



Influence of Antioxidant Supplementation on High Fat Diet-Streptozotocin (HFD-STZ) Induced Type 2 Diabetes Mellitus in Albino Rats

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Authors' contributions

This work was carried out in collaboration between both authors. Author LCC designed the study and performed the statistical analysis. Author NCC wrote the protocol, the first draft of the manuscript, managed the literature review and analyses of the study. Both authors read and approved the final manuscript.

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ABSTRACT

The influence of antioxidant supplementation on high fat diet-streptozotocin (HFD-STZ) induced type 2 diabetes mellitus in Wistar albino rats was investigated. Appropriate (RDA) proportions of some antioxidant rich substances which includes; vitamins (A, B₃, B₆, B₁₂, C and E), minerals (calcium, selenium, chromium, magnesium, potassium and zinc), α-lipoic acid, cinnamon powder, curcumin (Meriva®), cordyceps, resveratrol, quercetin, D-ribose-L-cysteine were pulled together in corn oil and stored at 4°C for use. Serum glutathione (GSH) and malondialdehyde (MDA) levels, as well as activities of antioxidant enzymes: superoxide dismutases (SOD), catalase (CAT), glutathione-s-transferase (GST), glutathione peroxidase (GPx) and glutathione reductase (GR) were measured using standard methods. Data analysis was done with SPSS version 20.0 and significant level was set at $P \leq 0.05$. Results of *in vitro* oxidative stress indices and antioxidant enzyme activity indicate that after 4 weeks of treatment, there was no significant change ($p \geq 0.05$) in serum FBS levels of treated groups compared to the normal control group, but there was a significant decrease

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($p \leq 0.05$) after 8 and 12 weeks of treatment when compared to the diabetic control group. There was no significant difference ($p \geq 0.05$) in the activities of antioxidant enzymes when compared to the normal control group, while in the diabetic control there was significant increase ($p \leq 0.05$) compared to the other groups. The results after 4, 8 and 12 weeks of treatment showed a significant increase ($p \leq 0.05$) in serum GSH level of normal and treated groups compared to diabetic control group, whereas there was a significant decline ($p \leq 0.05$) in serum MDA level of treated and normal control groups when compared to diabetic control. The results therefore suggest that the supplement may possess significant ($p \leq 0.05$) free radical scavenging potentials which could be beneficial to health.

Keywords: Antioxidant supplement; catalase; glutathione; oxidative stress; serum.

1. INTRODUCTION

Oxidative stress is a known component of molecular and cellular tissue damage mechanisms, relating to a wide spectrum of human diseases of which diabetes mellitus is one of them [1].

The localization and effects of oxidative stress, as well as information regarding the nature of the reactive oxygen species, may be gleaned from the analysis of discrete indices of oxidative stress/damage in tissues and biological fluids. Oxidative stress causes alterations in major biomolecules in the cell and status of plasma antioxidant potential in diabetes mellitus.

High fat diet and low dose streptozotocin induced diabetes in animal model is often characterized by increased serum blood glucose level, lipid peroxidation and free radical generation [2].

Antioxidants are class of compounds perceived to play vital role in preventing certain types of damages chemically induced by excess free radicals. They are molecules whose reactions are very crucial for life such that they inhibit the oxidation of other molecule of which in their absence the actions of free radicals resulting from oxidative stress can be life threatening. Hence, imbalance in antioxidant levels against free radicals results to oxidative stress which damages cells and cell contents [3].

Several studies have been conducted on the role of antioxidant agents (supplements) in the management of free radical related problems and some are already in use for routine treatment and management of oxidative stress related complications. The use of some of these antioxidant supplements arose from clinical evidence and extensive investigations [4].

A few examples of these dietary food supplements include; Cellgevity, Detoxi-Green, Edaravone, Emergen-C, Idobene, RiboCeine and Trevo, which are widely available for use.

However, this study investigates the therapeutic effect of antioxidant supplementation in the management of induced diabetes mellitus (type 2) in rat model.

2. METHODS

2.1 Animal Procurement and Care

Wistar rats (albino) aged 3-4 weeks (70-110 g) were purchased from the animal house of the Faculty of Pharmacy and relocated to the animal house of the Department of Biochemistry, Madonna University, Nigeria, Elele campus, Rivers State. They were housed in well ventilated cages with access to water and food (chow) *ad libitum*. The animals were grouped into five with 10 rats each in stainless steel cages (34 x 47 x 18 cm) with soft wood shavings as bedding and maintained under normal laboratory conditions (temperature 24-28°C, relative humidity 60-70%, and 12 hour light-dark cycle).

2.2 Preparation of High Fat Diet

The high fat diet was compounded according to the method of Srinivasan *et al.*, (2005) using growers mash, lard and sucrose in the ratio of 3:1:1 respectively. The diet was carefully homogenised, pelleted, and then fed to the animals with exception to the normal control group.

