Content available at: https://www.ipinnovative.com/open-access-journals

International Journal of Clinical Biochemistry and Research

Journal homepage: https://www.ijcbr.in/

Original Research Article

Effect of trimetazidine in experimental animals with coronary heart disease or and in combination with diabetes mellitus

C Uthira Kumar[1], A Suma Preethi[2,*], Y V Ramakrishna Reddy[2], K Kalyan Kumar[2], B Nageshwar Rao[3]

[1] *UK Institute of Health Sciences and Research, Chennai, Tamil Nadu, India*
[2] *Dept. of Biochemistry, Mamata Medical College, Khammam, Telangana, India*
[3] *Dept. of Microbiology, Mamata Medical College, Khammam, Telangana, India*

ARTICLE INFO

Article history:
Received 21-01-2022
Accepted 29-01-2022
Available online 11-03-2022

Keywords:
Streptozotocin
Trimetazidine
Blood glucose
Serum insulin
HbA1c and Haemoglobin

ABSTRACT

Introduction: The epidemic of cardiovascular disease especially coronary heart disease (CHD) is emerging in rural India and accelerating in urban India. Coronary heart disease is the leading cause of death in India; diabetes accounts for a significant burden of CHD events. Trimetazidine (TMZ) is a piperazine derivative with anti-ischaemic properties. It is the first in a new class of metabolic agent, available for clinical use.

Aim: To determine the effect of TMZ on blood parameters in normal, hyperlipidemic, diabetic and Streptozotocin induced diabetic hyperlipidemic rats.

Materials and Methods: Adult male albino rats of Wistar strain, weighing approximately 150 to 180 g, were used in the present study. After various treatments, blood was collected from the rats by sinocular puncture for the blood parameters like Fasting blood glucose, urine sugar, HbA1c and Haemoglobin with standard protocols.

Results: The levels of blood glucose, HbA1c and urine sugar were found to be elevated in the entire diseased control group than normal control.

Conclusion: TMZ has the potential effect against the STZ induced syndromes with an optimum dose of 40 mg/kg in rats.

1. Introduction

Coronary artery disease (CAD) is the leading cause of cardiovascular morbidity and mortality worldwide and two-thirds of all cardiovascular fatalities occur in developing countries.[1] To date, data from myriad research sources have documented several hundred risk factors that are statistically associated with the development of CVD. Hyperlipidemia is a major cause of cardiac illness and deaths.[2] A causal relationship between the elevated plasma lipids and the development of atherosclerotic plaques has been well established. It is also found that diabetes substantially increases prevalence of atherosclerotic heart disease and cardiovascular mortality even in the absence of hyperlipidemia, smoking and hypertension.[3]

Over the past century, significant advances have been made in the development of animal models for atherosclerosis. Ignatowski[4] first reported that rabbits fed with a diet rich in animal proteins developed thickening of the intima with formation of large clear cells in the aorta. However, other investigators believed that the causal factor for atherosclerosis is lipids, but not proteins of the animal tissues. Cholesterol theory still remains at the centre of atherosclerotic vascular disease development. However,

* *Corresponding author*.
E-mail address: nageshmsc@gmail.com (A. S. Preethi).

https://doi.org/10.18231/j.ijcbr.2022.004

administering certain drugs like thiazides, glucocorticoids, anabolic steroids, and triton X-100 are also known to induce hyperlipidemia.

Dietary cholesterol is needed for induction of atherosclerosis in experimental animal models,[5] because of a strong association between certain types of dyslipidemia and development of atherosclerotic lesions. This indicates an inevitable role for cholesterol feeding in atherosclerosis research. The atherogenic role of cholesterol has been tested in an ever-increasing number of laboratory animals including wild-types, naturally defective or genetically modified animal models of atherosclerosis. The results of almost all these animal studies demonstrate increased plasma cholesterol level, and therefore is a reliable method for induction of atherogenesis.

