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Comparative Effect of Metformin Alone or in Combination with Glibenclamide on Oxidative Stress Markers of Patients with Type 2 Diabetes: A Randomized Open Clinical Trial

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Abstract

Background: Oxidative stress has been implicated in incidence of Type 2 Diabetes (T2D) and its related vascular complications. This study is aimed at investigating the comparative effect of add on glibenclamide treatment to metformin on oxidative stress markers in patients with T2D.

Materials and Methods: An open label randomized clinical trial was conducted on 95 patients (58.9% female) with T2D. Participants were allocated randomly to either metformin (1,000 mg/day) monotherapy or add on glibenclamide (10 mg/day) to metformin (1,000 mg/day) treatment. Oxidative stress markers were investigated at baseline and following 3 months.

Results: Following just 3 months' treatment with either metformin or metformin and glibenclamide coupled with life style modification, oxidative stress markers of observers improved significantly. Serum concentration of AGE, AOPP, MDA and Ox-LDL decreased significantly (p-value <0.001) compared to baseline.

Conclusion: In comparison with no medication, metformin in patient diabetes (p <0.05 in all analyses) the metformin monotherapy provided greater reductions than combination with glibenclamide. Three months' treatment with Pioglitazone is beneficial in reduction anti-oxidant capacity.

Keywords: Sulfonylurea compounds; Metformin; Antioxidants; Clinical trial

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Introduction

Despite the global efforts to reduce the prevalence and burden of Type 2 Diabetes (T2D), this silent but debilitating disease is still growing [1]. Recent study by non-communicable diseases risk factor collaboration estimated that the worldwide prevalence of T2D has been approximately doubled with the greater slope in men and reached to 442 million in 2014 [1]. The costs attributable to T2D which accounts to $242 billion in 2012 only in United States highlights high burden of this disease [2]. Pathophysiology of T2D is still an area of active research and many aspects are discovered day by day which enables invention of better preventive and therapeutic approaches. Oxidative stress is defined as disturbance in pro-oxidant and anti-oxidant milieu towards the former [3]. Lipid peroxidation products, protein oxidation products, damaged DNA bases and reducing enzyme activities are measured as surrogate of oxidative stress status in the body. Oxidative stress has been implicated in incidence, and vascular complications of T2D, and is directly associated with duration of T2D [4-7]. It has been shown that chronic hyperglycemic state leads to production of Reactive Oxygen Species (ROS) thereby making a vicious cycle of oxidative stress in the body [8]. Hyperglycemia induced oxidative stress augments cascades leading to production of pro-inflammatory and pro-coagulant factors, impaired nitric oxide release and underlies endothelial dysfunction that is pivotal in vascular complications of T2D [7,8]. Anti-hyperglycemic therapy in patients with T2D hypothetically may break this vicious cycle. We have recently demonstrated that metformin, the preferred initial agent for T2D, decreases pro-oxidant factors and enhance the anti-oxidant reserve in patients with T2D just after 3 months [9]. Sulfonylureas, another group of anti-hyperglycemic agents, are also capable to reduce the pro-oxidant products [10]. Studies on diabetic rats have shown that glibenclamide, the second generation of Sulfonylurea, is able to enhance activity

Table 1: Baseline characteristics of participants in each arms.

Variables	Metformin, (N=47)	Metformin+Glibenclamide (N=48)	P-value
Age (years)	52.66 ± 8.70	52.29 ± 7.75	0.828
Gender (Female)	28 (59.5%)	28 (58.3%)	0.903
Weight (kg)	77.45 ± 11.44	77.35 ± 14.52	0.973
Waist circumference (cm)	99.45 ± 9.61	99.21 ± 10.64	0.909
BMI (Kg/m^2)	29.55 ± 4.42	29.60 ± 4.23	0.954
SBP (mm/Hg)	122.34 ± 12.59	127.18 ± 12.75	0.066
FPG (mg/dL)	164.36 ± 46.86	176.58 ± 55.90	0.252
A1C (%)	7.13 ± 0.90	7.68 ± 1.19	0.013
Serum insulin (IU/L)	11.31 ± 7.29	12.24 ± 7.44	0.538
HOMA-IR	4.87 ± 3.09	4.76 ± 3.11	0.864
Total cholesterol (mg/dL)	191.88 ± 41.87	192.66 ± 49.33	0.934
LDL-C (mg/dL)	112.51 ± 38.27	104.02 ± 30.14	0.232
HDL-C(mg/dL)	46.91 ± 13.06	46.00 ± 11.33	0.716
Triglycerides (mg/dL)	170.79 ± 86.52	209.23 ± 87.55	0.034

