

Full Length Research Paper

Effect of aqueous leaf extract of *Senecio bialfrae* on hyperglycaemic and serum lipid profile of alloxan-induced diabetic rats

*Ajiboye B.O¹, Edobor G¹, Ojo A.O¹, Onikanni S.A¹, Olaranwaju O.I² and Muhammad N.O³

¹Department of Chemical Sciences, Biochemistry Unit, Afe Babalola University, Ado-Ekiti, Ekiti State, Nigeria.

²Department of Biochemistry, Ado-Ekiti State University, Ekiti State, Nigeria.

³Department of Biochemistry and molecular Biology, Federal University, Dustin-ma, Kastina State, Nigeria.

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Diabetes mellitus has been characterized by increased levels of oxidative stress, hyperglycemia and hyperlipidemia which are implicated in the development of diabetic complications. Therefore, the aim of this study is to assess the effects of aqueous leaf extract of *Senecio bialfrae* on hyperglycemic and hyperlipidemia on alloxan-induced diabetic rats. Twenty five male and female rats weights between 140-200g were used for the study and divided into five groups of five rats in each. Group A were non-diabetic rats, the remaining four groups were induced interperitoneally with 150mg/kg of alloxan monohydrate. Group B were diabetic control, Group C were metformin treated group, Group D were treated 200mg/kg of *Senecio bialfrae* aqueous leaf extract while Group E were treated with 400mg/kg of *Senecio bialfrae* aqueous leaf extract. The experiment period was 15 days, with the determination of their glucose level every two days. At the end of the experimental period blood sample were collected and total cholesterol, triglycerides, very low density lipoprotein, low density lipoprotein and high density lipoprotein were assayed. Statistical analyses of the results shows significant reduction in the blood glucose, total cholesterol, triglycerides, low density lipoprotein and very low density lipoprotein levels of diabetic rats treated with *Senecio bialfrae* aqueous leaf extract (with highest performance at 400mg/kg) compared to diabetic control. Also, *Senecio bialfrae* aqueous leaf extract treated diabetic rat's shows significant increase in HDL levels compared to the diabetic control. Therefore these results suggested that the aqueous leaf extract of *Senecio bialfrae* possess both hypoglycaemic and hypolipidaemic effects.

Key words: *Senecio bialfrae*, leaf, aqueous extract, diabetic rats, hypoglycaemia, hypolipidaemia.

INTRODUCTION

Diabetes mellitus is among the most common disorder in developed and developing countries (Udenze et al., 2012; Makund et al., 2008). The disease is increasing rapidly in most parts of the world (Kumar et al., 2008). In 2000, according to WHO, at least 171 million people worldwide suffer from diabetes which corresponds to 2.8% of population (Wild et al., 2004), this figure is expected to double in the year 2030. Hyperglycaemia in diabetic patients is associated with alterations in glucose and lipid metabolism and modification in liver enzyme levels

(Jenson and Stender, 1998). Diabetes mellitus is consistently characterized by abnormalities in lipid profile (Andallu et al., 2009) and an increase in atherogenic index (Mazumder et al., 2009). Diabetes mellitus has been recognized as a major risk factor for cardiovascular Diseases (CVD), such as atherosclerosis, heart attacks, stroke (Mazumder et al., 2009). About 75% of deaths among men and 57% of death among women with diabetes are attributable to CVD (Moller, 2004). The use of plants in traditional medical practice has a long drawn history and remains the mainstay of primary health care in most of the third world (Prescott-Allen and Prescott-Allen, 1982). Traditional medicines are used by about 60% of the world population in both developing and developed countries (Mythilypriya et al., 2007).

*Corresponding author. E-mail: bash1428@yahoo.co.uk

Senecio bialfræ (with local name “worowo” by Yoruba tribe in Nigeria or bologni in Sierra Leone) belong to the group of vegetables that grow in large quantity in undercover tree crop plantation (most especially under cocoa trees). Moreover, fresh succulent leaf of *Senecio bialfræ* is used in Sierra Leone, Ghana, Benin, Nigeria, Cameroon and Gabon. They are highly popular in south-western part of Nigeria. They are usually cooked with pepper, tomato and onions. In such dishes there is no need for meat or fish because of the excellent properties of the vegetable, reflected in the Yoruba proverb “vegetable soup prepared with *Senecio bialfræ* does not need meat” (Aji boye et al., 2013a; Stevels, 1990). This plant has been speculated to be endowed with medicinal properties. Moreover, frequent uses of the synthetic anti-diabetic drugs are characterized by side effects and toxicity (Luo et al., 2004). Therefore, there is need to discover more effective hypoglycaemic agents with minimum side effects becomes apparent. The aim of this study is to evaluate the effect of aqueous leaf extract of *Senecio bialfræ* on hyperglycemia and serum lipid profile of alloxan-induced diabetic rats and to ascertain if it could protect against coronary heart disease (CHD) which is secondary to diabetes.

