INTERNATIONAL JOURNAL OF PHARMACOLOGY AND THERAPEUTICS
eISSN 2249 – 6467

Research Article

EFFECT OF VERAPAMIL IN ALLOXAN INDUCED DIABETES

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ABSTRACT

The prevalence of diabetes mellitus is rapidly increasing all over the world and it has become a global public health crisis. Progressive loss of functional beta cell mass by apoptosis is a critical step in pathogenesis of diabetes and drugs to address these issues are unavailable at present. Thioredoxin-interacting protein (TXNIP) has emerged as a key player in pancreatic cell biology and elevated TXNIP levels in diabetes is responsible for beta-cell apoptosis. Recent evidence suggests that verapamil can inhibit TXNIP expression and thereby prevent beta-cell apoptosis. This pilot study was conducted to assess whether verapamil can prevent alloxan induced diabetes in rats. We compared blood glucose in 6 control rats receiving alloxan alone with 6 study rats receiving alloxan plus verapamil. Alloxan was given as single dose in both groups and verapamil was given by oral feeding tube for 10 days. At the end of the study significantly relatively fewer rats in verapamil group had diabetes and there was also reversal of alloxan induced diabetes with verapamil. Blood glucose at the end of the study was significantly lower in verapamil group than the control group. The results of the study are promising and further studies with larger sample size are needed.
Key words: Alloxan, Diabetes mellitus, Thioredoxin-interacting protein (TXNIP), Verapamil.

INTRODUCTION

The prevalence of diabetes mellitus is rapidly increasing all over the world and it has become a global public health crisis (1). According to international diabetes federation 387 million people worldwide have diabetes and it is projected to reach 592 million by 2035 (2). Progressive loss of functional beta cell mass by apoptosis is a critical step in pathogenesis of both type 1 and type 2 diabetes mellitus (3). Even though several medications are available for diabetes management, approaches preventing beta cell loss are lacking at present. Thioredoxin-interacting protein (TXNIP) has emerged as a key player in pancreatic cell biology (4). Hyperglycemia up regulates beta-cell TXNIP expression and elevated TXNIP levels induce beta-cell apoptosis (5). TXNIP induces oxidative stress by inhibiting thioredoxin (5). Both over-expression of thioredoxin and TXNIP deficiency promotes beta-cell survival and were shown to protect against development of diabetes in animal models (6, 7). This makes TXNIP as an attractive target and current research is looking at drugs which can inhibit TXNIP. In this regard verapamil was noted to inhibit TXNIP expression in cardiomyoctyes (8). Anath Shalev et al have shown that verapamil inhibits TXNIP expression in INS-1 cell line and human islets and orally administered verapamil rescued mice from streptozotocin induced diabetes (9). These data makes verapamil as a promising drug

in addressing the basic pathophysiology in diabetes. Hence we hypothesized that verapamil can prevent alloxan induced diabetes and conducted this pilot study in rats to test the same.

AIM:

To assess whether verapamil can prevent alloxan induced diabetes in animal model and to assess the effect of verapamil on blood glucose levels.

MATERIALS AND METHODS:

Animals:

Healthy albino wistar rats were used in this study. 12 rats weighing 150-200gms were selected for the study and they were randomly divided into 2 groups (6 controls and 6 cases). Both groups were housed under identical conditions in polypropylene cages at room temperature with natural dark-light cycle. Rats were marked with picric acid for identification. During the entire period of study, animals were given standard pellet diet and water ad libitum. The rats were fasted overnight before the day of study but had free access to water. The experimental procedure and protocol were approved by Institutional Animal ethical committee (Ethical clearance Ref.No: Roc No 12677/E1/5/2012).

Induction of diabetes:

Alloxan was used for induction of diabetes in rats. All study rats received a single intraperitonial injection of freshly prepared alloxan monohydrate dissolved in saline [120mg/kg] (10).

