



Hematological Indices in Alloxan-Induced Diabetic Rats: Effect of Supplementation with the Antioxidant Dimethyl Sulfoxide

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Authors' contributions

This work was carried out in collaboration between both the authors. Author MKD designed the study. Author FUB did the literature searches. Authors FUB and MKD designed the protocol. The experiment and analysis of data were handled by both the authors jointly. The first draft of the manuscript was written by author FUB. Both authors read, reviewed and approved the final manuscript.

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ABSTRACT

Background: Antioxidants are substances that protect the cell damage caused by free radicals. Dimethyl sulfoxide (DMSO) has radical-scavenging activity thus acting as antioxidant. In this study, we evaluated haematological changes in alloxan-induced diabetic rats treated or not with DMSO.

Materials and Methods: 24 adult wistar albino rats, aged 9-11 weeks, were divided into three groups of eight rats each: control, diabetics and diabetics supplemented with DMSO (0.5 ml/kgbw/day for two weeks). Diabetic groups received a single intraperitoneal injection of freshly dissolved alloxan monohydrate (150 mg/kgbw) in normal saline. 5% glucose dissolved in clean water and same volume of normal saline served as drinking water for diabetic rats and controls, respectively. Hematological parameters (RBC, HCT, HGB, WBC, LYM, GRA, MCH, MCHC, MCV and PLT) were determined by automated hematology analyzer.

Results: The mean values of RBC, HCT, HGB, GRA and PLT (5.58 ± 0.21 , 34.84 ± 1.96 , 10.35 ± 0.69 ,

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8.21±1.20 and 608.89±61.0, respectively) of the diabetic DMSO-supplemented rats were significantly different ($P<0.05$) when compared to the diabetic unsupplemented rats (14.16±7.18, 52.18±1.17, 15.21±0.39, 11.58±2.03 and 626.88±56.0 respectively). Moreover, the mean value of PLT (76.20±1.10) in DMSO-supplemented rats was significantly higher ($P= 0.01$) than that of the diabetic unsupplemented animals (56.21±1.9). Statistically significant difference was also observed ($P<0.05$) in blood glucose of diabetic DMSO-supplemented rats (15.38±1.56 and 10.30±2.13 mmol/L after, respectively, 72 hours and 2 weeks of diabetes induction) compared to diabetic unsupplemented rats (24.75±3.06 and 22.93±2.82 mmol/L after, respectively, 72 hours and 2 weeks of diabetes induction).

Conclusion: Treatment with DMSO tends to reverse all the aforementioned hematologic anomalies to near control values in diabetic rats. Thus, diabetes mellitus predisposes to haematological abnormalities that may be prevented or reversed by treatment with the antioxidant compound DMSO.

Keywords: Diabetes mellitus; hematological parameter; dimethyl sulfoxide (DMSO); wistar albino rats.

1. INTRODUCTION

Diabetes mellitus is a multifactorial and heterogeneous disorder with both genetic and environmental factors contributing its pathogenesis [1]. Diabetes mellitus is a disorder of carbohydrate, fat and protein metabolism characterized by high blood sugar levels (hyperglycemia) and presence of sugar in the urine (glycosuria) [2]. Diabetes is due to either the pancreas not producing enough insulin or the cells of the body not responding properly to the insulin produced or both [3]. The chronic hyperglycemia of diabetes mellitus is associated with long-term damage, dysfunction, and failure of various organs, especially eyes, kidneys, nerves, heart and blood vessels.

Alloxan is a toxic glucose analogue, which selectively destroys insulin-producing β -cells in the pancreas when administered to rodents and many other animal species. This causes an insulin-dependent diabetes mellitus (alloxan diabetes), with characteristics similar to type 1 diabetes in humans [4]. Alloxan in the presence of intracellular thiols, generates reactive oxygen species (ROS) in a cyclic reaction with its reduction product, dialuric acid. The β -cell toxic action of alloxan is initiated by free radicals formed in this redox reaction. Antioxidants are substances that may protect cells from the damage caused by these free radicals. Antioxidants can interact with, stabilize and scavenge free radicals, preventing their noxious effects [5].

Dimethyl sulfoxide (DMSO) is an amphipathic molecule with a high polar domain and two apolar methyl groups making it soluble in both

aqueous and organic media. In 1978, it received approval by the United State Food and Drug Administration (FDA) for use in the treatment of interstitial cystitis by instillation [6]. Moreover, DMSO has been used successfully in the treatment of dermatological [5,7], urinary [8], pulmonary [9], rheumatic and renal manifestations of amyloidosis [4].

DMSO has an anti-inflammatory and radical-scavenging action [10]. Its use has been proposed in gastrointestinal diseases [11]. DMSO crosses the blood-brain barrier [12], so that it may be used in the treatment of traumatic brain oedema [13]. It has also been in the treatment of musculoskeletal disorder [14], pulmonary adenocarcinoma [15], rheumatological diseases [16], chronic prostatitis [17] dermatologic diseases [18], schizophrenia [19] and as a topical analgesic [20]. In addition, it has been suggested for the treatment of Alzheimer's disease [21].