The combination of coconut oil and cholesterol has been shown to be one of the most effective atherogenic diets when used on an experimental model to induce atherosclerosis in non-human primates.[6] Several other studies also indicate that dietary saturated fat and cholesterol supplementation causes hyperlipidemia in rabbits,[7] chicks,[8] hamsters,[9] and rats.[10] Rats are generally hypo-responsive to dietary cholesterol; thus, hyperlipidemia and atherogenesis may only be induced in rats by high cholesterol/high fat diet containing cholic acid. Cholic acid increases cholesterol absorption and in addition, suppresses cholesterol 7 α-hydroxlyase activity that results in decreased cholesterol excretion.[11]

Many chemicals are used for the induction of diabetes mellitus in the animal models for testing new antihyperglycemic drugs. The most commonly used chemicals for the induction of diabetes in the experimental animals are alloxan and streptozotocin (STZ).

Trimetazidine (TMZ) is a piperazine derivative (1-(2,3,4-trimethoxybenzyl)-piperazine dihydrochloride) with anti-ischaemic properties. It is the first in a new class of metabolic agent, available for clinical use. In conditions of hypoxia or induced ischaemia, TMZ maintains homeostasis and cellular functions by selectively inhibiting3-ketoacyl-CoA-thiolase.[12] So far no study has been reported on the TMZ potential against hyperlipidemia. The purpose of this preclinical study was to investigate the impact of TMZ on blood glucose, haemoglobin, HbA1c and Urine sugar in normal, hyperlipidemic, diabetic and STZ-diabetic hyperlipidemic rats.

2. Materials and Methods

2.1. Experimental animals

Adult male albino rats of Wistar strain, weighing approximately 150 to 180 g, were acclimatized for 7 days at room temperature (28 ± 3 °C) and relative humidity (55%) in a 12-hour light/dark cycle in a room under hygienic condition. The animals reared in King Institute of Preventive Medicine, Guindy, Chennai, Tamilnadu, India, were used for the experiment. Males were used throughout the investigation to avoid complications due to the oestrous cycle. The animals were allowed free access to water and standard pellet diet (Tetragon Chemie Pvt. Ltd., Yelahanka New Town, Bangalore, India). Animal handling and experimental procedures were approved by the Institutional Animal Ethics Committee, International Centre for Cardio Thoracic and Vascular Diseases (A Unit of Frontier Life Line Pvt. Ltd), (Registration Number: 871/ac/05/CPCSEA, Proposal No. SA – 02/2008) and animals were cared in accordance with the "Committee for the purpose of control and supervision on experimental animals" (CPCSEA, 2005).

2.2. Source of chemicals

Streptozotocins, Trimetazidine, were obtained from Sigma-Aldrich Co. (St. Louis, Missouri, USA). All other chemicals and solvents were of analytical grade and purchased from S.D. Fine Chemicals, Mumbai, Himedia Laboratories Pvt. Ltd., Mumbai, India, Qualigens Fine Chemicals, Dr. Annie Basant Road, Mumbai, India, Merck & Co., INC., Whitehouse Station, USA.

2.3. Experimental design for assays

The animals were randomly divided into 6 groups of six animals each as shown below. Different concentrations of the TMZ (20, 40 & 80 mg/kg body weight were suspended in distilled water and were fed to the rats by intubation.

Group I served as control and received standard pellet diet. Group II received Control + TMZ (80 mg/kg BW); Group III- Combination (HFD + DM); Group IV: Combination (HFD + DM) + TMZ (20 mg/kg BW); Group V- Combination (HFD + DM)+ TMZ (40 mg/kg BW); Group VI- Combination (HFD + DM)+ TMZ (80 mg/kg BW) respectively. Total duration of the experiment was 60 days. The experimental groups received 2% Cholesterol, 20% Coconut oil, 0.125 cholic acid, all mixed with the diet and On the 31^{st} day, the animals were rendered diabetic by a single intraperitoneal injection of streptozotocin (40 mg/kg BW) in freshly prepared citrate buffer (0.1M, pH 4.5) after an overnight fast. TMZ were given orally by intragastric tube to different groups from 46^{th} day onwards.

