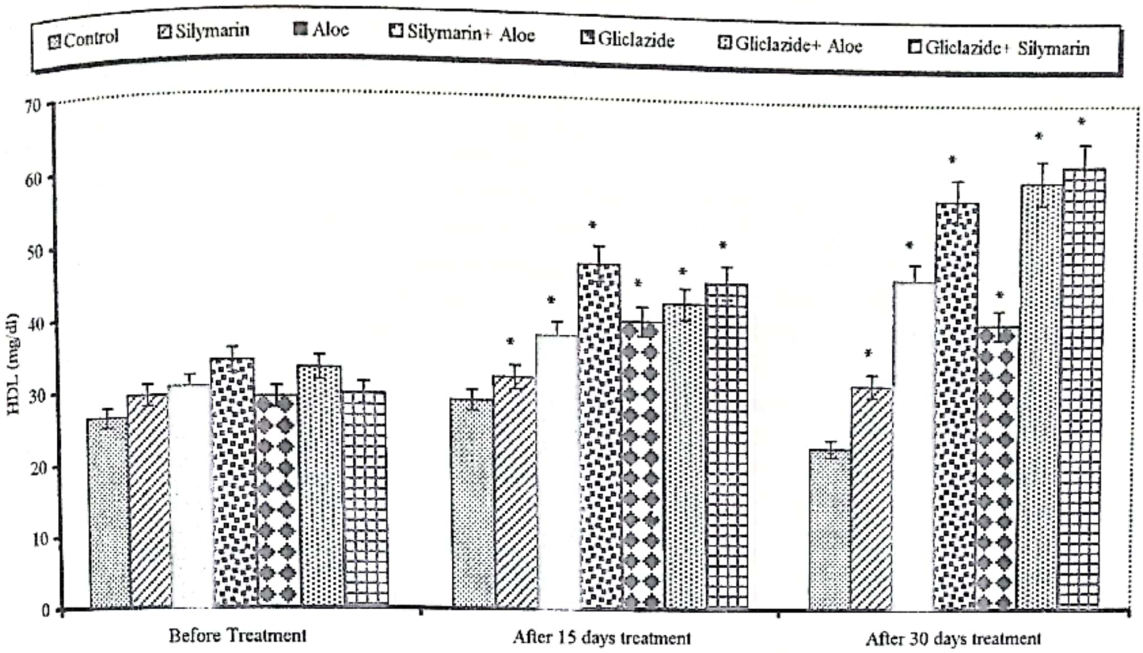

Fig. (7): Effect of oral administration of gliclazide (7.2mg/kg), silymarin (100mg/kg), aloe (500mg/kg) and their combinations on serum high denesity lipoprotien cholesterol (HDL) level (mg/dl) of adult male diabetic rats.

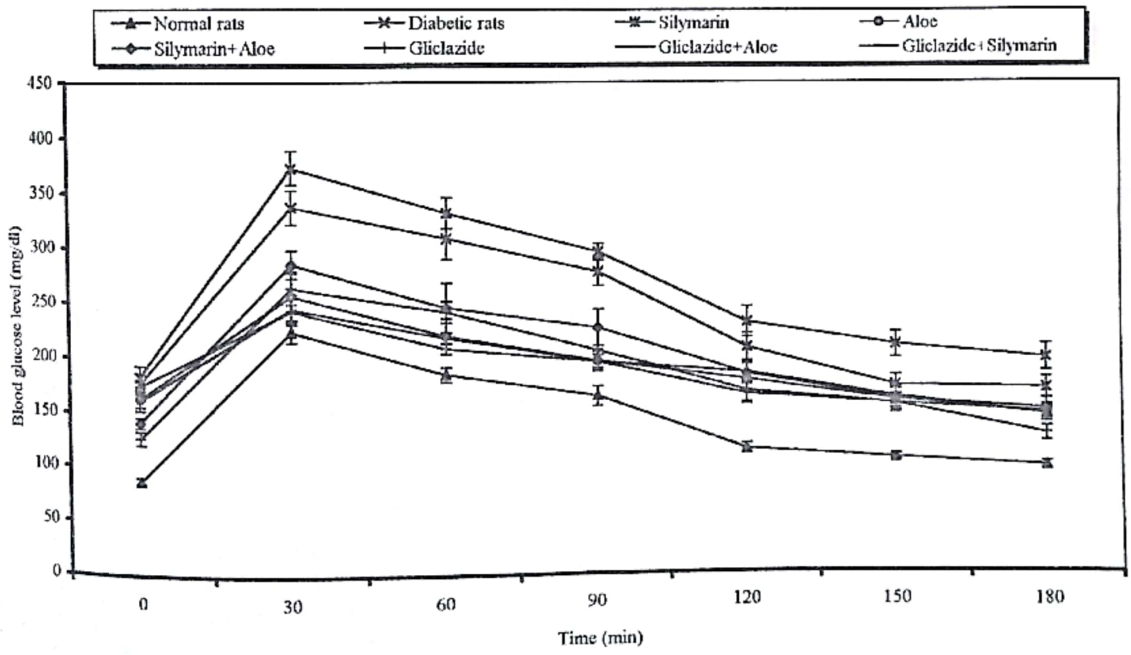

Fig. (8): Time course of changes in the blood glucose level (mg/dl) in diabetic rats after oral administration of gliclazide (7.2mg/kg), silymarin (100mg/kg), aloe (500mg/kg) and their combinations for 30 days.

