

EFFECT OF AQUEOUS LEAF EXTRACT OF *SENECIO BIAFRAE* ON HYPERGLYCAEMIC AND HAEMATOLOGICAL PARAMETERS OF ALLOXAN-INDUCED DIABETIC RATS

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Abstract

Aim of this study is to evaluate the effect of aqueous leaf extract of *Senecio biafrae* on hyperglycemic and haematological parameters of alloxan-induced diabetic rats for fifteenth day.

Twenty five rats, grouped into five with five rats per each group. Group A non-diabetic rats, the remaining twenty rats were induced interperitoneally with 150mg/kg of alloxan monohydrate and grouped into four. Group B diabetic control, Group C metformin treated group, Group D were treated with 200mg/kg body weight of *Senecio biafrae* aqueous leaf extract while Group E were treated with 400mg/kg body weight of *Senecio biafrae* aqueous leaf extract. Their blood glucose were monitored every two days and haematological parameters such as haemoglobin (Hb), red blood cell (RBC), platelet, packed cell volume (PCV), white blood cell (WBC), Lymphocyte (L), monocytes (M), neutrophils (N), Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were determined.

Statistical analysis: the extracts show significant decrease ($p < 0.05$) in the blood glucose, while there were significant increase in all haematological parameters determined when compared with diabetic control especially 400mg/kg body weight.

These results suggested that the aqueous leaf extract of *Senecio biafrae* have both hypoglycaemic and antianaemic properties.

Keywords: *Senecio biafrae*, leaf, aqueous extract, diabetic rats, hypoglycaemia, haematological parameters

Introduction

Diabetes is a disease associated with glucose metabolism resulting from defects in insulin secretion and action [1]. It is characterized by hyperglycemia, glucosuria and several microvascular and macrovascular complications [2-3].

The complications of diabetes are linked to oxidative stress induced by hyperglycemia which overcomes the body's natural anti-oxidant system [4-5]. The incidence of diabetes is on the rise and is estimated to be over 150 million worldwide [6]. Moreover, assessment of haematological parameters can be used to assess the extent of destructive effects on blood constituents of animals due to diabetes mellitus [7]. As a result of glycosylation and stiffening of red blood cells, this may be responsible for, or associated with, large vessel disease in diabetes [8]. There is yet no effective cure for diabetes. The currently available drugs and insulin used in managing the disease are associated with several undesirable side effects [8-9-10] and its high cost has led to search for plants with normoglycemic properties in the management of diabetes [11-12]. Several species of medicinal plants used in traditional treatment and management of diabetes worldwide have been evaluated [13-14]. The hypoglycemic properties of plants used in management of diabetes are reported to be due to their content of flavonoids, glycosides, alkaloids terpenoids, plant polysaccharides and other bioactive compounds [15]. *Senecio biafrae* (called "worowo" in Western part of Nigeria) is a vegetable; grow mostly under cocoa trees plantation. This plant has been believed to be endowed with medicinal usefulness. Therefore, the aim of this study is to evaluate the effect of aqueous leaf extract of *Senecio biafrae* on hyperglycemia and haematological parameters of alloxan-induced diabetic rats

Material and methods

Senecio biafrae (figure 1) leaf was purchased from Oja-Oba Market, Ado-Ekiti, Ekiti State, Nigeria. Authentication was done in the University of Ilorin where a voucher number was given. The sample was then dried using an oven at 50°C, subsequently followed by milling in an automatic electrical blender (model MS-223, China) to form powder.

Chemicals and drugs

Alloxan monohydrate was obtained from sigma

chemical company, St Louis Mo, USA and metformin from Merck Sante, France.

Preparation of Aqueous extraction

50g of *Senecio biafrae* leaf powder was weighted into a conical flask; 500ml of distilled water {i.e. 1:10} (at normal room temperature) was then added, stirred and plugged with cotton wool. The mixture was filtered after 24hours using cheese cloth and then through Whatman No.1 filters paper. The filtrate was concentrated using rotary evaporator and followed by freeze drier [16].

