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COMPARATIVE EFFECTS OF METFORMIN AND GLIBENCLAMIDE ON OXIDATIVE STRESS BIOMARKERS IN MALE WISTAR RATS

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ABSTRACT

Metformin and glibenclamide are widely used glucose-lowering agents, but their effects on oxidative stress and antioxidant defence may vary according to tissue and duration of exposure. This study investigated changes in selected oxidative stress and antioxidant defence biomarkers in the serum and liver of male Wistar rats administered either drug for different durations. Thirty-five mature male Wistar rats were assigned to seven groups of five animals each: a control group, three metformin-treated groups receiving 500 mg/kg/day for 7, 14 or 21 days, and three glibenclamide-treated groups receiving 5 mg/kg/day for the same durations. Serum and liver homogenates were analysed for malondialdehyde (MDA), reduced glutathione (GSH), ascorbic acid, glutathione peroxidase (GPx), glutathione S-transferase (GST), catalase (CAT) and superoxide dismutase (SOD). The reported serum MDA concentrations declined across treatment durations in both metformin-treated rats (2.67 ± 0.08, 1.17 ± 0.17 and 0.84 ± 0.32 µmol/ml) and glibenclamide-treated rats (1.43 ± 0.74, 0.91 ± 0.18 and 0.61 ± 0.10 µmol/ml). Serum GSH increased from 0.77 ± 0.04 to 5.58 ± 0.89 mmol/ml in the metformin groups and from 0.81 ± 0.05 to 2.77 ± 0.15 mmol/ml in the glibenclamide groups. Serum and liver ascorbic acid generally declined by the third week, while GPx, GST, CAT and SOD exhibited variable responses across treatment groups and tissues. These findings suggest that administration of metformin and glibenclamide was associated with changes in oxidative stress and antioxidant defence biomarkers in male Wistar rats. However, the observed responses varied with tissue and treatment duration, indicating that neither drug demonstrated a uniform effect across all measured biomarkers. The reported values require verification against the complete animal-level dataset and appropriate statistical reanalysis before definitive conclusions can be drawn.

Keywords: Metformin; glibenclamide; oxidative stress; malondialdehyde; reduced glutathione; antioxidant enzymes; Wistar rats

1. Introduction

Oxidative stress reflects an imbalance between reactive oxygen species and antioxidant defence mechanisms. Excessive reactive species can promote oxidation of membrane lipids, proteins and nucleic acids, potentially disrupting cellular function (Demirci-Çekiç *et al.*, 2022). Malondialdehyde (MDA), a product associated with lipid peroxidation, is commonly used as an indirect marker of oxidative damage, although thiobarbituric acid-based measurements require careful interpretation. The antioxidant system includes non-enzymatic components such as reduced glutathione (GSH) and ascorbic acid, together with enzymes including glutathione peroxidase (GPx), glutathione S-transferase (GST), catalase (CAT) and superoxide dismutase (SOD). These components act through complementary mechanisms in cellular antioxidant defence (Demirci-Çekiç *et al.*, 2022). Metformin, a biguanide, primarily reduces hepatic glucose production and improves insulin sensitivity. Glibenclamide, a sulfonylurea, stimulates insulin secretion from pancreatic beta cells. Their distinct pharmacological actions provide a rationale for examining their biochemical effects. However, findings from non-diabetic animals cannot be assumed to represent responses in diabetic models or patients. This study compared selected serum and liver oxidative-stress-related biomarkers in male Wistar rats exposed to metformin or glibenclamide for 7, 14 and 21 days.

2. Materials and methods

2.1 Animals and experimental design

Thirty-five mature male Wistar albino rats weighing 120–150 g were obtained from Dr Chris' Center for Veterinary Research, Mgbakwu, Anambra State, Nigeria. Animals were housed five per cage under a 12-hour light/dark cycle at 22±2 °C and allowed one week of acclimatisation. Animals were allocated to a control group (n=5), a metformin group (n=15) and a glibenclamide group (n=15). Each drug group was subdivided into three groups of five animals. Metformin hydrochloride was administered orally at 500 mg/kg body weight daily for 7, 14 or 21 days. Glibenclamide was administered orally at 5 mg/kg body weight daily for the corresponding durations. The control group received standard feed and water for 21 days. Feed and water were provided.

