



Research Articles

Amelioration of Haematological Alterations in Streptozotocin-induced Diabetic Rats by Aqueous Leaf Extract of *Senna fistula*

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SUMMARY

Objectives: This study investigated the effects of aqueous leaf extract of *Senna fistula* on some haematological parameters, glucose-6-phosphate dehydrogenase activity, lipid peroxidation and osmotic fragility changes in streptozotocin-induced diabetic rats.

Methods: Thirty albino rats were divided into six groups (A-F). such that A was the control non-diabetic, B was the diabetic that received distilled water, C was the diabetic group treated with 2.5 mg/kg body weight of glibenclamide while animals in groups D-F were made diabetic and treated with different doses (28.57, 57.14 and 114.28 mg/kg body weight) of the extract for 28 days.

Results: Packed cell volume (PCV), red blood cell count (RBC), haemoglobin (Hb) and platelets count were decreased in diabetic untreated rats, but administration of the extract significantly increased ($p < 0.05$) these values. Treatment with aqueous leaf extract of *Senna fistula* also increased the resistance of erythrocytes to lysis as evidenced by the median mean corpuscular fragility (MCF50) of 0.72% in diabetic rats and 0.58%, 0.57% and 0.58% after administration of the extract. Similarly, treatment with aqueous leaf extract of *Senna fistula* decreased the elevated Malondialdehyde level ($p < 0.05$) of diabetic rats. Glucose-6-phosphate dehydrogenase activity increased significantly in the extract-treated groups and these compared favourably with the control non-diabetic and glibenclamide-treated groups.

Conclusion: This study concludes that the extract might improve the diabetic-induced disturbances of some haematological parameters by reducing lipid peroxide level and susceptibility of red blood cells to haemolysis.

INTRODUCTION

Diabetes mellitus (DM) is a multi-factorial disease which leads to abnormalities in lipid, carbohydrate and protein metabolism. The disease is often associated with increased risk of systemic complications which include retinopathy, ischemic heart disease, neuropathy, nephropathy, ulceration and gangrene of extremities.[1-2] Diabetes mellitus could occur alone, but most often usually co-exists with other systemic disease like hypertension. As a result, DM and its complications have major impact on health, quality of life and life expectancy of its sufferers. In 2012 and 2013 the disease resulted in 1.5 to 5.1 million deaths per year worldwide, making it the 8th leading cause of death,[3-4] and more than eighty percent of deaths due to diabetes occur in low and middle-income countries.[5] Overall, diabetes doubles the risk of death.[6] Diabetes mellitus occurs all over the world, but is more common (especially type 2) in developed countries. The highest increase in incidence is, however, expected to occur in Africa and Asia continents, where most people with the disease will most likely be found by 2030.[7]

Medicinal plants have been used to treat wide varieties

of diseases before the advent of orthodox medicine.[8] According to WHO, medicinal plants have been defined as any plant which one or more of its organ contains substances that can be used for therapeutic purpose or which are precursors for the synthesis of useful drugs.[9] The traditional medicine system has continued to play a major role in health care. Studies have reported that more than 75% of the world population depends mainly on medicinal plants for their primary health care,[10] even those who live in developed countries like Europe and America still make use of plant-derived products for their health care.

Senna fistula Linn. Family (Leguminosae) referred to as golden shower (English), Aidantoro (Yoruba) is a deciduous tree with greenish grey bark, compound leaves; the tree is known for its beautiful bunches of yellow flowers.[11] The plant has been used for centuries in India, Sri-Lanka, West Africa among others for the treatment of pain and inflammation, ulcer, diabetes mellitus, infection and constipation.[12-15]

Some of the constituents of *Senna fistula* include Rhein, glycosides- Sennosides A and B,[16] Tannins, Anthraquinone and Volatile oils.[18] Other constituents

include Carbohydrates, Fats, Protein, Minerals such as K, Ca, Mg, Fe, Zn, Mn, P and Vitamin C.[17] Hyperglycaemia as it occurs in DM has been reported to cause alterations in some haematological parameters.[18-19] This study was carried out to determine whether aqueous extract of *Senna fistula* leaves could restore changes in haematological values, lipid peroxidation and osmotic fragility due to diabetes mellitus.

