World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Published by Atom and Cell Publishers
Available online at: http://www.wjpsonline.org/
Original Article

Effect of garlic Extract and Citrus aurantifolia (lime) juice and on Blood Glucose level and Activities of aminotransferase Enzymes in streptozotocin-induced diabetic rats

Akbar Karimi, Nahid Khayam Nasab

Department of Biology, Payame Noor University, Tehran, Iran

Received: 14-06-2014 / Revised: 01-07-2014 / Accepted: 16-07-2014

ABSTRACT

Diabetes is a chronic disorder of carbohydrate, fat and protein metabolism characterized by defects in insulin secretion, insulin action, or both. Garlic (*Allium sativum, Liliaceae*) has been reported to have many medicinal properties including antidiabetic and anti lipidemic activities. Lime *(citrus aurantifolia*) contains flavonoids such as Eriocitrin and Hespridin with antioxidant effects. We studied the effects of fresh juice of lime and garlic extract on glucose level and Activities of aminotransferase enzymes in streptozotocin- induced diabetic rats. Streptozotocin was administered as a single dose (60mg/kg BW) to induce diabetes. STZ induced diabetic rats were treated with garlic extract at three different doses (500mg/100gBW, 250mg/100gBW and 125mg/100gBW) for 21 days. Other groups of diabetics were treated with fresh lime juice with different concentration (100, 50 and 25%) by gavage during the study period. As well as the other diabetic groups were treated with combination of garlic extract and lime juice. Further, the results are comparable with metformin, an oral standard drug. Diabetic control rats, blood glucose level was significantly ($p<0.05$) increased as compared to the control glucose level. The maximum hypoglycemic response between the groups treated with garlic, was observed with highest doses of garlic extract (500mg/100gBW), while in diabetic rats treated with lime juice, 50% concentration produced a better effect than other concentration. Blood glucose level also significantly decreased in diabetic groups were treated with combination of garlic extract and lime juice, so this combination has additive effect on reducing blood glucose level. Also, garlic and lime juice could not inhibit liver damage. These results suggest that lime and garlic exhibit antihyperglycemic effects for a brief period in experimental diabetic rats.

Keywords: Diabetes, Garlic, Lime, metformin, rat

INTRODUCTION

Diabetes is a chronic disorder of carbohydrate, fat and protein metabolism characterized by defects in insulin secretion, insulin action, or both [1] and it is considered to be 1 of the 5 leading causes of death in the world [2]. Chronic hyperglycemia in diabetes leads to auto-oxidation of glucose, non-enzymatic protein glycosylation, impaired glutathione metabolism, alteration in antioxidant enzymes and formation of lipid peroxides; the events accelerate production of free radicals and weaken the antioxidant defense [3,4,5]. Management of diabetes without any side effects is still a challenge to the medical system. There is an increasing demand by patients to use the natural products with anti diabetic activity, because insulin and oral hypoglycemic drugs possess undesirable side effects [6]. Consumption of garlic (*Allium sativum, Liliaceae*) has been reported to have many medicinal properties including antidiabetic and anti lipidemic activities [7]. Garlic's strong odour is largely due to sulphur-containing compounds (e.g. s-allylcystein sulphoxide), which are believed to account for most of its medicinal properties. S-allyl cysteine prevents the occurrence of various complications of diabetes in rats by changing the blood glucose and protein metabolism [8]. SAC also normalizes hepatic carbohydrate metabolism and plasma and tissue glycoprotein level in diabetic rats[9]. Lime (*citrus aurantifolia*) is one of the most popular citrus fruits in the word. Lime juice contains a number of nutrients such as citric acid, ascorbic acid, minerals and flavonoids [10]. Eriocitrin (eriodictyol 7-o-β-rutinoside) and hespridin (hesperetin 7-o-β-rutinoside) among the flavanone glycosides contained abundantly in lime juice [10]. Many clinical studies have been

***Corresponding Author Address: Akbar, Department of Biology, Payame Noor** University (PNU), P.O.Box.19395-3697, Tehran , Iran, E-mail: karimiakbar38@yahoo.com*

investigated the effect of garlic and its active ingredients on diabetes. In addition, hypoglycemia and hypolipidemic effects of garlic were been approved on diabetic rats. However, not any comparative and longitudinal study was done on effect of both garlic and lime on type I diabetes and there is not any printed study shows the effect of lime on blood parameters in diabetic animals. Therefore, the aim of the present study was to evaluate the effect of garlic aqueous extract and lime juice on the levels of blood glucose and the activities of pathophysiological enzymes such as aspartate transaminase (AST), alanin transaminase (ALT), and alkaline phosphatase (ALP) in STZ-induced diabetic rats. Additionally, in this research, comparative assessment of effects for both plants extract were done with Methformin; a common medicine for diabetes treatment.

