

Full Length Research Paper

Effect of thespesone-vanadium complex in alloxan induced diabetic rats

Phanse Mohini^{1*}, Padhye Subhash³, Patil Manohar⁴, Takawale Abhijit² and Navghare Vijay²

¹Department of Pharmacognosy, Pad. Dr. D. Y. Patil Institute of Pharmaceutical Sciences and Research, Pimpri, Pune-411018, Maharashtra, India.

²Department of Pharmacology, Pad. Dr. D. Y. Patil Institute of Pharmaceutical Sciences and Research, Pimpri, Pune-411018, Maharashtra, India.

³Department of Pharmaceutical Chemistry, Pad. Dr. D. Y. Patil Institute of Pharmaceutical Sciences and Research, Pimpri, Pune-411018, Maharashtra, India.

⁴Marathwada Mitra Mandal College of Pharmacy, Kalewadi, Pune, Maharashtra, India.

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In the present study, we isolated “thespesone”, a pyranonaphthaquinone, from *Thespesia populnea*, complexed with vanadium (TVa) evaluated for diabetes mellitus in alloxan induced diabetic rats. Diabetes was induced by pre-standardized dose of alloxan in the animals. Diabetic animals showed significant increase ($p < 0.01$) in the levels of plasma glucose, serum triglyceride, cholesterol, and blood pressure significant decrease in serum HDL-cholesterol level ($p < 0.01$) when compared with control animals. Treatment with TVa for 42 days significantly lowered the plasma glucose and serum cholesterol ($p < 0.01$) when compared with diabetic animals. Also, diabetic animals treated with TVa showed significant decrease in elevated mean arterial blood pressure ($p < 0.01$). Hence, the results obtained in the present study indicated that thespesone vanadium complex has the potential to treat diabetes mellitus owing to its insulin mimetic potential.

Key words: Insulin mimetic, thespesone vanadium complex, glycemic control.

INTRODUCTION

Diabetes mellitus (DM) is a heterogeneous group of disorders, characterized by hyperglycemia, alterations in carbohydrate, lipid metabolism, and vascular and neurological complications. These changes result due to abnormal insulin secretion, insulin receptor or post receptor events which affect metabolism of carbohydrates, proteins and fats in addition to damaging liver, kidney and β -cells of pancreas. Therefore, treatment of DM involves administration of exogenous insulin or oral hypoglycemic drugs. However, this approach is not completely satisfactory in a large proportion of patients; hence continuous effort is on for new antidiabetic agents with high potency and little or no side effects.

Renewed interest has been observed in recent years on multiple activities of natural molecules and metallopharmaceuticals. Since 1980, many researchers have reported that metal ions such as vanadium, zinc, copper, chromium and tungsten exhibit *in vitro* and *in vivo* insulin mimetic activity in experimental animals. Among these metal ions, vanadium, zinc, oxovanadium (IV) and zinc (II) complexes are found to treat diabetes better (Yoshikawa et al., 2007; Qian et al., 2000; Ho et al., 2001; Rehder, 2003). Vanadium ions and complexes have been demonstrated to exert various insulin mimetic and anti-diabetic effects, such as increase glucose transport and metabolism in adipocytes, hepatocytes and skeletal muscle, stimulating glycogen synthesis and lipogenesis, inhibiting lipolysis and protein catabolism in animal models.

Compound with 1, 4-naphthaquinones pharmacophore are reported for insulin mimetic potential. Thespesone

*Corresponding author. E-mail: phanse_mohini@yahoo.co.in.

present in bark of *T. populnea* contains 1, 4-naphthaqionone moiety. Taking into consideration the antidiabetic activity of 1, 4-naphthaqionones and role of vanadium as an insulin mimetic agent, in the present investigation, thesopesone-vanadium complex was synthesized and its antidiabetic potential was assessed in alloxan induced diabetic rats.

MATERIALS AND METHODS

Drug and chemicals

Vanadium sulphate and sodium acetate were obtained from Sigma Chemicals. Analytical solvent were employed during all synthesis and which were distilled prior to their use.

Synthesis of thesopesone-vanadium (TVa) complex

The bark of *T. populnea* were collected and cut in to small pieces and dried in hot air oven at 55°C. The hexane extract was prepared using soxhlet apparatus and concentrated under vacuum. Isolation of thesopesone rich fraction was done by column chromatography on silica gel having mesh sizes of 60 to 120, by using chloroform and methanol solvent system. It was characterized by ultraviolet (UV), infrared (IR) and nuclear magnetic resonance (NMR) spectra.