Group	Treatment	Duration	N
Control	No drug reported	21 days	5
M1	Metformin 500 mg/kg/day	7 days	5
M2	Metformin 500 mg/kg/day	14 days	5
M3	Metformin 500 mg/kg/day	21 days	5
G1	Glibenclamide 5 mg/kg/day	7 days	5
G2	Glibenclamide 5 mg/kg/day	14 days	5
G3	Glibenclamide 5 mg/kg/day	21 days	5

2.2 Sample preparation

Blood was collected into plain tubes, allowed to stand for one hour and centrifuged at 3,000 rpm for five minutes. Serum supernatant was retained for biochemical analysis. Liver tissue was homogenised in ice-cold 0.25 M sucrose solution diluted with 0.9% normal saline. Homogenates were centrifuged at 3,000 × g for one hour at 0–4 °C, and supernatants were used for analysis.

2.3 Biochemical assays

MDA was determined by the thiobarbituric acid colorimetric method attributed to Gutteridge and Wilkins (1982). GSH was determined by a modified Jollow *et al.* (1974) method using DTNB. Ascorbic acid was determined by a modified Roe and Kuether (1943) method. Total protein was measured using Lowry et al. (1951). GPx, GST, CAT and SOD activities were determined using the methods attributed respectively to Rotruck et al. (1973), Habig et al. (1974), Claiborne (1985), and Misra and Fridovich (1972).

2.4 Statistical analysis

SPSS version 19 was used for one-way and two-way ANOVA, with least significant difference post-hoc testing and $p<0.05$.

3. Results

Table 1. Reported MDA concentrations

Duration	Metformin serum µmol/ml	Glibenclamide serum µmol/ml	Control liver µmol/g	Metformin liver µmol/g	Glibenclamide liver µmol/g
7 days	2.67±0.08	1.43±0.74	—	0.64±0.48	0.53±0.23
14 days	1.17±0.17	0.91±0.18	—	0.46±0.10	0.54±0.08
21 days	0.84±0.32	0.61±0.10	0.69±0.24	1.86±0.87	1.19±0.45

Table 2. Reported GSH concentrations

Duration	Serum metformin mmol/ml	Serum glibenclamide mmol/ml	Control liver mmol/g	Metformin liver mmol/g	Glibenclamide liver mmol/g
7 days	0.77±0.04	0.81±0.05	—	0.24±0.01	0.34±0.06
14 days	1.49±0.22	2.09±0.47	—	1.63±0.29	2.18±0.41
21 days	5.58±0.89	2.77±0.15	0.86±0.32	1.13±0.20	1.43±0.23

Table 3. Reported ascorbic acid concentrations

Duration	Serum metformin mg/ml	Serum glibenclamide mg/ml	Control liver mg/g	Metformin liver mg/g	Glibenclamide liver mg/g
7 days	20.87±4.63	11.89±1.64	—	11.96±1.32	8.40±0.20
14 days	7.79±0.93	8.74±1.53	—	7.40±0.55	7.00±0.00
21 days	6.45±1.89	6.20±0.82	6.01±0.52	5.40±0.55	4.40±0.54

Table 4. Reported GPx activities

Duration	Serum metformin U/mg protein	Serum glibenclamide U/mg protein	Control liver U/mg protein	Metformin liver U/mg protein	Glibenclamide liver U/mg protein
7 days	0.64±0.05	0.40±0.03	—	0.38±0.03	0.31±0.33
14 days	2.99±0.23	3.85±0.24	—	2.15±0.12	1.94±0.23
21 days	0.75±0.08	1.10±0.10	0.39±0.88	0.50±0.06	0.58±0.10