MATERIALS AND METHODS

Plant material and Authentication

Senna fistula leaves (locally called Aidantoro) were purchased from Itoku Market in Abeokuta, Ogun State. The identification of the plant leaf was carried out in the Department of Plant Biology of University of Ilorin with voucher specimen (UIH 1020), which was earlier deposited in the herbarium of the Department. Fresh leaves of the plant were air-dried at room temperature for about 2 weeks.

Animals

Male albino rats of Wistar strain, weighing between 120-130 g were used for this study. They were obtained from the animal holdings of the Department of Biochemistry, University of Ilorin. The animals were fed on rat pellets (Premier Feed Limited, Ibadan) and water *ad libitum*. All animals were maintained under standard laboratory conditions of temperature ($22\pm 2^{\circ}\text{C}$), humidity ($45\pm 5\%$) and natural photoperiod of about 12h light:dark cycle. All experimental protocols and handling were carried out in compliance with standard procedures and regulations.

Animal Grouping and Treatment

Thirty male albino Wistar rats were divided into 6 groups of 5 rats each:

Group A – (Control) non-diabetic, received 0.5 ml of distilled water orally

Group B- Streptozotocin-induced diabetic rats, received 0.5 ml of distilled water orally

Group C- diabetic and treated with 2.5 mg/kg b.w. of glibenclamide [20]

Group D- diabetic and treated with 28.57 mg/kg b.w. of aqueous extract of *Senna fistula* leaves

Group E- diabetic and treated with 57.14 mg/kg b.w. of aqueous extract of *Senna fistula* leaves

Group F- diabetic and treated with 114.28 mg/kg b.w. of aqueous extract of *Senna fistula* leaves.

The extract was suspended in vehicle (distilled water) solution. It was administered orally using oral canula three times daily (morning 8 am, afternoon 2 pm and evening 8 pm) for a period of 28 days.

Preparation of aqueous extract

Air-dried powdered leaves (150 g) of *S. fistula* was exhaustively extracted with 2 litres of water by maceration for 24 hours, after which it was filtered and the filtrate was evaporated to dryness using water bath regulated at 400°C . A dark green extract was obtained weighing about 42.6g (28.40%). The extract was stored in the refrigerator till the commencement of the study. Calculated amount of the extract was reconstituted in distilled water to give the required doses of 28.57, 57.14 and 114.28 mg/kg body weight. The doses used in this study were as obtained from the survey carried out among the users of the plant.[17]

Haematological Parameters

These parameters (PCV, Hb, RBC count and Platelets) were measured using automated haematological analyser k-x-21, (Symex Corporation, Japan).

Determination of erythrocyte Osmotic Fragility

Erythrocyte osmotic fragility test was carried out according to the method of Dacie and Lewis.[21] Briefly, 10% buffered NaCl solution was prepared by dissolving 90g of NaCl, 2.43g of NaH_2PO_4 and 13.6g of NaH_2PO_4 in distilled water and made up to 1 litre, to make stock solution. 1% buffered saline solution was then prepared and its pH adjusted to 7.4 using a pH meter. Lower dilution of NaCl solution (range 0.1%-0.9%) was then prepared in 9 centrifuge tubes and 10th tube contained distilled water. 5ml of each concentration of NaCl was put in each of the centrifuge tube (9 in all) and 5ml of distilled water in 10th tube. To each of the tube, 0.02ml of blood was added. The content was thoroughly mixed by gentle inversion and allowed to stand for 30 minutes and then centrifuged for 10minutes at 2000 rpm. The optical density of the supernatant was read in a photospectrometer at a wavelength of 540nm. Erythrocyte fragiligram was obtained by plotting the mean percentage haemolysis against the concentration of NaCl solution.

Determination of Glucose-6-Phosphate Dehydrogenase

The enzyme activity of Glucose-6-Phosphate Dehydrogenase (G6PDH) was determined by measurement of the rate of absorbance change at 340nm due to the reduction of NADH^+ , basically, 0.2ml of the blood was washed with 2ml of 0.9% NaCl solution and then it was centrifuged after each wash for 10 minutes at 3000rpm. This process was carried out 3 times. The washed and centrifuged erythrocyte was suspended in 0.5ml of solution 4 (Digitonin) and stood for 15mins at 4°C and then centrifuged again.