TVa was synthesized by interaction of methanolic solutions of vanadium sulfate and thesopesone in 1:2 metal: ligand stoichiometry. The reaction mixture was maintained at neutral pH and stirred for 8 h at room temperature. The precipitating metal complex was collected by filtration, washed with cold methanol and dried under vacuum. The compounds were characterized by UV, IR and electron paramagnetic resonance (EPR).

Experimental animals

Albino rats of Wistar strain weighing 160 to 200 g were obtained from National Toxicology Center (NTC), Pune. Animals of either sex were housed under standard laboratory conditions: temperature of $22 \pm 3^\circ\text{C}$, relative humidity of 30%, 12 h light and dark cycle was maintained, and free access to standard pellet diet and water *ad libitum*.

Induction of diabetes

Diabetes was induced by a single intraperitoneal injection of alloxan monohydrate in citrate buffer (pH 4.5) at a dose of 140 mg/kg (Reshmi et al., 2001), body weight of the rat. The diabetic state was confirmed 48 h after alloxan injection by hyperglycemia. Surviving rats with fasting blood glucose level higher than 250 mg/dl were included in the study (Murali et al., 2002; Ghosh and Suryawanshi, 2001).

Treatment schedule

Total of 40 diabetic surviving wistar rats were used for study. The rats were divided in to 8 groups (n = 5) as follows:

1. Group I (Control): The animals of this group were nondiabetic and received only 1% gum acacia (1 ml/kg/day, p. o.) for six weeks.
2. Group II (D-Control): The animals of this group were diabetic and received 1% gum acacia (1 ml/kg/day, p. o.) for six weeks.

3. Group III (D + Glimepride): The animals of this group were diabetic and received Glimepride (0.09 mg/kg/day, p.o.) for six weeks.

4. Group IV (D + Losartan): The animals of this group were diabetic and received Losartan (2 mg/kg/day, p. o.) for six weeks.

5. Group V (D + TVa 5): The animals of this group were diabetic and received TVa (5 mg/kg/day, p. o.) for six weeks.

6. Group VI (D + TVa 10): The animals of this group were diabetic and received TVa (10 mg/kg/day, p.o.) for six weeks.

7. Group VII (D + TVa 20): The animals of this group were diabetic and received TVa 20 (20 mg/kg/day, p. o.) for six weeks.

8. Group VIII (D + Va): The animals of this group were diabetic and received vanadium sulphate (0.20 mg/kg/day, p. o.) for six weeks.

Biochemical parameters from blood

Animals were anesthetized with anesthetic ether, and blood was withdrawn by puncturing retro-orbital plexus by using fine glass capillary and collected in epindorff tubes. Estimation of plasma glucose was done by GOD/POD method and total cholesterol (COD/POD method), triglyceride (GPO/POD method), high-density lipoprotein (HDL) using standard diagnostic kits from Biolabs Diagnostic Pvt. India Ltd.

Study of hemodynamic parameters

At the end of the six-week treatment, blood pressure was measured according to procedure described by Balaraman et al. (1989). Rats of all experimental groups were anaesthetized by urethane (1.75 gm/kg, i. p.); temperature was maintained at 37°C throughout the experiment. The necks of the rats were opened, and common carotid artery was cannulated with polyethylene tube which was filled with 0.9% v/v heparinized saline. Mean arterial blood pressure was directly measured by connecting the polyethylene tubing to precalibrated pressure transducer SS13L (BIOPAC system, Inc., CA, USA) connected to Biopac MP-30 data acquisition system (BIOPAC Systems, Inc) (Balaraman et al., 1989).

Statistical analysis

The results were expressed as mean \pm SEM, and statistically analyzed by ANOVA followed by Dunnett test, with level of significance set at $p < 0.05$.

RESULTS

Effect of thesopesone-vanadium complex on plasma glucose level (Figure 1)

Diabetic control group showed significant increase ($p < 0.01$) in plasma glucose level when compared with control group. TVa-20 treated groups showed significant decrease ($p < 0.01$) in plasma glucose level on day 29 onwards. TVa treated group showed significant decrease ($p < 0.05$) in plasma glucose level on day 22 and ($p < 0.01$) on day 29 onwards when compared with diabetic control. TVa-10 and TVa-20 treated groups showed significant decrease ($p < 0.05$) in plasma glucose level on day 22 and ($p < 0.01$) on days 29, 36, and 43, when compared with D-control.

