Influence of antidiabetic drug Biguanide against Alzheimer's induced rats

ABSTRACT

Amis: Alzheimer's disease (AD) is one of the most rapidly growing diseases in recent times. Despite extensive research to find an appropriate medicine, there has been no effective drug until now.

Study Design: The present study was designated to investigate the therapeutic impact of antihyperglycemic biguanide on Alzheimer's disease symptoms.

Methodology: Alzheimer's disease was induced in male rats by $AlCl_3$ and D-galactose at doses of 50 and 120 mg/kg daily for one month. Then, for the next four weeks, patients were given oral metformin (200 mg/kg daily).

Results: The obtained data indicated an increase in the arrival time of the AD rat group (G2) compared to the control group (G1). In addition, the AD rat group showed an elevation in glucose level, oxidative stress, liver, and kidney function. Importantly, metformin was able to enhance these unpleasant outcomes in G3. Interestingly, metformin was able to reduce GFAB immunoreactivity in the metformin-treated group compared to the AD group (G2).

Conclusion: Anti-diabetic drugs could be effective against Alzheimer's disease.

Keywords: Antidiabetic drug; Alzheimer's disease; D-galactose; $AlCl_3$.

1. INTRODUCTION

Alzheimer's disease (AD) is a neurological condition that worsens over time and is brought on by hereditary, epigenetic, and environmental factors **[1, 2]**. There are two different types of AD, including the more prevalent late-onset AD and an early-inception AD. There are currently no effective therapies for AD [3]. Indeed, no brand-new medications have accomplished Food and Drug Administration (FDA) or European Medicine Agency (EMA) endorsement **[1]**.

The medicine Biguanide or Metformin, a member of the biguanide family of antihyperglycemics, is currently the most used for treating type 2 diabetes mellitus (T2DM) **[4]**. Previous biomedical reports have even shown that metformin has several valuable impacts, including cardiovascular protection, anti-cancer activity, and anti-inflammatory properties **[5]**. Based on the anti-inflammatory effect of metformin, we and others suggested that it can protect against AD **[6, 7]**. The aim of this study was to investigate the impact of metformin against Alzheimer's disease.

2. MATERIAL AND METHODS

2.1. Chemicals

Biguanide, D- galactose were gained from Sigma-Aldrich (St. Louis, MO, USA). $AlCl_3$ was obtained from Loba chemie, India. Calretinin antibody (No. IR627) and polyclonal GFAB (NO. Z0334) were provided by Agilent Dako, Denmark. All other chemicals obtained in highly pure grade.

2.2. Experimental section

2.2.1. Alzheimer's disease induction design

The current research was performed corresponding to the general guidelines of Faculty of Science, Tanta University Egypt (Approved ethical No. IACUC-SCI-TU-0259) for handling of laboratory animals. 200- 220 g on average, fifty male Wistar rats were bought from Faculty of Pharmacy, Cairo University, Egypt. Rats were maintained according to general care guidelines (free access to water and food *ad libitum*/ 12 h day cycle at 25 ^{0}C) **[8]**. Five groups were generated (n= 10/ each group). G1; is normal rat. G2; is AD rats that received 50 mg/Kg of $AlCl_3$ and 120 mg/ Kg of D- galactose daily for constitutive 4 weeks **[9]**. G3; Biguanide treated that firstly received $AlCl_3$ and D-galactose as the same as G4 for one month, then treated with 150 mg/Kg Biguanide for one month **[10]**. Finally, the rats were slaughtered by decapitation and the skulls were opened with fine scissors and the brains were excised. Hippocampus were quickly removed and divided into two segments; one was fixed in 10% neutral buffer formalin, for histopathological examination and the remaining was washed and stored at -80 °C for preparation of tissue homogenates.

2.2.2. Biochemical assessment in serum

Fasting blood glucose (FBG), liver function (ALT and AST) and kidney function (urea and creatinine) were conducted according to the instruction of commercial kits procured from (Bio diagnostic, Egypt).

2.2.3. Evaluation oxidative and anti-oxidative parameter in hippocampus

Glutathione and glutathione peroxidase were assessed employing profitable kits from (Bio diagnostic, Egypt). Further, Malondialdehyde (MDA), lipid peroxidation indicator, was measured according to **[11]**.

2.2.4. Histopathological investigation

Brains were excised and fixed in 10% formalin in phosphate buffered saline pH 7.4 for 24 h at 4°C. Fixed tissues were dehydrated through a graded series of ethanol and embedded in paraffin according to standard procedures. Paraffin sections (5µm thick) were mounted on gelatin chromalum–coated glass slides and used for Haematoxylin and eosin stains as a routine method **[12]**.