BMI: Body Mass Index; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; FPG: Fasting Plasma Glucose; LDL-C: Low Density Lipoprotein-Cholesterol; HDL-C: High Density Lipoprotein-Cholesterol.

of anti-oxidant enzymes and reduce the ROS formation [10,11], while these significant results have not been confirmed by clinical trials [12-14]. We hypothesized that glibenclamide may augment the efficacy of metformin in reducing of ROS formation. In this study we aimed at investigating the add-on efficacy of glibenclamide to metformin compared to metformin monotherapy in mitigating the oxidative milieu in patients with T2D.

Materials and Methods

Study design

An open label, parallel-group, randomized clinical trial was conducted on patients with T2D. Participants were recruited from newly diagnosed patients with T2D attended to the outpatient diabetes clinic of Valiasr Hospital (Tehran, Iran) from October 2010 to March 2011. Diagnosis of T2D was made based on American Diabetes Association (ADA) recommendations [15]. This study was originally designed for comparison of lifestyle modification alone, with metformin plus life style modification on oxidative stress markers of patients with T2D (NCT01521624) [9]. The trial was extended to include a group of patients with T2D who treated with add on glibenclamide to metformin and life style modification. This study is part of the original trial and compares the add-on efficacy of glibenclamide to metformin and life-style modification compared to metformin monotherapy with life style modification on oxidative stress markers of patients with T2D over a 3-month trial. Institutional review board approved the protocol of this clinical trial. All interventions were in accordance with Helsinki declaration guidelines. Participants received detailed information about study procedures and possible adverse outcomes and signed the consent forms prior to enrollment.

Eligibility criteria

Inclusion criteria were: (1) patients newly diagnosed with T2D, and (2) Age greater than 40 years. Box sexes were eligible to participate. Exclusion criteria were: (1) known chronic heart, kidney or lung diseases, cancer; (2) had been previous treatment with oral anti-diabetic agents either for diabetes or other conditions associated with insulin resistance; (3) consumption of antioxidants such as Vitamin C and E in the past year; (4) use of aspirin in the past year; (5) mean alcohol consumption of ≥ 20 gr/day in men, and ≥ 10 gr/day in women over the past year; (6) current or past history of cigarette smoking; (7) participants who lost during follow-up or had missing data.

Randomization and masking

Participants were allocated to metformin and life style modification (Metformin group) or glibenclamide plus metformin and life style modification (Metformin-Glibenclamide group), randomly. First individual was allocated to metformin and life-style modification group and second to life-style modification alone and third to life-style modification with metformin and add-on glibenclamide therapy in original cohort. As demonstrated in Figure 1 metformin group and add-on glibenclamide therapy were compared in this study. No masking was done.

Interventions

Life style modification and metformin was prescribed to both groups. Recommendations for life style modification were according to ADA [15]; (1) weight loss in a gradual trend (about 7% of body weight); (2) maintaining of balanced diet (emphasis on limited consumption of simple carbohydrates and saturated fatty acids followed by rising intake of whole grains and dietary fibers); and (3) performing a regular exercise with moderate intensity (50% to 70% of maximum heart rate), at least 30 minutes a day, five times a week as a minimum. In addition, resistance training was advised if there were no contraindications. Tablets of metformin 500 mg were prescribed two times per day orally. Participants allocated to the group with add on glibenclamide therapy treated with glibenclamide 10 mg per day divided in two separate doses coupled with life style modification and metformin as described above.

Anthropometric measurements

At the initial visit and the second one, a medical interview and complete physical examination were performed by the same physician. Height was measured in standing position by a standard stadiometer; and with the approximation of 0.1 cm. Weight was measured by a digital scale (Beurer, GS49, Germany). Body Mass Index (BMI) was

Table 2: Mean changes in Oxidative stress markers and antioxidant reserve.