Verapamil:

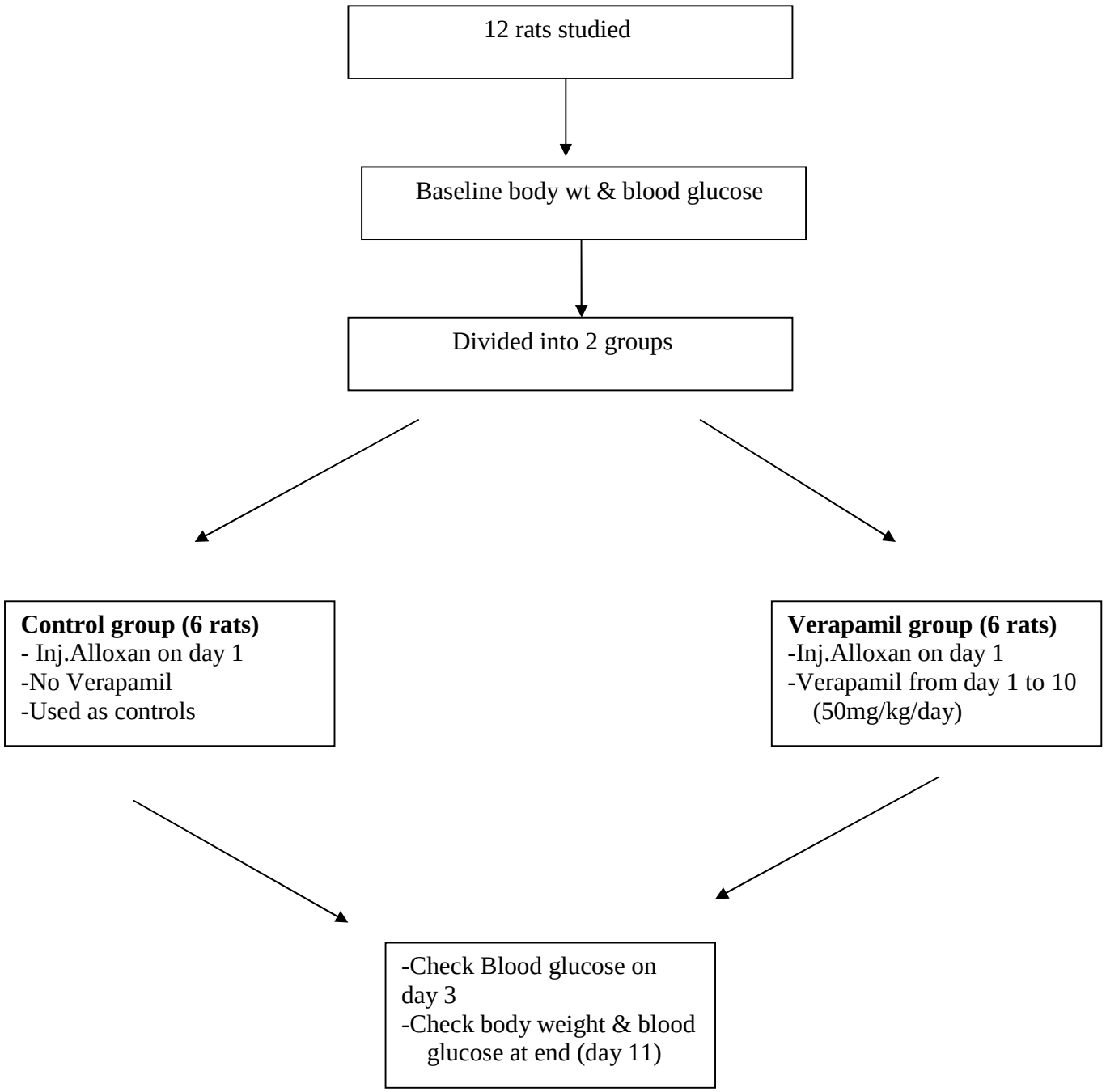
Commercially available verapamil tablets (Calaptin 40mg, Piramal Health Care) were used for the study. Verapamil solution was prepared by dissolving the tablets in water. Verapamil was given at a dose of 50mg/kg, once daily in morning by oral feeding tube for 10 days.