2.2.5. Glial fibrillary acidic protein (GFAP) immunohistochemistry

Expression of GFAB-ir (GFAB immunoreactivity) in brain sections (hippocampus) were detected using the avidin biotin peroxidase complex method. Briefly, sections were incubated with Polyclonal rabbit anti-GFAP immunoglobulin (Z0334, Dako) **[13]** at a dilution of 1:1000 for 16 h at room temperature.

2.3. Statistical analysis

GraphPad Prism v. 6 (GraphPad Software, San Diego, CA, USA) was used in the current study. Using one way ANOVA and multiple comparison the significances between groups were statistically presented.

3. RESULTS

3.1 Biguanide ameliorate memory, glucose level, liver kidney function in AD induced rats

Table 1 indicated that there is a significant increase in the time taken during Labyrinth in the AD - induced rats (G2). This time was obviously decreased in Biguanide post treated groups (G3). Further, the liver function including (ALT and AST) and Kidney parameters as creatinine and urea were remarkedly elevated in G2 which ameliorated after Biguanide administration in G3.

Table 1: Impact of metformin on biochemical parameters in AD induced rats

parameters	Rat groups		
	G1	G2	G3
Arrival time (sec)	43±4.51	150.4±6$^{\#}$	92.3±3.2$^{\#,*}$
FBG (mg/dl)	79.3±1.23	214.23±3.23$^{\#}$	126.2±2.16$^{\#,*}$
ALT (IU)	35.2±2.1	66.72±1.54$^{\#}$	44.3±1.98$^{\#,*}$
AST (IU)	44.3±1.2	98.2±2.3$^{\#}$	65±1.78$^{\#,*}$
Urea(mg/dl)	24.8±2.54	57.2±1.97$^{\#}$	35.4±1.1$^{\#,*}$
Creatinine (mg/dl)	0.8±0.02	2.7±0.1$^{\#}$	1.6±0.13$^{\#,*}$

Data was presented as mean± SD, P<0.05 was considered as significant. #,* are the significancy compared to control (G1) and AD group (G2) respectively.

3.2. Impact of metformin on the hippocampal oxidative stress

Administration of $AlCl_3$ and D-galactose induced oxidative stress in hippocampus with visible decline in antioxidant parameters in G2 compared to control (G1). Indeed, the treated group with Biguanide showed a magnificent drop in MDA and an elevation of GPx and GSH as antioxidant parameters as shown in Figure 1.

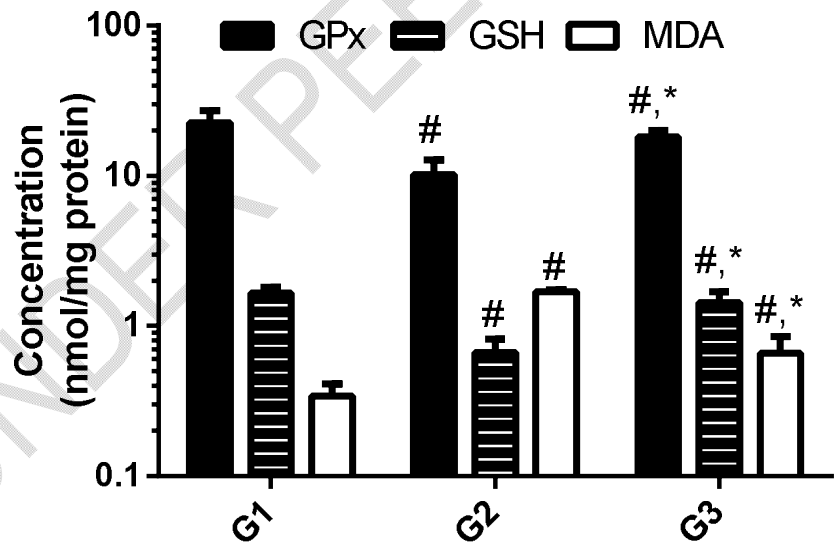

Figure 1. The antioxidant/ oxidative stress parameters in treated rats. Data was presented as mean± SD, P<0.05 was considered as significant. #,* are the significancy compared to control (G1) and AD group (G2) respectively.

3.3. Histopathology assessment

Histopathology examination of hippocampus of treated rats demonstrated a typical structure with tightly packed layers of pyramidal cells in control groups (Figure 2A). Further, AD-induced rats (G2) revealed oedema, disseminate vacuolar deterioration, vacuolated neurocytes and degenerated, reduction and alteration pyramidal cells (Figure 2B). On the other hand, Biguanide treatment rats (G3) showed little tissue damage with a few neuronal injuries without extensive vacuolar atrophy (Figure 2C).