Variable	Metformin (N=47)				Metformin+Glibenclamide (N=48)			
	Baseline	At 3 months	Mean difference	P-value	Baseline	At 3 months	Mean difference	P-value
AGE (%)	67.96 ± 8.45	65.64 ± 8.43	-2.32 (-2.49, -2.15)	P <0.001	72.26 ± 4.86	70.24 ± 4.83	-2.02 (-2.21, -1.82)	P <0.001
AOPP (µmol/L)	138.77 ± 20.48	134.94 ± 20.62	-3.83 (-4.11, -3.55)	P <0.001	135.51 ± 29.43	133.01 ± 29.41	-2.5 (-2.77, -2.23)	P <0.001
Ox-LDL (U/L)	16.80 ± 0.88	16.47 ± 0.88	-0.33 (-0.36, -0.30)	P <0.001	16.08 ± 0.96	15.81 ± 0.97	-0.27 (-0.31, -0.23)	P <0.001
MDA (nmol/L)	2.81 ± 0.24	2.87 ± 0.24	-0.029 (-0.049, -009)	P <0.001	3.31 ± 0.23	3.29 ± 0.23	-0.021 (-0.024, -0.017)	P<0.001
CAT (U/ml)	2.18 ± 0.32	2.32 ± 0.33	0.14 (0.12, 0.17)	P <0.001	1.98 ± 0.33	2.09 ± 0.36	0.11 (0.088, 0.132)	P <0.001
FRAP (µmol/L)	1083.47 ± 253.47	1145.70 ± 279.08	62.23 (6.72, 48.71)	P <0.001	1034.52 ± 123.00	1082.40 ± 150.32	47.88 (37.88, 57.87)	P <0.001
GPX (U/ml)	90.57 ± 6.22	92.08 ± 6.15	1.51 (1.32, 1.70)	P <0.001	83.44 ± 12.54	85.87 ± 6.15	2.44 (-0.53, 5.41)	P <0.001
SOD (U/ml)	4.12 ± 0.31	4.30 ± 0.32	0.183 (0.154, 0.212)	P <0.001	3.82 ± 0.40	3.92 ± 0.41	0.106 (0.09, 0.122)	P <0.001

AGE: Advanced Glycation Products; AOPP: Advanced Oxidation Protein Products; Ox-LDL: Oxidized Low-Density Lipoprotein; MDA: Malondialdehyde; CAT: Catalase; FRAP: Ferritin Reducing the Ability of Plasma; GPX: Glutathione Peroxidase; SOD: Superoxide Dismutase.

calculated by dividing weight in kilograms by square of height in meters. Waist Circumference (WC) was measured at the middle place between lower costal margin and iliac crest by using an inflexible measurement tape. Hip circumference was measured at the widest location of the bone. After resting for about 5 minutes in a quiet place blood pressure was measured using standard sphygmomanometer (Riester, Big Ben adults, Germany). The measurement was repeated and the mean value was recorded.

Laboratory evaluation

After 8 to 10 hours of overnight fasting 10 milliliters of blood sample was drawn from each participant at baseline and at the 3-month visit. Fasting Plasma Glucose (FPG) was measured by the glucose oxidase test (Pars Azmoon kits, Iran). Fasting serum insulin was investigated by immuno radiometric assay (immunotech, Prague, Czech Republic). Glycated Hemoglobin A1c (HbA1C) was investigated by High-Performance Liquid Chromatography (HPLC, DREW, DS5, United Kingdom). Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) was calculated as follows; Fasting Insulin (IU) × FPG (mg/dl) divided by 405. To determine the concentration of the lipid profile, including triglycerides (TG), total cholesterol, LDL and HDL enzymatic methods were applied (Pars Azmoon kits, Iran). Serum enzymatic activity of catalase (CAT) and Glutathione Peroxidase (GPX) was investigated by the colorimetric method (Biocore Diagnosik Ulm GmbH kits, Germany). Enzymatic activity of Super Oxide Dismutase (SOD) was also determined by Colorimetric method (BioVision kits, USA). The aforementioned measurements were carried out by an auto-analyzer device (BT-3000 (plus), Biotechnica, Italy). The serum concentration of Malondialdehyde (MDA) was measured by the colorimetric method (Cayman, USA). ELISA (Sandwich) method (Mercodia kits, Sweden), was applied to investigate serum oxidized low-density lipoprotein (Ox-LDL). Serum concentration of AGE, AOPP and FRAP were assessed in accordance with previous protocol [9]. AGE concentration was determined with spectrophotometric techniques (Shimadzu, RF-5000, Japan). Advanced glycation products (AGE), advanced oxidation protein products (AOPP) and Ferritin Reducing the Ability of Plasma (FRAP) concentration were measured by spectrophotometry (Shimadzu, UV-3100, UV-3100, Japan).