2.3 Preparation of Antioxidant Supplement

Recommended proportions of some antioxidant rich substances which include; vitamins A (14.3 mcg/kg), B₃ (0.214 mg/kg), B₆ (0.03 mg/kg), B₁₂ (0.03 mg/kg), C (0.9 mg/kg) and E (0.14 mg/kg); minerals like calcium (11.4 mg/kg), selenium

(0.79 mcg/kg), chromium (0.2 mg/kg), magnesium (1.9 mg/kg), potassium (0.05 g/kg) and zinc (0.07 mg/kg), α -lipoic acid (8.57 mg/kg), cinnamon powder (43 mg/kg), curcumin [Meriva®] (3 mg/kg), cordyceps (7.5mg/kg), resveratrol (0.5 mg/kg), quercetin (2.5 mg/kg), D-ribose-L-cysteine [Ribocine®] (30 mg/kg) were pulled together in corn oil and stored at 4°C for use.

2.4 Induction of Type 2 Diabetes Mellitus

Experimental diabetes mellitus (type 2) was modeled in the animals (excluding normal control group) by feeding with high fat diet for a period of 8 weeks, after which a single dose IP injection of 35 mg/kg bw of streptozotocin was administered.

2.5 Treatment

Treatment with standard drugs and antioxidant supplement commenced 7 days after fasting blood glucose levels were analysed and hyperglycaemia established. The treatment lasted for a period of 12 weeks with analysis carried out on the 4th, 8th and 12th week of study. The treatment grouping is illustrated in the Table 1.

Table 1. Treatment grouping

Groups	Definition
Group 1:	Normal control
Group 2:	Diabetic control
Group 3:	Standard antidiabetic drug (Actovista)
Group 4:	Antioxidant supplement (formulation)
Group 5:	Standard supplement (Riboceine®)

2.6 Assays

The glucose level was determined using glucose oxidase method Trinder [1969], malondialdehyde (MDA) level by Fraga et al., [5], and glutathione level by the method of [6] Ellman (1959). The antioxidant enzyme activities assayed for were superoxide dismutase (SOD) using the method of Misra and Fridovich [7], catalase (CAT) by Beers and Sizer [8], glutathione-s-transferase by the method of Jocelyn [9], glutathione peroxidase by Wood [10], and glutathione reductase activity which was determined according to the method of Kakkar et al., [11].

2.7 Statistical Analysis

Data obtained from the study was analysed using the statistical package for social sciences (SPSS) version 20.0 for windows (SPSS Institute, Inc., Chicago, IL). One-way analysis of variance (ANOVA) was used to compare means, followed by the Tukey's test correction.

Values were considered significant at $p \leq 0.05$. Post hoc multiple comparisons for the differences between groups were established by least significance differences (LSD). All the data are expressed as mean \pm Standard error of the mean (SEM).

3. RESULTS

From the result shown in Figs. 1, after 4, 8 and 12 weeks treatment, the serum blood glucose levels was significantly high ($p \leq 0.05$) in the group 2 (152.0 ± 5.0 , 136.0 ± 1.0 and 143.5 ± 0.5 mg/dL) when compared with the group 1 (89.5 ± 0.5 ; 96.5 ± 0.5 and 85.5 ± 0.5 mg/dL), Group 3 (102.50 ± 12.5 , 83.50 ± 9.5 and 93.50 ± 0.50 mg/dL), group 4 (107.0 ± 2.0 , 98.0 ± 4.0 and 96.0 ± 2.0) and group 5 (126.0 ± 1.0 , 92.0 ± 6.0 and 54.0 ± 29.0) for the respective weeks. The significantly low ($p \leq 0.05$) serum blood glucose level observed in the treated (groups 3 to 5) especially on the 12th week of group 5 is evident of a hypoglycaemic potential of the treatments.

The serum malondialdehyde (MDA) level as shown in Fig. 2, was significantly higher ($p \leq 0.05$) in the group 2 compared to the group 1 and treated (groups 3 to 5) across the respective weeks. However, group 5, week 12 showed a significantly high ($p \leq 0.05$) level of MDA compared to group 1.

The serum reduced glutathione (GSH) level was significantly high ($p \leq 0.05$) in the normal control (group 1) and treated (groups 3 to 5) when compared with the diabetic control (group 2) which recorded a significantly low ($p \leq 0.05$) level throughout the study period (12 weeks). However, there was no statistically significant difference ($p \geq 0.05$) between the serum GSH levels of the treated (groups 3 to 5), when compared to that of the normal control (group 1) as shown in Fig. 3.

For the serum antioxidant enzyme activities (Figs. 4-8), there was no statistically significant changes ($p \geq 0.05$) in the SOD activity of both the normal control (group 1), diabetic control (group

2) and treated (groups 3 to 5) for the study period (4, 8 and 12 weeks). Invariably, there was an observed significantly high ($p \leq 0.05$) difference in catalase (CAT), glutathione-s-transferase and glutathione peroxidase activities among the normal control (group 1) and the treated (groups 3 to 5). Furthermore, a significant reduction ($p \leq 0.05$) in CAT activity was recorded in the

diabetic control (group 2) and these changes were consistent with time/duration of study (4, 8 and 12 weeks). The glutathione reductase activity revealed a significant increase ($p \leq 0.05$) in the normal control (group 1) and treated (groups 3 to 5), with the exception of the diabetic control (group 2) which only recorded a significant change ($p \leq 0.05$) on the 8th week of the study.

