Type 2 Diabetes Mellitus in Rats on a High-Fat Diet with Streptozotocin Induction: Evaluation of the Model

Alexander A. Spasov, Denis A. Babkov, Diana R. Prilepskaya, Olga Yu. Zakharyashcheva

Volgograd State Medical University, Volgograd, Russia.

ABSTRACT

Introduction: Type 2 diabetes mellitus (T2DM) is a complex heterogeneous disease linked with genetic conditions and lifestyle, especially dietary component. Development of the accessible experimental animal model of T2DM is indispensable for elucidation of its pathogenesis and identification of novel antidiabetic agents. **Methods:** Modeling of experimental T2DM were performed on male Wistar rats aged 9-12 weeks with 2 weeks of dietary manipulation (58% of calories from fats) followed with 35 mg·kg^{-1} i.p. streptozotocin injection. To evaluate the model, we used intraperitoneal glucose and insulin tolerance test and determined fasting blood concentration of glucose, triglycerides, total cholesterol, and insulin. Additional evaluation of the model was performed by administration of pioglitazone (10 mg/kg^{-1} p.o.) for 7 days. Student's *t*-test was used for single variable comparison between two groups. One-way ANOVA followed by Dunnett post hoc test was used for multiple comparisons versus the control group. **Results:** T2DM model obtained reproduced key metabolic disorders of the disease, including hyperglycemia ($p<0.05$), hyperinsulinemia ($p<0.05$), dyslipidemia ($p<0.05$) and impaired glucose and insulin tolerance ($p<0.05$). Administration of pioglitazone significantly improved these metabolic disorders. Thus, we obtained a suitable rat model of T2DM for screening of antidiabetic agents. **Conclusion:** In comparison of our results with other authors' data, we have found decreased severity of plasma glucose and basal total cholesterol levels impairment, which highlights the need for careful monitoring of biochemical parameters in high-fat diet and streptozotocin-treated rats.

KEYWORDS: type 2 diabetes, streptozotocin, high-fat diet, pioglitazone

INTRODUCTION

Type 2 diabetes mellitus (T2DM), which comprises approximately 90-95% of all cases of diabetes, is a complex heterogeneous disease linked with genetic make-up and lifestyle, especially dietary intake [1]. T2DM generally features a loss of glucose homeostasis attributable to either a subnormal pancreatic insulin secretory response to glucose stimulation, or to development of insulin resistance (the latter generally associated with obesity), or both. There are several widely used animal models of T2DM. Spontaneous models, such as the models of Zucker Diabetic Fatty (ZDF) rats [2] and Goto-Kakizaki (GK) rats [3], employ genetic manipulations to induce diabetic phenotype. Chemically-induced models generally use relatively high doses of streptozotocin (STZ) (>30 mg·kg^{-1}) [4], which leads to β-cell loss, thus developing type 1 diabetes mellitus, even when a combination of 30-35 mg·kg^{-1} STZ and a high-fat diet is used [5]. These models differ from typical T2DM in many ways, including pathogenesis and clinical symptoms. In most T2DM cases, impairment of lipid metabolism and peripheral insulin action precede the development of hyperglycemia [6]. Therefore, a combination of a high-fat diet (HFD) with a low-dose injection of STZ is considered as a more suitable approach to model T2DM [7, 8]. Since obesity is caused more by environmental factors rather than by genetic impairment, this model is therefore also considered to be more suitable than genetically induced obesity models. The weight gain is associated with insulin resistance, and lack of β-cell compensation leads to impaired glucose tolerance [9, 10].

Yu et al. [11] demonstrated that high concentration of free fatty acids in plasma leads to an increase in intracellular concentration of acyl-CoA and diacylglycerol, and subsequent activation of PKC-θ and phosphorylation of Ser307 IRS-1. These changes inhibit the activation of the IRS-1-associated PI3 kinase by suppressing insulin-stimulated glucose uptake. In turn, hyperglycemia and oxidative stress deplete the insulin pool of pancreatic β-cells and

induce their death, closing the vicious circle of pathogenesis. The reduction of β-cell functional mass down to 50-75 % manifests the development of T2DM [12].