Statistical analysis

Using SPSS version 19.0 for windows (IBM Corporation, New York, United States) statistical analysis was accomplished. Quantitative variables were represented as mean ± standard deviation of mean and nominal variables as frequency. Continues variables were compared between two groups using independent t-test. Paired t-test was used to compare the baseline and final values of continues variables including oxidative stress markers in each group. Analysis of variance (ANCOVA) method was employed to compare the mean difference of oxidative stress markers between two study groups. For adjustment for possible confounding variables, multivariate models were applied. Variables with significant correlation (Pearson correlation coefficient >0.70) were not situated in a single model, in order to avoid from collinearity bias. Effect size was estimated by the use of partial eta squared. In accordance with Cohen's recommendations, eta squared values of 1%, 6%, and 13.8% imply small, medium, and large effect sizes, respectively. All the tests were two-sided with probability less than 0.05 was considered statistically significant.

Results

Characteristics

Characteristics of participants in this clinical trial have been demonstrated in Table 1. Age and gender distribution was the same in metformin and metformin-glibenclamide groups. However, participants assigned to metformin-glibenclamide group had significantly higher baseline serum triglyceride (209.23 ± 87.55 *vs.* 170.79 ± 86.52, p-value=0.034) and A1C (7.68 ± 1.19 *vs.* 7.13 ± 0.90, p-value=0.013) compared to metformin group. There was no significant difference in mean of oxidative stress markers between two groups at baseline. Data on baseline oxidative stress markers have

Table 3: Adjusted 3 month mean and (95% CI).
Squared values of 1%, 6%, and 13.8% indicate small, medium, large effect sizes respectively.

Variables	Metformin	Metformin+Glibenclamide	P-value	Effect size (%)
AGE (%)	66.98	71.07	0.01	7.70%
	(64.87, 69.08)	(68.99, 73.15)		
AOPP (µmol/L)	138.03	133.12	0.385	0.90%
	(130.35, 145.72)	(125.51, 140.71)		
Ox-LDL (U/L)	16.63	15.96	P=0.002	11.10%
	(16.34, 16.91)	(15.68, 16.24)		
MDA (nmol/L)	2.79	3.31	p <0.001	52.40%
	(2.72, 2.86)	(3.23, 3.38)		
CAT (U/ml)	2.24	2.04	P=0.008	8.20%
	(2.14, 2.35)	(1.93, 2.14)		
FRAP (µmol/L)	1119.22	1053.92	P=0.176	2.20%
	(1054.00, 1184.44)	(989.44, 1118.41)		
GPX (U/ml)	91.06	84.91	P <0.001	14.30%
	(88.82, 93.31)	(82.69, 87.13)		
SOD (U/ml)	4.22	3.87	P <0.001	18.90%
	(4.11, 4.32)	(3.76, 3.97)		

AGE: Advanced Glycation Products; AOPP: Advanced Oxidation Protein Products; Ox-LDL: Oxidized Low-Density Lipoprotein; MDA: Malondialdehyde; CAT: Catalase; FRAP: Ferritin Reducing the Ability of Plasma; GPX: Glutathione Peroxidase; SOD: Superoxide Dismutase.

been shown in Table 2.

Oxidative stress markers

Following just 3 months treatment with either metformin or metformin and glibenclamide coupled with life style modification, oxidative stress markers of participants improved significantly. Serum concentration of AGE, AOPP, MDA and Ox-LDL decreased significantly (p-value <0.001) compared to baseline values as shown in Table 3. On the other hand, enzymatic activities of CAT, FRAP, GPX, and SOD increased significantly compared to baseline values (p-value <0.001). It was clarified that in controlling oxidative markers, treatment with metformin alone was preferable to treatment with metformin plus glibenclamide. In multivariate assessment and after adjusting confounding variables, the highest rate of efficacy was on MDA (effect size=52.4%, p-value <0.001).