The animals were fasted for 12 h, anaesthetized using ketamine (24 mg/ kg^{-1} BW, intramuscular injection) and sacrificed by cervical dislocation on 61^{st} day morning. The serum and plasma were used for the biochemical analysis. Since the TMZ at a 40 mg dose gave a maximum effect on the, HbA1c, hemoglobin and it was fixed as the optimum dose for further work. We also assessed the plasma glucose levels on fasting study after giving the TMZ (20, 40 and 80 mg/kg BW). The TMZ at a dose of 40 mg/kg BW that gave a maximum reduction of plasma glucose level was used for

further study.

2.4. Estimation of biochemical parameters

Fasting blood glucose was estimated by using auto analyzer, (BeneSphera, H33s). Serum insulin and C-peptide was assayed by multiple analyte plate system using reagent kits obtained from Monobind Inc., Lake Forest., CA 92630 USA. HbA1c was estimated by Cobas Integra 400 plus analyzer with hemolyzing reagent. Haemoglobin in the blood was estimated by the method of Drabkin and Austin (1932).

2.5. Statistical analysis

The collected data analysis was done using SPSS software. The results were presented as mean ± standard deviation (SD) and percentages. Mean, and SD was computed for all continuous variables and comparisons were done using Student's t-test.

3. Results

3.1. Dose dependent efficacy of TMZ on blood glucose

Table 1 shows the effect of the TMZ on the blood glucose levels of the experimental rats. There is significant change in the blood glucose levels between the control and all other groups from day 0 to day 60. Animals treated with various doses, TMZ (40 mg/kg BW) treated group of animals showed a significant decrease in blood glucose levels when compared with control groups and all other drug treated groups up to day 60 respectively. It is evident from the results that TMZ has its effect in the regulating the blood glucose levels at effective drug dosage concentration of 40 mg/kg BW.

3.2. Dose dependent efficacy of TMZ on hemoglobin, HbA1c and Urine sugar excretion

Table 2 and Figure 1 shows the effect of the TMZ on the HbA1c and Urine sugar onexperimental rats.

3.2.1. Hemoglobin

There is significant change in the Hemoglobin levels among the normal control and drug control to the diseased control and drug treated groups and the significant change is also noted among all the diseased control and diseased TMZ treated groups.

3.2.2. HbA1c

There is significant change in the HbA1c levels among the normal control and drug control to the diseased control and the drug treated groups and the significant change is also noted among all the diseased control and diseased TMZ treated groups.

3.2.3. Urine sugar excretion

The excretion of the sugars in the urine occurs when the blood glucose levels reaches the renal threshold levels, it is evident from the Table 2 that the TMZ has the effect in controlling the blood glucose levels at a effective drug dosage of 40 mg/kg BW on the 60^{th} day of the drug treatment and the effect of the drug failed in regulating the blood glucose homeostasis on the 46^{th} day of the treatment.

It is evident from the results that TMZ has its effect in the regulating the blood glucose levels during the drug treatment has started at 46^{th} day at effective drug dosage concentration of 40 mg/kg BW.

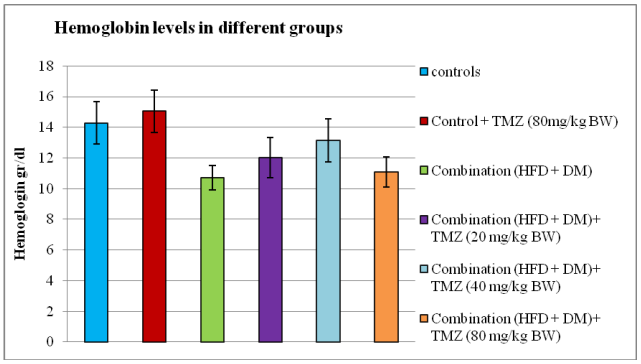

Fig. 1: Changes in HbA1c, levels after exposure to various treatments of TMZ. Values are mean ± SD from six mice per group

.