Srinivasan et al. [7] reported a rat model of T2DM, which combines HFD with 35 mg·kg^{-1} STZ treatment. Thus, this study attempts to reproduce this methodology and assesses its ability to model the key components of the metabolic syndrome associated with T2DM, including lipotoxicity, glucotoxicity, and peripheral insulin resistance, and to evaluate its ability to screen pharmacological substances with antidiabetic potential.

METHODS

Animals

Animal studies were conducted after approval from the VolgSMU Animal Care and Use Committee. Male, Wistar rats, aged 9-12 weeks were procured from "Pushchino" facility and housed 4 per cage in a room with a 12/12-hour light/dark cycle at an ambient temperature of 25°C. Animals had free access to food and water before the study.

High-fat Diet

Rats were given a standard pellet diet (13 000 kJ/kg, protein 19%, fats 5%, fibre 4%, lysine 1.2%, methionine + cysteine 0.7%, calcium 0.6-0.9%, phosphorus 0.6-0.9%, sodium 0.20-0.25%), pork lard, casein, methionine, and a vitamin-mineral premix "Ushastik" (Russia), containing vitamin A – 1 000 000 IU, vitamin D_3 – 300 000 IU, vitamin E – 1.0 g, vitamin B_2 – 0.6 g, vitamin B_{12} – 12 mg, iron – 20 g, copper – 4 g, manganese – 6 g, zinc – 10 g, cobalt – 0.08 g, iodine – 0.4 g in 1 kg. Their proportions of these in one kilogram of diet are as listed in Table 1 based on the calculation of mixture of 30 g per animal per day. The components were ground and mixed until homogeneous. Diet mixture was stored at 4°C.

Table 1 High-fat diet components [7]

Component	Weight, g·kg^{-1}
Standard pellet diet	370
Pork lard	313
Casein	253
Vitamin-mineral composition	61
Methionine	3

Induction of Type 2 Diabetes

The rats were divided into 2 groups. The experimental group (12 animals) received a high-fat diet (58% fat, 25% protein, 17% carbohydrate of total calories), control group (10 animals) received a normal diet in the same amount with free access to water. Animals were weighed and blood glucose levels were analyzed after 1 week of dietary treatment. After 2 weeks of dietary manipulation, rats in the experimental group were given intraperitoneal injection of STZ (35 mg·kg^{-1}) in citrate buffer whereas rats in the control group were given 1 ml·kg^{-1} of citrate buffer intraperitoneally [13]. After one week of STZ-treatment, animals were weighed and blood glucose levels were recorded again.

Intraperitoneal Glucose and Insulin Tolerance Test

For this, four animals from each group were deprived of food for 6 hours before the start of the study while maintaining free access to water. All animals were administered with 1 mg·kg^{-1} glucose and 0.175 U·kg^{-1} of insulin in isotonic sodium chloride solution intraperitoneally. Blood samples were taken from the tail vein before injection and then at the 5th, 10th, 15th and 30th minute after the injection of glucose and insulin.

Analysis of Blood Samples

Blood samples were taken from the tail vein with an "end-to-end" capillary of 20 μl volume and hemolyzed with 1 ml of commercial glucose/lactate hemolytic solution. The level of glucose in plasma was determined using Biosen C_Line biochemical analyzer (EKF Diagnostics, Germany). For biochemical assays, blood samples from the tail vein were allowed to clot and centrifuged at 10000 g for 15 min. Serum samples were analyzed using commercial kits in order to determine triglycerides (TG 250, Erba Lachema), total cholesterol (CHOL 250 S, Erba Lachema) and insulin levels (Rat Insulin ELISA kit, Cusabio) according to the manufacturer's manual.

Pioglitazone Administration

After a week of STZ treatment animals that developed type 2 diabetes mellitus (fasting blood glucose >12 mM) were randomly divided into two experimental

groups. The animals in the experimental group (n = 4) were given pioglitazone (10 mg·kg^{-1} *per os*) in 1% aqueous solution of carboxymethylcellulose once a day at the 09:00 hr for 7 days. The dose of pioglitazone was adopted from published literature [14,15]. Animals in the control group (n = 4) were orally administered an equal volume of 1% aqueous solution of carboxymethylcellulose. Blood glucose level was monitored daily. Serum triglycerides, total cholesterol and insulin levels were determined prior to the administration of pioglitazone and again on the day after.

