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ORIGINAL ARTICLE

COMPARATIVE STUDY: N-ACETYLCYSTEINE AND METFORMIN ON ATHEROGENIC INDEX IN DIABETIC RATS

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ABSTRACT

Background: Diabetes Mellitus (DM) is a global health issue with severe systemic health effects. A key driver of these complications is prolonged high blood sugar, which enhances the lipolysis and leads to dyslipidemia. Atherogenic index plasma (AIP) significantly heightens the risk of cardiovascular diseases (CVS) in diabetic patients. The objectives of this study were to check the novel use of N- acetylcysteine (NAC) in diabetic rats and to evaluate the comparative effects of NAC and Met on diabetes induced rats.

Materials & Methods: This randomized control trial study was undertaken at the Department of Pharmacology & Therapeutics, Army Medical College Rawalpindi in association with the National Institute of Health (NIH) Islamabad from August 2021 to October 2021. There was a total of twenty-five rats, with five in each group randomized. An intraperitoneal (IP) injection of Streptozotocin (STZ) (35 mg/kg) was given to induce a diabetic condition in the animals. Rats with blood sugar levels in excess of 300 mg/dl after 48 hours were considered to be diabetic. The rats with Diabetes were used as a diabetic control group in one group while the remaining DM rats in three groups were treated with N-acetylcysteine (NAC), Metformin (Met), and Metformin plus N-acetylcysteine (Met + NAC) respectively. The treatment lasted for four weeks. The Atherogenic Index plasma (AIP) of the groups was compared at the end of the trial period. In the statistical analysis, the whole data array was handled with the SPSS 25.0 statistical software package. The Analysis of Variance (ANOVA Test), which compares the variations within all the groups, has been employed, followed by post hoc Tukey test.

Results: There was a total of twenty-five rats, with five in each group randomized. There was a marked improvement in the Atherogenic index plasma (AIP) for the N-acetylcysteine (NAC) group, as well as for the Metformin + N-acetylcysteine (Met + NAC) group having a p value <0.001in these groups.

Conclusion: The use of antioxidants, including N-acetylcysteine, caused a significant decrease in the Atherogenic Index Plasma in a diabetes-induced model produced by Streptozotocin.

KEY WORDS: Atherogenic Index Plasma; Cardiovascular Diseases; Dyslipidemias; Hyperglycemia; Metformin; N-acetylcysteine; Streptozotocin.

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INTRODUCTION

The morbidity and mortality rates worldwide are enormously influenced by the complicated inter-relationship existing between DM and CVD, which are commonly experienced together. Dyslipidemia is considered one of the prominent risk factors for CVD in diabetic patients, apart from other factors. In this respect, a useful concept that helps to make a clearer interpretation of the complicated relationship that exists between Dyslipidemia, DM, and CVD is that of the AIP, a marker that indicates the

relationship that exists in the bloodstream between atherogenic and protective lipids.[1] DM is commonly experienced together with Dyslipidemia, characterized by high values of triglycerides (TG), Low Density Lipoprotein cholesterol (LDL-C), and reduced values of High Density Lipoprotein cholesterol (HDL-C). Insulin resistance, which is a prominent characteristic of Type 2 DM, leads to increased production of triglyceride-rich lipoproteins in the liver, as well as alterations in lipid metabolism, which contribute to the condition of Dyslipidemia. This condition that occurs in Diabetic patients is liable to increase the risks of atherosclerosis as well as Cardiovascular problems.[2] The AIP provides a complete assessment of the balance that exists in the bloodstream between atherogenic lipids, apart from the protective ones. High atherogenic potential, apart from an unfavorable profile, is a significant characteristic of high AIP, which makes a person prone to atherosclerosis as well as other serious CVD.[3,4] Calculated as: Atherogenic Index plasma (AIP) = Log 10 (TG / HDL).[5]

The naturally existing amino acid is cysteine, from which NAC, recognized for possessing high antioxidant properties, is derived. Quite a number of researches have investigated the impact of NAC on the AIP, as well as other atherosclerosis indices, for people with diabetes. It has been proven that NAC reduces oxidative stress by eliminating free radicals, thereby increasing intracellular glutathione. This has been linked to a reduction in inflammation and lipoperoxidation, which might retard the development of atherosclerosis.[6] Among the contributing factors to the development of atherosclerosis is chronic inflammation. It has been shown that NAC has anti-inflammatory properties because it inhibits signal transduction pathways and pro-inflammatory cytokines. NAC can help a patient with diabetes lessen the threat of atherosclerosis because of the reduced inflammation.[7] It has been reported that NAC improves the function of the endothelium, which is a significant component of overall vascular protection. Endothelial dysfunction is a primary causative agent in atherosclerosis. The AIP can be reduced by NAC in diabetics because it improves endothelium function.