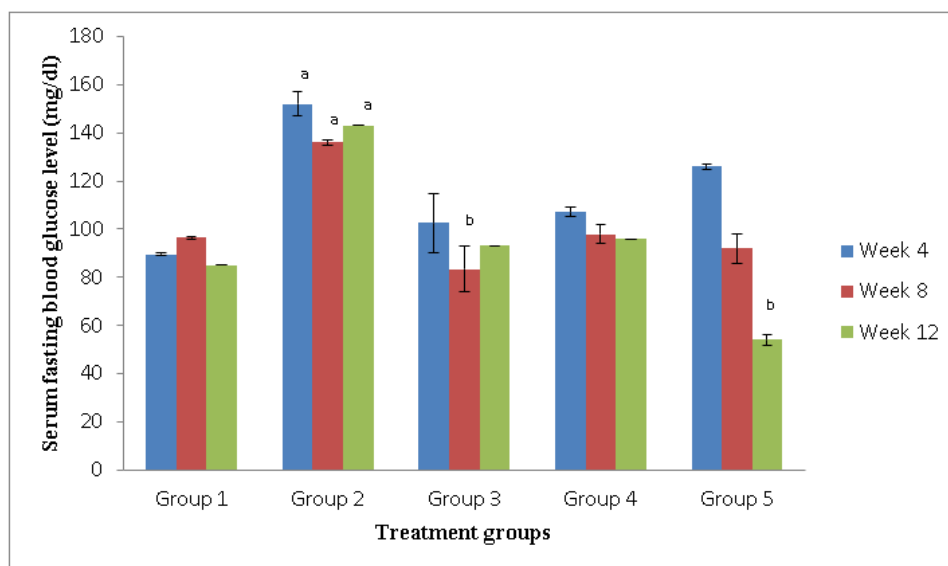


Fig. 1. Effect of antioxidant supplement on serum fasting blood glucose (FBG) level in normal and HFD/STZ-induced diabetic rats

(Data represented as Mean \pm SEM; ^a = significantly higher relative to all other groups; ^b = significantly lower compared to all other groups)

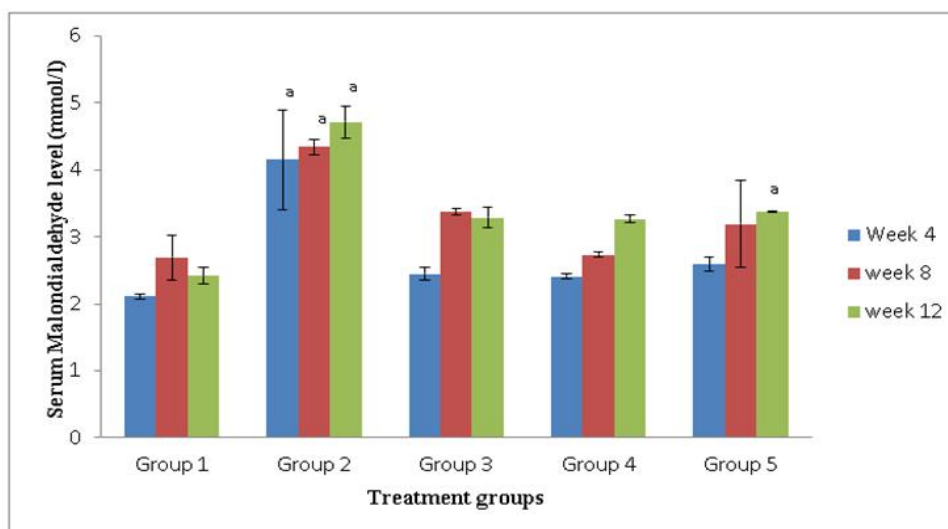


Fig. 2. Effect of antioxidant supplement on serum malondialdehyde (MDA) level in normal and HFD/STZ-induced diabetic rats

(Data represented as Mean \pm SEM; ^a = significantly higher relative to all other groups; ^b = significantly lower compared to all other groups)