Statistical Analysis

Data analyses were performed using GraphPad Prism version 7.00 for Windows, GraphPad Software, La Jolla California USA, www.graphpad.com. Student's *t*-test was used for single variable comparison between two groups. One-way ANOVA followed by Dunnett post hoc test was used for multiple comparisons versus the control group. $P < 0.05$ was considered to be statistically significant and is presented as $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, or $^{****}p < 0.0001$.

RESULTS

Body weight and blood glucose levels of the animals in the control group remained stable throughout the experiment. Diet-induced metabolic changes within Wistar strain varies, and diet-induced obesity and diet-resistant subpopulations are generally recognized. In our study, relatively short 2-week dietary intervention increased body weight significantly, whereas hyperglycemia was not observed. After STZ treatment, animals of the HFD+STZ group demonstrated a significant increase in plasma glucose level compared to both intact and HFD only animals (Table 2). The mortality rate for the first 4 days was 33% and could be attributed to severe hypoglycemia caused by insulin release after massive β-cell destruction and STZ toxicity *per se* [16]. In the following days, the body weight and blood glucose levels remained stable in both groups, and death of the animals was not observed (Table 2). After 3 weeks from the beginning of the experiment, a statistically significant increase in the levels of insulin, glucose, triglycerides and blood cholesterol was evident (Figure 1).

Table 2 Effect of high-fat diet and streptozotocin on animal's weight and blood glucose level

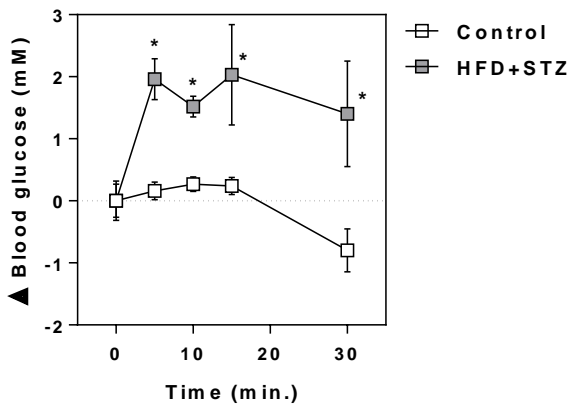| Parameters	Before HFD (day 0)	Before the STZ administration (day 14)		7 days after the STZ administration (day 21)	
		Control	HFD	Control	HFD+STZ
Weight (g)	336.2±5.9	353.8±8.3	426.3±13.3*	353.6±8.0	381.5±17.5
Blood glucose (mM)	5.57±0.34	5.89±0.12	5.64±0.12	3.95±0.12	13.06±1.10*