MATERIAL AND METHODS

Animals: Male Wistar rats of body weight 180-200 g were used for this study. The animals were housed in clean cages under conditions of controlled temperature (25±2 ^{o}C) with a 12/12-h day-night cycle, during which time they had free access to food and water ad libitum. The animals were maintained in the animal house, Esfahan payamnor University.

Induction of diabetes: Diabetes was induced in overnight fasted adult Wister male rats by a single intraperitoneal injection of 60 mg/kg streptozotocin. Hyperglycaemia was confirmed by elevated glucose levels (above 250 mg/dl) in blood, determined on day 3 after injection [11].

Extract preparation: To prepare garlic extract in different concentrations, respectively 150,75 and 37.5 gr of garlic bulbs were prepared from Hamadan, a city in Western Iran and famous for its fresh garlic. Then garlic bulbs Egypt and cut into small pieces. About 300 ml of distilled water were added and crushed in a mixcing machine. The resultant slurry was filtered through a fine cloth and the filtrate was quickly frozen until used. The concentration of this garlic preparation was considered to be 500mg/ml, 250mg/ml and 125 mg/ml on the basis of the weight of the starting material. The aqueous extract of garlic was stored in small aliquots at-20°C until use. Lime (*Citrus Aurantifolia*) obtained and daily lime juice taken manually. It is noteworthy, a voucher specimen is maintained at the Herbarium of the Isfahan University with herbarium codes (5527).

Tretment of Diabetic Rats: The experimental rats were divided into ten groups each comprising five rats. Treatment groups were classified according to the following table 1.

Groups 2,3 and 4 recived 1 ml garlic juice/100gr BW by gavage for three weeks. Groups 5,6 and 7 recived 1.1 cc of fresh lime juice with different concentration (100, 50 and 25%) by gavage during the study period, respectively. Metformin 500mg tablets were used at human dose. Metformin dissolved in distilled water, 1cc administrated, daily by gavage. Metformin is one of the most common drugs to control hyperglycemia and the result in this study is comparable with metformin, an oral standard drug. A seven days extract of garlic and lime administrated to rats by gavage, after three days rest period, this extract administrated to rats during fourteen days again. Overall period for experiments was 21 days.

Assays: 12 hours fasting period was applied before samplings the subjects. Sampling was done between 8-10am. Glucose level was measured three times by using an automated blood glucose analyzer (01-mini glucometer). At the end of the experimental period, rats were fasted for 12h, and blood samples were collected from retro–orbital vein plexus of the animal's eyes by hematocrit tubes (Herck, 2001). Plasma samples were obtained by centrifugation at 3000g for 15 min and stored at -20 ^{0}C till measurements.

The aminotransferase enzymes (ALT, ALP and AST) for all the ten groups of animals were performed using commercially available kits (pars, Iran) and according to the guidelines.

Statistical analysis: The data are expressed as mean ± SEM. Readings within a group were compared using the one-way ANOVA analysis and readings between groups were compared using independent sample test. A level of $p < 0.05$ was considered to be significant.

RESULTS

The effects of oral administration of garlic and lime juices on blood glucose level are presented in figures 1-8. Figure 1 shows changes in the blood glucose levels in diabetic rats in response to 500mg/ml on the basis of the weight of the starting material of garlic extract administration. This dose leads to a decrease in blood glucose on days 7 and 21 experiments. The present study shows that administration of high dose (500 mg/100gBw) of garlic extract is more effective than that of moderate (250mg/100gBw) and low dose (125mg/100gBw) (Figures 1,2 and3). Also, the data from this study show that administration of lime juice (50%) leads to significantly ($p< 0.05$) reduce blood glucose levels but this beneficial effect on

the hyperglycemia of diabetic rats have short-term effect. The administration of lime juice (100% ,25%) in rats diabetes did not reduce blood glucose level (Figures4,5 and6). Results showed that the hypoglycemic effect of extract- combined -treated diabetic groups have been much more than other groups and this hypoglycemic effect was more than rats diabetic treated with Metformin (Figure7 and8). Table 2 shows the activities of serum AST, ALT and ALP in control and experimental groups of rats. There was a significant elevation in serum AST, ALT and ALP in STZ diabetic rats when compared with the control rats.