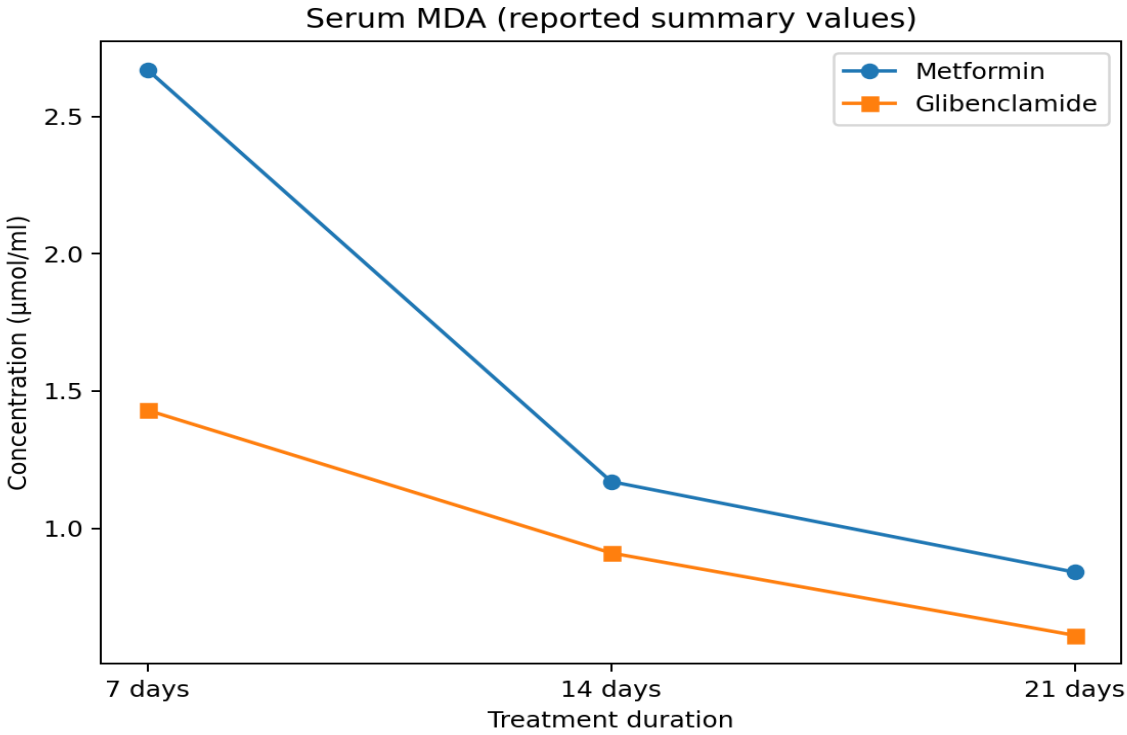

Figure 1. Serum MDA across treatment durations

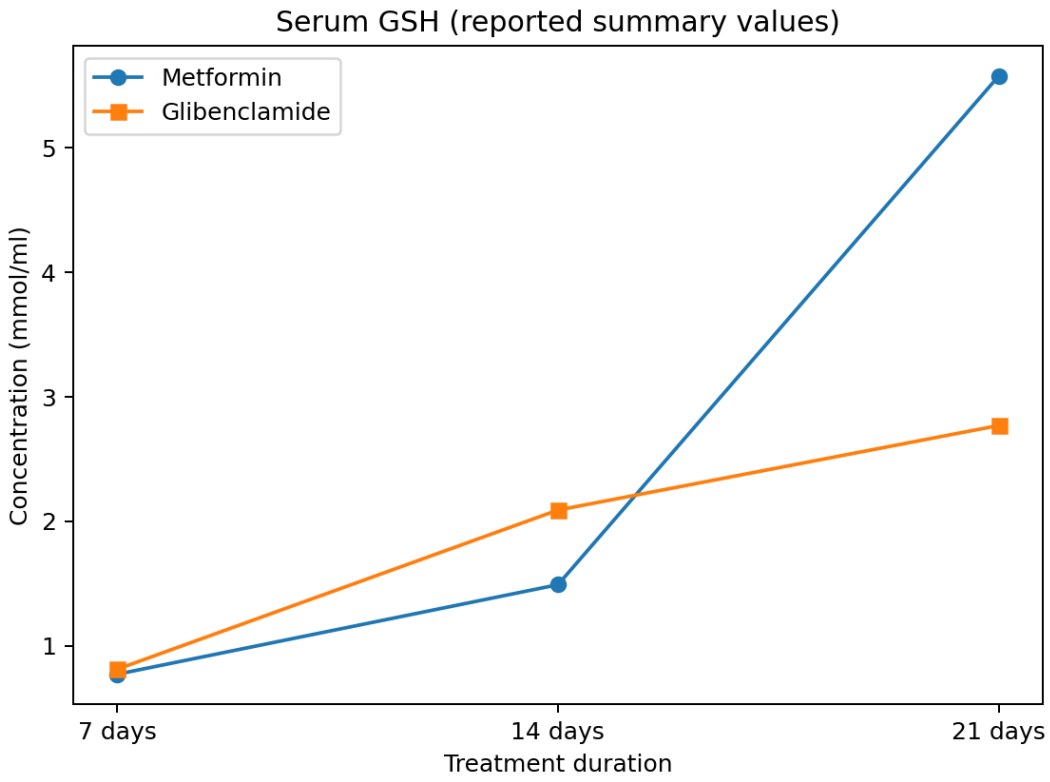

Figure 2. Serum GSH across treatment durations

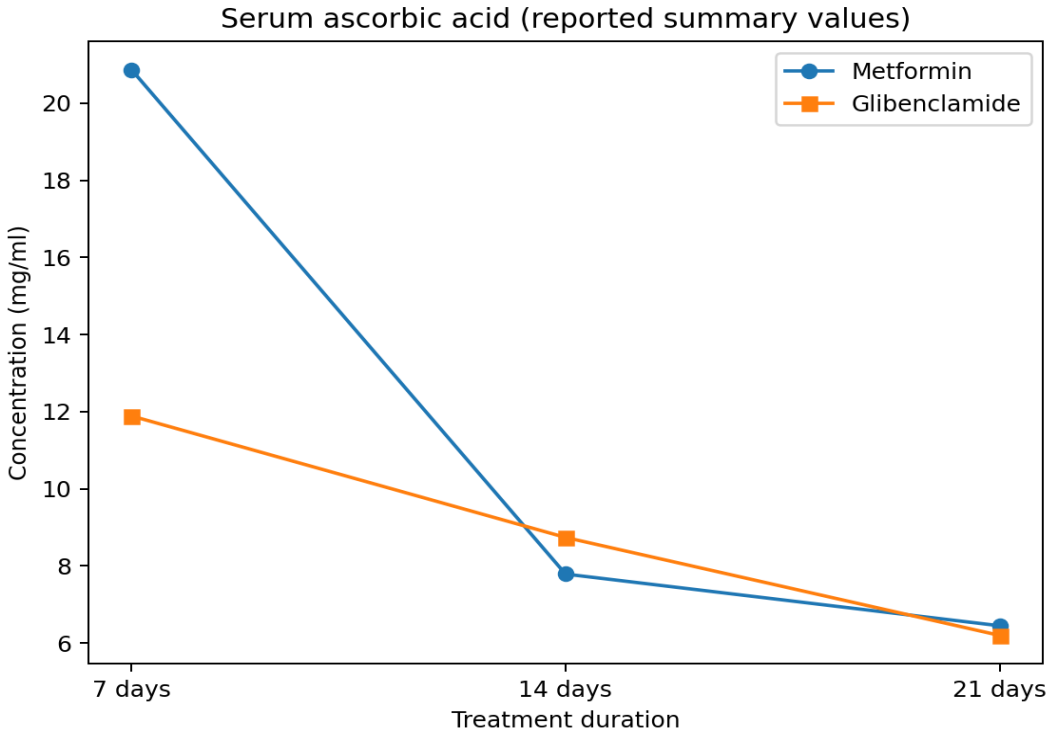

Figure 3. Serum ascorbic acid across treatment durations

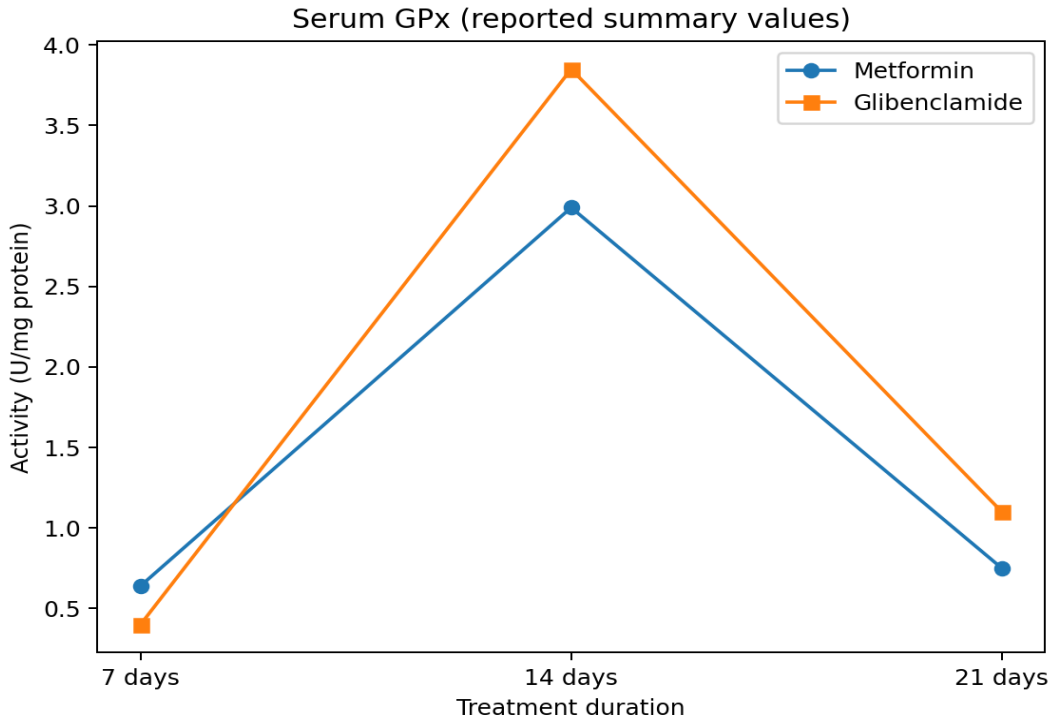

Figure 4. Serum GPx across treatment durations.

4. Discussion

The present study indicates that metformin and glibenclamide administration was associated with changes in oxidative stress and antioxidant defence biomarkers in the serum and liver of male Wistar rats. Serum MDA declined across treatment durations in both treatment groups, whereas hepatic MDA was higher at 21 days than at 14 days. This divergence suggests that circulating MDA may not accurately represent hepatic lipid peroxidation and that responses may differ between tissues. MDA is widely used as an index of lipid peroxidation, although thiobarbituric acid-based assays are not entirely specific and should be interpreted cautiously (Demirci-Çekiç *et al.*, 2022). Serum GSH increased across treatment durations, while hepatic GSH peaked at 14 days and declined by 21 days. GSH is a major component of intracellular antioxidant defence and participates in the detoxification of reactive species. Similar drug-related changes in antioxidant indices have been reported in diabetic rat models treated with metformin and glibenclamide (Obi *et al.