3.4. Immunohistochemistry of GFAB

GFAB immunoreactivity in AD rats (G2) showed moderately positive immunoreactivity compared to control (G1), which significantly dropped in the Biguanide post-treatment group (G3) as shown in Figure 3.

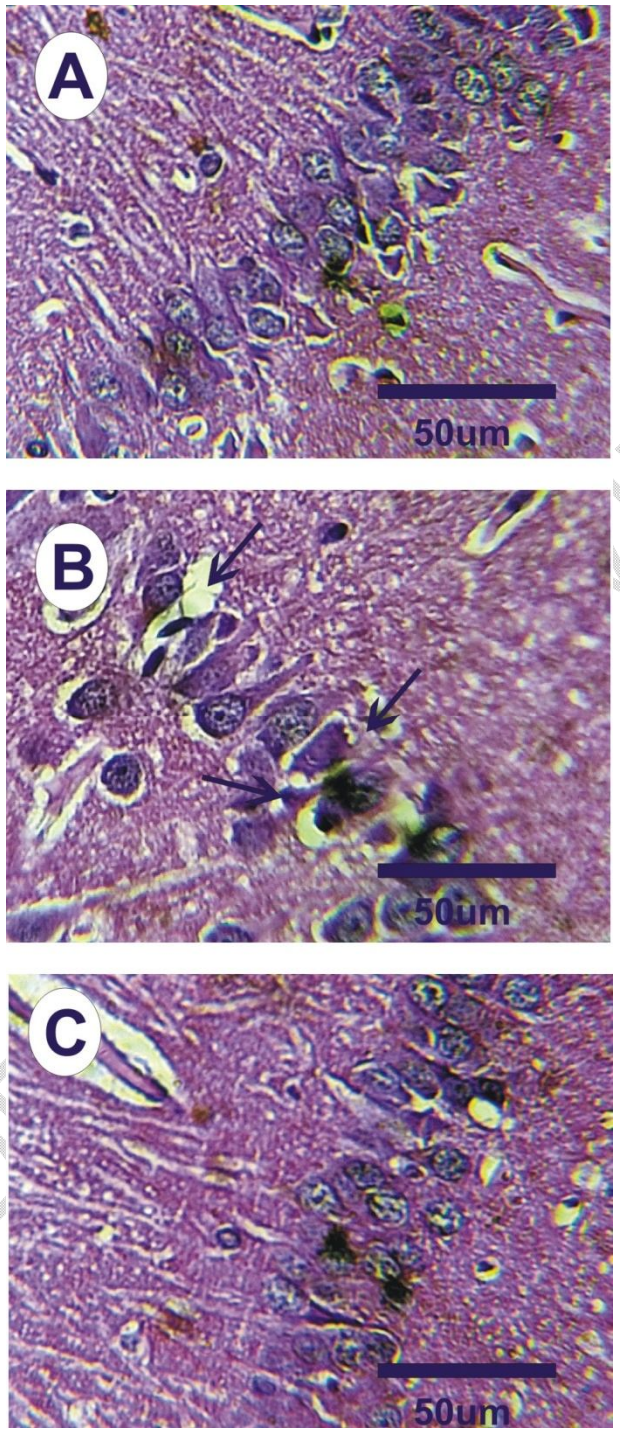

Figure 2: Histopathological Changes in hippocampus structure in different groups. A: Control, B: Alzheimer's disease (AD) revealed oedema, disseminate vacuolar deterioration, vacuolated neurocytes (arrows) and degenerated, reduction and alteration pyramidal cells, C: Biguanide post-treatment group.