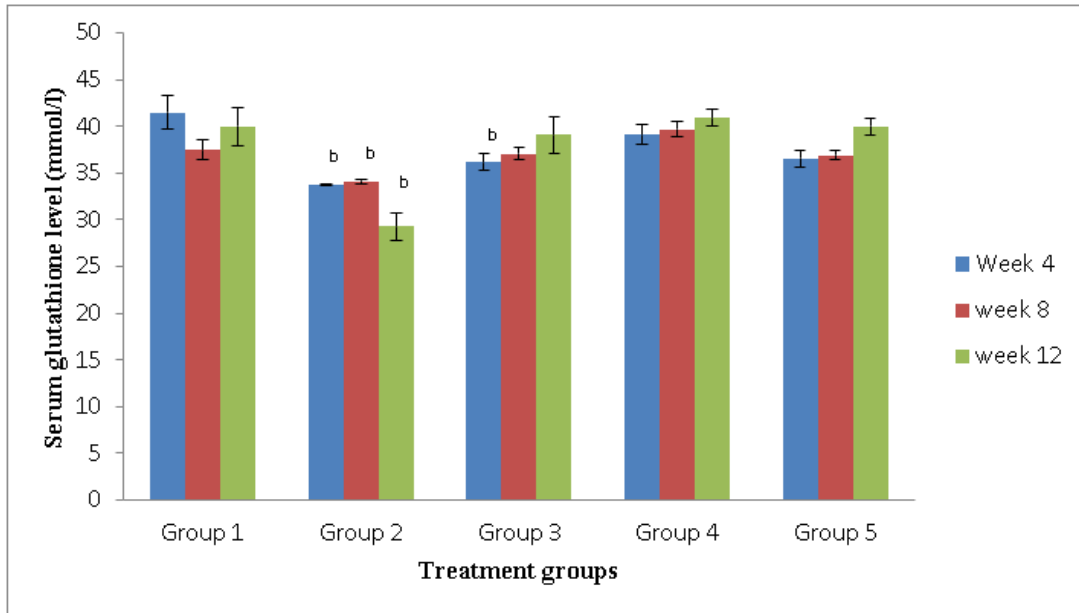


Fig. 3. Effect of antioxidant supplement on serum reduced glutathione (GSH) level in normal and HFD/STZ-induced diabetic rats
 (Data represented as Mean \pm SEM; ^a = significantly higher relative to all other groups; ^b = significantly lower compared to all other groups)

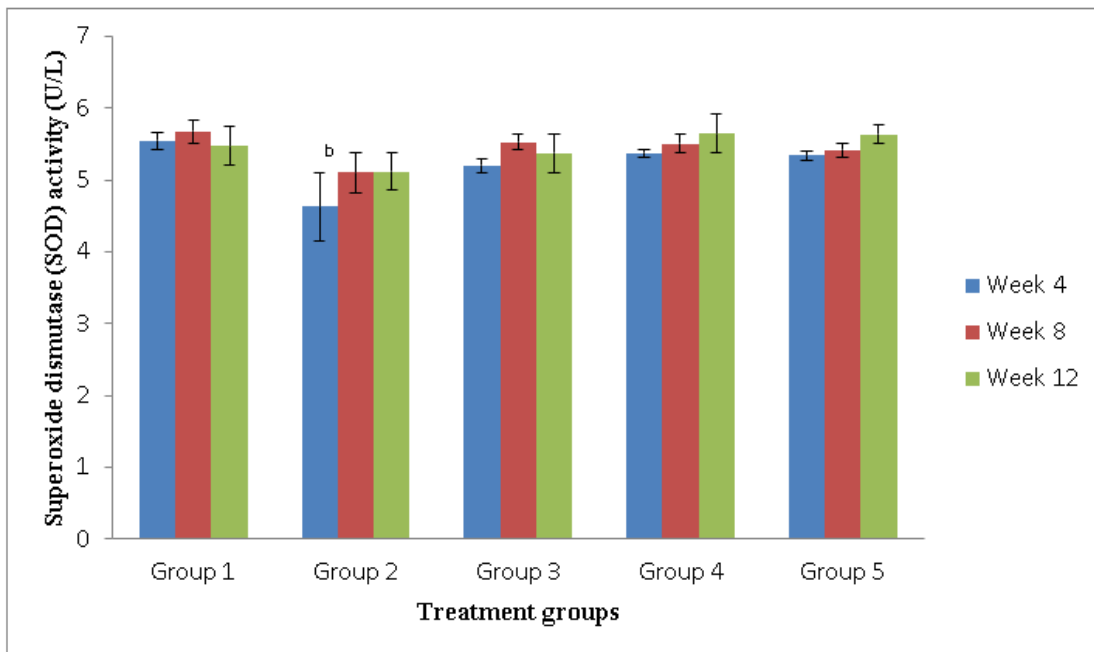


Fig. 4. Effect of antioxidant supplement on superoxide dismutase (SOD) activity in normal and HFD/STZ-induced diabetic rats
 (Data represented as Mean \pm SEM; ^a = significantly higher relative to all other groups; ^b = significantly lower compared to all other groups)

