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**ORIGINAL ARTICLE**

# Comparative Study of Metformin, N-Acetylcysteine and L-Arginine on Hyperglycemia in Streptozotocin-Induced Diabetic Rats

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## ABSTRACT

Diabetes mellitus (DM) is a chronic metabolic syndrome which results from deficiencies/defects in the pancreatic insulin secretions or insulin action on the target tissues.

**Purpose**: To compare the beneficial effects of Metformin, N -acetylcysteine and L-arginine on dyslipidemia and hyperglycemia in Streptozotocin-induced diabetic rats.

**Study Design:** Randomized controlled trial.

**Methodology:** A total of 35 rats were used with 5 rats randomly allocated in 7 groups. Diabetic model was created by using streptozotocin (35 mg/kg) single dose intraperitoneal (IP). After 48 hours the animals with blood sugar concentration more than 300 mg/dl were considered diabetic. First group and second group were controls with Diabetic Mellitus (DM) rats. The group three, four and five with diabetic rats were given Metformin, N -acetylcysteine (NAC) and L-arginine (L-Arg) respectively. The sixth group of diabetic rats was given combination of Metformin and NAC while the seventh group of diabetic rats was given Metformin and L-Arg. All groups were treated for four weeks. At the end of the experimental period, groups were compared for fasting blood glucose.

**Statistical analysis**: SPSS 25.0 statistical software was used to analyze the whole data. The difference between all the groups was analyzed using One-Way Analysis of Variance (ANOVA).

**Results:** Significant improvement, with regards to the BSF test in L-Arginine Group, Metformin plus L-Arginine Group and the Metformin plus N-acetylcysteine group.

**Conclusion**: It was concluded that in streptozotocin induced diabetic model, hyperglycaemia was significantly attenuated by the use of novel antioxidants; L-Arginine and N-acetylcysteine.

**Keywords**: Hyperglycemia, L-arginine, Metformin, N-acetylcysteine and Streptozotocin.

## INTRODUCTION

Diabetes mellitus (DM) is defined as a chronic metabolic syndrome which results from deficiencies/defects in the pancreatic insulin secretions or insulin action on the target tissues. The enhanced rate of mortality and morbidity linked to DM is generally connected to macro and micro-vascular complications[1]. Glucose remains the main oxidizable substrate in number of cell categories. The catabolic pathways like gluconeogenesis and glycogenolysis, in a diabetic state, are activated in an attempt to keep homeostasis. In this case, the lipolysis is increased thus activating dyslipidemia, a risk factor for atherosclerosis that affects almost 97% of diabetic patients[2]. DM and dyslipidemia are significant cardiovascular risk factors and diabetic atherosclerosis may grow due to rise in "Reactive Oxygen Species" (ROS) and a decline in "Nitric Oxide" (NO) bioavailability owing to high level of plasma glucose[3].

The International Diabetes Federation (IDF) predicted in 2015 that 415 million diabetic population of the World may increase to 642 million by 2040. Despite access to diverse anti diabetic agents which control hyperglycaemia, the therapeutic options targeting other disorders mostly related to DM, like oxidative stress and dyslipidemia have also been a major emphasis in research. It is therefore important to explore new agents as well as the strategies to manage DM related dyslipidemia, hyperglycemia and other DM complications[4]. The usage of natural substances with hypoglycemic and anti-oxidant properties have proved to improve chronic degenerative disease such as DM and the related complications.

Metformin is presently one of the most commonly recommended medicine for treatment of Type-2 DM[5]. Metformin decreases oxidative stress and the superoxide free radical in the platelets. Metformin may also lessen the development of advanced glycation end-products directly through an insulin independent mechanism and indirectly through decline of hyperglycaemia[6]. Nevertheless, Metformin remains controversial because as per few human studies, it exhibited harmful effects on renal function, resulting in increased rates of acute dialysis and mortality[7].

L-arginine can be labelled as a provisionally crucial amino acid used by human body for function of immune cell, urea cycle, protein synthesis and tissue repairing. Besides, insufficiency of L-arginine is related to an oxidative process and a number of inflammatory actions in the vascular endothelium, which may result into blood flow dysfunction. The L-arginine's antioxidant effects may be owing to the dropping strength of radical reactions or reduced superoxide anion which are released from the

endothelial cells, thus lessening the oxidative stress during the process[8].

L-arginine is a precursor of NO that affects insulin release as demonstrated in animals in vivo, vitro and in diabetic and healthy humans. There are number of mechanisms of NO on glucose homeostasis proposed in various studies. Through vasodilation of pancreatic islet blood flow, the nitric oxide enhances the insulin secretions. It also enhances the GLUT4 translocation which is insulin independent hence causing increased uptake of glucose by adipose tissue and skeletal muscles. These major effects of L-Arg on lipid metabolism show its ability to increase NO level and also regulate the expression of lipid metabolic genes. L-Arg has numerous useful effects on cardiovascular system as well. Hence supplying L-Arg may lead to development of NO and improve endothelium in atherosclerotic disorder[9].