Some studies have shown that antioxidants are effective and cheaper than conventional therapy in management of certain diseases [22,23]. Therefore, antioxidants or nutrients with high antioxidant capacity may offer additional health benefits with potential for limiting the progression of diabetes and its related complications [24,25,26]. The aim of this study was to evaluate hematological parameters in rats with alloxan-induced diabetes treated or not with DMSO.

2. MATERIALS AND METHODS

All chemicals, equipments and reagents used in the study were of analytical grade.

2.1 Ethical Consideration and Clearance

An ethical clearance for this study was sought and obtained from the relevant ethical committee (faculty of veterinary medicine, UDUS) prior to the commencement of this study. The ethical clearance number of the study is: VETUDUS/FEC/198.

2.2 Experimental Animals

Twenty four Wistar Albino rats were used in the study. Apparently healthy animals were kept at normal environmental temperature of 27 - 30°C. They were housed in well ventilated wire mesh cages and fed *ad libitum* with standard commercial pelleted feed purchased from Vital Feed Nigeria, with free access to clean drinking water.

2.3 Experimental Design

Rats were divided into 3 groups of 8 rats each, viz: Group 1: the control group, neither diabetes-induced nor supplemented, group 2: rats rendered diabetic by alloxan and not supplemented with DMSO, and group 3: rats with alloxan-induced diabetes supplemented with DMSO.

2.4 Induction of Diabetes

To rats fasted overnight, diabetes was induced by a single intraperitoneal injection of freshly dissolved alloxan monohydrate (150 mg/kg body weight) in normal saline maintained at 37°C. 5% glucose dissolved in clean water served as their drinking water. Control rats received the same volume of normal saline only [27].

2.5 Administration of DMSO

Using an oral cannula, rats of group 3 were treated with dimethyl sulfoxide (DMSO, 0.5 ml/kg body weight) daily in distilled water for two consecutive weeks.

2.6 Blood Collection

Blood samples were collected from the tail ends of the rats using a lancet in basal conditions and after 72 hours of diabetes induction for glucose analysis using glucometer. At the end of the supplementation period, the rats in all the three groups were fasted overnight and blood was

collected in the same manner for glucose analysis using glucometer. The rats were then anaesthetized by dropping each in a transparent jar saturated with chloroform vapour. Incision was made on the abdomen and blood sample was collected through cardiac puncture into ethylene diamine tetraacetic acid (EDTA) container for the haematological parameters analysis.

2.7 Inclusion Criteria

Apparently healthy adult rats weighing 110-160 g with fasting blood glucose levels greater or equal to 7.0 mmol/L (126 mg/dl) were included in this study.

2.8 Exclusion Criteria

Wistar albino rats weighing below 110 g and above 160 g were excluded from the study. Rats with fasting blood sugar levels less than 7.0 mmol/L were also excluded.

2.9 Statistical Analysis

Statistical analysis was performed using SPSS version 20.0. Data are presented as mean \pm standard error of mean (SEM). Statistical comparison between groups was made using analysis of variance (ANOVA) with post hoc Bonferroni comparison test to identify differences in mean where appropriate. A P -value < 0.05 was considered as statistically significant.

3. RESULTS

The mean \pm SEM values of fasting blood glucose of control, diabetic unsupplemented and diabetic supplemented are shown in Table 1. Fasting blood glucose values after 72 hours and 2 weeks of DMSO treatment were different ($P < 0.05$) between the control, unsupplemented diabetic and DMSO-supplemented diabetic rats (Table 1). In particular, DMSO supplementation reduced significantly fasting glycemia in comparison with the unsupplemented diabetic rats (Table 1).

Table 2 shows the haematological parameters in the control, unsupplemented diabetic and DMSO-supplemented diabetic groups. Statistically significant differences ($P \leq 0.05$) were observed for RBC, LYM, HGB, HCT, GRA and PLT between unsupplemented and DMSO-supplemented rats (Table 2).

Table 1. Fasting blood glucose of control, unsupplemented diabetic and DMSO-supplemented diabetic rats

	Parameter		
	Initial FBG (mmo/L)	After 72 hours FBG (mmo/L)	Final FBG (mmol/L)
CG (n=8)	7.20±1.07	7.78±1.01	7.05±1.03
UDG (n=8)	7.10±1.06	24.75±3.06	22.93±2.82
SDG (n=8)	7.10±1.06	15.38±1.56	10.30±2.13
P-value			
	0.056	0.001	0.001
	0.057	0.001	0.001
	0.755	0.01	0.01

Data are expressed as mean±SEM. CG: control group; UDG: unsupplemented diabetic group; SDG: DMSO-supplemented diabetic group; FBG: Fasting blood glucose. The first, second and third P-values are between CG and UDG, CG and SDG, and between DUG and SDG, respectively.