There have been researches on the effects of Met, a medication used in the treatment of type 2 diabetic patients, on lipids as well as overall cardiovascular health. The AIP, which is an indication of the possibility of atherosclerosis, a condition characterized by the deposition of plaque within the arteries. Metformin has been investigated with variable success in researches concerning its effect on lipid profiles, together with the AIP. Met has been proven in some clinical researches to exert a favorable effect on lipid profiles by increasing high-density lipoprotein cholesterol (HDL-C), with a corresponding reduction in triglycerides, overall cholesterol, as well as low-density lipoprotein cholesterol (LDL-C).[8] The AIP might decrease because of the change in the said lipids, with a corresponding reduced threat of atherosclerosis. In addition, the effect of Met on Atherogenic indexes might vary with regards to different factors such as treatment doses, treatment periods, as well as certain patient-specific factors.[9] In conclusion, the most significant contribution of Met to a healthy cardiovascular system is from the mechanism of enhancing the sensitivity of insulin, although the drug might also have a positive impact on the AIP, even though there are no significant effects on lipid profiles. Thus, patients with type 2 diabetes not only need to manage glycemia but need to manage all of their factors that exert a negative impact on the cardiovascular system, such as diet, exercise, as well as all medications.[10] The objectives of this research were to evaluate the comparative and coupled effects of NAC and Met on diabetes induced rats to draw conclusions on possible use of these. Given its antioxidant properties, novel use of NAC may offer substantial and preventive relief in managing diabetes and related complications, for future shaping of diabetic regimen.

MATERIALS AND METHODS

The study design was randomized controlled trial which was undertaken at the Department of Pharmacology & Therapeutics, Army Medical College Rawalpindi in association with the National Institute of Health (NIH) Islamabad.

Twenty-five Sprague Dawley rats only female (to avoid pregnancy) were used for research purposes at NIH, randomly allocated to five different groups. The freshly prepared injection of Streptozotocin (IP, 35 mg/kg)[11]in a single dose was given to rats intended for inducing DM. Rats with blood sugars greater than 300 mg/dl, 48 hours following injection, were considered to be diabetic. The blood samples obtained at the onset, as well as at the completion of the research, were assessed via biochemical evaluation. Prior to the induction of DM, the animals were fasted for a period of eight hours, while towards the end of the research, the animals were fasted for a period of twelve hours, with subsequent sacrifice via inhalation of chloroform. The blood samples at onset were obtained via tail vein, while subsequently, via heart puncture.

The entire dataset has been processed with the SPSS 25.0 statistical software. The quantitative values have been expressed in mean + Standard Deviation form. For comparing the variations in all the groups, the One-Way Analysis of Variance (ANOVA) has been employed, which has further been corrected with Tukey's Post Hoc Test. The cut-off value for statistical significance has been set at $P < 0.05$.

Table 1: The Intervention Protocols Groups Categories

Groups	Interventions protocols
Group I: Group NC (Negative Control)	Rats were fed on standard diet and clean drinking water.
Group II: Group DC (Positive Control)	DM rats were fed on standard diet and clean drinking water.
Group III: DM+ Met	DM rats treated with Metformin (250 mg/kg/day)[12] administered by oral gavage for 28 days.
Group IV: DM + NAC Group	DM rats treated with NAC (25 mg/kg/day)[13] administered by oral gavage, for 28 days.
Group V: DM+ Met+ NAC Group	DM rats treated with Metformin. (250 mg/kg/day) +NAC (25 mg/kg/day) administered by oral gavage for 28 days.

RESULTS

There was a total of twenty-five rats, with five in each group randomized. An intraperitoneal (IP) injection of Streptozotocin (STZ) (35 mg/kg) was given to induce a diabetic condition in the animals. Rats with blood sugar levels in excess of 300 mg/dl after 48 hours were considered to be diabetic. TG test results showed little variations in NC group, NAC group and Met+NAC groups, while slight raised in values were observed in Met, whereas DC group had very high value of TG. All the results were significant statistically with value of p less than 0.001. As shown in table 2.