N-acetylcysteine (NAC) generates amino acid L-cysteine and later the antioxidant glutathione (GSH). NAC has anti-oxidant features; its structure contains a sulph-hydryl radical, which causes neutralization of ROS or direct sifting of oxidizing agents. It controls state of redox of NMDA (N-methyl-D-aspartate) and AMPA (amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptors which are the neuro-transmitters and hinders the nuclear factor kappa-light-chain-enhancer of the activated B cells (NF-B) to control cytokine synthesis (anti/pro-inflammatory). In contrast to GSH, NAC has enhanced topical and oral bio-availability. Studies have concluded that NAC, which is an enhancer of NO, is also capable of lessening blood glucose scales, possibly due to its potential to induce secretion of insulin. Moreover, few other reports suggest that NAC lowers the severity of atherosclerosis and reduces the cholesterol concentration, its triacylglycerols and lipoprotein fractions through stabilizing the development of atherosclerotic plaque[10].

NAC and L-Arg with antioxidant features have a significant value for the prevention, management and treatment of diabetes related problems. This is because of their ability to maintain glucose homeostasis, lessen oxidative stress and dyslipidemia, improving production of NO and arterial endothelium in the atherosclerotic disorder[11].

**Objectives:**To compare the beneficial effects of Metformin, N -acetylcysteine and L-arginine on dyslipidemia and hyperglycemia in Streptozotocin-induced diabetic rats**.**

## METHODOLOGY

Total of 35 rats were used, with 5 rats randomly allocated in 7 groups. Diabetic model is created by using streptozotocin (35 mg/kg) single dose intraperitoneal (IP). After 48 hours the animals with blood sugar concentration more than 300 mg/dl were considered diabetic.

Blood samples were taken for biochemical assessment at the start and end of the experimental period. Initial samples were taken from the tail vein after keeping the rats fasted for 8 hrs, before the induction of DM, while at the end of experimental period, animals were kept fasted over twelve hours, euthanized with inhaled chloroform. The sample were then obtained via cardiac puncture[12].

**Statistical analysis**: SPSS 25.0 statistical software was used to analyze the whole data. The quantitative variables were summarized using mean + SD. The difference between all the groups was analyzed using One-Way Analysis of Variance (ANOVA) which was followed by Tukey's Post Hoc correction for multiple comparisons. A value of $P < 0.05$ was considered statistically significant.

Table 1: The Intervention Protocols

| Groups | Categories | Intervention |
|---|---|---|
| I | Negative control | Fed on standard diet |
| II | Positive control | Fed on standard diet |
| III | DM+Metformin | Metformin (250mg/kg/day) for 28 days given |
| IV | DM+L-Arg | L-Arg (200mg/kg/day) for 28 days given |
| V | DM+NAC | NAC (25mg/kg/day) for 28 days given |
| VI | DM+Metformin+ L-Arg | Metformin (250mg/kg/day) + L-Arg (200mg/kg/day) for 28 days given |
| VII | DM+Metformin+ NAC | Metformin (250mg/kg/day) + NAC (25mg/kg/day) for 28 days given |

## RESULTS

With regards to the BSF test, the results show significant improvement, in comparison to Diabetic Control (DC) which had highest values, in L-Arginine Group (L-Arg), Metformin plus L-Arginine Group (Met+L-Arg) and the Metformin plus N-acetylcysteine (Met+NAC) group, while N-acetylcysteine (NAC) group, and Metformin Group (Met) showed little improvement in BSF. All the results were statistically significant with p value less than 0.001 (Table-2).

Table 2: COMPARISON OF MEANS – BSF*

| | NC | DC | Met | L-Arg | NAC | Met+ L-Arg | Met+NAC |
|---|---|---|---|---|---|---|---|
| BSF | 109.00 | 379.40 | 183.80 | 147.80 | 198.00 | 151.00 | 139.20 |
| p-value <0.001* | | | | | | | |

* Statistically Significant

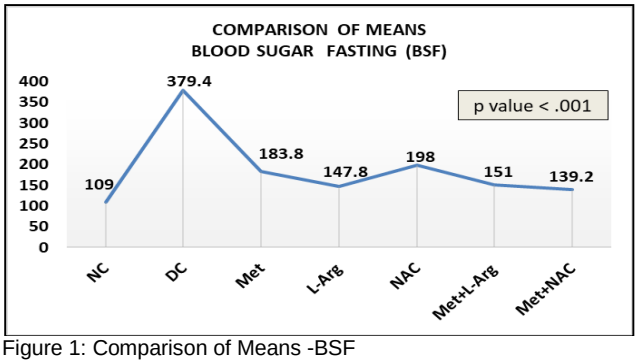


Figure 1: Comparison of Means -BSF

## DISCUSSION

DM has become a leading public health problem. Currently over 8.8% of global population is suffering from DM. Protracted blood glucose level is the major reason of various complications such as nephropathy, retinopathy, neuropathy, metabolic disorders, weakened antioxidant defence system and variations in lipid profile and atherosclerosis[13]. Complex aetiology of diabetes has

affected shifting patterns of medication, i.e. starting from monotherapy to a set of combination therapies. Numerous studies substantiate the fact that the combination therapy of synthetic drug and antioxidant drug is far better than a single therapy approach[14].