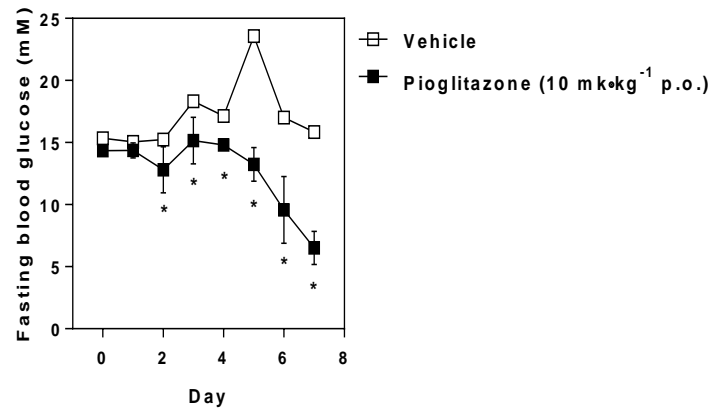Differences between groups were analyzed by Student's *t*-test. $^*p< 0,05$ vs. lean control.
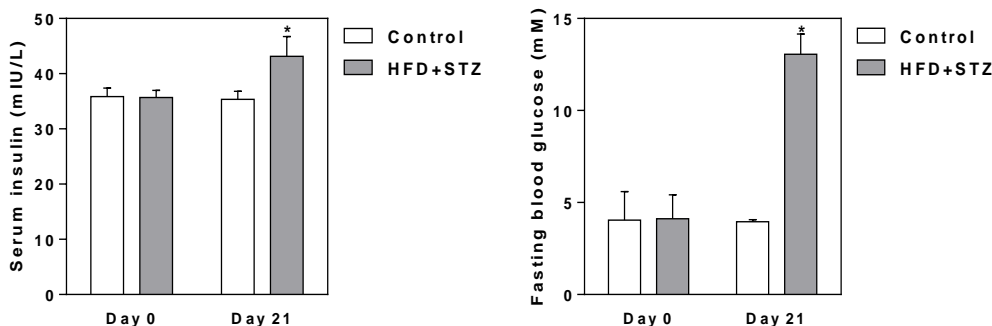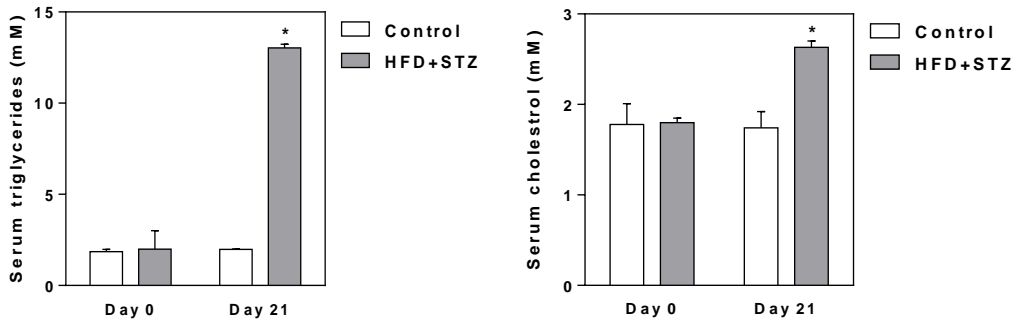
Figure 1 The effect of a 3-week high-fat diet and 35 mg·kg⁻¹ STZ treatment on biochemical parameters of Wistar rats (*$p < 0.05$ vs. lean control).

In order to determine the insulin resistance of the both groups, an intraperitoneal glucose and insulin tolerance test was performed. Blood glucose levels of the rats of the experimental group were statistically higher than those of the control group at each time point ($p < 0.0001$). Also, for the experimental group, glucose levels throughout the test significantly exceeded the basal level at time point 0 ($p = 0.032$), while for the control group such differences were not observed (Figure 2).

Figure 3 Blood glucose levels during 7-day oral administration of pioglitazone to HFD+STZ rats (*$p < 0.05$ vs. HFD+STZ control).
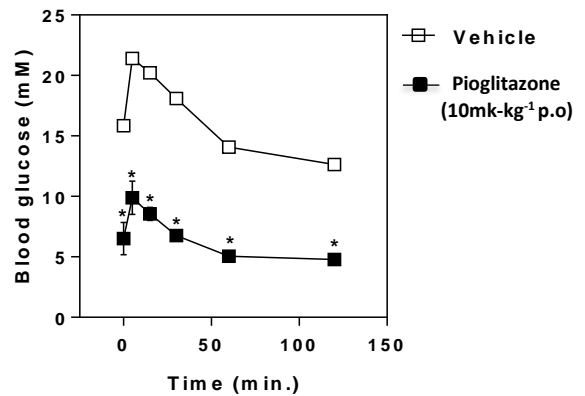

Figure 2. Intraperitoneal glucose and insulin tolerance test for the control group and the group receiving the high-fat diet, 7 days after the administration of 35 mg·kg⁻¹ STZ. Data points reported as mean values ± SEM, n = 4; *$p < 0.0001$ vs. control).

We nest evaluated the antidiabetic activity of pioglitazone in this model of T2DM. Pioglitazone was administered *per os* at a dose of 10 mg·kg·day⁻¹ for 7 consecutive days. Blood glucose level was monitored daily (Figure 3). Pioglitazone decreased glucose levels from the 5th day onwards resulting in a near normoglycemic state by day 7.


Figure 4 Intraperitoneal glucose and insulin tolerance test after chronic pioglitazone administration to HFD+STZ Wistar rats (*$p < 0.05$ vs. HFD+STZ control).