Table 2: Comparison of means – TG

COMPARISON OF MEANS - TG					
	NC	DC	Met	NAC	Met+NAC
TG	0.66	2.63	1.16	1.01	0.63
p value <0.001*					

*** Significant p value ≤ 0.05**

The tests results of HDL obtained from the samples of various groups showed little variation in NC group, NAC group, and Met+NAC groups, while HDL results were found to be high in 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 table 3.

Table 3: Comparison of Means – HDL

COMPARISON OF MEANS - HDL					
	NC	DC	Met	NAC	Met+NAC
HDL	1.38	1.74	0.79	1.12	1.13
p value <0.001*					

*** Significant p value < 0.05**

In terms of AIP, Met+NAC group showed a substantial improvement over DC group. In contrast, the NAC group showed little improvement and the Met showed no such improvement in AIP. Table 4 along with the Figure 1 display all of the statistically significant outcomes, with p values less than 0.001.

Table 4: Comparison of Means – Atherogenic Index Plasma (AIP)

COMPARISON OF MEANS - ATHEROGENIC INDEX PLASMA					
	NC	DC	Met	NAC	Met+NAC
AIP	0.64	1.50	1.49	0.90	0.56
p value <0.001*					

*** Significant p value < 0.05**

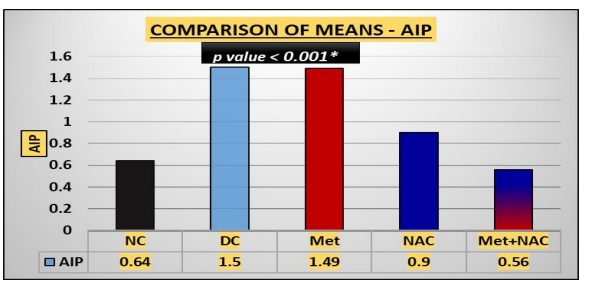

Figure 1: Comparison of Means – AIP

DISCUSSION

Diabetes has been identified as a serious health issue. It is a fact that over 8.8% of the population worldwide is suffering from this disease. The World Health Organization forecasts that the number of patients suffering from DM can increase to twice the existing numbers. Elevated blood glucose is the fundamental cause of a variety of conditions, such as metabolic disorders, renal problems, retinopathy, neuropathy, changes in the lipids profile and atherosclerosis.[14]

The use of these antioxidants, especially when used alongside medications, is distinct. It has the potential to halt, at a minimum, the emergence of the condition linked with metabolic syndromes, as well as side effects. Antioxidants have, in this case, been shown to reduce the glycemic indices of DM. The complicated etiology of DM triggers a shift in medication use from single medication to a cocktail approach as enunciated by Al-Nami.[15] Various other research findings also indicate that taking a combination of

antioxidant and synthetic medications for treatment is far more efficacious than merely taking one medication; as concluded by Sun.[16] This research was intended to examine the novel use of antioxidant NAC in comparison and coupled with Met. The empirical evidence showed anti-dyslipidemia effect of both NAC and Met stalling the emergence of the conditions linked with metabolic syndromes, like cardiovascular complications, found consistent with the findings of Abdel[17] and Li.[18]

In a normal state, the body's defense mechanisms are capable of destroying adequate ROS; hence, the extra production of ROS together with DM further reduced the use of carbs by cells, thus resulting in problems related to the metabolism of proteins and fats, thus proved to contribute towards fluctuating redox states of cells as enunciated by Ghasemi.[19] On other hand, an increase in ROS causes the deficiency of endogenous antioxidants leading to a rise in lipid peroxidation as well as a reduction in Glutathione Peroxide Activity (GSH-Px) and Superoxide Dismutase (SOD), which further increases the oxidative stress.[20] In this study, the antioxidant like NAC because of its potency in protecting membrane-bound lipoprotein lipase from lipid peroxides effectively reduced the serum TG and LDL. This decrease in TG and LDL might have also attributed to better glycemic control; while on the other hand, increase in HDL was observed, most probably linked to mechanisms involving increased activity of insulin supported by antioxidant-NAC. These empirically supported effects of antioxidant have also been endorsed in the studies of Xing.[21]

The findings of this study on the risk stratification and therapeutic targets affirmed that the AIP provided valuable insights into how persons with diabetes are stratified in terms of cardiovascular risk and atherosclerosis, consistent with the study of Cui.[22] Similarly, the primary target for the treatment of dyslipidemia in patients with diabetes is the reduction of AIP values, which cannot be achieved only by typical pharmacologic interventions (Metformin) therefore warranting rigorous modification strategies like adding some antioxidant which not only improves dyslipidemia, AIP but also the glycemic indices Blahova[23] and Panahi.[24]