Laboratory animals

Twenty five albino rats (*Rattus norvegicus*) of both sexes weighing between 140-200g were obtained from the animal holding section of the Department of Biochemistry, Afe Babalola University, Ado Ekiti, Ekiti State, Nigeria. The animals were kept in cages to acclimatize with ambient temperature of 26-28°C, standard environmental conditions of 12hrs light and 12hrs dark and adequate ventilation for two weeks. The ethical guidelines of the University were followed strictly throughout the experimental period.

Induction of diabetes mellitus in experimental animals

Rats were fasted for 12 hours (their blood glucose levels were recorded) before being induced by the administration of single intraperitoneal dose of alloxan monohydrate (150mg/kg) (dissolved in 0.9% sterile NaCl solution of pH 7) [17-18] (type II diabetes mellitus), except rats in Group A which were non-diabetic rats. Two days after alloxan injection, rats with blood glucose level of 200 mgdL⁻¹ and above were used for the study. All animals were allowed free access to water and pellet diet, while the aqueous extract (*Senecio biafrae*) was administered orally at concentrations of 200 and 400mg/kg body weight per day for 15 days for the appropriate Groups

Mechanism of alloxan action

Alloxan is a toxic glucose analogue, which selectively destroys insulin-producing cells in the pancreas (that is beta cells). This is because it preferentially accumulates in beta cells through uptake via the GLUT2 glucose transporter. Alloxan, in the presence of intracellular thiols, generates reactive oxygen species (ROS) in a cyclic reaction with its reduction product, dialuric acid. The beta cell toxic action of alloxan is initiated by free radicals formed in this redox reaction [19].

Experimental Design

A total of twenty five rats with twenty diabetic rats and five normal rats, were divided into five groups with five rats each.

Group A: Non-diabetic rats

Group B: Diabetic control rats

Group C: Diabetic rats given standard drug metformin

Group D: Diabetic rats given 200mg/kg body weight of *Senecio bialfrae* leaf extract

Group E: Diabetic rats given 400mg/kg body weight of *Senecio bialfrae* leaf extract

Blood Glucose level Determination

Fasting rat's blood glucose (12 hours) was determined by glucose oxidase method with the aid of Accu chek glucometer (Roche diagnostics, Germany). This was done by cutting the tail end of the rat's tail with sharp blade and a drop of blood was squeezed onto the marked point of the strip inserted into the glucometer, this was repeated every 48 hrs till the end of the experiment (15 days).

Collection and analysis of samples

At the end of the experiment (15day), the animals were fasted for 12 h, anaesthetized with diethyl ether with the aid of cotton wool. The animals were then sacrificed, with the collection of their blood by cardiac puncturing. Their blood was collected into EDTA tubes, used for the analysis.

Haematological parameters determination

The parameters such as packed cell volume (PCV), haemoglobin (Hb), white blood cell (WBC), red blood cell (RBC), platelets, neutrophil, lymphocytes, monocytes, mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular volume (MCV) were analyzed using an automated analyzer (Sysmex K-2 IN, Japan)

Statistical analysis

All the data were analyzed by statically using students' T- test and one way ANOVA. Values of $p < 0.05$ were considered significant.

Results

The effect of diabetic animals on body weight was depicted in Table 1, in which there was significant

different ($p < 0.05$) in final body weight of all the Groups. Although, there was significant increase ($p < 0.05$) in final body weight of Group E (400mg/kg body weight) when compared with diabetic control Group (Group B). Table 2 shows significant increases ($p < 0.05$) in the fasting blood glucose levels of diabetic control (Group B) when compared to other Groups, with 400mg/kg body weight (Group E) performs best among the two extract treated Groups. Table 3 shows significant reduction ($p < 0.05$) in the activities of haemoglobin (Hb), packed cell volume (PCV), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV), platelet (PL), red blood cell (RBC), white blood cells (WBC), neutrophil (N), lymphocyte (L) and monocytes (M) in diabetic untreated Group when compared to control, metformin, 200mg/kg and 400mg/kg with Groups.

