DOI: https://doi.org/10.53350/pjmhs2216596

ORIGINAL ARTICLE

Effects of Metformin and N-Acetylcysteine on Dyslipidemia in Streptozotocin-Induced Diabetic Rats

NOVERA SOHAIL BAJWA[1*], SAVEELA SADAQAT[2], WAJAHAT ULLAH KHAN[3], MEHWISH MOHSIN[4], MAHAK MEMON[5], M. WAQAR ASLAM[1], RIZWAN MASUD[6]

[1]*Department of Pharmacology, CMH Medical College, Kharian- Pakistan*
[2]*Department of Biochemistry, M. Islam Medical and Dental College, Gujranwala- Pakistan*
[3]*Department of Biochemistry, Mohi-Ud-Din Islamic Medical College, Mirpur, AJ&K- Pakistan*
[4]*Department of Biochemistry, CMH Medical College, Kharian- Pakistan*
[5]*Department of Biochemistry, PUMHSW-SBA, Nawabshah-Pakistan*
[6]*Department of Physiology, Mohammad College of Medicine, Peshawar- Pakistan*
Correspondence to Dr. Novera Sohail Bajwa, Email: noverasb@gmail.com Tel:+92-333-8725118

ABSTRACT

Background: Diabetes is a chronic health issue globally that has a high incidence.
Aim: To see the effects of metformin and N-acetylcysteine on dyslipidemia in streptozotocin-induced diabetic rats.
Study Design: Randomized controlled trial.
Methodology: A total of 25 rats were divided into 05 equal groups. Diabetes was induced into rats by streptozotocin (35 mg/kg) single doze (i.p). Rats having blood sugar >300 mg/dl after 48hrs were considered as diabetic. Negative and positive control groups were fed on standard diet. Treatment groups were given Metformin, N-acetylcysteine and combination of Metformin and N-acetylcysteine respectively for 4 weeks. Groups were compared for lipid profile thus efficacy of treatment given was evaluated. SPSS 25.0 was used to analyze the whole data. The difference between all the groups was analyzed using One-Way Analysis of Variance (ANOVA).
Results: There was significant improvement in a treatment group that received both Metformin plus N-acetylcysteine while other treatment groups had little improvement.
Conclusion: It was concluded that dyslipidemia was significantly reduced by use of N-acetylcysteine in combination with metformin among diabetic rats.
Keywords: Diabetes Mellitus, Dyslipidemia, Metformin, N-acetylcysteine and Streptozotocin.

INTRODUCTION

DM (Diabetes Mellitus) signifies a heterogeneous/ mixed collection of chronic metabolic conditions and disorders. Diabetes remains one of top five causes of deaths in the world. Its patho-physiology involved lack of insulin secretion from beta pancreas or decreased sensitization of insulin receptors toward insulin thus resulting in hyperglycemia[1]. According to an estimate, this disease has affected almost 171 million people globally and will get double by 2030[2]. Glucose remains the main oxidizable substrate in number of cell categories. Catabolic processes (gluconeogenesis & glycogenolysis) thus become activated among diabetic rats in-order to maintain homeostasis. Thus lipolysis occurs that cause dyslipidemias. As a result atherosclerosis (97%) develops among diabetic patients. Both DM and dyslipidemias carry a significant risk factor for cardiovascular diseases. Literature review has shown that there is an increase in ROS whereas NO bioavailability decreases owing to a high level of plasma glucose[3].

According to UK prospective diabetes study, dyslipidemias cause both deadly and non-fatal cardiovascular events in diabetic patients. Suitable administration targeting glycaemic and lipid control is significant for declining mortality and morbidity thus contributing towards better quality of life in the long term for diabetic patients[4]. In modern era, health issues like oxidative stress and dyslipidemias have gained importance. Antioxidants have proven to be good alternative to dyslipidemia and other diabetic complications[5].

Metformin is a biguanide in addition to anti hyperglycemic effectiveness, also shows variable results in improving dyslipidemia[6]. One previous study reported that an improvement in LDL cholesterol, triglycerides and total cholesterol was seen among subjects that received metformin while HDL-cholesterol remained unchanged[7]. One other study showed that free fatty acids decreased significantly after receiving Metformin treatment. Metformin also impeded lipid peroxidation of HDL and LDL, decreased oxidative stress and the superoxide free radical in the platelets[8].