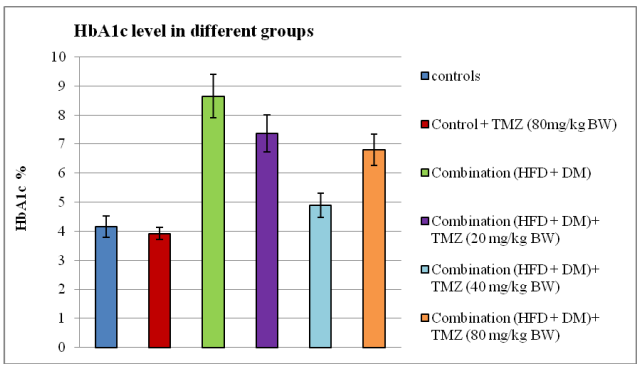

Fig. 2: Changes in blood glucose levels after exposure to various treatments of TMZ. Values are mean ± SD from six mice per group

4. Discussion

Diabetes is a chronic disease which is relatively common throughout the world, which may lead to abnormalities in fluid and electrolyte balance and consequently affect blood volume and blood pressure and persistence of hyperglycemia causes an increase in the production of oxygen free radicals through auto oxidation and nonenzymatic glycation.[13] In addition, it has been observed

Table 1: Changes in the levels of glucose in control and experimental rats

Groups	Blood glucose (mg/dl)				
	0 day	15^{th} day	30^{th} day	45^{th} day	60^{th} day
Group I	68.73 ± 5.21	72.57 ± 4.47^{a}	81.24 ± 5.71^{a}	84.52 ± 7.91^{a}	91.35 ± 8.15^{a}
Group II	66.14 ± 4.56	69.83 ± 4.23^{a}	80.61 ± 5.68^{a}	83.59 ± 6.43^{a}	87.52 ± 7.68^{a}
Group III	71.23 ± 6.34	75.42 ± 5.08^{b}	86.84 ± 5.16^{b}	342.26 ± 16.17^{b}	353.92 ± 17.32^{b}
Group IV	69.11 ± 5.69	71.27 ± 4.59^{ab}	82.27 ± 4.82^{b}	338.49 ± 15.34^{b}	195.28 ± 12.34^{c}
Group V	68.29 ± 5.14	73.94 ± 5.79^{ab}	83.94 ± 5.18^{ab}	332.82 ± 15.92^{b}	120.71 ± 11.56^{d}
Group VI	70.58 ± 6.17	72.47 ± 5.73^{ab}	83.81 ± 6.13^{ab}	335.28 ± 15.36^{b}	156.34 ± 13.53^{e}

Values are expressed as mean ± S.D. for six rats in each group. Values not sharing a common superscript differ significantly at p<0.05. The significant levels: a = p < 0.05, b = p < 0.01, c = p < 0.001 and No symbol = Non-significant, when compared with the respective control group.

Table 2: Changes in the levels of Hemoglobin, HbA1C and urine sugar in control and experimental rats

Groups	Hemoglobin (g/dl)	HbA1c (%)	Urine sugar		
			0 day	46^{th} day	60^{th} day
Group I	14.27 ± 1.38^{a}	4.16 ± 0.36^{a}	Nil	Nil	Nil
Group II	15.05 ± 1.39^{a}	3.92 ± 0.21^{a}	Nil	Nil	Nil
Group III	10.72 ± 0.79^{b}	8.65 ± 0.74^{b}	Nil	+++	++++
Group IV	12.02 ± 1.30^{c}	7.36 ± 0.63^{c}	Nil	+++	++
Group V	13.14 ± 1.39^{a}	4.89 ± 0.42^{a}	Nil	+++	Nil
Group VI	11.08 ± 0.99^{d}	6.81 ± 0.54^{c}	Nil	+++	+

Values are expressed as means ± S.D. for six rats in each group. Values not sharing a common superscript differ significantly at p<0.05. The significant levels: a = p < 0.05, b = p < 0.01, c = p < 0.001 and No symbol = Non-significant, when compared with the control group