Diabetes control

No significant differences were observed in SBP, FPG, serum insulin, HOMA-IR between metformin and add on glibenclamide groups (Table 4). However, those with add on glibenclamide therapy had significantly higher A1C values compared to metformin group (7.5 ± 1.7 *vs.* 6.8 ± 0.9, p-value=0.010).

Discussion

A growing body of evidence reveals that oxidative stress plays a crucial role in development of several features of T2D, including hyperglycemia, insulin resistance, hyperinsulinemia [16-18]. Regular stepwise treatment for diabetes treatment, including using of an oral medication titrated at a maximum dosage that each of which targets a single pathological deficiency of T2D depends on its primary mode of action, with the prerequisite of inadequate glycemic control. Combination therapy with Metformin plus Glibenclamide tablets concurrently is used for treatment of insulin resistance and insulin deficiency of T2D [19]. In this study, a combination therapy with Metformin plus Glibenclamide was used and compared with Metformin only to evaluate an oxidative profile in T2D patients. In addition, oxidative stress markers, including AOPP, AGEs, ox-LDL, MDA, and FRAP as well as antioxidant enzymatic activities were elevated in T2D patients. In this study, we found that 1,000 mg of Metformin in two separated dosage had a significant effect on ameliorate oxidative stress compared with Metformin plus Glibenclamide co-treatment. On the other hand, Metformin alone improved oxidative stress markers, including AGE and MDA compared to combination therapy with Metformin plus Glibenclamide. At the same time, the enzymatic activities of CAT, SOD and GPX were increased in Metformin group compare to Metformin plus Glibenclamide combination therapy. Surprisingly, treatment with Metformin alone caused a significant diminishing in the oxidation of LDL compared to Metformin plus Glibenclamide. However, the rate of AOPP and FRAP was not different from both treatment groups. It is well accepted that lipid peroxidation reflects the impact of oxidative damage of cell membrane and macromolecules. In T2D, insulin deficiency leads to elevation of fatty acyl coenzyme an oxidase, an enzyme that causes oxidation of fatty acids [20,21]. In the present study, MDA concentrations were significantly decreased in Metformin alone group. This is similar to what was previously described [9,22]. Although diminished MDA concentrations in Metformin mono-therapy group suggest the effectiveness of implication of this drug for the prevention of T2D. It can be speculated that Metformin exerts its pharmacological effects by its anti-peroxidative properties more efficient than that of Metformin plus Glibenclamide co-treatment [9]. An interacting network of antioxidant enzymes, such as SOD, CAT and GPX are employed to neutralize the adverse effects of ROS in biological systems. These enzymes disintegrate deleterious reactive compounds such as hydrogen peroxide into oxygen and water [23]. As a result, administration of Metformin was found to be more effective than Metformin plus Glibenclamide to elevate the activity of SOD, CAT and GPX in the T2D patients. SOD accelerates the dismutation of superoxide (O_2•−) radical anions to hydrogen peroxide (H_2O_2) [24]. The role of SOD is assuredly substantial in the amelioration of

Table 4: No significant differences were observed in SBP, FPG, serum insulin, HOMA-IR.

Variables	Metformin (N=47)	Metformin+Glibenclamide (N=48)	P-value
SBP (mm/Hg)	122.3 ± 12.5	127.1 ± 12.7	0.066
FPG (mg/dL)	138.6 ± 38.7	155.3 ± 56.3	0.098
A1C (%)	6.8 ± 0.9	7.5 ± 1.7	0.01
Serum insulin (IU/L)	12.2 ± 7.4	11.3 ± 7.2	0.539
HOMA-IR	4.3 ± 2.3	4.2 ± 2.8	0.793

oxidative stress in T2D. Therefore, elevated SOD activity might be taking into account for enhanced generation of superoxide anions. GPX merges another major role to reduce lipid hydroperoxides in response to alcohols and the other oxidants. It is obvious that, GPX plays a critical role in metabolism of hydrogen and lipid's peroxides and prevention of intracellular pathogenic processes [23,25]. Increased activity of antioxidant enzymes has been reported as an adaptive mechanism to protect cells against oxidative stress [26]. In a normal condition, CAT metabolizes H_2O_2 to H_2O and oxygen. CAT, as an endogenous antioxidant enzyme has to be renewed. Although, in the event of elevated production of H_2O_2. CAT might be induced to scavenge adequately the high levels of H_2O_2. Alongside, it is reported that CAT is highly sensitive to increased superoxide anions [27].