Intraperitoneal glucose and insulin tolerance test was performed immediately after 7 days of pioglitazone oral treatment to evaluate insulin sensitivity (Figure 4). Areas under glucose-time curves indicate that pioglitazone improved glucose tolerance significantly.
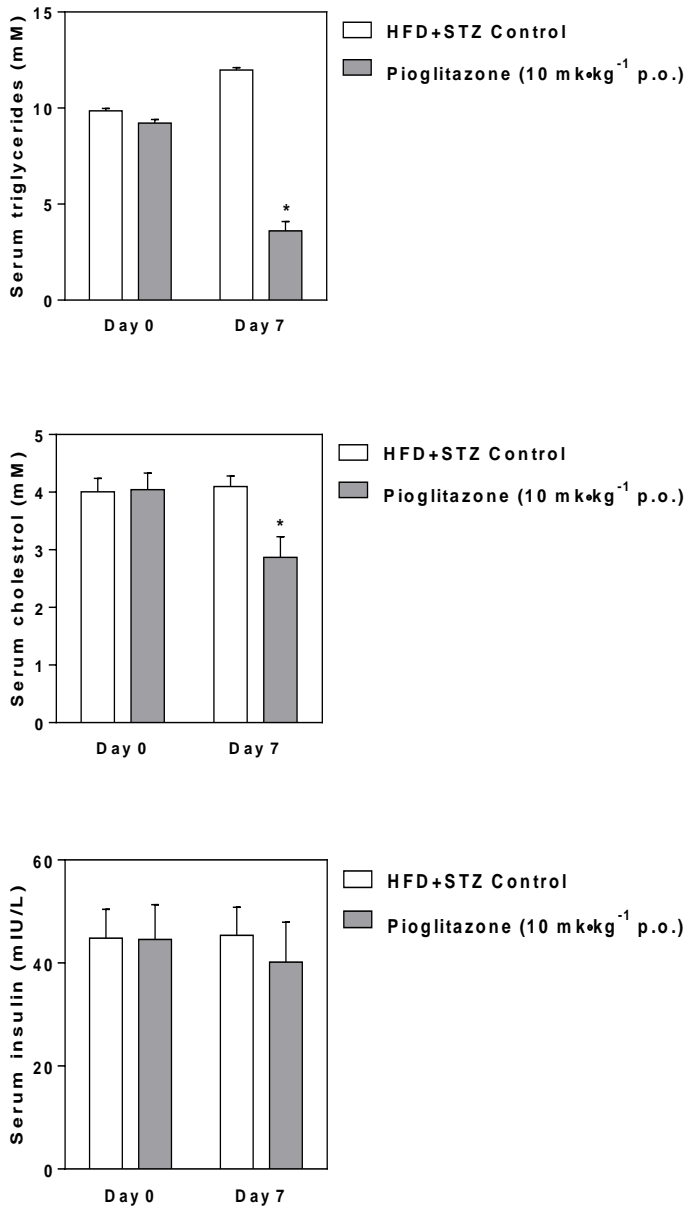
Figure 5 Effect of pioglitazone (10 mg·kg⁻¹, *p.o.* for 7 days) on biochemical parameters in HFD and STZ-treated rats (*$p < 0.05$ vs. day 0).

To evaluate the effect of pioglitazone on fasting serum concentrations of triglycerides, total cholesterol, and insulin, these were measured before and immediately after 7 days of treatment with pioglitazone. As Figure 5 shows, pioglitazone significantly decreased the levels of serum triglycerides and total cholesterol.

DISCUSSION

Type 2 diabetes mellitus is a heterogeneous disease characterized by a progressive decline in insulin action (insulin resistance), followed by inability of β-cells to compensate for insulin resistance (pancreatic β-cell dysfunction), while type 1 diabetes mellitus is characterized by a progressive loss of β-cell function over a period of years (pancreatic β-cell dysfunction only). We initiated this study with the objective to develop a suitable type 2 diabetic rat model that would closely mimic the natural history of the disease events (from insulin resistance to β-cell dysfunction) as well as key metabolic features of human type 2 diabetes. Reproduction of the disease pathogenesis was achieved by combining the high-fat diet (HFD), which resulted in hyperlipidemia and insulin resistance with low dose streptozotocin (STZ) treatment that caused the initial β-cell dysfunction and, subsequently, marked hyperglycemia in experimental animals.