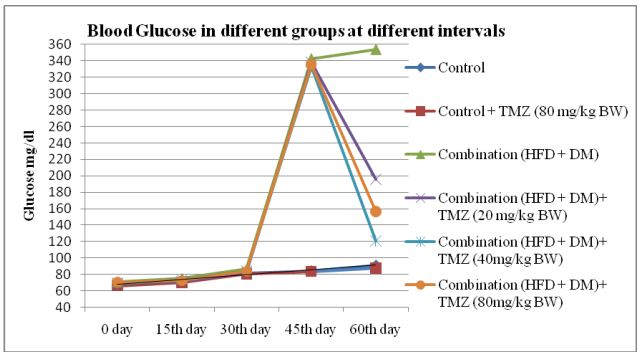

Fig. 3:

that reactive oxygen species can cause an increase in lipid peroxidation, alterations in structure and function of enzymes and deficits in the antioxidant defense systems.[14] Experimental diabetes mellitus has been induced in laboratory animals by several methods. Out of which injecting drugs such as alloxan or Streptozotocin which ultimately degenerate the Langerhans islets beta cells, 60mg/kg body weight Streptozotocine dose results in the toxicity of beta cells with emergence of clinical diabetes within 2-4 days.[15] Streptozotocin induced hyperglycemia has been described as a useful experimental model to study diabetes mellitus.

Streptozotocin administration to rats increased plasma glucose and decreased insulin and C-peptide levels.[16] It is evident from the results that TMZ has its effect in the regulating the blood glucose levels at effective drug dosage concentration of 40 mg/kg BW by decreasing in plasma glucose with propionate rise in insulin and C-peptide levels. TMZ Partially inhibits fatty acid oxidation and increases glucose and pyruvate oxidation and decrease lactate production. The metabolic and functional effects in particular to diabetes mellitus where glucose metabolism is impaired and metabolism is shifted towards utilization of fatty acids for energy. TMZ acts at cellular level by shifting the energy substrate preference from fatty acid oxidation to glucose oxidation, secondary to selective inhibition of 3-ketoacylCoA thiolase.[17]

C-peptide is formed in the biosynthesis of insulin and the two peptides (insulin and C-peptide) are subsequently released in equimolar amounts to the circulation.[18] TMZ administration to diabetic rats increased the levels of C-peptide. An increase in C-peptide levels in diabetic rats treated with TMZ correlates well with the increased insulin secretion (endogenous secretion) thereby possibly regenerating β-cells of the islets of Langerhans.

Animals treated with various treatments resulted in continuous decline in the hemoglobin levels due to damage caused to the RBC's and bone marrow. Diabetic group of animals with TMZ (40mg/kg) showed the significant change among all the control and TMZ treated groups. HbA1c, increased while blood total hemoglobin decreased in diabetic, hyperlipidemic and diabetic hyperlipidemic rats. The levels of insulin and c-peptide increased in hyperlipidemic rats while decreased in diabetic and diabetic hyperlipidemic rats. Oral administration of TMZ reversed above parameters towards normalcy. The excretion of the

sugars in the urine occurs when the blood glucose levels reaches the renal threshold levels, TMZ has the effect in controlling the blood glucose levels at an effective drug dosage of 40 mg/kg BW on the 60^{th} day of the drug treatment and the effect of the drug failed in regulating the blood glucose homeostasis on the 46^{th} day of the treatment. Similar observations were also reported for other studies.[19,20]

5. Conclusion

Our studies for the first time demonstrated that TMZ has the potential effect against the STZ induced syndromes with an optimum dose of 40 mg/kg in rats. However, further studies may be needed before advocating its potential for human application.

6. Source of Funding

None.

7. Conflict of Interest

The authors declare no conflict of interest.