Data represents the mean + SE of 6rats / group.
*: significantly different from the mean value of control rats at $p<0.05$.

This hypothesis is in agreement with Sheppard, et al.,[33] who suggested that the healthy liver produces a "factor" that facilitates glucose uptake in extrahepatic tissues and lack of this factor may be the cause of insulin resistance. This was documented by the elevation in serum HDL and the reduction in LDL in the present study.

Diabetes, as a disease, is a state of an increased oxidative stress based on increased peroxidation[5,20,24] and reduced antioxidant reserves[25,26]. Furthermore, in diabetic animal models auto-oxidation of non-enzymatic glycated proteins generate a superoxide anion radical[27].

Diabetes increases oxidative stress by several mechanisms. One of the most important is the increased advanced glycation end products which progressively accumulate in diabetes and can generate free radicals[28]. Moreover, hyperglycemia catalyzes lipid peroxidation *in vitro*. Second, the reduction of antioxidants defenses including glutathione[2], vitamin E[29] and vitamin C[30] have been reported to be reduced in diabetic patients. The above two mechanisms may play an important role in the pathogenesis of diabetes vascular complications[31], neurovascular dysfunction[32], liver and beta cell damage in the pancreas[13], atherosclerotic peripheral arterial disease[34,35] and teratogenesis[36].

According to the results of this investigation, oral administration of aloe significantly reduced glycated hemoglobin, LDL, cholesterol, MDA and increased HDL after 30 days compared with diabetic control rats. This effect was more pronounced when it is concurrently administered with gliclazide. These evidence suggest that the ingestion of aloe may inhibite lipoxidation processes since there were an elevation in HDL level and reductions of elevated LDL and MDA levels.

Our results revealed that silymarin reduced blood level of glucose, LDL, total cholesterol, glycated heamoglobin and increased HDL after 30 days. Some of these effects of silymarin were more pronounced when given with gliclazide. These effects may be attributed to the prevention of the oxidative effects produced by diabetes and to its protective antioxidant activities on both pancreatic and liver tissues[9].

Our results showed that, the antidiabetic effect of silymarin might be attributed to the improvement in the liver status. This improvement may be due to its antioxidant activity which may leads to increased protein synthesis. This also may be due to increased regeneration of the hepatocyte membrane, which in-turn increased the glucose transporters and insulin receptors in the liver tissues. This postulation was documented from our results by the reduction in the AUC of the OGTT, in addition to reduction in glycation end products, which progressively accumulate in diabetes and can generate free radicals capable of oxidizing lipids[28]. This is in harmony with our results that reveal significant decreases in MDA, LDL, glycoheamoglobin and TC and a significant increase in HDL in rats.

Silymarin was reported to increase pancreatic and blood glutathione levels which may play an important role in its protective effect on pancreatic damage in experimental animals[28]. These effects may be due, in part, to the stimulation of protein synthesis by increasing the activity of ribosomal RNA, which may lead to enhancement of hepatocyte regeneration[37].

Conclusion: From this study, it could be concluded that aloe has an antidiabetic and antihyperlipidemic effects. Further studies should be carried out to investigate the clinical effects in diabetic patients. Adaptation of dose and dose regimen is required for the adjustment of blood glucose levels in these patients. Due to its antioxidant activity against STZ-induced diabetes, silymarin should be subjected for further studies to investigate its protective effect against some environmental and other agents that may participate in the incidence of diabetes.

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تغيير تأثير الجليكلازيد المخفض للجلوكوز بواسطة الصبر والسيليمارين فى الجرذان المصابة بمرض البول السكرى

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يعتبر مرض البول السكرى من الأمراض الخطيرة التى تصيب العديد من البشر. وتعتبر المنتجات الطبيعية هذه الأيام هدفاً للباحثين للتوصل لمضاد للبول السكرى ، له القليل من الآثار الجانبية ويعتبر الصبر واحداً من المنتجات الطبيعية الذى يستخدم بكثرة فى الطب الشعبى فيما يعتبر السيليمارين من المنتجات الطبيعية التى تشكل العلاج الأمثل لأمراض الكبد. وقد اختيرت هذه المنتجات الطبيعية لقياس تأثيرها المخفض للسكر عند إعطائها بصورة مفردة أو مع الجليكلازيد فى الجرذان المصابة بمرض البول السكرى.

هذا وقد أحدث الجليكلازيد ، الصبر ، السيليمارين عند إعطاء كل منهم منفرداً انخفاضا معنوياً فى مستوى الجلوكوز فى الدم ، الجلايكوهيموجلوين ، المالون داى ألدهيد ، الدهون عالية الكثافة والدهون منخفضة الكثافة فى حين أحدثوا جميعاً ارتفاعاً فى مستوى الإنسولين فى مصل الدم والدهون عالية الكثافة وهذه التأثيرات كانت واضحة بعد ثلاثين يوماً ، وقد أحدث كل من الصبر والسيليمارين زيادة فى تأثير الجليكلازيد.