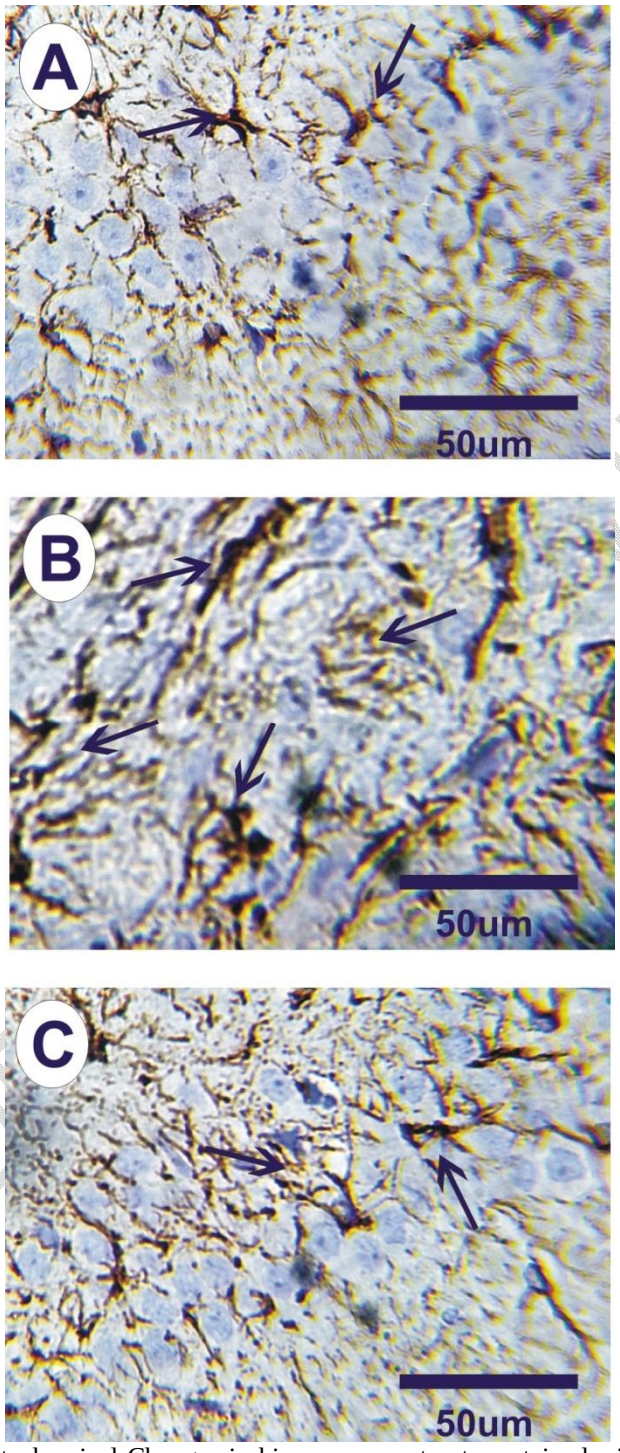

Figure 3: Immunohistochemical Changes in hippocampus structure stained with GFAB in different groups. A: Mild positive reactions (arrows) in Control, B: Moderate positive reactions in Alzheimer's disease (AD) (arrows), C: Mild positive reactions (arrows) in Biguanide post-treatment group.

4. DISCUSSION

The current aim of this study is to investigate the curing role of the antidiabetic metformin against Alzheimer's disease. The data collected revealed that the arrival time of AD rats was 3.48 times longer than that of control rats. This high-fold change was dropped after metformin uptake. Moreover, AD rats showed higher glucose levels than the control rats, which were further ameliorated by metformin treatment. Our data agreed with previously published reports, which indicated an elevation in arrival time with hyperglycemia **[14-16]**. Furthermore, the alteration in liver and kidney functions in AD syndrome was well-known due to impairment in lipid metabolism with elevated inflammatory markers **[17, 18]**. Our findings were completely consistent with this phenomenon.

Later research found a buildup of free radicals to be involved in the etiology of AD or moderate cognitive impairment [19]. Furthermore, an elevation of the oxidative stress marker MDA, along with a considerably declining antioxidant system (GPx and GSH), were clearly presented in our data. Previously published reports revealed similar findings **[20, 21]** .

On the level of histopathology changes, our results indicated a harmful alteration with distortion in the hippocampus region of AD rats that was greatly ameliorated with metformin medication. Previous reports suggested that the brain is shielded from harm by metformin. The findings of our investigation are consistent with these studies **[22-25]**, and we found that the rats' hippocampus significantly reduced the amount of neuronal tissue damage.

In our work, AD model rats showed substantial hippocampus astrogliosis (heightened GFAP intensity with a reduction in calretinin). In these rats, a dose of 200 mg/kg metformin resulted in significant improvement. *Our findings are completely in line with those of* ***Pilienko et al.*** *(***2020)** who indicated that metformin improved GFAB levels in AD rats **[26]**.

Conclusion

Antihyperglycemic metformin was able to improve neural damage caused by Alzheimer’s disease and other cognitive disorders. As a result, Alzheimer’s disease pathogenesis is more complicated and related to other metabolic disorders, such as diabetes mellitus type 2. More research is needed to determine whether they share the same root or common pathways.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

Not applicable.

ETHICAL APPROVAL

Animal Ethic committee approval has been collected and preserved by the author(s).

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