In the present study, a significant decrease as the AOPPs concentrations after three months with Metformin in comparison with Metformin plus Glibenclamide and control group was noted. In agreement with our results, previous studies have demonstrated a same pattern in AOPPs levels following Metformin therapy [9]. AOPPs are oxidative modified proteins that are recognized as markers of the oxidative damage of proteins. Noteworthy high level of AOPPs was found in many chronic disorders, including, uremia, diabetic nephropathy, and retinopathy and especially in T2D [28,29]. In line with our findings, Piwowar et al. [30] documented that, combination therapy with Metformin plus Glibenclamide was less effective compared to Metformin therapy in reducing oxidative stress markers. As a result of chronic hyperglycemia in diabetes, a series of non-enzymatic reactions in the setting of decreasing glucose or other carbohydrates takes place with amino acids, lipids and nucleic acids and ensuing oxidation of product compounds. These molecules, by stimulating oxidation of LDL, have an important role in generating Ox-LDL [31]. Also these molecules (AGEs) through binding to their receptors (RAGEs) cause diabetic vascular complications [32]. As a result, a number of cytokines, growth factors and pro-inflammatory agents are released; which cause inflammation and tissue damages [32]. A significant decrease of AGEs compared to Metformin plus Glibenclamide group were found, which is in agreement of the results with earlier studies. It has been proposed that Metformin exerts its biological role by reacting with dicarbonyl compounds [33,34]. In addition, Metformin is able to activate AMP-activated protein kinase that suppresses generating AGE receptors (RAGEs) [32,35]. Although, Glibenclamide was found to cause insulin secretion improvement but had no significant effect in glucose tolerance and reducing of A1C. FRAP as a determinant of anti-oxidant capacity index, robustly is associated with oxidative stress in T2D [36]. Some studies showed significant low levels of FRAP in T2D patients with worsening glycemic control [37]. In this trial study, we found a considerable increase in the FRAP levels of Metformin group alone in comparison with Metformin plus Glibenclamide. In agreement with our results, Esteghamati et al. [9] demonstrated the same pattern.

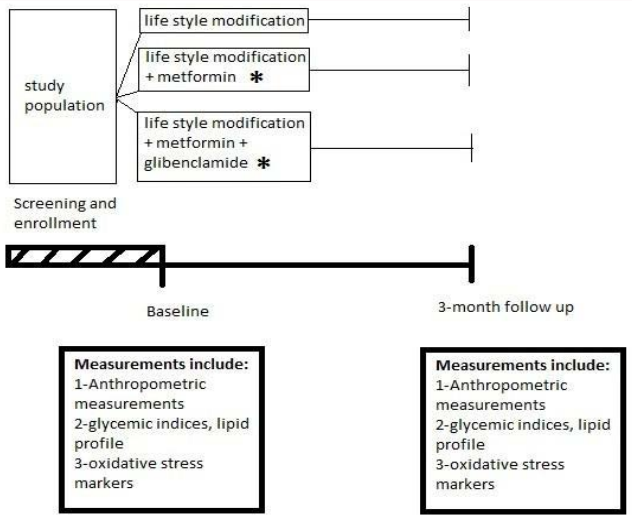

Figure 1: Study timeline: following initial screening participants with T2D were allocated to 4 arms with equal ratio. In this study which is part of the main trial two arms which are highlighted by asterisk are compared to find the comparative efficacy of add-on treatment with glibenclamide to metformin compared to metformin monotherapy on oxidative stress markers.

Conclusion

Therapy with the metformin or co-treatment with metformin and glibenclamide was assessed for efficacy and evaluation of oxidative stress profile in patients with type 2 diabetes who had inadequate glycemic control. In this study, all active treatments produced clinically meaningful reductions in oxidative stress markers compared with baseline values, and the metformin monotherapy provided greater reductions of AGE, ox-LDL, MDA markers and increased activity of CAT, SOD and GPx antioxidant enzymes in relative to the metformin and glibenclamide co-administration. Although some previous studies suggested that co-administration of other anti diabetic agents with metformin had better efficacy compared with metformin monotherapy, our results are not proved them. Though, our study provides further evidence that metformin monotherapy significantly diminishes oxidative stress and restores antioxidant capacity, failed to reduce AOPP and enhance enzymatic activity of FRAP.

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