MATERIAL AND METHODS

Sample of *Senecio bialfræ* leaf was obtained from Oja-Oba Market in Ado-Ekiti, Ekiti State, Nigeria. It was then authenticated in the University of Ilorin where a voucher number was given. The leaf was dried in an oven at 50°C, which was milled using an automatic electrical blender (model MS-223, China) to powder.

Chemicals and Drugs

Alloxan and all kits used for this study were obtained from sigma chemical company, St Louis Mo, USA. All other chemicals used were of analytical grade.

Aqueous extraction

10 g of *Senecio bialfræ* leaf powder was added to 100 ml of distilled water (at normal room temperature) inside a conical flask and plugged with cotton wool. After 24 hours the mixtures was filtered using cheese cloth and then through Whatman No.1 filter paper. The filtrate was then concentrated using combination of rotary evaporator and freeze drier (Iweala and Okeke, 2005).

Laboratory Animals

Twenty five albino rats (*Rattus norvegicus*) of both sexes

weighing between 140-200g were purchased from the animal holding unit of the Department of Biochemistry, Afe Babalola University, Ado Ekiti, Ekiti State. The animals were kept in cages to acclimatize with conditions of the animal housing facility, ambient temperature of 26-28°C, standard environmental conditions of 12hrs light and 12hrs dark and adequate ventilation for two weeks. The rats were fed with standard rat pellet mash and clean water *ad libitum*.

Induction of Diabetes Mellitus

A single dose freshly prepared alloxan monohydrate of 150mg/kg bodyweight (dissolved in 0.9% sterile NaCl of pH 7) (Ebong et al., 2008; Osinubi et al., 2004; Habib et al., 2005) was administered intraperitoneally to rats in group B to E to induced type II diabetes. Prior to this, their blood glucose levels have been determined. After 48hrs rats that had blood glucose level above 200 mgdL⁻¹ were considered diabetic and selected for the study. Thereafter aqueous leaf extract of *Senecio bialfræ* was administered orally at concentrations 200 and 400mg/kg body weight rats per day for 15 days.

Experimental Design

The animals were divided into five groups with five rats per group. The groups are as follows:

Group A: Non-diabetic rats

Group B: Diabetic control rats

Group C: Diabetic rats treated with metformin

Group D: Diabetic rats treated with *Senecio bialfræ* leaf extract (200mg/kg)

Group E: Diabetic rats treated *Senecio bialfræ* leaf extract (400mg/kg)

Determination of blood glucose level

Fasting blood glucose was determined by glucose oxidase method using Accu chek glucometer (Roche diagnostics, Germany). The tail of the rat was cut swiftly with sterile scalpel and a drop of blood was squeezed onto the test area of strip inserted into the glucometer. The animals were fasted for 12hours before each glucose determination, which was repeated every 48hrs till the end of the experiment (15 days).

Collection and analysis of samples

At the end of the 15-day period, the animals were fasted for 12 h, anaesthetized with chloroform and then sacrificed and blood collected by cardiac puncture. Blood was collected into non heparinised sample tubes and

Table 1. Effect of *Senecio bialfræ* leaf aqueous extract on body weight (g) of alloxan-induced diabetic rats.

Group	Initial Weight	Final Weight
Control	196.21±2.30 ^a	211.12±2.1 ^a
Diabetic control	158.10 ±1.62 ^c	98.01±1.09 ^e
Metformin (treated)	170.03±3.1 ^b	143.90±1.20 ^d
<i>Senecio bialfræ</i> (200mg/kg)	184.11 ±2.8 ^a	158.62±2.0 ^c
<i>Senecio bialfræ</i> (400mg/kg)	163.06±3.20 ^b	165.16±1.20 ^b

Row values with different superscripts are significantly ($p < 0.05$) different
Each values is a mean of five determination ± SEM

allowed to clot for two hours and later centrifuged at 3000 rpm for 10 min, after which serum was recovered for analysis.