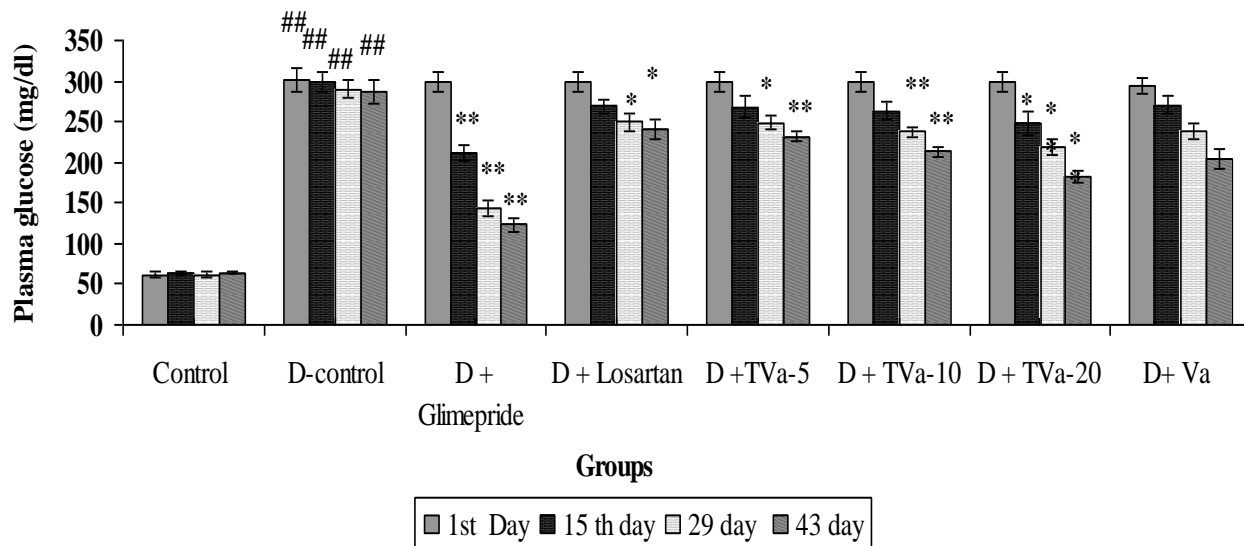


Figure 1. Effect of thesposone-vanadium complex (TVa) on plasma glucose level in alloxan-induced diabetic rats. Values are expressed as mean \pm SEM. (n=5), ANOVA followed by Dunnett test *p < 0.05, ##p < 0.01 when compared with control. *p < 0.05 and **p < 0.01 when compared with D-control.

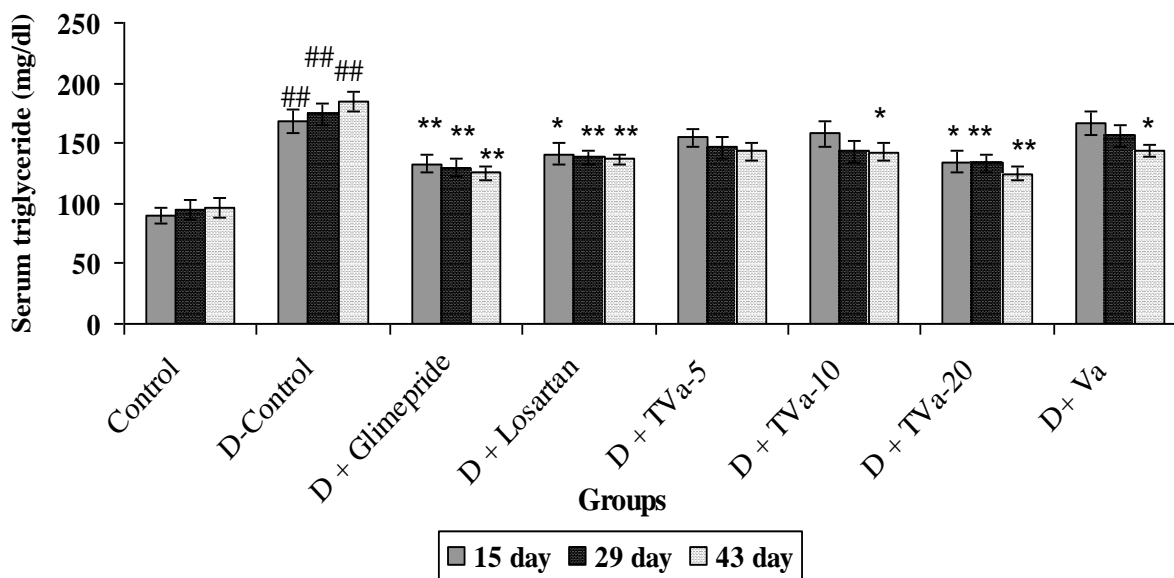


Figure 2. Effect of thesposone-vanadium complex (TVa) on serum triglyceride level in alloxan-induced diabetic rats. Values are expressed as Mean \pm SEM. (n = 5), ANOVA followed by Dunnett test. ##p < 0.01 when compared with control; *p < 0.05 and **p < 0.01 when compared with D-control.