DISCUSSION

The STZ-induced diabetic rat is one of the animal models of human diabetes mellitus. Diabetes arises from the destruction of pancreatic β-cells, causing degranulation and reduction of insulin secretion [12]. In the present study, the intraperitoneal administration of streptozotocin effectively induced diabetes mellitus in rats. It is clear from the data that the blood glucose levels of the control diabetic animals continued to increase during the 21 days of the experiment compared to the post-STZ injection level.

In contrast, the diabetic rats treated with garlic and lime juice showed significantly reduced blood glucose levels during the treatment period when compared to the control diabetic rats, but this beneficial effect on the hyperglycemia of diabetic rats was dose-dependent and time-dependent. Saravanan (2011) reported that s-allyl cysteine, isolated from garlic, had antihyperglycemic and antioxidant effect[13]. Further, it is also suggested that decreas the level of blood glucose, coud be to potentiation of the insulin effect of plasma by increasing the pancreatic secretion of insulin from existing β-cells. So garlic is one of the effective medicinal plants in lowering blood glucose level in the STZ-induced diabetic rats and the results of plasma glucose are consistent with the finding of Thomson (2007) and El-Demerdash (2005). The hypoglycaemic potency of garlic has been attributed to allicin derived organosulphur compounds, which protect insulin from –SH inactivation by reacting with endogenous thiolcontaining molecules such as cysteine, glutathione and serum albumin [14,15,16]. Also, According to the data obtained in the present study, the greatest effect of lowering blood glucose was observed in diabetic rats that received the garlic and lime mixture and this hypoglycemic effect was more than rats diabetic treated with Metformin. In a study by SHER et al (2012) found that in normal as well as diabetic rabbits, garlic extract produced hypoglycemia as well as hypolipidaemia like metformin[17]. Although these changes in blood glucose level were significant ($p<0.05$) in diabetic rats, none of the doses (500, 250 and 125 mg/ 100g BW) was able to decrease glucose level to the normal rang.

There are very few reports available regarding effect of fresh lime juice on blood sugar level. However, there have been very extensive studies concerning effect of lemon bioflavonoids on biochemical parameters of diabetic and hypercholesterolemic rats. Hesperidin (20.5mg/100ml) and Eriocitrin (16.7mg/100ml) are bioflavonoids in lime juice, exhibits biological and pharmacological properties (Sattuso et al, 2007). Satoko (2010) investigated the effect of hesperidin on blood glucose level for 4 weeks. Dietary hesperidin decreased blood glucose in diabetic rats by altering the activity of glucose-regulating enzymes, such as glucokinas (Enzyme of glucose catabolism) and decrease the level of G_6pase[18,19]. Administration of lime juice (50%) leads to reduce blood glucose levels and this effect may be due to the antioxidant property of vitamin C available in lime juice and Continuous supplementation of lime juice (50%) for 21 days resulted no significant difference in plasma glucose level of diabetic rats. Serum AST, ALT and ALP, are widely used as markers for acute and chronic hepato-cellular damage. In the present study, treatment of STZ diabetic groups with both garlic and lime for 21 days could not restore the activities of the above enzymes to their normal levels. The increase in the activities of plasma AST, ALT and ALP indicated that diabetes may be induced due to liver dysfunction. Supporting our finding it has been found by Ohaeri et al. (2001) that liver was necrotized in STZ-induced diabetic rats[20]. Therefore, an increase in the activities of AST, ALT and ALP in serum may be mainly due to the leakage of these enzymes from the liver cytosol into the blood stream [20] which gives an indication of the hepatotoxic effect of STZ induced diabetic rats. These manifestations are a consequence of a metabolic alteration, with an increase of glyconeogensis and of cetogenesis and/or of hepatic lesions that occur in diabetic animals[21]. In this study, although lime and garlic mixture has no effect on reducing the levels of liver enzyme, it significantly and dose-dependently decreased the plasma glucose level of STZ-induced diabetic rats .From the above results, it could be concluded that garlic and lime are able to decrease the blood glucose levels. In addition, combination of garlic extract and lime juice have Further lowering hypoglycemic effects, So we can use these two plant extracts as anti-diabetic drugs, Although further biochemical and pharmacological research should be consider for use.

