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# Effect of Drugs Used in the Treatment of Diabetes & Hypertension in Urea & the Creatinine Level in Streptozotocin Induced Diabetes in Rats

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***Author's contribution***

*The sole author designed, analyzed and interpreted and prepared the manuscript.*

***Article Information***

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## ABSTRACT

Co-morbidity of diabetes and hypertension have severe effects on kidney function, these effects are due to the elevated pressure in the kidney due to hypertension and the oxidative stress caused by hyper glycemic. Uncontrolled hyperglycemia and hypertension lead to the growth of micro and macro vascular complications. Patients suffering from these diseases usually are treated with calcium channel blocking agents, aspirin and metformin. A combination of medications may have beneficial or adverse effects on the patients. The objective of this study is to see the effect of this combination on kidney function by measuring the serum levels of urea and creatinine in diabetic rats.

Blood samples were studied before treatment and after 10 days and analyzed for urea and creatinine. ANOVA test was done to compare the urea and creatinine levels in rats with STZ induced hyperglycemia after 10 days treatment. In case of urea and creatinine, there was a significant difference ($p < 0.000$) between the group treated with metformin alone and the control group, (Metformin + Nifedipine) group, and (Metformin + Aspirin) group. Again, there was no significant difference ($P > 0.197$) in creatinine level between the groups treated with (metformin +

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aspirin), (metformin + Nifedipine). These results conclude that using metformin when there is kidney dysfunction may aggravate the situation while the combination of metformin with aspirin and or with nifedipine as in case of diabetes associated with hypertension may be beneficial in attenuating the complications of diabetes and hypertension on the kidney, a further study may be needed to confirm this finding.

*Keywords: Hypertension; hyperglycemia; calcium channel blockers; streptozotocin.*

## 1. INTRODUCTION

Type 2 diabetes is usually complicated by other medical conditions, these include obesity, dyslipidemia, hypertension, albuminuria, cardiovascular disease and chronic kidney disease. Up to 75% of adults with diabetes also suffer from hypertension, and patients with hypertension alone often show evidence of insulin resistance [1].

Diabetes mellitus results from a defect in insulin secretion, insulin action, or both. Insulin deficiency leads to chronic hyperglycemia (very high blood glucose levels) [2]. Diabetes mellitus (DM) is classified into two class type 1; insulin dependent (IDDM) and type -2 non -insulin dependent (NIDDM). Type 1 DM is characterized by destruction of the pancreas β cells [3]. Individuals with type 2 DM exhibits a gradual change in glucose homeostasis due to insulin resistance and/or decreased insulin secretion [4].

Sustained hyperglycemia leads to the progressive development of micro vascular and macro vascular complications [5,6]. Glycemic control is the mainstay for preventing the progression of diabetic complications, but there is far less evidence that these interventions reverse diabetic complications [7]. Also, limitations in intensive glycemic treatment such as difficulty in achieving and/or maintaining tight glycemic control [8].

Micro vascular complications manifested by diabetic nephropathy, neuropathy and retinopathy, while macro vascular complications cause coronary artery disease, peripheral arterial disease and stroke [6].

Furthermore, some patients with diabetes may continue to experience complications despite subsequent glycemic control, a phenomenon termed metabolic memory which can be a major challenge in clinical management.

## 2. JUSTIFICATION OF THE STUDY

As diabetes, induce micro-vascular and macro-vascular complications and as diabetic patients may suffer from other chronic disease like hypertension we would like to see the effect of the combination of these medications on the complications associated with diabetes mellitus and hypertension, especially the plasma levels of urea & creatinine.

## 3. MATERIALS AND METHODS

### 3.1 Chemicals

Streptozocin (Manufactured by Sigma- Aldrich, USA) was purchased from a local distributor in KSA; Sodium Citrate (Manufactured by LobelChemie, laboratory reagents & fine chemical, India) was availed from the laboratory stock at the Northern Border University. Metformin (Glucophage 500 mg, Merck santé, France), Aspirin 100 mg (Bayer, Germany) and Nifedipine (Adalat 20 mg, Bayer, Germany) were purchased from the local market.