We compared our results with reported data from other authors, specifically with those of Srinivasan K. *et al.* (2005) in their paper entitled "Combination of high-fat diet-fed and low-dose streptozotocin-treated rat: A model for type 2 diabetes and pharmacological screening" [7]. Our findings are comparable to those of Srinivasan et al (200%). Like in our study, they administered STZ (35 mg·kg⁻¹ i.p.) after 2 weeks of dietary manipulation and found significantly ($p < 0.05$) increased plasma glucose, basal total cholesterol levels and basal plasma triglycerides in HFD rats. Similar findings have also been reported by other researchers albeit with slightly differing durations of dietary manipulation and the dose of STZ. For example, Sharma et al. [17] (15 weeks of dietary manipulation, 40 mg·kg⁻¹ STZ), Hu et al. [18] (12 weeks, 30-35 mg·kg⁻¹ STZ) and Lu et al.[19] (8 weeks, 30 mg·kg⁻¹ STZ). Given that comparable changes were evident plasma glucose, triglycerides and cholesterol despite the varying duration of dietary manipulation in some studies, it might be concluded that a long duration of diet manipulation might not be necessary and that a 2-week HFD is sufficient to reproduce T2DM in male Wistar rats. Chronic treatment of HFD+STZ rats with 10 mg·kg·day⁻¹ pioglitazone significantly ameliorated metabolic impairments of experimental animals, including fasting glucose, insulin and glucose tolerance, serum triglycerides and cholesterol, thus confirming the validity of the reported diabetes model.

A combination of a 2-week high-fat diet followed by administration of 35 mg·kg⁻¹ streptozotocin intraperitoneally leads to the development of severe hyperglycemia and impaired insulin sensitivity and glucose intolerance in male Wistar rats, which could be corrected by pioglitazone treatment. It should be noted that the revealed severity of the glucose utilization disruption is inferior to that described by the authors of similar methods. This could be attributed to the variable response of different rat strains and subpopulations to dietary manipulation. Thus, careful monitoring of lipid metabolism parameters and insulin and glucose tolerance should be performed in order to ensure the manifestation of experimental type 2 diabetes.

CONCLUSION

In conclusion therefore, it appears that a 2-week high-fat diet followed with 35 mg·kg⁻¹ streptozotocin treatment is a suitable and readily accessible model for type 2 diabetes mellitus, which could be employed to identify and test new substances with antidiabetic activity.

Conflict of Interest

Authors declare none.

Acknowledgement

REFERENCES

1. Spasov AA, Petrov VI, Chepliaeva NI, Lenskaia KV. Fundamental bases of search of medicines for therapy of a diabetes mellitus type 2. Vestn. Ross. Akad. meditsinskikh Nauk. 2013; 43–9. Available from: http://www.ncbi.nlm.nih.gov/pubmed/238193

2. Peterson RG, Shaw WN, Neel M-AM, Little LA, Eichberg J. Zucker diabetic fatty rat as a model for non-insulin-dependent diabetes mellitus. Ilar News. 1990; 32(3): 16–9. Availablefrom: http://ilarjournal.oxfordjournals.org/cgi/doi/10.1093/ilar.32.3.16

3. Cheng ZJ, Vaskonen T, Tikkanen I, Nurminen K, Ruskoaho H, Vapaatalo H, Muller D, Park JK, Luft FC, Mervaala VM. Endothelial dysfunction and salt-sensitive hypertension in spontaneously diabetic Goto-Kakizaki rats. Hypertension. 2001; 37(2): 433–9. Available from: http://hyper.ahajournals.org/cgi/doi/10.1161/01.HYP.37.2.433

4. Kim BM, Han YM, Shin YJ, Min BH, Park IS. Clusterin expression during regeneration of pancreatic islet cells in streptozotocin-induced diabetic rats. Diabetologia. 2001; 44(12): 2192–202. Available from: http://link.springer.com/10.1007/s001250100029

5. Reed MJ, Meszaros K, Entes LJ, Claypool MD, Pinkett JG, Gadbois TM, Reaven GM. A new rat model of type 2 diabetes: the fat-fed, streptozotocin-treated rat. Metabolism. 2000; 49(11): 1390–4.