Table 1. Grouping rats studied.

Group1: normal rats	Get regular food and water	
	Get the aqueous extract of garlic	Group2: concentration of 500mg/100gBw
		Group3: concentration of 250 mg/100gBw
		Group4:concentration of 125 mg/100gBw
		Group5: ratio 100%
		Group6: ratio 50%
		Group7: ratio 25 %
	Get combination of garlic extract and lime juice	Group8:concentration of garlic extract 250 mg/100gBw + lime juice 50%
	Get drug Metformin	Group9: metformin 500mg
Group10: control diabetic rats	Get regular food and water	

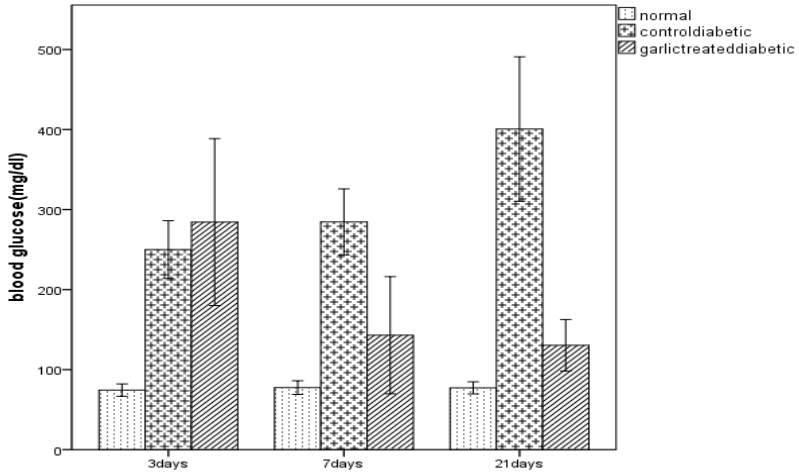

Figure 1: Blood glucose levels in STZ-induced diabetic rats treated with aqueous extract of garlic with 500mg/100gBW. *: Significantly different from diabetic control.P<0.05.

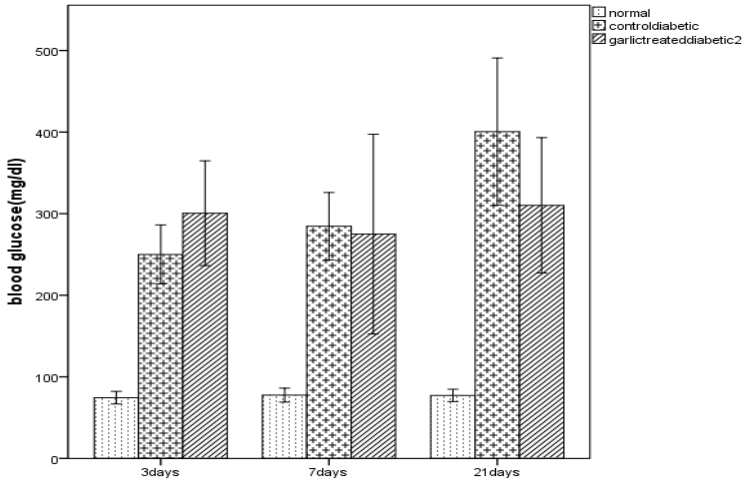

Figure2: Blood glucose levels in STZ-induced diabetic rats treated with aqueous extract of garlic with 250mg/100gBW. *: Significantly different from diabetic control.P<0.05.