Discussion

The examination of blood has been described as a good way of assessing the health status of animals, because it plays an important role in the physiological, nutritional and pathological status of organisms [20-21]. This can also be used to determine the degree of damage due to single dose of alloxan-induced diabetic on blood constituents of the animals [22]. Also, during diabetes the excess glucose level observed in the blood might react with haemoglobin to form glycosylated haemoglobin [23].

In this study, the aqueous leaf extract of *Senecio bialfrae* significantly increased the final body weight (Table 1) of alloxan-induced diabetic rats, especially at 400mg/kg body weight; this may be attributed to the availability of glucose, as a source of energy in the tissues [24] and probably the nutrients levels in the plants as earlier reported [25-26]. The aqueous leaf extract of *Senecio bialfrae* significantly reduced ($p < 0.05$) the blood glucose level (Table 2) in alloxan-induced diabetic rats especially at higher dosage of 400mg/kg body weight. This is possible that the plant extract may increase glucose removal from blood, decrease the release of glucagon or increase that of insulin, stimulate directly glycolysis in peripheral tissues or reduce glucose absorption from the gastrointestinal tract [27-28]. It may also be due to inhibitory action of the extract on α -glucosidase which is an enzyme found in the brush border of the intestine and is responsible for the conversion of polysaccharides into simple sugars. The inhibition of

this enzyme slows the elevation of sugar after carbohydrate meal, which is one the way to decrease postprandial increase in blood glucose level [29].

The antioxidant minerals and vitamin present in the plant as reported earlier by Ajiboye *et al* [25] might contribute towards the antidiabetic effects of the extract by providing protection against cytotoxic effect of free radicals generated by alloxan.

Moreover, haematological parameters provide information regarding the status of bone marrow activity and hemolysis [30]. Furthermore, it has been revealed by the present study that haematological parameters in diabetic control (untreated group) showed abnormalities. This might be due to the destruction of matured red blood cells leading to low haemoglobin count (Hb) (because of reaction of excess glucose with the haemoglobin to give rise to glycosylated haemoglobin) with decrease in red blood cell (RBC) [31] (an indication of imbalance between its synthesis and destruction) and packed cell volume (PCV) normally being affected by alloxan-induced diabetic, an indication of anaemia [32]. The decrease in the platelet count might indicate that the oxygen carrying ability of the blood has been altered [33].

In addition, decrease in the MCV, MCHC and MCH relates to individual red blood cells while decrease in WBC and its indices (lymphocytes, neutrophil, monocytes) in diabetic control (Group B) might indicate decrease in immune system in fighting foreign substances [24]. Excellent performance of the aqueous extract (especially at 400mg/kg) in reversing all these irregularities in the haematological parameters may be ascribed to the presence of iron in the plant extract as reported by Ajiboye *et al* [25], an essential component of many enzymes in cells and parts of heme group in haemoglobin. Most iron in the body is stored, within the red blood cells where iron is very crucial for haemoglobin synthesis [30]. Also, the presence of other antioxidant vitamins (vitamin A, B, C and E) and minerals (Zn, Se, Fe, Cu etc), total flavonoid and total phenol reported by Ajiboye *et al* [26] might also be responsible in improving the immune system being weak due to the generation of reactive oxygen species as a result of alloxan-induction and shows that the aqueous extract may not have a negative effect on the bone marrow, kidney and haemoglobin metabolism [34].

Conflict of Interest

The authors wish to declare that there was no conflict of interest before and after this research work.

Conclusion

The oral administration of aqueous leaf extract of *Senecio bialfrae* significantly demonstrated normoglycemic effects in alloxan-induced diabetic rats. Also, the extract might ameliorate haematological parameter disturbances as a result of alloxan-induced.