### 3.2 Method of Analysis

Samples were analyzed for serum creatinine and urea using the kits of Human Diagnostic Worldwide. Max –Planck-ring 21. 650205 Wiesbaden-Germany.

### 3.3 Statistical Analysis

Data was analyzed using SPSS software applying one way ANOVA. Data is considered significant for $P < 0.05$.

### 3.4 Experimental Animals

Wistar albino rats weighing 150-250 GM of either sex were obtained from the animal house of the faculty of pharmacy, at Northern Border University. The animals were housed in a standard controlled animal care facility. The animals were maintained under standard nutritional and environmental conditions throughout the experiment as per the guidelines of the ethics committee at the northern border university, KSA. Water was given ad libitum.

### 3.5 Induction of Diabetes

Rats were fasted overnight before inducing diabetes with streptozotocin. Streptozotocin is used to induce both types I and Type II diabetes based on the dose, i.e. it is concentration dependent, for the induction of type I, 60 mg/kg is given intraperitoneally or intravenously, and for type II induction 40-50 mg/kg is the usual dose used [9-11]. In this study the rats were given an intraperitoneal injection of streptozotocin (50 mg kg−1) freshly prepared in 0.1 M sodium citrate buffer. The diabetic state was confirmed 72 h after streptozotocin injection. A threshold value of fasting blood glucose was taken as >200 mg $dL^{-1}$. Diabetic rats were weighed and matched for body weight and divided into following groups consisting five animals each:

- Group 1: Diabetic control
- Group 2: Diabetic rats administered Metformin 100 mg/kg (10 days, p.o.)...
- Group 3: Diabetic rats administered Metformin 100 mg/ kg (10 days, p.o.) + Aspirin 100 mg/kg (10 days, p.o.).
- Group 4: Diabetic rats administered Metformin 100 mg/kg (10 days, p.o.) + Nifedippine 20 mg/kg (10 days, p.o.).

## 4. RESULTS

Table 1 shows the mean, the standard deviation, and the standard error for different groups regarding the creatinine level and urea level. Table 2 shows an ANOVA test for creatinine. The result shows that there is a significant difference between and within the different groups. Table 3, shows Post Hoc Test; multiple comparison for creatinine level in the different groups. The result in this table shows that there is a significant difference between the individual groups. From this table it is clear that there is a significant difference ($P < 0.000$) between the group treated with metformin + Aspirin and the group with Metformin only. There is also a significant difference ($P < 0.000$) between the group treated with Metformin + Nifedipine (group-4) and the group treated with Metformin only (group 2), and again, there is a significant difference ($P< 0.000$) between the group treated with metformin (Group 2) and the group with induced hyperglycemia using STZ (Group 1) which are not treated with any medication. Finally, this table shows that there is no significant difference ($P > 0. 197$) between the group treated with Metformin + Aspirin (Group 3) and the group treated with Metformin + Nifedipine (Group 4). Table 4 shows an ANOVA test for urea level in the different groups. The result shows that there is a significant difference between and within the different groups. Table 5 shows the Post Hoc Test, multiple comparisons for urea level for the different groups. The result in this table shows that there is a significant difference ($P < 0.001$) between the groups administered Metformin only (Group 2) and the group with induced diabetes using STZ (Group 1). Also the result shows that there is no significant difference ($P > 0.955$) between the group treated with metformin + Nifedipine (Group 4) and the diabetic group not treated with any medication STZ (Group 1). Again, there is no significant difference ($P > 0.349$) between the group administered Metformin + Nifedipine (Group 4) and the group treated with Metformin + Aspirin (Group 3).