CONCLUSION

It has been concluded that the use of the antioxidant-NAC coupled with traditional treatment Met, significantly lowered the AIP in the STZ-induced diabetic rats. The use of the antioxidant-NAC additionally exhibited a hypo-lipidemic effect, which is also known to be very effective for treating paracetamol toxicity and as mucolytic with reduced side effects and safety profile. The anti-atherogenic effect of NAC has been proven to be significantly better compared to the established medication Met. The stand-alone use of Met lacks the anti-atherogenic effect in comparison when used together with the antioxidant-NAC.

Restrictions: The sample size, time constraints, available resources and budgetary restrictions were among the limitations.

REFERENCES

1. Al-Shaer MH, Elzaky MM, Farag ESM, Saad MOM. Atherogenic index of plasma as a marker of coronary artery disease in type 2 diabetes mellitus patients. Indian J Clin Cardiol. 2021;2(4):217-221. https://doi.org/10.1177/26324636211031362
2. Tenório MCS, Graciliano NG, Moura FA, Oliveira ACM, Goulart MOF. N-acetylcysteine (NAC): impacts on human health. Antioxidants (Basel). 2021;10(6):967. https://doi.org/10.3390/antiox10060967
3. Fu L, Zhou Y, Sun J, Zhu Z, Xing Z, Zhou S, et al. Atherogenic index of plasma and major adverse cardiovascular events in patients with type 2 diabetes mellitus. Cardiovasc Diabetol. 2021;20:1-11. https://doi.org/10.1186/s12933-021-01393-5
4. Domínguez MAM, González MLZ, Muñiz ERZ, Varela VAM, Nájera CAI, Robledo NSF, et al. Inflammation and atherogenic markers in type 2 diabetes mellitus. Clin Investig Arterioscler (Engl Ed). 2022;34(3):105-112. https://doi.org/10.1177/00033197211012129
5. Zhou K, Qin Z, Tian J, Cui K, Yan Y, Lyu S. Atherogenic index of plasma as a predictor for coronary artery disease in type 2 diabetes patients. Angiology. 2021;72(10):934-941.
6. Kalamkar S, Acharya J, Kolappurath Madathil A, Gajjar V, Divate U, Karandikar-Iyer S, et al. Long-term glutathione supplementation improves oxidative damage and HbA1c in elderly type 2 diabetic patients: a randomized clinical trial. Antioxidants (Basel). 2022;11(5):1026. https://doi.org/10.3390/antiox11051026
7. Johnson R, Sangweni NF, Mabhida SE, Dludla PV, Mabasa L, Riedel S, et al. Combination effect of metformin and N-acetylcysteine against hyperglycaemia-induced cardiac damage: an in vitro study. Nutrients. 2019;11(12):2850. https://doi.org/10.3390/nu11122850
8. Poznyak AV, Litvinova L, Poggio P, Moschetta D, Sukhorukov VN, Orekhov AN. From diabetes to atherosclerosis: potential of metformin for cardiovascular disease management. Curr Pharm Des. 2022;28(16):1324-1336. https://doi.org/10.2174/1381612828666220310142620
9. Kheniser KG, Kashyap SR, Kasumov T. Effects of metformin on lipoprotein modification and function: a systematic review. Obes Sci Pract. 2019;5:36-45. https://doi.org/10.1002/osp4.323
10. Sundararajan S, Jayachandran I, Pandey GK, Venkatesan S, Rajagopal A, Gokulakrishnan K, et al. Metformin reduces atherogenesis via the Sestrin2-mTOR pathway in obese and diabetic rats. J Lipid Atheroscler. 2023;12(3):290-306. https://doi.org/10.12997/jla.2023.12.3.290