Blood glucose:

Blood was obtained by tail vein puncture by restraining rats in rat-holder. Blood glucose was checked using one touch glucose meter (glucose oxidase method). Blood glucose levels higher than 250mg/dl were considered as diabetes (11).

Experimental design:

The selected 12 rats were randomly divided into 2 groups with 6 rats in each group.
Control group: Received alloxan on day 1 and was followed-up.
Verapamil group: Received alloxan on day 1 and verapamil (50mg/kg) from day 1 to day 10.
Body weight was checked at baseline and at end of study (day 11).
Blood glucose was checked baseline, on day 3 and at the end of study (day 11)


12 rats studied
Baseline body wt & blood glucose
Divided into 2 groups
Control group (6 rats)
- Inj.Alloxan on day 1
-No Verapamil
-Used as controls
Verapamil group (6 rats)
-Inj.Alloxan on day 1
-Verapamil from day 1 to 10 (50mg/kg/day)
-Check Blood glucose on day 3
-Check body weight & blood glucose at end (day 11)

STATISTICAL ANALYSIS:

Chi-square test was used to test the association between verapamil and diabetes. Paired T-test was used to compare weight and blood glucose values between the two groups. T-test with Welch's correction was used to compare day 11 blood glucose in view of unequal variance of the values.

RESULTS:

The weight and blood glucose at baseline and at end of study (day 11) for both groups are shown in table I and II.

Table I. Control group

S.No	Baseline Wt (in gms)	Baseline BG (in mg/dl)	BG on day 3 (in mg/dl)	BG on day 11 (in mg/dl)	Wt on day 11 (in gms)
1	175	105	584	461	150
2	200	110	>600	459	150
3	150	101	451	562	130
4	175	110	>600	420	140
5	120	98	96	107	150
6	200	108	580	Died on day 3	-

*Wt: Weight; BG: Blood glucose.

Table II. Verapamil group

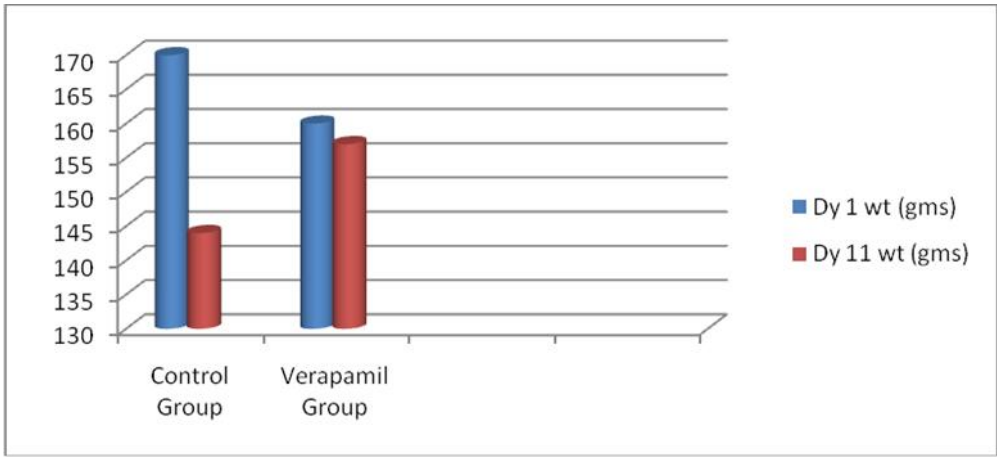
S.No	Baseline Wt (in gms)	Baseline BG (in mg/dl)	BG on day 3 (in mg/dl)	BG on day 11 (in mg/dl)	Wt on day 11 (in gms)
1)	175	103	125	90	150
2)	150	80	105	59	160
3)	150	94	>600	134	150
4)	160	96	592	79	175
5)	150	86	511	425	150
6)	175	78	140	Died on day 4	-

*Wt: Weight; BG: Blood glucose.

A) **Change in weight of rats:**

The mean weights of rats at baseline were comparable (P-Value 0.24). Weight at end of the study was significantly lower in control group when compared to verapamil group (P-value 0.036). The results are shown in Figure I.