**Table 1. Mean, standard deviation and standard error of creatinine and urea for the different groups**

| Group | Mean | Std deviation | Std error |
|---|---|---|---|
| **Creatinine** | | | |
| Group 1 | 0.0200 | 0.0000 | 0.0000 |
| Group 2 | 0.5350 | 0.02121 | 0.01500 |
| Group 3 | 0.3200 | 0.02646 | 0.01183 |
| Group 4 | 0.2767 | 0.03512 | 0.02028 |
| **Urea** | | | |
| Group 1 | 83.5300 | 5.20967 | 3.00781 |
| Group 2 | 112.0000 | 6.00797 | 3.46870 |
| Group 3 | 73.5367 | 5.77426 | 3.33377 |
| Group 4 | 81.2923 | 5.92769 | 2.96385 |

*Group 1 = Diabetic control, Group 2= group treated with metformin, Group 3= Metformin + Aspirin, Group 4 = Metformin + Nifedipine*

**Table 2. Analysis of variance (ANOVA) for the creatinine**

| | Sum of Squares | Sig |
|---|---|---|
| Between groups | 0.271 | 0.000 |
| Within groups | 0.006 | |
| Total | 0.276 | |

## 5. DISCUSSION

Abdulqadir et al. [12] reported that Metformin attenuates streptozotocin-induced diabetic complications in rats through modulation of oxidative stress gene expression.

**Table 3. Post Hoc Tests; multiple comparisons for creatinine**

| (1) Factor | ( j) Factor | Mean difference (1- j) | Std. error | Sig. |
|---|---|---|---|---|
| | Group 3 | 0.21500 | 0.02237 | 0.000 |
| Group 2 | Group 1 | 0.51500 | 0.02673 | 0.000 |
| | Group 4 | 0.25833 | 0.02440 | 0.000 |
| | Group 2 | 0.21500 | 0.02237 | 0.000 |
| Group 3 | Group 1 | 0.30000 | 0.02237 | 0.000 |
| | Group 4 | 0.04333 | 0.01952 | 0.197 |
| | Group 2 | 0.51500 | 0.02673 | 0.000 |
| Group 1 | Group 3 | 0.30000 | 0.02237 | 0.000 |
| | Group 4 | 0.25667 | 0.02440 | 0.000 |
| | Group 2 | 0.25833 | 0.02440 | 0.000 |
| Group 4 | Group 3 | 0.04333 | 0.01952 | 0.197 |
| | Group 1 | 0.25667 | 0.02440 | 0.000 |
| | Group 3 | 0.21500 | 0.02237 | 0.000 |
| Group 2 | Group 1 | 0.51500 | 0.02673 | 0.000 |
| | Group 4 | 0.25833 | 0.02440 | 0.000 |
| | Group 2 | 0.21500 | 0.02237 | 0.000 |
| Group 3 | Group 1 | 0.30000 | 0.02237 | 0.000 |
| | Group 4 | 0.04333 | 0.01952 | 0.255 |
| | Group 2 | 0.51500 | 0.02673 | 0.000 |
| Group 1 | Group 3 | 0.30000 | 0.02237 | 0.000 |
| | Group 4 | 0.25667 | 0.02440 | 0.000 |
| | Group 2 | 0.25833 | 0.02440 | 0.000 |
| Group 4 | Group 3 | 0.04333 | 0.01952 | 0.255 |
| | Group 1 | 0.25667 | 0.02440 | 0.000 |

- *The mean difference is significant at the 0.05 level*
- *Group 1= Diabetic control, Group 2= Metformin, Group 3= Metformin +Aspirin, Group 4= Metformin + Nifedipine.*

Abdulqadir finding does not go online with this research finding regarding the effect of metformin on kidney function. In this finding diabetic rats induced by STZ treated with metformin alone (Group 2) have shown elevated levels of urea and creatinine as compared to the control group (Group 1), (Group 3), and (Group 4). But our findings in this work are in agreement with Omotayo et al. [13] findings, which stated that significantly increased serum levels of creatinine, urea and bilirubin were observed in the diabetic control rats compared to non-diabetic rats. Serum levels of creatinine and bilirubin remained elevated in the diabetic rats treated with glibenclamide or metformin.