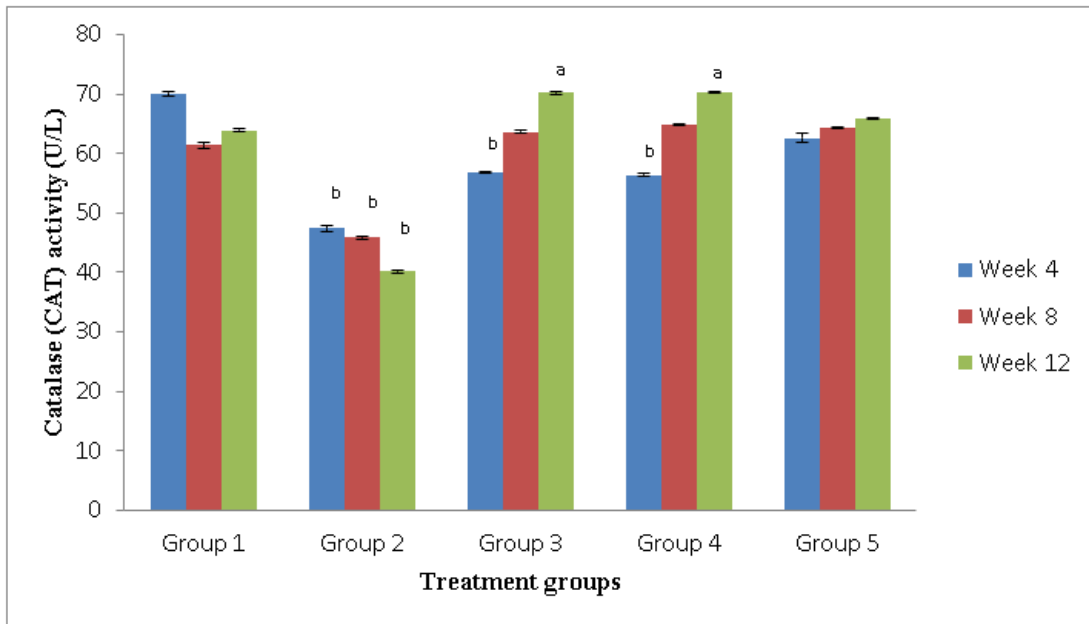


Fig. 5. Effect of antioxidant supplement on catalase (CAT) activity in normal and HFD/STZ-induced diabetic rats

(Data represented as Mean \pm SEM; ^a = significantly higher relative to all other groups; ^b = significantly lower compared to all other groups)

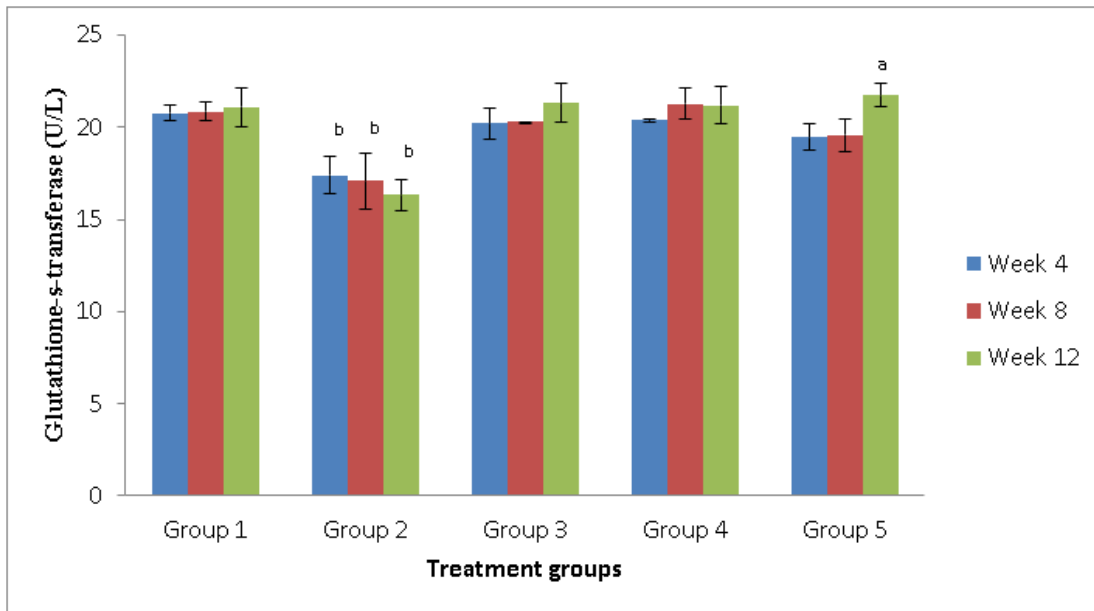


Fig. 6. Effect of antioxidant supplement on glutathione-s-transferase (GST) activity in normal and HFD/STZ-induced diabetic rats

(Data represented as Mean \pm SEM; ^a = significantly higher relative to all other groups; ^b = significantly lower compared to all other groups)