Analyses of the samples

The method describes by Trinder (1969) and Roeschlau et al (1974) was employed for determination of cholesterol concentration. The method of Tietz (1995) was employed for determination of triglycerides concentration and high density lipoprotein (HDL)

Concentration of LDL-Cholesterol

$$= \text{Total Cholesterol} - \frac{\text{Triglycerides}}{2.2} - \text{HDL Cholesterol}$$

(mmol/l)

Statistical Analysis

The data were analysed with students' T-test and one way ANOVA. Values of $p < 0.05$ were considered significant.

RESULT

Table 1 shows the effect of the *Senecio bialfræ* leaf aqueous extract on the body weight of alloxan-induced diabetic rats with significant decrease in the body weight of diabetic control group when compared to metformin group, 200mg/kg and 400mg/kg groups. Although

concentration. The method described by Richmond (1973) and Friedwald equation (Friedwald et al., 1972) was used for determining very low density lipoprotein (VLDL) concentration and low density lipoprotein (LDL) concentration.

Concentration of VLDL-Cholesterol

$$= \frac{\text{Triglycerides}}{2.2} \quad (\text{mmol/l})$$

400mg/kg perform better than 200mg/kg. Also, there were significant increase in the glucose concentration of diabetic control when compared to other groups, with higher performance at 400mg/kg (Table 2). In addition, the lipid profiles shows (Table 3) there were significant increase in cholesterol concentration, low density lipoprotein (LDL), Very low density lipoprotein (VLDL), and triglycerides concentrations, with significant decrease in high density lipoprotein (HDL) of diabetic control group when compared to extract treated groups with 400mg/kg perform better than 200mg/kg.

DISCUSSION

Diabetes mellitus is characterized by elevated level of oxidative stress indices, decreased level of antioxidants defenses and lipid abnormalities due to lipid peroxidation (Wali et al., 2013; Asayama et al., 1993).

In the present study, diabetes induced in the experimental animal by alloxan produced significantly decreased in body weight (Abed, 2002). The reduction of

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

Group	Initial glucose level	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 bialfræe</i> (200mg/kg)	88±2.41 ^b	546±2.10 ^d	180±4.0 ^d
<i>Senecio bialfræe</i> (400mg/kg)	82±1.81 ^a	520±1.30 ^c	120±3.0 ^c

Row values with different superscripts are significantly ($p < 0.05$) different
Each values is a mean of five determination \pm SEM

Table 3: Effect of *Senecio bialfræe* leaf aqueous extract on the lipid profile (mmol/l) of alloxan-induced diabetic rats

Group	Cholesterol	Triglyceride	High Density Lipoprotein (HDL)	Low Density Lipoprotein (LDL)	Very Low Density Lipoprotein (VLDL)
Control	11.12±0.12 ^a	1.45±1.20 ^a	8.21±0.21 ^a	2.24±0.01 ^a	0.67±0.01 ^a
Diabetic control	20.48±2.10 ^d	5.42±1.20 ^c	7.28±2.1 ^d	15.72±2.48 ^d	2.48±0.04 ^c
Metformin	10.40±1.10 ^a	1.99±1.20 ^b	5.20±0.01 ^c	4.29±0.21 ^b	0.91±0.02 ^b
<i>Senecio bialfræe</i> (200mg/kg)	14.89±0.21 ^c	1.88±1.18 ^b	5.86±0.81 ^b	8.18±2.96 ^c	0.85±0.10 ^b
<i>Senecio bialfræe</i> (400mg/kg)	12.96±0.21 ^a	2.43±1.10 ^b	6.20±0.10 ^b	5.66±1.20 ^b	1.10±0.03 ^b

Row values with different superscripts are significantly ($p < 0.05$) different
Each values is a mean of five determination \pm SEM

body weight in diabetic rats is due to dehydration and catabolism of fats and proteins (Hakim et al., 1992), increased catabolic reaction leading to muscle wasting can be the cause of the reduced body weight, body weight gain in 400mg/kg of alloxan induced-diabetic group is an indication that it support weight gain.