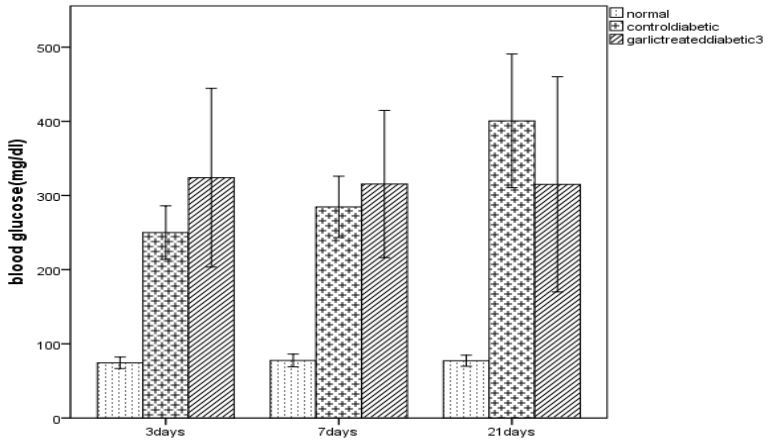

Figure3: Blood glucose levels in STZ-induced diabetic rats treated with aqueous extract of garlic with 125mg/100gBW. *: Significantly different from diabetic control.P<0.05.

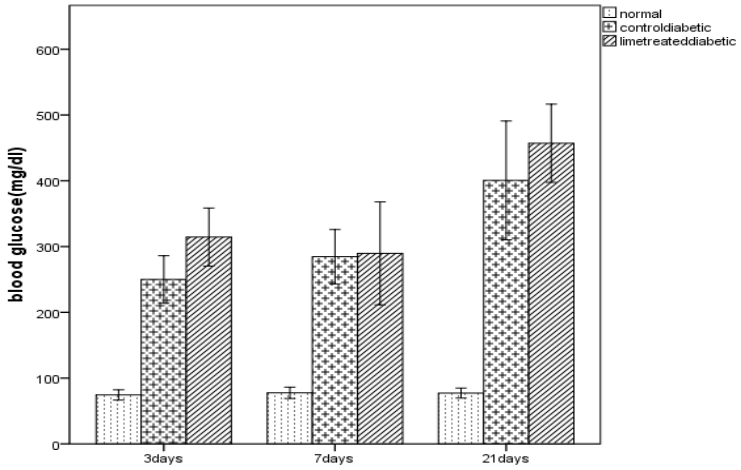

Figure4: Blood glucose levels in STZ-induced diabetic rats treated with fresh lime juice100%.

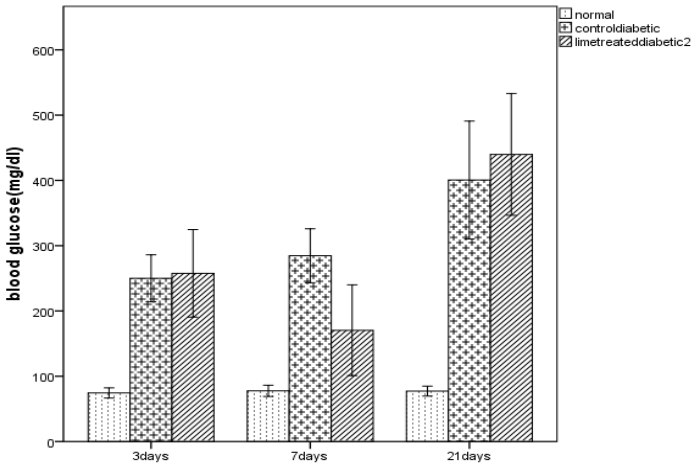

Figure5: Blood glucose levels in STZ-induced diabetic rats treated with fresh lime juice50%.
*: Significantly different from diabetic control.P<0.05.

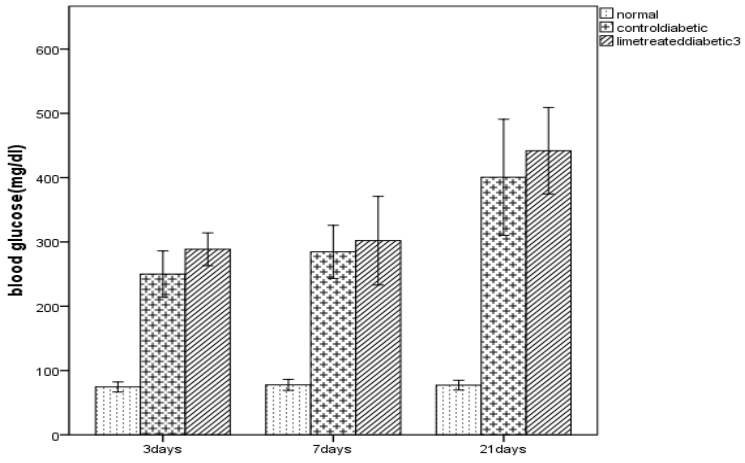

Figure6: Blood glucose levels in STZ-induced diabetic rats treated with fresh lime juice25%.