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Figure 1: Sample of *Senecio biafrae***Table 1:** Effect of *Senecio biafrae* leaf aqueous extract on body weight of alloxan-induced diabetic rats

Group	Initial Weight	Final Weight
Control	190±2.30 ^a	218±2.1 ^a
Diabetic control	186±1.62 ^a	98±1.09 ^e
Metformin	184±3.10 ^a	158±1.20 ^d
<i>Senecio biafrae</i> (200mg/kg)	188±2.80 ^a	178±2.0 ^c
<i>Senecio biafrae</i> (400mg/kg)	185±3.20 ^a	186±1.20 ^b

Each values is a mean of five determination ± SEM
 Column values with different superscripts (a, b, c, and d) are significantly (p<0.05) different

Table 2: Effect of *Senecio bialfræ* leaf aqueous extract on blood glucose level (mg/dl) of alloxan-induced diabetic rats

Group	Initial glucose level before alloxan induction	Glucose level after alloxan induction	Final glucose level
Control	82±1.42 ^a	76±2.10 ^a	73±2.10 ^a
Diabetic control	88±2.41 ^b	513±2.1 ^c	520±3.1 ^e
Metformin	83±1.21 ^a	490±1.20 ^b	113±2.10 ^b
<i>Senecio bialfræ</i> (200mg/kg)	88±2.41 ^b	546±2.10 ^d	180±4.0 ^d
<i>Senecio bialfræ</i> (400mg/kg)	82±1.81 ^a	520±1.30 ^c	120±3.0 ^c

Each values is a mean of five determination ± SEM

Column values with different superscripts (a, b, c, and d) are significantly (p<0.05) different

Table 3: Effect of oral administration of aqueous leaf extract of *Senecio bialfræ* on haematological parameters in alloxan-induced albino rats

Groups	Hb (g/100ml)	PCV (%)	MCHC (g/l)	MCH (pg)	MCV (fl)	PL (X10 ⁹ /l)	RBC (X10 ⁶ /µL)	WBC (X10 ³ /mm ³)	N(%)	L(%)	M (%)
Control	15.4±0.02 ^a	47.01±0.03 ^a	0.44±0.01 ^a	4.12±0.13 ^a	10.20±0.12 ^a	169.00±0.02 ^a	5.60±1.20 ^a	9.80±0.03 ^a	40.01±0.01 ^a	45.00±0.01 ^a	16.00±0.12 ^a
Diabetic control	13.1±0.05 ^b	30.23±0.40 ^c	0.21±0.02 ^c	2.14±0.23 ^b	4.45±0.02 ^d	120.01±0.04 ^b	2.50±0.02 ^c	4.2±0.01 ^d	31.02±0.03 ^b	34.10±0.01 ^c	10.2±0.31 ^c
Metformin	15.90±0.06	48.22±0.01 ^a	0.39±0.04 ^a	3.94±0.12 ^a	9.40±0.04 ^b	158.00±1.20 ^a	4.89±0.1 ^a	7.20±0.11 ^b	39.01±0.23 ^a	45.01±0.0 ^a	14.23±0.21 ^b
<i>Senecio bialfræ</i> (200mg/kg)	16.01±0.02	44.01±0.02 ^b	0.32 ^b ±0.23	3.68±0.13 ^a	6.48±0.05 ^c	160.01±1.20 ^a	3.89±0.03 ^b	5.40±0.04 ^c	40.10±0.02 ^a	43.01±0.0 ^b	13.00±0.34 ^b
400mg treated	14.9±0.03 ^a	48.23±0.03 ^a	0.38±0.04 ^a	3.98±0.03 ^a	6.12±0.12 ^c	168.00±1.32 ^a	4.50±0.05 ^a	5.60±0.01 ^c	41.20±0.02 ^a	44.34±0.3 ^a	13.23±0.5 ^b

Values are expressed as mean of five replicates ± SEM

Column values with different superscripts (a, b, c, and d) are significantly (p<0.05) different