According to the prediction of World Health Organization (WHO), the figure of diabetic patients will grow double by year 2025 worldwide; i.e. reaching to new figures of around 300 million from the existing 150 million cases. DM is concomitant with ROS (Reactive Oxygen Species), leading to oxidative stress, which stimulates variation in the cellular redox state in chronic hyperglycaemia and reduces the capability of tissues to use carbohydrates, leading to metabolic disorders in proteins and fats[15].

Furthermore, this aetiology is coupled with discrepancy between the oxidant and antioxidant status, i.e., amplified production of ROS and/or weakening in an antioxidant protection systems. In this perspective, experimental data supports that prolonged higher blood glucose levels add to the production of ROS, through numerous processes such as oxidation of proteins, the non-enzymatic glycation of protein and glucose autoxidation, hence worsening oxidative stress[16].

While many of the studies suggest that the hyperglycaemia in DM adds to oxidative stress, it is also being proposed that nutritional supplement and augmentation of antioxidants may lessen the oxidative stress and may also guard tissues against from ROS damage. Such supplementation has been associated with a lowering in the occurrence of several degenerative diseases like DM and its complexities[17]. This study is designed to evaluate the effects of N-acetylcysteine (NAC) and L-arginine (L-Arg) with antioxidant and hypoglycemic characteristics and compare with Metformin on STZ – induced diabetic model.

In this respect, treatment with antioxidants like N-acetylcysteine and L-arginine especially in combination with standard antidiabetic drugs is novel. This may also preclude or delay development of metabolic syndrome and its complications.

The present data showed that antioxidants lowered the glycaemic index in diabetes exhibiting consistency with the studies done by Yousefi[18] and Kumawat[19]. Improved glycaemic control could be associated with the reduction in both TG and LDL levels however an increase in HDL is most likely linked to mechanisms involving increased insulin functions promoted by antioxidants.

The efficacy of antioxidant with respect to reducing serum TG and LDL can be linked to its safeguarding of membrane-bound lipoprotein lipase against lipid peroxide[20]. Decrease in the concentration of VLDL (Very Low Density Lipoprotein) and subsequently the LDL levels, might be due to the anti-hyperglycaemic effects of antioxidants hence improved diabetic state. These together showed that better glycemic control on the rise in oxidized LDL is promoted by ROS in DM.

The antioxidant defence systems removed adequate amounts of ROS under usual conditions however the extra creation of ROS, due to a depleted endogenous antioxidant system contributes to a raise in lipid peroxidation and reduction in Glutathione per oxide activity (GSH-Px) and Superoxide Dismutase (SOD). It aggravates oxidative stress, mediated mainly by hyperglycaemia.

The reduction in antioxidant enzyme activity under diabetic circumstances could be attributed to glycation of these enzyme, which is taking place at persistently raised blood glucose levels. Glycation of SOD reduces its activity, leading to inadequate dismutation of superoxide anions ($O^-_2$), established a correlation between the inhibition of protein glycation and improved glycemic control thus causing a raise in SOD activity as previously enunciated by Teixeira and Alves[21].

Oxidative stress is the outcome of a redox disparity between the production of ROS and the compensatory response from network of endogenous antioxidant. Meanwhile consensus is still lacking regarding the changes in the actions of antioxidant enzymes in rats with DM. Though few studies following the activities of SOD in DM exhibited drops in the levels of these enzymes, yet other studies reported enhanced activities in streptozotocin-induced diabetic rats. This raise in SOD activity could be attributed to enhanced production of superoxide, and $H_2O_2$ acting as an inducer of tissue SOD[22].

Lipid hydroperoxide is enhanced in the serum of Type-1 DM experimental models and is also used as an indirect biomarker to measure the level of oxidative stress. The enhanced levels of hydroperoxide in the serum increased the generation of ROS in DM[23].

Antioxidant treatment could preclude a disorder in the protection mechanism against the harmful cellular and biomolecular effects which could lead to modifications in cell functions. As the DM is related to enhanced oxidative stress as a result of persistent hyperglycaemia, augmenting antioxidant to regulate glycaemia, had a shielding impact against lipid peroxidation in DM. This finding is pursuant to the study of Aldini[24].

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

## CONCLUSION

It was concluded that that in streptozotocin induced diabetic model, the hyperglycaemia is significantly attenuated by the use of novel antioxidants; L-Arg and NAC. L-Arg is an anti-oxidant supplement known for safety profile and minimal adverse effects, has significant anti-hyperglycaemic activity. NAC is an antioxidant known for the treatment of paracetamol poisoning with safety profile and minimal adverse effect, also has significant anti-hyperglycaemic activity. When comparing the anti-hyperglycaemic activity with standard drug Metformin, L-Arg proved to possess better activity. When the standard drug Metformin is given in the combination with the antioxidants (L-Arg and NAC), a better anti-hypoglycaemic activity is observed than Metformin given standalone.

**Author's contribution:** NSB&MWA: Conceptualized the study, analyzed the data, and formulated the initial draft.

RUA&AMA: Contributed to the proof reading.

SK&QUA: Collected data.

TL: Contributed to the proofreading the manuscript for intellectual content.

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