Received on 07-11-2021
Accepted on 27-04-2022

N-acetylcysteine releases L-cysteine (amino acid) and an oxidizing agent (glutathione). NAC contains a sulpha-hydryl radical, which causes neutralization of Reactive Oxygen Species[9]. It controls state of redox of neuro-transmitters such as NMDA and AMPA receptors. In contrast to GSH, NAC has enhanced topical and oral bio-availability[10,11]. In view of the significant character of oxidative stress and NO in growth of diabetic complexities and growth of the disease, the study is aimed at exploring the novel uses of these drugs and contribute to reconstruct the diabetic regime.

The objective of the study was to see the effects of metformin and N-acetylcysteine on dyslipidemia in streptozotocin-induced diabetic rats.

METHODOLOGY

Twenty five (25) adult Sprague Dawley rats only female (to avoid pregnancy) weighing about ±250 g, were procured from National Institute of Health (NIH), Islamabad and were retained in the animal house of NIH during the study period. The standard lab conditions were maintained in animal house of NIH. Room was well ventilated and twelve hourly cycles of light and dark were maintained. Room temperature was maintained at 22-24º C. The animals had free access to standard diet and clean drinking water. Acclimatization of the rats was carried out for one week prior to the induction of DM. Diabetes was induced into rats by streptozotocin (35mg/kg) single doze (i.p). Rats having blood sugar >300mg/dl after 48hrs were considered as diabetic. Negative and positive control groups were fed on standard diet. Treatment groups were given Metformin, N-acetylcysteine and combination of Metformin and N-acetylcysteine respectively for 04 weeks. Groups were compared for lipid profile thus efficacy of treatment given was evaluated. Permission was granted by Institutional Ethical Review Board.

Statistical analysis: SPSS 25.0 was used to analyze the whole data. The quantitative variables were described as mean+SD. The difference between all the groups was analyzed using One-Way Analysis of Variance (ANOVA) with P- value of < 0.05 was considered statistically significant.

Table-1: Intervention Protocol

Groups	Treatment Given
Negative Control	Standard diet and clean drinking water
DM control (positive)	Standard diet and clean drinking water
DM + Metformin Group	Metformin (250mg/kg/day) for 28days by o.g
DM + NAC Group	NAC (25mg/kg/day) for 28days by o.g
DM+Metformin +NAC Group	Metformin (250mg/kg/day) + NAC (25mg/kg/day) for 28days by o.g

RESULTS

Treatment with metformin, N Acetylcysteine and combination of MET plus NAC showed significant improvement in the lipid profile after the 28 days of treatment.

Comparison of groups based on variables/ parameters: The behaviour of various groups in each category of parameters can be summarized in terms of their mean values as under:

Total cholesterol (T-Chol): With regards to the T-Chol test, the result showed little variations in Normal Control Group (NC) and Metformin plus N-acetylcysteine Group, while Metformin and N-acetylcysteine showed little high values, whereas Diabetic Control (DC) had very raised values of T-Chol with significant p-value (0.001*) as shown in table-2.

Table-2: Comparison of means – T Chol

	T-Chol
NC	1.32
DC	3.56
Met	1.61
NAC	1.64
Met+NAC	1.35

Significant p value < 0.05 p value <0.001

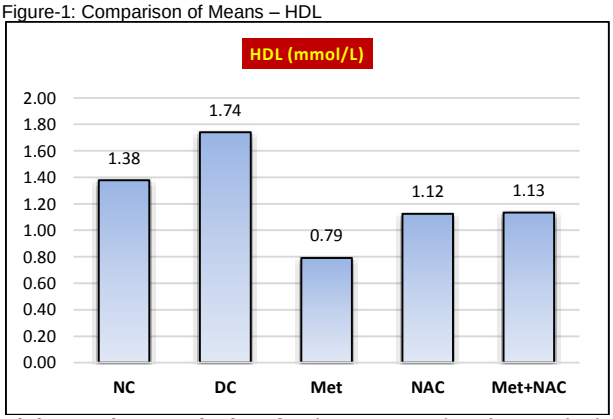
TRIGLYCEROL (TG): TG test results showed little variations in Control Group (NC), and Metformin plus N-acetylcysteine (Met+NAC) groups, while slight raised in values were observed in Metformin (Met) and N-acetylcysteine (NAC), whereas Diabetic Control group (DC) had very high value of TG with significant p-value (0.001*) as shown in table-3.

Table-3: Comparison of means – TG

	TG
NC	0.66
DC	2.63
Met	1.16
NAC	1.01
Met+NAC	0.63

Significant p value < 0.05 p value <0.001

Figure-1: Comparison of Means – HDL



High Density Protein (HDL): The tests results of HDL obtained from the samples of various groups showed little variation in Normal Control group (NC), N-acetylcysteine (NAC) and Metformin plus N-acetylcysteine (Met+NAC) groups, while HDL results were found to be high in Diabetic Control (DC) group and significantly low in Metformin group. Overall, the results were significant statistically with value of p less than 0.001 as shown in fig-1.