Effect of thesposone-vanadium complex on serum triglyceride level (Figure 2)

Diabetic control group showed significant increase (p < 0.01) in serum triglyceride level on day 15 onwards when compared with control group. TVa-20 treated groups showed significant decrease (p < 0.05) in serum triglyceride level on day 15 onwards.

Effect of thesposone-vanadium complex on serum cholesterol level (Figure 3)

Diabetic control group showed significant hypercholesterolemia (p < 0.01) when compared with control. Diabetic TVa-20 and TVa-10 treated groups showed significant decrease (p < 0.05) in serum cholesterol level on day 15, day 29 and (p<0.01) on day

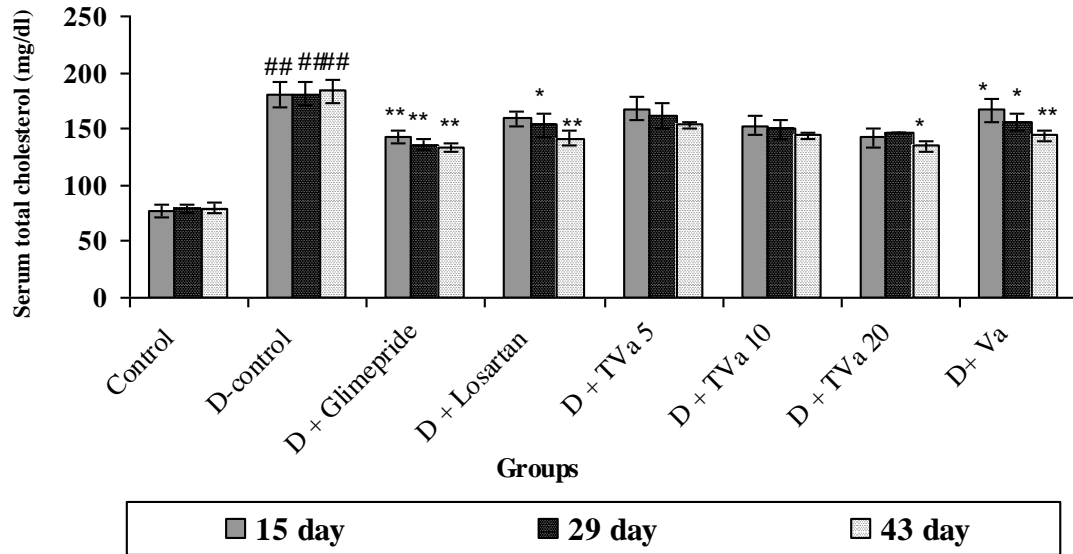


Figure 3. Effect of thespesone-vanadium (TVa) complex on serum total cholesterol level in alloxan-induced diabetic rats. Values are expressed as mean \pm SEM (n = 5), ANOVA followed by Dunnett test. ##p < 0.01 when compared with control; *p < 0.05 and **p < 0.01 when compared with D-control.