*, 2016; Erejuwa *et al.*, 2011). However, those studies used diabetes-induced animals and therefore provide contextual rather than directly equivalent comparisons with the non-diabetic animals used in the present study. The general decline in serum and hepatic ascorbic acid by the third week may reflect altered utilisation or metabolism, although the mechanism was not investigated. GPx, GST, CAT and SOD activities showed variable responses across treatment duration and tissue. These enzymes have complementary roles in antioxidant defence, including superoxide dismutation, peroxide removal and glutathione-dependent detoxification (Habig *et al.*, 1974; Rotruck *et al.*, 1973; Demirci-Çekiç *et al.*, 2022). Changes in enzyme activity may represent adaptive responses to altered oxidant exposure and should not automatically be interpreted as

improved antioxidant protection. Alterations in hepatic CAT and SOD have also been reported following glibenclamide treatment in diabetic rats (Elmalı *et al.*, 2004). An important limitation is that the present animals were not rendered diabetic; consequently, the findings cannot be interpreted as evidence that either drug reverses diabetes-associated oxidative stress. In addition, the control group was maintained for 21 days only, leaving no time-matched controls for the 7- and 14-day treatment endpoints. The reported summary values also require verification against the original animal-level observations, with each animal treated as the experimental unit and technical replicates averaged before inferential analysis. These limitations restrict causal and comparative interpretation. Overall, metformin and glibenclamide administration was associated with changes in lipid peroxidation, non-enzymatic antioxidants and antioxidant enzyme activities. The responses varied across biomarkers, tissues and treatment durations, and the available results do not establish that either drug has uniformly superior antioxidant effects under the experimental conditions.

5. Conclusion

The findings demonstrate that metformin and glibenclamide administration was associated with alterations in serum and hepatic oxidative stress and antioxidant defence biomarkers in male Wistar rats. The observed responses varied according to the biomarker, tissue and duration of treatment, indicating that the effects of both drugs on redox balance were not uniform. Neither drug could therefore be conclusively established as having superior antioxidant effects under the experimental conditions. Further studies involving time-matched controls and validated animal-level statistical analyses are necessary to confirm these findings and clarify the effects of treatment duration on oxidative stress and antioxidant defence.

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