Low Density Protein (LDL): With regards to lipid profiling, LDL test results showed little variations in Control Group (NC) and Metformin plus N-acetylcysteine (Met+NAC) groups, while slight raised in Metformin (Met) group and N-acetylcysteine (NAC) group, however a significantly raised level in Diabetic Control (DC) group as shown in table-4.

Table-4: Comparison of means – LDL

	LDL
NC	0.13
DC	0.71
Met	0.38
NAC	0.33
Met+NAC	0/28

Significant p value < 0.05 p value <0.001

DISCUSSION

The occurrence of DM (diabetes mellitus) is on increase globally which is associated to a certain lifestyle and high rate of obesity. Persistent hyperglycemia leads to microvascular complications like retinal, renal and neural damage. Furthermore, diabetic hyperglycemia may also lead to dyslipidemia linked to a number of macrovascular problems like myocardial infarction, atherosclerosis, risk of stroke and even diabetic coma[12]. Usually people with diabetic conditions suffer misery and disturbing complications which may result into morbidity and mortality. According to the recent report of IDF (International Diabetes Federation), around 5 million deaths were noted due to various diabetes complications and almost 2.5% people have diabetes globally[13]. Thus with its rising prevalence, the requirement is to have effective strategies for its control and management. Hyperglycemia-induced oxidative stress in terms of an increased generation of ROS/RNS, GSH-Px (Glutathione per Oxide) perform a vital role in the pathogenesis of diabetes related complications[14,15].

In the study, hyperglycemia was induced STZ injection. The anti hyperglycemic effects of NAC may signify prevention of free radical formation which is STZ induced. This may also be due to increase in insulin secretion from pancreatic ß-cells however with the reduction of tissue resistance to insulin[16].

Moreover, MET is a strong anti-hyperglycemic medicine that decreases hepatic gluconeogenesis and the rate of intestinal glucose absorption. It is through enhancing glucose uptake by peripheral tissues, such as adipocytes and muscle cells that the MET raises peripheral insulin sensitivity. Numerous investigations have shown significant variations in the lipid profile, which is one of the major reasons of diabetic complexities like cardiovascular problems[17].

In line with other studies, NAC displayed significant hypolipidemic activity due to improved glycaemic control associated with the reduction in both TG and LDL levels.[18,19] Moreover, MET also showed a considerable lipid-lowering effect in rats with DM by affecting lipoprotein synthesis in the intestine by decreasing mRNA expression of genes linked with intestinal lipid homeostasis, which is also reinforced by other studies[20,21]. In this study, MET plus NAC treatment significantly improved hyperglycaemia and dyslipidaemia due to attenuating oxidative stress and raising NO. The data obtained through our study is found compatible with the findings of authors like Gupta[22] and Kashyap[23].

Limitations: Limitations included limited sample size, time frame, resources and financial constrains.

CONCLUSION

It was concluded that dyslipidemia was significantly reduced by use of N-acetylcysteine in combination with metformin among diabetic rats.

Author's contribution: NSB&SS: Conceptualized the study, analyzed the data, and formulated the initial draft, **WUK&MM:** Contributed to the proof reading, **MM, MWA & RM:** Collected data.
Conflict of interest: None
Funding: None