Table 2. Haematological parameters in the control, unsupplemented diabetic and DMSO-supplemented diabetic rats at the end of the experiment

	CG(n=8)	UDG	SDG(n=8)	P-values
RBC (10 ¹² /u L)	5.34±0.44	14.16±7.18	5.58±0.21	0.001, 0.56, 0.001
HCT (%)	34.51±2.04	52.18±1.17	34.84±1.69	0.01, 0.59, 0.01
HGB (g/dl)	10.48±0.21	15.21±0.39	10.35±0.69	0.001, 0.051, 0.001
WBC (10 ⁹ /u L)	7.77±0.9	18.24±1.10	8.10±1.21	0.05, 0.05, 0.05
LYM (10 ³ /u L)	78.94±1.55	56.21±1.90	76.20±1.10	0.001, 0.051, 0.01
GRA (10 ³ /u L)	8.54±1.27	11.58±2.03	8.21±1.20	0.01, 0.05, 0.01
MCHC (g/dL)	31.15±0.73	30.91±0.97	31.36±0.63	0.05, 0.05, 0.05
MCH (pg)	20.78±4.02	19.58±0.29	19.18±0.28	0.05, 0.05, 0.05
MCV (fl)	63.40±4.63	62.43±1.59	63.30±1.56	0.051, 0.051, 0.051
PLT (10 ⁹ /uL)	606.88±60	626.88±56.0	608.89±61.0	0.01, 0.052, 0.01

Data are expressed as mean ± SEM; P-value ≤ 0.05 is statistically significant. RBC: red blood cells; HCT: haematocrit; HGB: haemoglobin; WBC: white blood cells; LYM: lymphocytes; GRA: granulocytes; MCHC: mean cell haemoglobin concentration; MCH: mean cell haemoglobin; MCV: mean cell volume; PLT: platelets; CG: control group; UDG: unsupplemented diabetic group; SDG: DMSO-supplemented diabetic group. P-value: first, second and third P-values are between CG and UDG, CG and SDG, and UDG and SDG respectively.

4. DISCUSSION

Recent research evidences suggest that haematological indices are altered in diabetes [28]. In this study the mean fasting blood glucose concentration of the unsupplemented diabetic group was found to be higher than that of the DMSO-supplemented diabetic group. This is in contrast to the study by Adeneye and Agbaje [29], and by Kianbakht and Kajiaghaee [30], which reported higher fasting blood glucose in supplemented than unsupplemented diabetic animals. The variation may be due to difference in the dose of alloxan and DMSO and also the method of glucose estimation. In this study we used 150 mg/Kg of alloxan, 0.5 ml/Kg body weight of DMSO and 150 mg/Kg of alloxan, 10 ml/Kg of DMSO and Trinder oxidase method for glucose analysis [28]. Kianbakht and Kajiaghaee [30] used 125 mg/Kg of alloxan, 3 ml/Kg of

DMSO and oxidase method for serum glucose analysis [30].

In this study the mean fasting blood glucose of the unsupplemented and DMSO-supplemented diabetic groups was found to be higher compared to the control group, a finding in agreement with the study by Kianbakht and Kajiaghaee [30]. The LYM (10³/u L) of the control and DMSO-supplemented diabetic group was found to be higher compared to the unsupplemented diabetic group. This is in line with the results of the study by Muhammad et al. [31].

Several haematological changes affecting the RBCs, WBCs and the coagulation factors are shown to be directly associated with diabetes mellitus [5]. Notably, the current study shows that the mean RBC, HGB, GRA, and PLT values of

the DMSO-supplemented diabetic group are near control values compared to the unsupplemented diabetic group, a finding apparently in contrast to the study by Azmi and Qureshi [32].

Vascular complications resulting from high platelets count can be prevented through the use of DMSO as it reduces the platelets count of the supplemented group. Also complications resulting from anemia such as diabetic nephropathy could be prevented through supplementation with DMSO since it maintains the haematocrit, hemoglobin and the red cell count of the supplemented group to near control values. The supplement may also burst the immune system by increasing the lymphocytes count thereby preventing immune disorders associated with diabetes mellitus. Hyperglycemia has also been reduced by this supplement [33].

DMSO has a broad spectrum of biological activities suggesting efficacy as neuroprotective agent. These activities include hydroxyl radical scavenging, reduced platelet aggregation and adhesiveness and prevention of glutamate-induced neuronal cell death [34].

The unique capability of DMSO to penetrate living tissues without causing significant damage is most probably related to its relatively polar nature, its capacity to accept hydrogen bonds, and its relatively small and compact structure [33]. This property combination results in the ability of DMSO to associate with water, proteins, carbohydrates, nucleic acid, ionic substances, and other constituents of living systems. Of foremost importance to our understanding of the possible functions of DMSO in biological systems is its ability to replace some of the water molecules associated with the cellular constituents, or to affect the structure of the omnipresent water [34].

5. CONCLUSION

Treatment of diabetic rats with DMSO, a potent radical-scavenging antioxidant compound, tends to reverse all the aforementioned haematological anomalies to near control values. Thus, in the experimental setting diabetes mellitus predisposes to haematological abnormalities that can be prevented or reversed by treatment with DMSO.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the author.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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