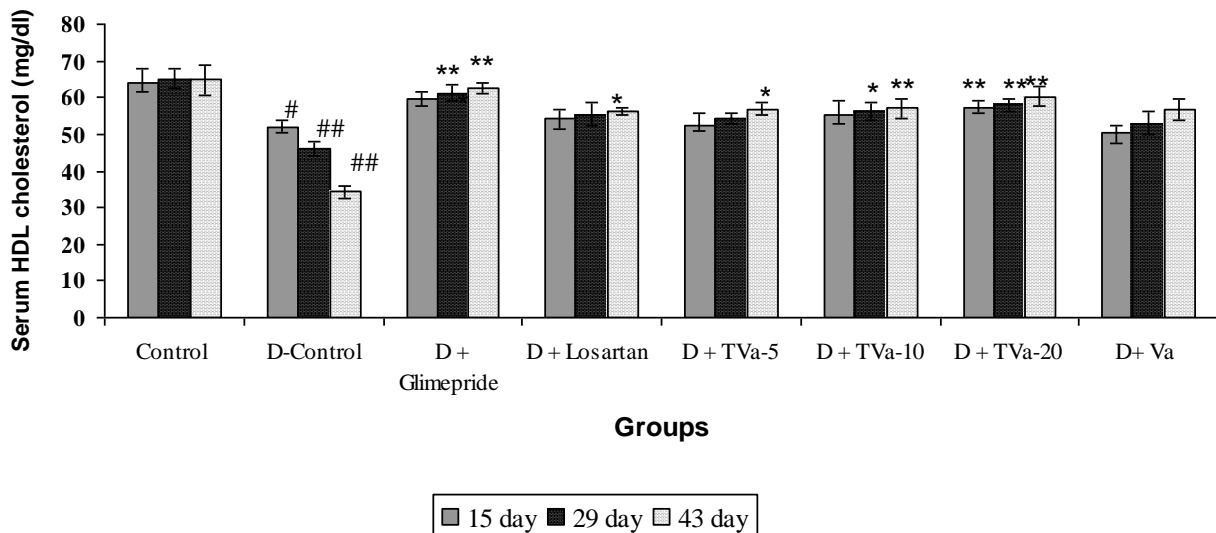


Figure 4. Effect of thespesone-vanadium complex (TVa) on serum HDL-cholesterol level in alloxan-induced diabetic rats. Values are expressed as Mean \pm SEM (n = 5), ANOVA followed by Dunnett test. #p < 0.05 and ##p < 0.01 when compared with Control; *p < 0.05 and **p < 0.01 when compared with D-control.

43 when compared with D-control.

Effect of thespesone-vanadium complex on serum HDL cholesterol level (Figure 4)

Diabetic control group showed significant decrease in serum HDL cholesterol (p < 0.05) levels on day 15 onwards (p < 0.05) when compared with control group.

Diabetic TVa 20 treated groups showed significant increase (p < 0.01) in serum HDL cholesterol levels on day 29 and 43 when compared with diabetic control. TVa 10 treated group showed significant (p < 0.05) increase in serum HDL-cholesterol level on days 29 and 43 when compared with diabetic control group, respectively. TVa 20 treated group showed significant increase (p < 0.01) in serum HDL-cholesterol level on days 29 and 43 when compared with diabetic control group.