Figure I. Mean weight of rats in both groups at baseline and at the end of study:



B) **Diabetes mellitus on day 3:**

Development of alloxan induced diabetes was based on blood glucose 48 hrs after giving alloxan. As shown in Table III more rats in verapamil group remained non-diabetic on day 3 when compared to control group. However the difference was not statistically significant (P-Value 0.2)

Table III. Diabetes on day 3

Group (no.)	DM	Non-DM
Control group (6)	5/6 (83.3%)	1/6 (16.7%)
Verapamil group (6)	3/6 (50%)	3/6 (50%)

C) **Diabetes mellitus at end of the study:**

The data on diabetes at the end of study (Day 11) are shown in table IV. 80% of rats in verapamil group did not have diabetes but only 20% in control group did not have diabetes at the end of the study and the difference was nearly statistically significant (P-Value 0.057)

Table IV. Diabetes at end of study (day 11)

Group	DM	Not-DM
Control group (5)	4/5 (80%)	1/5 (20%)
Verapamil group (5)	1/5 (20%)	4/5 (80%)

* 1 rat from each group died before the end of the study

D)Reversal of diabetes:

83.3% of control rats and 50% of rats in verapamil group developed diabetes on day 3. We analyzed whether there was remission of diabetes among these rats. Two-third of the rats in verapamil group which developed diabetes on day 3 had remission of diabetes on day 11. None of the control rats had remission of diabetes at the end of study. However the difference was not statistically significant.

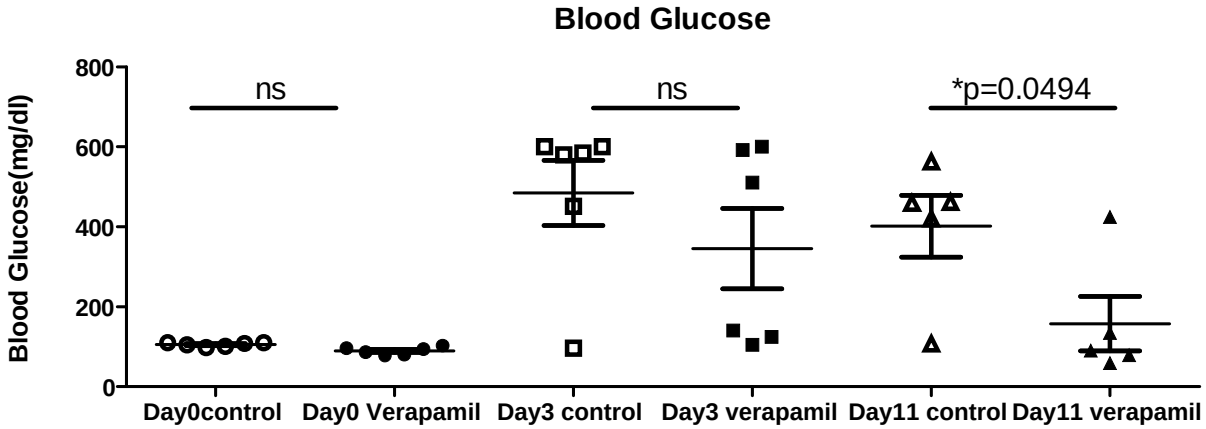
E) Blood glucose changes:

We compared the blood glucose at baseline, day 3 and end of the study between the verapamil group and control group. In view of large standard deviation, T-test with Welch's correction was used. The results are shown is table V and figure II. As seen in the figure the blood glucose at the end of study was significantly lower in verapamil group when compared to control group (P-Value 0.0494).