Capsi et al. [14] reported that at the lowest dosage, aspirin caused a 15% decrease in the rate of uric acid excretion, which was associated with a slight but significant increase in serum levels of uric acid. These effects on uric acid levels were gradually reduced with increasing dosages of aspirin. Generally, creatinine and uric acid clearance rates paralleled each other during aspirin treatment. This finding goes online with the finding in this study, where giving Aspirin (100 mg/kg) simultaneously with metformin has significantly ($p<0.000$) reduced creatinine levels in plasma as compared to the case of taking metformin alone.

Again the findings in this study comply with our earlier findings in which the combination of (metformin + nifedipine) and (metformin + aspirin) was found to have a highly significant Antidiabetic effect [15].

**Table 4. Analysis of variance (ANOVA) for urea**

| | Sum of squares | Sig |
|---|---|---|
| Between groups | 2585.076 | 0.000 |
| Within groups | 298.569 | |
| Total | 2883.646 | |

**Table 5. Post hoc tests; multiple comparisons for urea**

| (1) | (1) Factor | ( j) Factor | Mean difference (1- j) | Std. error | Sig. |
|---|---|---|---|---|---|
| | | Group 3 | 38.46333 | 4.70279 | 0.000 |
| | Group 2 | Group 1 | 28.47000 | 4.70279 | 0.001 |
| | | Group 4 | 30.70775 | 4.39906 | 0.000 |
| | | Group 2 | -38.46333 | 4.70279 | 0.000 |
| | Group 3 | Group 1 | -9.99333 | 4.70279 | 0.216 |
| Tukey HSD | | Group 4 | -7.75558 | 4.39906 | 0.349 |
| | | Group 2 | -28.47000 | 4.70279 | 0.001 |
| | Group 1 | Group 3 | 9.99333 | 4.70279 | 0.216 |
| | | Group 4 | 2.23775 | 4.39906 | 0.955 |
| | | Group 2 | -30.70775 | 4.39906 | 0.000 |
| | Group 4 | Group 3 | 7.75558 | 4.39906 | 0.349 |
| | | Group 1 | -2.23775 | 4.39906 | 0.955 |
| | | Group 3 | 38.46333 | 4.70279 | 0.000 |
| | Group 2 | Group 1 | 28.47000 | 4.70279 | 0.002 |
| | | Group 4 | 30.70775 | 4.39906 | 0.001 |
| Scheffe | | Group 2 | -38.46333 | 4.70279 | 0.000 |
| | Metformin + Asp | Group 1 | -9.99333 | 4.70279 | 0.278 |
| | | Group 4 | -7.75558 | 4.39906 | 0.422 |
| | Group 1 | Group 2 | -28.4700 | 4.70279 | 0.002 |
| | | Group 3 | 9.99333 | 4.70279 | 0.278 |
| | | Group 4 | 2.23775 | 4.39906 | 0.966 |
| | | Group 2 | -30.70775 | 4.39906 | 0.001 |
| | Group 4 | Group 3 | 7.75558 | 4.39906 | 0.422 |
| | | Group 1 | -2.23775 | 4.39906 | 0.966 |

- *The mean difference is significant at the 0.05 level*

*Group 1= Diabetic control, Group 2= Metformin, Group 3= Metformin +Aspirin, Group 4= Metformin + Nifedipine.*

## 6. CONCLUSION

From this study, we conclude that, when using metformin alone in treating type 2 diabetes in patients suffering from kidney dysfunction, this may aggravate the kidney dysfunction while the combination of metformin with aspirin and or nifedipine as in case of diabetes associated with hypertension may be beneficial. Further work may be required to support the findings.

## CONSENT

It is not applicable.

## COMPETING INTERESTS

Author has declared that no competing interests exist.

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