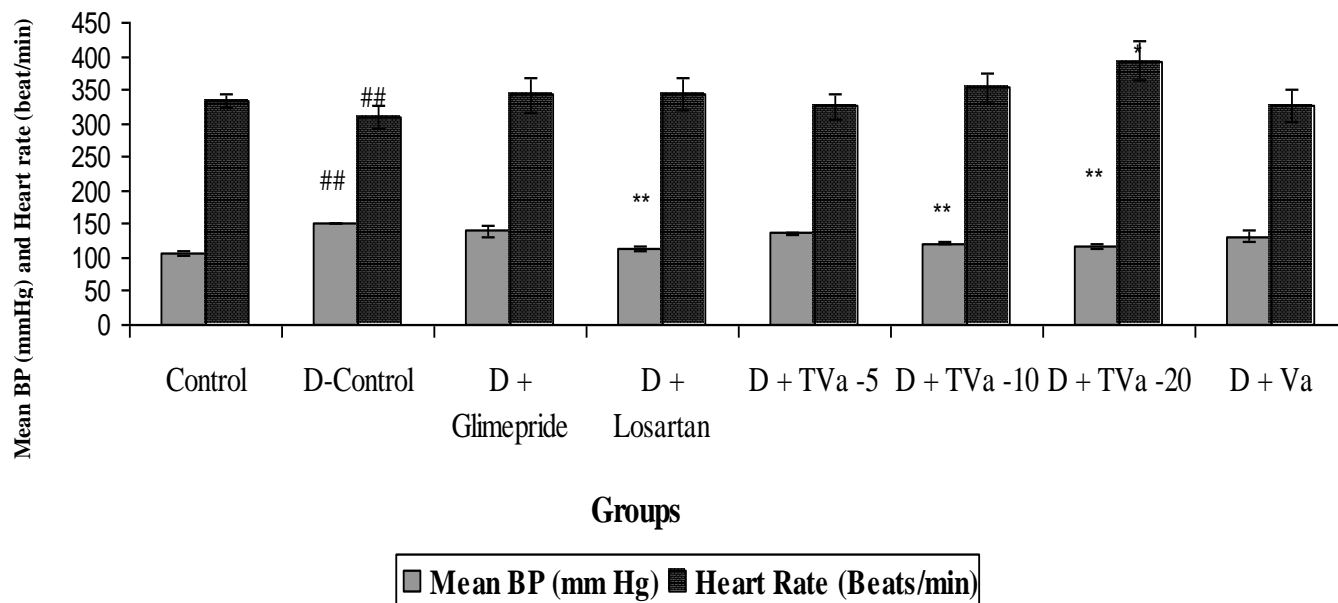


Figure 5. Effect of thesesone-vanadium complex (TVa) on Blood pressure and heart rate in alloxan-induced diabetic rats. Values are expressed as mean \pm SEM (n = 5), ANOVA followed by Dunnett test. ##p < 0.01 when compared with Control; *p < 0.05 and **p < 0.01 when compared with D-control.

Effect of thesesone-vanadium complex on blood pressure and heart rate (Figure 5)

Diabetic control group showed significant increase in blood pressure and heart rate ($p < 0.01$) when compared with normal control. TVa 10 and 20 mg/kg treated group showed significant decrease ($p < 0.01$) in blood pressure and heart rate when compared with D-control.

DISCUSSION

Alloxan induced diabetes in a model of type-II diabetic rat, is characterized by hyperglycemia, alterations in carbohydrate and lipid metabolism. In the present study, diabetic animals showed significant increase in blood glucose level from days 1 to 42 which indicated development of hyperglycemia. Treatment with TVa significantly reduced plasma glucose level in dose dependant manner (Figure 1). This may be attributed to insulin mimetic potential of TVa because vanadium is reported for its insulin mimetic potential by inducing insulin receptor tyrosine kinase and by inhibiting phosphotyrosine phosphatases enhanced basal rates of glucose uptake and presumed metabolism of glucose by liver and muscle.

Diabetic patients may encounter hyperinsulinemia and dyslipidemia which may cause elaboration of vasoactive factors, defective glucose transport, increased myocytes free acid uptake and altered calcium uptake thereby development of dyslipidemia, which leads to vascular

changes. In the present study, we observed hypertriglyceridemia and hypercholesterolemia, and decrease in serum HDL in diabetic rats which indicates development of dyslipidemia which was in accordance with previous reports (Majithiya et al., 2005). Vanadium inhibits lipolysis and stimulates lipogenesis in adipocyte, and mimics insulin isolated hepatocytes to inhibit VLDL release. These actions are probably mediated by signal trasduction pathways, by activation of PI-3K kinase. The treatment with TVa significantly reduced hypertriglyceridemia and hypercholesterolemia, while it increased serum HDL concentration (Figures 2, 3 and 4). Therefore, these results indicate that TVa has the potential to treat dyslipidemia associated with diabetes. This may probably be due to insulin mimetic potential of TVa, and due to the effect of vanadium on PI-3K pathway (Rehder, 2003; Tsiani and Fantus, 1997).

Long term exposure to hyperglycemia induces biochemical manifestations, such as non-enzymatic glycation, sorbitol-myoinositol-mediated changes, redox potential alterations, protein kinase C (PKC) activation and increase in free fatty acid (FFA) metabolism in monocytes (Scheetz and King, 2002; Cavajal and Moreno-Sanchez, 2003; Khan, 2003; Bell, 1995). These changes lead to particular sequence of events that include heamodynamic and structural changes. The hemodynamic modifications like increased permeability and decreased blood flow, leads to endothelial dysfunction (Khan, 2003). These changes results due to altered vasoactive factors such as increased endothelin-1 (ET-1) expression and decreased nitric oxide bioavailability,

leading to vasoconstriction and impaired vasodilatation leading to increase in blood pressure (Khan, 2003; Savage et al., 1998; Shehadeh and Regan, 1995; Lewinter, 1996). In the present study, diabetic animals showed increase in blood pressure and heart rate (Figure 5). Treatment with TVa significantly reduced blood pressure and heart rate significantly. These effects of TVa indicate that thesesone-vanadium complex has potential to treat hypertension associated with diabetes which might be due to glycemc control offered by TVa.

Conclusively, thesesone-vanadium complex has antidiabetic potential due to its insulin mimetic potential, ability to improve dyslipidemia and decrease blood pressure.

Conclusion

Conclusively, thesesone-vanadium complex has antidiabetic potential due to its insulin mimetic potential, ability to improve dyslipidemia and decrease blood pressure.

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