Table V. Blood glucose at baseline, day 3 and day 11 in both groups:

Mean blood glucose	Control group (mg/dl +/- SD)	Verapamil group (mg/dl +/- SD))
Baseline	105 +/- 4.97	90 +/- 9.79
Day 3	485 +/- 198.87	346 +/- 245.61
Day 11	402 +/- 172.97	157 +/- 152.09

Figure II. Blood glucose changes in control & Verapamil group:



(Open boxes – Control group; Closed boxes – Verapamil group)

DISCUSSION:

Alloxan is a cytotoxic glucose analogue that preferentially accumulates in pancreatic beta-cells via GLUT2 glucose transporters (12). An initial transient phase of hyperglycemia lasting for 2-4 hrs after alloxan is due to functional inhibition of insulin secretion. Permanent hyperglycemia gets established by 12-48hrs after alloxan as a result of selective beta-cell destruction (12). Mechanism of action of alloxan is by generation of reactive oxygen species (ROS). Its reduction product dialuric acid generates superoxide and hydrogen peroxide and finally hydroxyl radicals are formed by fenton reaction (13). These hydroxyl radicals ultimately cause beta-cell death resulting in permanent diabetes (12).

Thioredoxin is a thiol-oxidoreductase and is important in protecting cells against oxidative stress (12). TXNIP binds to thioredoxin. By inhibiting thioredoxin, TXNIP plays a major role in inducing oxidative stress and thereby causing apoptosis. TXNIP deficient Hcb-19 mice have

been found to be protected against stretozotocin induced diabetes (15). Verapamil has been shown to reduce beta-cell TXNIP expression and protect against streptozotocin induced diabetes (9). Even if verapamil was started after overt streptozotocin induced diabetes, it was able to normalize blood glucose and increase beta-cell mass in mice (9). Yu JR et al had studied the protective effect of verapamil against alloxan induced diabetes in rats (16). When verapamil was given before alloxan, serum glucose decreased from 412mg/dl to 158mg/dl in 48 hrs (16).
Verapamil is an L-type calcium channel blocker and reduces cellular calcium influx. Since release of insulin from beta-cell is dependent on calcium influx one would expect that, inhibiting calcium influx would block beta-cell insulin secretion. Shalev et al (9) had shown that insulin levels were significantly higher in alloxan + verapamil mice when compared to alloxan control mice. The protection against alloxan induced diabetes was accompanied by 80% reduction in TXNIP levels in isolated islets of verapamil treated mice (9). In the same study, immunohistochemistry of streptozotocin only mice showed severely disrupted pancreatic islets, but verapamil treated mice had significantly higher beta-cell mass (9). This emphasizes the significance of TXNIP pathway (i.e.) by lowering proapoptotic TXNIP level, verapamil promotes beta-cell survival and thereby prevents diabetes.
Beta-cell toxicity of alloxan is mediated by increase in intracellular calcium influx. Wollheim et al (17) studied membrane potential and changes in intracellular cytosolic calcium in INS-1D cells to clarify the mechanism of action of alloxan. The cellular depolarization correlated with rise of intracellular calcium after alloxan. However he had shown that verapamil was ineffective in preventing alloxan induced rise of intracellular calcium and postulated that different cation channels were involved in alloxan toxicity. Hence protective effect of verapamil is not by preventing alloxan induced calcium influx.

In this study relatively fewer rats in verapamil group developed diabetes 48 hours after alloxan when compared to control group. However the difference did not achieve statistical significance. However at the end of the study, only 20% of rats in verapamil group remained diabetic while 80% of control rats were diabetic and the difference was nearly statitstically significant (P-value 0.057). The difference in mean blood glucose at the end of the study was impressive. It was significantly lower in verapamil group than the control group (P-value <0.01).
Whether the protective effect of verapamil is temporary or long-standing is not clear at this point. Small sample size, lack of insulin /c-peptide assays and lack of pancreatic histopathology are limitations of our study. However this was only a pilot study and since the results are promising we are planning to do further similar studies in future.

CONCLUSION:
With verapamil relatively fewer rats developed alloxan induced diabetes and relatively greater number of rats had normoglycemia at the end of the study. Blood glucose was significantly lower in rats which received verapamil when compared to the control group. Further studies with larger sample size and longer follow-up are needed before definitive conclusions can be arrived

Article information:
Funding: There are no funders to report for this submission.
Duality of interest: No potential conflicts of interest relevant to this article were reported.