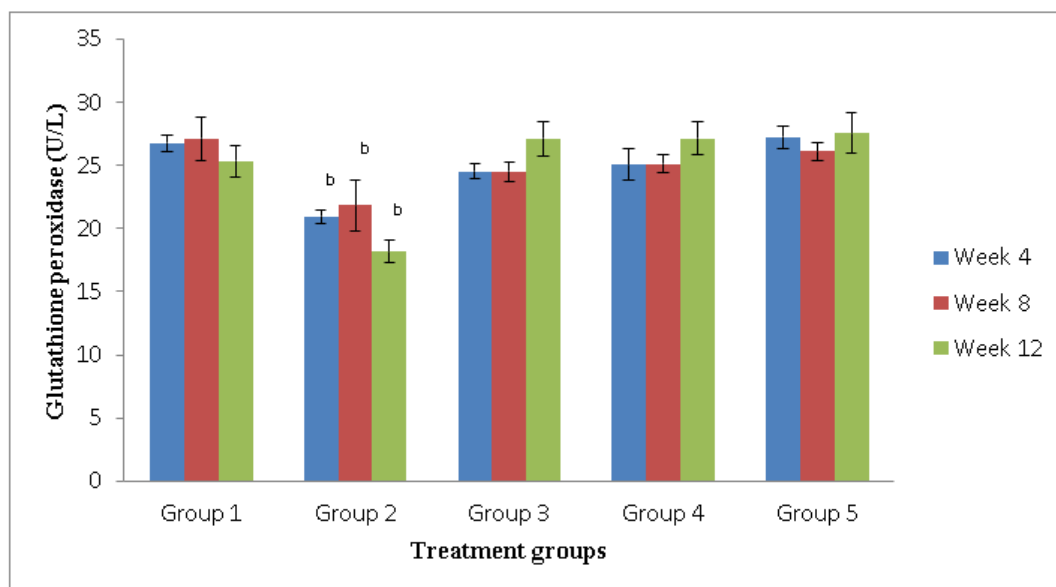


Fig. 7. Effect of antioxidant supplement on glutathione peroxidase (GPx) activity in normal and HFD/STZ-induced diabetic rats
 (Data represented as Mean \pm SEM; ^a = significantly higher relative to all other groups; ^b = significantly lower compared to all other groups)

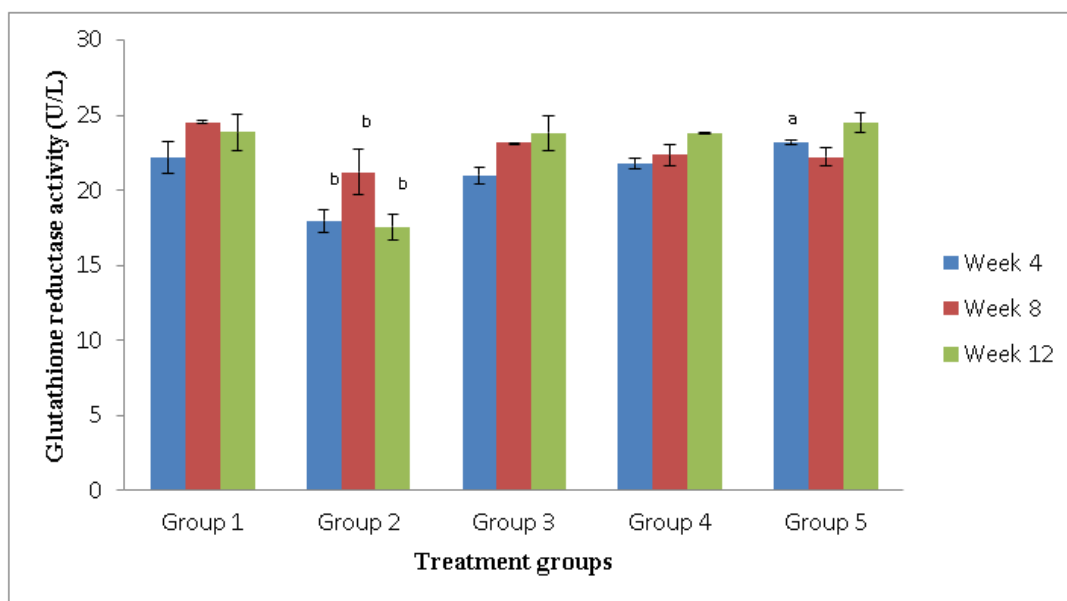


Fig. 8. Effect of antioxidant supplement on glutathione reductase (GR) activity in normal and HFD/STZ-induced diabetic rats
 (Data represented as Mean \pm SEM; ^a = significantly higher relative to all other groups; ^b = significantly lower compared to all other groups)

4. DISCUSSION

Oxidative stress is widely accepted to be associated with dysfunction of pancreatic β -cells

as well as insulin resistance in type 2 diabetes [12]. Experimental evidences suggest the involvement of free radicals in the onset of diabetes and more importantly

in the development of diabetic complications [13].

In type 2 diabetes mellitus, hyperglycemia induces generation of reactive oxygen species (ROS) and nitric oxide (NO) radicals, which induced pancreatic β -cell oxidative damage [14]. Mitigation of free radicals offers a unique therapeutic approach for the management of diabetes.

In the present study, oxidative stress indices was monitored and the extent of free radical damage suspected to be orchestrated by diabetes was significantly reduced by the antioxidant supplement and the standard drug/supplement used. The significant increase in serum blood glucose level upon administration with high fat diet and streptozotocin was evident that diabetes was induced in the experimental animals. Treatment of high fat diet and STZ-induced diabetic rats with antioxidant supplements and some standard drugs caused significant ($P < 0.05$) reduction in serum fasting blood glucose after 4 weeks. This reduction was significantly ($P < 0.05$) lower than the diabetic control group and statistically non significant from normal control group after 8 and 12 weeks of treatment (Fig. 1).