6. Spasov AA, Voronkova MP, Snigur GL, Cheplyaeva NI, Chepurnova MV. Experimental model of type 2 diabetes mellitus. Biomedicine. 2011; 3:12–8.

7. Srinivasan K, Viswanad B, Asrat L, Kaul CL, Ramarao P. Combination of high-fat diet-fed and low-dose streptozotocin-treated rat: a model for type 2 diabetes and pharmacological screening. Pharmacol. Res. 2005; 52(4): 313–20.

8. Zhang F, Ye C, Li G, Ding W, Zhou W, Zhu H, Chen G, Luo T, Guang M, Liu Y, Zhang D. The rat model of type 2 diabetic mellitus and its glycometabolism characters. Exp. Anim. 2003; 52(5): 401–7.

9. King AJF. The use of animal models in diabetes research. Br. J. Pharmacol. 2012; 166(3): 877–94.

10. Mühlhausler BS. Nutritional models of type 2 diabetes mellitus. In: Stocker C, editor. Totowa, NJ: Humana Press; 2009. pp. 19–36. Available from:

http://link.springer.com/10.1007/978-1-59745-448-3.

11. Yu C. Mechanism by which fatty acids inhibit insulin activation of insulin receptor substrate-1 (IRS-1)-associated phosphatidylinositol 3-kinase activity in muscle. J. Biol. Chem. 2002; 277(52): 50230–6. Available from: http://www.jbc.org/cgi/doi/10.1074/jbc.M200958200

12. Tkachuk VA, Vorotnikov AV. Molecular mechanisms of insulin resistance development. Diabetes Mellit. 2014;17(2): 29–40. Available from:http://endojournals.ru/index.php/dia/article/view/6597

13. Furman BL. Streptozotocin-induced diabetic models in mice and rats. Curr. Protoc. Pharmacol. 2015; 70: 5–47.

14. Ikeda H, Taketomi S, Sugiyama Y, Shimura Y, Sohda T, Meguro K, Fujita T. Effects of pioglitazone on glucose and lipid metabolism in normal and insulin resistant animals. Arzneimittelforschung. 1990; 40: 156–62. Available from: http://www.ncbi.nlm.nih.gov/pubmed/2334455

15. Hayakawa T, Shiraki T, Morimoto T, Shii K, Ikeda H. Pioglitazone improves insulin signaling defects in skeletal muscle from Wistar fatty (fa/fa) rats. Biochem. Biophys. Res. Commun.1996; 223(2): 439–44. Available from:http://linkinghub.elsevier.com/retrieve/pii/S0006291X96909122

16. Huang F, Wu W. Antidiabetic effect of a new peptide from Squalus mitsukurii liver (S-8300) in streptozocin-induced diabetic mice. J. Pharm. Pharmacol. 2005; 57(12): 1575–80. Availablefrom: http://doi.wiley.com/10.1211/jpp.57.12.0007

17. Sharma AK, Bharti S, Ojha S, Bhatia J, Kumar N, Ray R, Kumari S, Arya DS. Up-regulation of PPARγ, heat shock protein-27 and -72 by naringin attenuates insulin resistance, β-cell dysfunction, hepatic steatosis and kidney damage in a rat model of type 2 diabetes. Br. J. Nutr. 2011; 106(11): 1713–23. Availablefrom: http://www.journals.cambridge.org/abstract_S000711451100225X

18. Hu SH, Jiang T, Yang SS, Yang Y. Pioglitazone ameliorates intracerebral insulin resistance and tau-protein hyperphosphorylation in rats with type 2 diabetes. Exp. Clin. Endocrinol. Diabetes. 2013;121(4): 220–4. Available from: http://www.thieme-connect.de/DOI/DOI?10.1055/s-0032-1333277

19. Lu HE, Jian CH, Chen SF, Chen TM, Lee ST, Chang CS,Weng CF. Hypoglycaemic effects of fermented mycelium of Paecilomyces farinosus (G30801) on high-fat fed rats with streptozotocin-induced diabetes. Indian J. Med. Res. 2010; 131: 696–701.