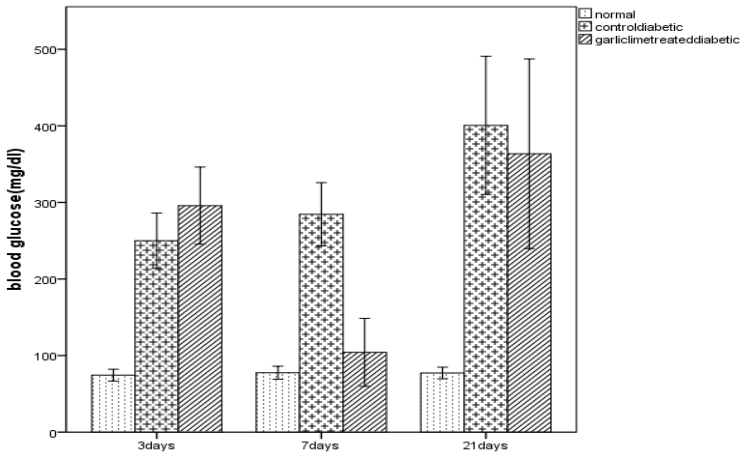

Figure7: Blood glucose levels in STZ-induced diabetic rats treated with combination of fresh lime juice and extract garlic. *: Significantly different from diabetic control.P<0.05.

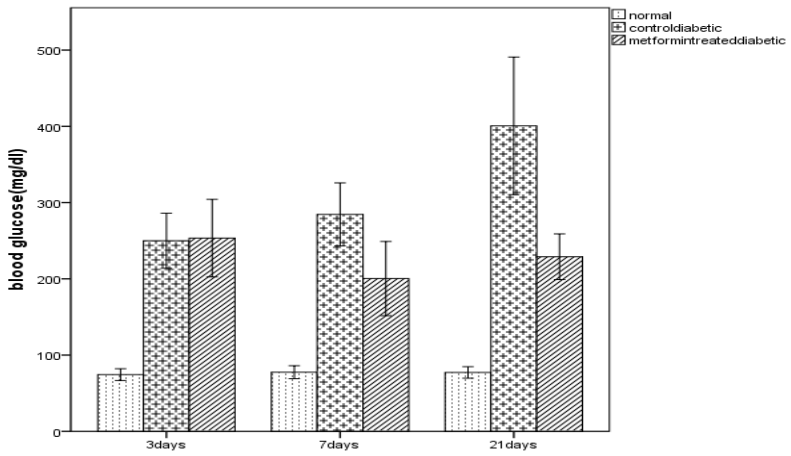

Figure8: Blood glucose levels in STZ-induced diabetic rats treated with metformin drug. *: Significantly different from diabetic control.P<0.05.

Table2**.** Effect of oral administration of garlic and lime on serum AST, ALT and ALP in control and experimental animals. Data are expressed as mean ± SEM.

Groups	**AST(U/dl)**	**ALT(U/dl)**	**ALP(U/dl)**
Control	133.33 ± 12.86	65.17 ± 15.52	539.67 ± 34.9
Diabetic control	189 ± 31.4	227.33 ± 95.29	3391 ± 777.71
Garlic 500mg/100gBW	194.6 ± 78.84	129.4 ± 51.13 *	2374 a 127.58
Garlic 250mg/100gBW	257.4 ± 77.26	164.4 ± 55.83	3518 ±
Garlic 125mg/100gBW	281 ± 65.15	186.2 ± 37.29	2980.02 ± 82.45
Lime 100%	218.4 ± 40.98	279.4 ± 105.18	3094.2 ± 236.19
Lime 50%	154.4 ± 37.66 *	140.2 ± 13.98	3763.4 ± 1347.5
Lime 25%	246.4 ± 36.61	138.2 ± 35.45	2215 ± 242.77
Lime 50%+ garlic250mg/100gBW	203 ± 60.49	148 ± 17. 93	2859.4 ± 1027.04
Diabetic + Metformin	296.4 ± 105. 06	248.4 ± 81.74	1869 ± 617.31 *

*: Significantly different from diabetic control. P<0.05.

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