The extract significantly lowers the blood glucose level in alloxan induced rat. This may be due to the fact that the plant extract increases glucose removal from the 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 (Marrif et al., 1995; Alamgeer et al., 2012), since increase in glucose level in alloxan-induced

rats made it unable to enter the cells due to lack of insulin lead to deficiency of energy for The abnormally high concentration of serum lipids profile (which are likely to increase the risk of coronary heart diseases) may be due to increase in the mobilization of free fatty acids from peripheral tissue as a result of activation hormone sensitive lipase during insulin insufficiency. Lack of insulin in diabetes is also known to be associated with increased synthesis of cholesterol which may be due to increased activity of HMG CoA reductase reductase (Ahmed et al., 2010). Insulin resistance in diabetic rats could increase the hepatic uptake of fatty acids released by lipolysis of adipose tissue, the intrahepatic synthesis of triglycerides and the over production and secretion of

VLDL particle that, in turn, leads to increased plasma levels of TG (Daniele et al., 2010). HDL-cholesterol is the smallest of the lipoprotein species containing approximately 20% cholesterol ester and very little triglyceride. Low concentration of HDL being an important predictor of cardiovascular disease (CHD) and high HDL concentration (in dose dependent manner) may be an indication that the extract may play an important role in protecting against CHD. In addition, it has been reported by Udenze et al. (2012) and Witztum et al. (1982) that non-enzymatic glycosylation of HDL accelerates its catabolism and same mechanism may be responsible for the low level of HDL observed in alloxan-diabetic rats. LDL-cholesterol concentration has strongly and positively been linked to risk of atherosclerosis and other CHDs (Udenze et al., 2012). In the present study, orally administered of *Senecio bialfræ* aqueous leaf extract to diabetic rats (for 15-days) reduced significantly the serum TC, LDL-cholesterol, TG, and VLDL-cholesterol index in the diabetic treated group compared to the diabetic control and this reduction was dose dependent manner. The underlying mechanism of lipid lowering effect of *Senecio bialfræ* aqueous leaf extract (especially at 400mg/kg) could be by inhibition of lipid absorption due to the presence of saponin and tannin in *Senecio bialfræ* aqueous leaf extract as earlier reported by Ajiboye et al., (2013b) or by inhibition of cholesterol esterase, activation of fatty acid synthase, acetyl-CoA carboxylase and production of triglyceride precursors such as acetyl-CoA and glycerol phosphate (Udenze et al., 2012; Sharmila et al., 2007).

Moreover, the mechanism of lipid lowering effect of *Senecio bialfræ* aqueous leaf extract could be modulated by the flavonoid content (Ajiboye et al., 2013b). Flavonoids (capable of decreasing the triacylglycerols and total cholesterol in blood of rats) from plants have been reported by Udenze et al., (2012) and Jung et al., (2006) variously implicated in the reduction of lipids by inhibiting hepatic HMG-CoA reductase. The decrease of LDL levels may occur due to the reduction of VLDL and increased hepatic depuration of LDL precursors (Knett et al., 2002). Also, increase in serum HDL-cholesterol in diabetic treated group in dose dependent is an advantage since HDL-cholesterol is responsible for transportation of cholesterol from peripheral tissues to the liver for metabolism. It also inhibits oxidation of LDL by transition metal ions, but also prevents 12-lipoxygenase-mediated formation of lipid hydroperoxides (Nofer et al., 2002). Furthermore, high soluble dietary fiber of *Senecio bialfræ* leaf (Ajiboye et al., 2013a) may also be responsible for reduced serum lipid profile (cholesterol, triglycerides, VLDL and LDL), most especially at 400mg/kg because of increase in the activity of HMG-coA reductase with concomitant excretion of bile acids and neutral sterols in faces (Ramulu et al., 2011).

Therefore, the antioxidant vitamins (such as vitamins C and E) and minerals (e.g. magnesium, selenium, zinc etc.), coupled with essential amino acids present in the

leaf as reported by Ajiboye et al (2013a) may have play an important roles in reducing the risk of oxidative stress being generated in diabetics (due to alloxan induction), which may be closely associated with insulin metabolism and help in maintaining proper blood glucose level (Chung et al., 1998). Also, vitamin E has the potentials to lowers LDL-oxidation, thus lowering the risk of diabetic cardiovascular complications (Ceriello et al., 1992; Fuller et al., 1996).

CONCLUSION

It can be concluded that *Senecio bialfræ* aqueous leaf extract could be very useful in the management of diabetes couple with its cardio protective potentials.

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