REFERENCES

1. Yang DK, Kang HS. Anti-Diabetic Effect of Cotreatment with Quercetin and Resveratrol in Streptozotocin-Induced Diabetic Rats. Biomol Ther (Seoul). 2018;26(2):130-8.
2. Kaga AK, Barbanera PO, Carmo NOLd, Rosa LRdO, Fernandes AAH. Effect of N-Acetylcysteine on Dyslipidemia and Carbohydrate Metabolism in STZ-Induced Diabetic Rats. International Journal of Vascular Medicine. 2018;2018:1-7.
3. Tsuboi T, Maeda M, Hayashi T. Administration of L-arginine plus L-citrulline or L-citrulline alone successfully retarded endothelial senescence. PLoS One. 2018;13(2):e0192252.
4. Jing S, Zhao Z, Wu J, Yan LJ. Antioxidative and Hypoglycemic Effect of Ta-ermi Extracts on Streptozotocin-Induced Diabetes. Diabetes Metab Syndr Obes. 2020;13:2147-55.
5. Schofield JD, Liu Y, Rao-Balakrishna P, Malik RA, Soran H. Diabetes Dyslipidemia. Diabetes Ther. 2016;7(2):203-19.
6. Markowicz-Piasecka M, Sikora J, Mateusiak Ł, Mikiciuk-Olasik E, Huttunen KM. Metformin and Its Sulfenamide Prodrugs Inhibit Human Cholinesterase Activity. Oxidative Medicine and Cellular Longevity. 2017;2017:1-11.
7. Bhatty S, Memon Z, Agha S, Ismail K, Hassan JA, Zafar U. Effectiveness of Probiotics, Metformin and Their Combination Therapy in Ameliorating Dyslipidemia Associated With PCOS. Journal of Pharmaceutical Research International. 2019:1-9.
8. Kaur M, Singh N. Synergistic effect of metformin and fenugreek on the lipid profile of type-II diabetic patients. International Journal of Basic & Clinical Pharmacology. 2019;8(8).
9. He Y, Zhou L, Fan Z, Liu S, Fang W. Palmitic acid, but not high-glucose, induced myocardial apoptosis is alleviated by Nacetylcysteine due to attenuated mitochondrial-derived ROS accumulation-induced endoplasmic reticulum stress. Cell Death Dis. 2018;9(5):568.
10. Aldini G, Altomare A, Baron G, Vistoli G, Carini M, Borsani L, et al. N-Acetylcysteine as an antioxidant and disulphide breaking agent: the reasons why. Free Radic Res. 2018;52(7):751-62.
11. Soleimani A, Habibi MR, Hasanzadeh Kiabi F, Alipour A, Habibi V, Azizi S, et al. The effect of intravenous N-acetylcysteine on prevention of atrial fibrillation after coronary artery bypass graft surgery: a double-blind, randomised, placebo-controlled trial. Kardiol Pol. 2018;76(1):99-106.
12. Faselis C, Katsimardou A, Imprialos K, Deligkaris P, Kallistratos M, Dimitriadis K. Microvascular Complications of Type 2 Diabetes Mellitus. Curr Vasc Pharmacol. 2020;18(2):117-24.
13. Stahel P, Xiao C, Nahmias A, Lewis GF. Role of the Gut in Diabetic Dyslipidemia. Front Endocrinol (Lausanne). 2020;11:116.
14. de Souza Cardoso J, Oliveira PS, Bona NP, Vasconcellos FA, Baldissarelli J, Vizzotto M, et al. Antioxidant, antihyperglycemic, and antidyslipidemic effects of Brazilian-native fruit extracts in an animal model of insulin resistance. Redox Rep. 2018;23(1):41-6.
15. John JJ, Nagar DP, Gujar NL, Bhattacharya R. Oxidative and histopathological alterations after sub-acute exposure of diisopropyl phosphorofluoridate in mice: Beneficial effect of Nacetylcysteine. Life Sci. 2019;228:98-111.
16. Song Y, Wang H, Huang H, Zhu Z. Comparison of the efficacy between NAC and metformin in treating PCOS patients: a meta-analysis. Gynecol Endocrinol. 2020;36(3):204-10.
17. Nandini HS, Naik PR. Antidiabetic, antihyperlipidemic and antioxidant effect of Vincamine, in streptozotocin-induced diabetic rats. Eur J Pharmacol. 2019;843:233-9.
18. Mada SB, Abarshi MM, Garba A, Sharehu KL, Elaigwu OP, Umar MJ, et al. Hypolipidemic effect of N-acetylcysteine against dexamethasone-induced hyperlipidemia in rats. Calabar Journal of Health Sciences. 2020;3:59-67.
19. Javanmanesh F, Kashanian M, Rahimi M, Sheikhansari N. A comparison between the effects of metformin and N-acetyl cysteine (NAC) on some metabolic and endocrine characteristics of women with polycystic ovary syndrome. Gynecol Endocrinol. 2016;32(4):285-9.
20. Wu B, Chen M, Gao Y, Hu J, Liu M, Zhang W, et al. In vivo pharmacodynamic and pharmacokinetic effects of metformin mediated by the gut microbiota in rats. Life Sci. 2019;226:185-92.
21. Hirano T. Pathophysiology of Diabetic Dyslipidemia. J Atheroscler Thromb. 2018;25(9):771-82.
22. Gupta M, Tummala R, Ghosh RK, Blumenthal C, Philip K, Bandyopadhyay D, et al. An update on pharmacotherapies in diabetic dyslipidemia. Prog Cardiovasc Dis. 2019;62(4):334-41.
23. Kashyap H, Gupta S. Analysis of Metformin on Endogenous Antioxidants and Oxidative Stress in Mice Brain Tissue of Alloxan-Induced Diabetes. International Journal of Pharmaceutical Sciences and Drug Research. 2019;11(05).