References

1. Mendis S, Pekka P, Norrving B. Global atlas on cardiovascular disease prevention and control. Mendis S, editor. Geneva: World Health Organization; 2011. Available from: https://apps.who.int/iris/handle/10665/44701.
2. Nelson RH. Hyperlipidemia as a risk factor for cardiovascular disease. *Prim Care*. 2013;40(1):195–211.
3. Després JP, Lemieux I, Dagenais GR, Cantin B, Lamarche B. HDL-cholesterol as a marker of coronary heart disease risk: the Quebec cardiovascular study. *Atherosclerosis*. 2000;153(2):263–72.
4. Ignatowski AC. Influence of animal food on the organism of rabbits. S Peterb Izviest Imp Voyenno-Med. *Akad*. 1908;16:154–73.
5. Katz LN. Experimental atherosclerosis. *Circulation*. 1952;5(1):101–4.
6. Wissler RW, Vesselinovitch D. Interaction of therapeutic diets and cholesterol-lowering drugs in regression studies in animals. In: InRegression of atherosclerotic lesions; 1984. p. 21–41.
7. Yanni AE. The laboratory rabbit: an animal model of atherosclerosis research. *Lab Anim*. 2004;38(3):246–56.
8. Toda T, Nishimori I, Kummerow F. Animal Model of Atherosclerosis Experimental Atherosclerosis in the Chicken Animal Model. *J Japan Atheroscl*. 1983;11(4):755–61.
9. Lin MH, Lu SC, Huang PC, Liu YC, Liu SY. A High-Cholesterol, n–3 Polyunsaturated Fatty Acid Diet Causes Different Responses in Rats and Hamsters. *Ann Nutr Metab*. 2005;49(6):386–91.
10. Matos SL, Paula HD, Pedrosa ML, Santos RC, Oliveira EL, Júnior JAC, et al. Dietary models for inducing hypercholesterolemia in rats. *Braz Arch Biol Technol*. 2005;48:203–9.
11. Joris I, Zand T, Nunnari JJ, Krolikowski FJ, Majno G. Studies on the pathogenesis of atherosclerosis. I. Adhesion and emigration of mononuclear cells in the aorta of hypercholesterolemic rats. *Am J Pathol*. 1983;113(3):341.
12. Banach M, Okonski P, Rysz J, Drozdz J, Zaslonka J. The role of trimetazidine in the treatment of heart diseases. Poznan: Termedia Publishing House; 2006.
13. Hebden RA, Gardiner SM, Bennett T, Macdonald IA. The influence of streptozotocin-induced diabetes mellitus on fluid and electrolyte handling in rats. *Clin Sci (Lond)*. 1986;70(1):111–7.
14. Singh N, Kamath V, Rjini PS. Attenuation of hyperglycemia and associated biochemical parameters in STZ-induced diabetic rats by dietary supplementation of potato peel powder. *Clin Chim Acta*. 2005;353(1-2):165–75.
15. Akbarzadeh A, Norouzian D, Mehrabi MR, Jamshidi S, Farhangi A, Verdi AA, et al. Induction of Diabetes by Streptozotocin in rats. *Indian J Clin Biochem*. 2007;22(2):60–4.
16. Barbosa N, Rocha JBT, Soares JCM, Wondracek DC, Goncalves JF, Schetinger MRC, et al. Dietary diphenyl diselenide reduces the STZ-induced toxicity. *Food Chem Toxicol*. 2008;46(1):186–94.
17. Rosano GMC, Vitale C, Sposato B, Mercuro G, Fini M. Trimetazidine improves left ventricular function in diabetic patients with coronary artery disease: a double-blind placebo-controlled study. *Cardiovasc Diabetol*. 2003;28:2–16.
18. Wahren J, Ekberg K, Samnegard B, Johansson BL. C-peptide: A new potential in the treatment of diabetic nephropathy. *Curr Sci*. 2001;1(3):261–6.
19. Meiszterics Z, Kónyi A, Hild G, Sárszegi Z, Gaszner B. Effectiveness and safety of anti-ischemic trimetazidine in patients with stable angina pectoris and Type 2 diabetes. *J Comp Eff Res*. 2017;6(8):649–57.
20. Li R, Tang X, Jing Q, Wang Q, Yang M, Han X, et al. The effect of trimetazidine treatment in patients with type 2 diabetes undergoing percutaneous coronary intervention for AMI. *Am J Emerg Med*. 2017;35(11):1657–61.

Author biography

C Uthira Kumar, -

A Suma Preethi, -

Y V Ramakrishna Reddy, -

K Kalyan Kumar, -

B Nageshwar Rao, -

Cite this article: Kumar CU, Preethi AS, Reddy YVR, Kumar KK, Rao BN. Effect of trimetazidine in experimental animals with coronary heart disease or and in combination with diabetes mellitus. *Int J Clin Biochem Res* 2022;9(1):17-21.