However, the finding is consistent with the works of Jeong-Sook, [15] and Roja et al., [16] which reported a significant ($p \leq 0.05$) reduction in serum blood glucose levels due to the effect of antioxidants and antioxidant enzyme activities.

Accumulation of free radicals such as superoxide anion and peroxynitrite during HFD/STZ metabolism resulted in the depletion of antioxidants, leading to oxidative stress and lipid peroxidation [17] (Esterbauer et al., 1991). In our study, a significant ($p \leq 0.05$) increase in serum MDA levels in the diabetic control rats suggests enhanced lipid peroxidation and failure of antioxidant defense mechanisms resulting in oxidative stress. The observed increase in the levels of serum MDA correlates with the decrease in the respective total SOD, CAT, and GPx activities of the diabetic control group (Figs. 2, 4, 5 and 7). The diabetic rats administered the various adopted treatments (antioxidant supplement and Actovista) showed significantly ($p \leq 0.05$) reduced levels of serum MDA compared to the diabetic control. Reduction in serum MDA levels of the treated groups may possibly be due to the ability of the active principles or compounds in the treatments to quench free radicals by the transfer of electrons to the

radicals [18] and possibly by activation of antioxidant enzymes [19]. The result of this study is consistent with previous findings [20], since it demonstrated a significant ($p \leq 0.05$) increase in TBARS level.

However, there was significant ($p \leq 0.05$) increase in serum GSH level of the treated groups with a concomitant decrease in the diabetic control group, when compared with the normal control group. The increase in serum GSH level of the treated groups, (with the exception of the normal control group) was continuous as time progressed from the 4th week through the 8th week to the 12th week (Fig. 3). The significant ($p \leq 0.05$) decrease observed in the diabetic control group was continuous, and as such was found to be consistent with the work of Aaseth and Stoe-Birketvedt [21], who reported reduced levels of serum glutathione in erythrocytes of subjects with obesity and poorly controlled type 2 diabetes. Similarly, decrease in intracellular glutathione levels in patients with type 2 diabetes was reported by Livingstone and Davis [22] in their recent review.

In type 2 diabetes, pancreatic β -cell membrane properties are altered thereby affecting signal transduction, ion exchange, and membrane fluidity, and ultimately cell damage [23,24,25,26]. The increase in levels of free radical production/accumulation in diabetic condition is suggested to be due to increased lipid peroxidation [27] and increased damage to the antioxidant defence system which results in increased production and or decreased scavenging of free radicals by antioxidants and antioxidant enzymes [28].

The protective effect of the different treatments were evident through significantly elevated ($p \leq 0.05$) activities of total SOD, CAT, GST, GPx and GR observed among the groups treated with the standard drug (Actovista), antioxidant supplement and known standard antioxidant supplement. The HFD/STZ-induced diabetic control rats had significantly lower ($p \leq 0.05$) SOD, CAT, GST, GPx and GR activities compared to the normal control and treated rats. The treatments may have significantly ($p \leq 0.05$) increased SOD, CAT, GST and GPx activities possibly by enhancing enzyme induction/synthesis, hence promoting their role in scavenging free radical generated due to the diabetes. The increase in antioxidant enzymes could be an indication of *in vivo* antioxidant enzyme activity of the treatments. As reported by

Patel et al. [29], studies in endothelial cells have shown that hyperglycemia increased hydrogen peroxide production and down-regulated the expression of CAT genes. As previously reported, biologically active compounds could also mediate *in vivo* antioxidant action by up-regulating the expression of genes for antioxidant enzymes synthesis [30], which might also be the case in this present study.

The observed correlation in the elevated level and activity of serum glutathione and antioxidant enzyme(s) measured is evident in affirming the interrelation of antioxidants and antioxidant enzymes in the antioxidant defense system.

5. CONCLUSION

Conclusively, the study examined the effect of a newly formulated antioxidant supplement on high fat diet and low dose streptozotocin induced type 2 diabetes mellitus in animal model (albino rats) and the results obtained suggest that the antioxidant supplement may be useful in elevating/enhancing the level and activities of antioxidant and antioxidant enzymes which are essential in the management of type 2 diabetes mellitus. It further reveals the potential of antioxidant composition in mitigating oxidative stress and free radical accumulation and resultant effect on cellular membrane and DNA integrity. Hence antioxidant rich diets and antioxidant supplements are recommended for regular use in type 2 diabetes related conditions due to its enormous benefits as demonstrated in this study.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All animals used in this study were treated in conformity to the National Institute of Health (NIH) guidelines for handling laboratory animals.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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APPENDIX

Table 2. Effect of antioxidant supplement on serum fasting blood glucose (FBG) level in normal and HFD/STZ-induced diabetic rats

Groups	Week 4	Week 8	Week 12
Group 1	89.50±0.50	96.50±0.50	85.0±0.00
Group 2	152.0±5.00 ^a	136.0±1.00 ^a	143.0±0.01 ^a
Group 3	102.50±12.50	83.50±9.50 ^b	93.0±0.10
Group 4	107.0±2.00	98.0±4.00	96.0±2.10
Group 5	126.0±1.00	92.0±6.00	54.0±2.90 ^b