11. Li M, Hu X, Xu Y, Hu X, Zhang C, Pang S. A possible mechanism of metformin in improving insulin resistance in diabetic rat models. Int J Endocrinol. 2019;2019:3248527. https://doi.org/10.1155/2019/3248527
12. Rosa LR, Kaga AK, Barbanera PO, Queiroz PM, do Carmo NO, Fernandes AA. Beneficial effects of N-acetylcysteine on hepatic oxidative stress in streptozotocin-induced diabetic rats. Can J Physiol Pharmacol. 2018;96(4):412-418. https://doi.org/10.1139/cjpp-2017-0598
13. Shen X, Wang L, Zhou N, Gai S, Liu X, Zhang S. Beneficial effects of combination therapy of phloretin and metformin in streptozotocin-induced diabetic rats and improved insulin sensitivity in vitro. Food Funct. 2020;11(1):392-403. https://doi.org/10.1039/c9fo01912b
14. Xu J, Zhou H, Xiang G. Association of atherogenic index of plasma with retinopathy and nephropathy in adult patients with type 2 diabetes mellitus aged> 18 years. Canadian journal of diabetes. 2022 Oct 1;46(7):708-14. https://doi.org/10.1016/j.jcjd.2022.03.006
15. Al-Nami MS, Al-Kuraishy HM, Al-Gareeb AI. Thioctic acid effects on glycemic indices and endothelial dysfunction in type 2 diabetes mellitus. Int J Crit Illn Inj Sci. 2020;10(Suppl 1):21-27. https://doi.org/10.4103/IJCIIS.IJCIIS_80_19
16. Sun M, Lu Z, Chen WM, Lv S, Fu N, Yang Yet al. N-acetylcysteine therapy reduces major adverse cardiovascular events in patients with type 2 diabetes mellitus. Atherosclerosis. 2025 Mar 1;402:119117. https://doi.org/10.1016/j.atherosclerosis.2025.119117
17. Abdel-Moneim AM, Lutfi MF, Alsharidah AS, Shaker G, Faisal Wet al. Short-term treatment of metformin and glipizide on oxidative stress, lipid profile and renal function in a rat model with diabetes mellitus. Applied Sciences. 2022 Feb 15;12(4):2019. https://doi.org/10.3390/app12042019
18. Li X, Zou J, Lin A, Chi J, Hao H, Chen H, Liu Z. Oxidative stress, endothelial dysfunction, and N-acetylcysteine in type 2 diabetes mellitus. Antioxidants & Redox Signaling. 2024 Jun 1;40(16-18):968-89. https://doi.org/10.1089/ars.2023.0278
19. Ghasemi-Dehnoo M, Amini-Khoei H, Lorigooini Z, Rafieian-Kopaei M. Oxidative stress and antioxidants in diabetes mellitus. Asian Pac J Trop Med. 2020;13(10):431-438. https://doi.org/10.4103/1995-7645.288651
20. Naz R, Saqib F, Awadallah S, Wahid M, Latif MF, Iqbal I, et al. Food polyphenols and type II diabetes mellitus: mechanisms and pharmacology. Molecules. 2023;28(10):3996. https://doi.org/10.3390/molecules28103996
21. Xing S, Guo Z, Lang J, Zhou M, Cao J, He H et al. N-Acetyl-l-cysteine ameliorates gestational diabetes mellitus by inhibiting oxidative stress. Gynecological Endocrinology. 2023 Dec 14;39(1):2189969. https://doi.org/10.1080/09513590.2023.2189969
22. Cui M, Xiao X, Zhao X, Wang Y, Gang X, Wang G. Association between the atherogenic index of plasma and chronic kidney disease: an evaluation of an adult population of American patients with diabetes mellitus. Renal Failure. 2025 Dec 31;47(1):2556495. https://doi.org/10.1080/0886022X.2025.2556495
23. Blahova J, Martiniakova M, Babikova M, Kovacova V, Mondockova V, Omelka R. Pharmaceutical and natural products for treatment of type 2 diabetes mellitus. Pharmaceuticals (Basel). 2021;14(8):806. https://doi.org/10.3390/ph14080806
24. Panahi Y, Ostadmohammadi V, Raygan F, Sharif MR, Sahebkar A. The effects of N-acetylcysteine administration on metabolic status and serum adiponectin levels in patients with metabolic syndrome: A randomized, double-blind, placebo-controlled trial. Journal of Functional Foods. 2022 Dec 1;99:105299. https://doi.org/10.1016/j.jff.2022.105299

CONFLICT OF INTEREST
Authors declare no conflict of interest.
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AUTHORS' CONTRIBUTION

The following authors have made substantial contributions to the manuscript as under:

Conception or Design:	NSB, ZI
Acquisition, Analysis or Interpretation of Data:	NSB, ZI, MM, SMA, MI
Manuscript Writing & Approval:	NSB, ZI, MM, SMA, QTAH

All the authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.