REFRENCES:

1. Hu FB. Globalization of diabetes: the role of diet, lifestyle and genes. Diabetes care. 2011 Jun;34(6):1249-57
2. International Diabetes Federation. IDF Diabetes Atlas 6th edition. Available from http://www.idf.org/. Accessed on 12 march 2015
3. Cnop M, Welsh N, Jonas JC, Jörns A, Lenzen S, Eizirik DL. Mechanisms of pancreatic beta-cell death in type 1 and type 2 diabetes: many differences, few similarities. Diabetes. 2005 Dec; 54 Suppl 2:S97-107.
4. Shalev A, Pise-Masison CA, Radonovich M, Hoffmann SC, Hirshberg B, Brady JN,Harlan DM. Oligonucleotide microarray analysis of intact human pancreatic islets: identification of glucose-responsive genes and a highly regulated TGFbeta signaling pathway. Endocrinology. 2002 Sep; 143(9):3695-8.
5. Shalev A. Minireview: Thioredoxin-interacting protein: regulation and function in the pancreatic β-cell. Mol Endocrinol. 2014 Aug;28(8):1211-20.
6. Chen J, Hui ST, Couto FM, Mungrue IN, Davis DB, Attie AD, Lusis AJ, Davis RA,Shalev A. Thioredoxin-interacting protein deficiency induces Akt/Bcl-xL signaling and pancreatic beta-cell mass and protects against diabetes. FASEB J. 2008 Oct;22(10):3581-94.
7. Hotta M, Tashiro F, Ikegami H, Niwa H, Ogihara T, Yodoi J, Miyazaki J. Pancreatic beta cell-specific expression of thioredoxin, an antioxidative and antiapoptotic protein, prevents autoimmune and streptozotocin-induced diabetes. J Exp Med. 1998 Oct 19;188(8):1445-51.
8. Chen J, Cha-Molstad H, Szabo A, Shalev A. Diabetes induces and calcium channel blockers prevent cardiac expression of proapoptotic thioredoxin-interacting protein. Am J Physiol Endocrinol Metab. 2009 May;296(5) :E1133-9.
9. Xu G, Chen J, Jing G, Shalev A. Preventing β-cell loss and diabetes with calcium channel blockers. Diabetes. 2012 Apr;61(4):848-56.
10. Srinivasan K, Ramarao P. Animal models in type 2 diabetes research: an overview. Indian J Med Res. 2007 Mar;125(3):451-72.
11. Rahul V Mandlik, Sandya K Desai, Suresh R Naik, Gaurav Sharma, R K Kohli. Antidiabetic activity of polyherbal formulation (DRF/AY/5001). Indian J Exp Biol. 2008 Aug;46(8):599-606.
12. Lenzen S. The mechanisms of alloxan- and streptozotocin-induced diabetes. Diabetologia. 2008 Feb;51(2):216-26.
13. Ankur Rohilla and Shahjad Ali. Alloxan Induced Diabetes: Mechanisms and Effects. International Journal of Research of Pharmaceutical and Biomedical Sciences. 2012 Apr:Vol.3(2):819-23
14. Holmgren A. Thioredoxin and glutaredoxin systems. J Biol Chem. 1989 Aug 25;264(24):13963-6.
15. Chen J, Hui ST, Couto FM, Mungrue IN, Davis DB, Attie AD, Lusis AJ, Davis RA, Shalev A. Thioredoxin-interacting protein deficiency induces Akt/Bcl-xL signaling and pancreatic beta-cell mass and protects against diabetes. FASEB J. 2008 Oct;22(10):3581-94.
16. Wei YJ, Yu GZ, Zhang GF, Yu JR. Protective effect of verapamil against alloxan-induced damage on pancreatic islet beta-cells in rats. Sheng Li Xue Bao. 1992 Apr;44(2):209-14.
17. Janjic D, Maechler P, Sekine N, Bartley C, Annen AS, Wolheim CB. Free radical modulation of insulin release in INS-1 cells exposed to alloxan. Biochem Pharmacol. 1999 Mar:15;57(6):639-48.