Data represented as Mean ± SEM; (^a = significantly higher relative to all other groups; ^b = significantly lower compared to all other groups)

Table 3. Effect of antioxidant supplement on serum malondialdehyde (MDA) level in normal and HFD/STZ-induced diabetic rats

Groups	Week 4	week 8	week 12
Group 1	2.11±0.04	2.69±0.33	2.42±0.12
Group 2	4.15±0.74 ^a	4.34±0.12 ^a	4.72±0.24 ^a
Group 3	2.45±0.09	3.37±0.05	3.29±0.16
Group 4	2.41±0.04	2.73±0.04	3.27±0.05
Group 5	2.59±0.10	3.19±0.65	3.38±0.01 ^a

Data represented as Mean ± SEM; (^a = significantly higher relative to all other groups; ^b = significantly lower compared to all other groups)

Table 4. Effect of antioxidant supplement on serum reduced glutathione (GSH) level in normal and HFD/STZ-induced diabetic rats

Groups	Week 4	week 8	week 12
Group 1	41.47±1.82	37.46±1.08	39.97±2.00
Group 2	33.78±0.12 ^b	34.12±0.25 ^b	29.25±1.46 ^b
Group 3	36.15±0.89 ^b	37.05±0.62	39.09±1.95
Group 4	39.12±1.01	39.69±0.81	40.87±0.91
Group 5	36.55±0.88	36.90±0.53	40.00±0.89

Data represented as Mean ± SEM; (^a = significantly higher relative to all other groups; ^b = significantly lower compared to all other groups)

Table 5. Effect of antioxidant supplement on superoxide dismutase (SOD) level in normal and HFD/STZ-induced diabetic rats

Groups	Week 4	Week 8	Week 12
Group 1	5.55±0.12	5.68±0.16	5.47±0.27
Group 2	4.63±0.48 ^b	5.10±0.29	5.12±0.26
Group 3	5.20±0.09	5.53±0.10	5.38±0.27
Group 4	5.37±0.06	5.51±0.13	5.65±0.27
Group 5	5.34±0.06	5.42±0.10	5.63±0.13

Data represented as Mean ± SEM; (^a = significantly higher relative to all other groups; ^b = significantly lower compared to all other groups)

Table 6. Effect of antioxidant supplement on catalase (SOD) level in normal and HFD/STZ-induced diabetic rats

Groups	Week 4	Week 8	Week 12
Group 1	70.12±0.37	61.39±0.43	63.89±0.32
Group 2	47.41±0.58 ^b	45.81±0.18 ^b	40.11±0.20 ^b
Group 3	56.87±0.15 ^b	63.60±0.28	70.18±0.35 ^a
Group 4	56.38±0.20 ^b	64.77±0.10	70.38±0.01 ^a
Group 5	62.62±0.75	64.24±0.15	65.89±0.22

Data represented as Mean ± SEM; (^a = significantly higher relative to all other groups; ^b = significantly lower compared to all other groups)

Table 7. Effect of antioxidant supplement on glutathione-s-transferase (GST) level in normal and HFD/STZ-induced diabetic rats

Groups	Week 4	Week 8	Week 12
Group 1	20.76±0.42	20.85±0.51	21.11±1.06
Group 2	17.39±1.03 ^b	17.08±1.49 ^b	16.32±0.82 ^b
Group 3	20.20±0.82	20.29±0.03	21.33±1.07
Group 4	20.37±0.06	21.28±0.84	21.18±1.01
Group 5	19.46±0.71	19.59±0.89	21.74±0.63 ^a

Data represented as Mean ± SEM; (^a = significantly higher relative to all other groups; ^b = significantly lower compared to all other groups)

Table 8. Effect of antioxidant supplement on glutathione peroxidase (GPx) activity in normal and HFD/STZ-induced diabetic rats

Groups	Week 4	Week 8	Week 12
Group 1	26.76±0.64	27.12±1.75	25.36±1.27
Group 2	20.95±0.52 ^b	21.84±2.06 ^b	18.17±0.91 ^b
Group 3	24.52±0.59	24.52±0.75	27.11±1.36
Group 4	25.06±1.22	25.14±0.75	27.15±1.27
Group 5	27.25±0.87	26.11±0.75	27.53±1.60

Data represented as Mean ± SEM; (^a = significantly higher relative to all other groups; ^b = significantly lower compared to all other groups)

Table 9. Effect of antioxidant supplement on glutathione reductase (GR) activity in normal and HFD/STZ-induced diabetic rats

Groups	Week 4	Week 8	Week 12
Group 1	22.18±1.06	24.52±0.09	23.86±1.19
Group 2	17.95±0.73 ^b	21.21±1.48 ^b	17.56±0.88 ^b
Group 3	21.00±0.57	23.14±0.02	23.81±1.19
Group 4	21.76±0.33	22.37±0.70	23.82±0.06
Group 5	23.19±0.12 ^a	22.22±0.63	24.53±0.64

Data represented as Mean ± SEM; (^a = significantly higher relative to all other groups; ^b = significantly lower compared to all other groups)

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