The supernatant in the assay was used as follows; 1ml of the solution R_1 , 0.03ml of solution R_2 , and 0.015ml of haemolysate (sample) were pipetted into each test tube and they were mixed, incubated for 5 minutes at 37°C , after which 0.015ml of solution R_3 was added and mixed.

Absorbance was read at 340nm, after 1, 2, and 3 minutes of the initial reading.

Activity of G6PDH was calculated using:

$$\text{M}\mu/\text{erythrocyte per ml blood} = 336506 \times \Delta A_{340\text{nm}/\text{min}} \text{ (Randox glucose-6-phosphate dehydrogenase manual E.C.1.1.1.49)}$$

Determination of Malondialdehyde (MDA) concentration

This assay is based on the reaction of Malondialdehyde (MDA) with thiobarbirturic acid (TBA) under acidic solution, forming an MDA-TBA. The estimation of MDA concentration was carried out using thiobarbirturic acid (TBA) procedure.[22]

Statistical Analysis

The results were analysed using Statistical package for the social science (SPSS) version 21 (SPSS Inc., Chicago, IL, USA). The test for significance was carried out using one-way analysis of variance (ANOVA) followed by Duncan new multiple range test (DMRT) post hoc test. All data are expressed as the mean $\pm$ SEM. Differences were considered statistically significant at $p < 0.05$.

RESULTS

Figures 1, 2, 3 and 4, depict the effect of administration of aqueous extract of *Senna fistula* leaves on packed cell volume, haemoglobin, red blood cell count and platelets count respectively. There were significant decreases in packed cell volume (PCV), haemoglobin (Hb), red blood cell (RBC) count and platelets count in STZ-induced diabetic animals. Administration of aqueous extract of *Senna fistula* leaves increased significantly the reduced PCV, Hb, RBC and platelet counts in diabetic rats. However, there were no significant differences in PCV, Hb, RBC and platelet counts in extract-treated rats and glibenclamide-treated rats when compared with the control ($p > 0.05$). Figure 5 shows the effect of administration of aqueous extract of *Senna fistula* leaves on erythrocytes osmotic fragility of streptozotocin-induced diabetic rats. Administration of the extract reduced the right shift of the fragiligram significantly ($p < 0.05$). However, there were no significant differences ($p > 0.05$) in osmotic fragiligram between the control, glibenclamide-treated and extract-treated groups. The median mean corpuscular fragility (MCF50) in the diabetic untreated group was 0.72% which was significantly higher than the one obtained for extract treated, glibenclamide-treated and the control groups.

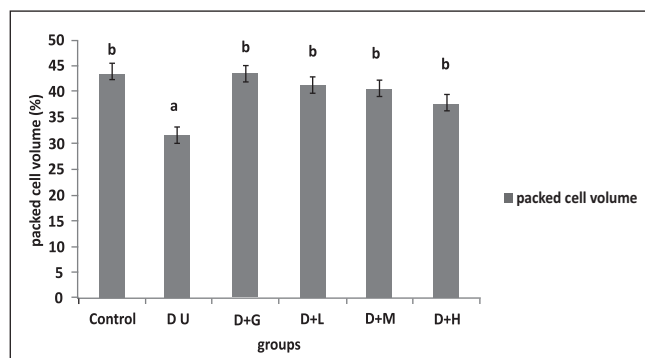


Figure 1: Effect of *S. fistula* leaf extract on packed cell volume of STZ-induced diabetic rats.

Control, DU = Diabetic untreated, D+G = Diabetic treated with glibenclamide (2.5mg/kg), D+L = Diabetic treated with low dose of the extract (28.57mg/kg b.w), D+M = Diabetic treated with moderate dose of the extract (57.14mg/kg b.w), D+H = Diabetic treated with high dose of the extract (114.28 mg/kg b.w). Bars with different alphabets are significantly ($p < 0.05$) different.

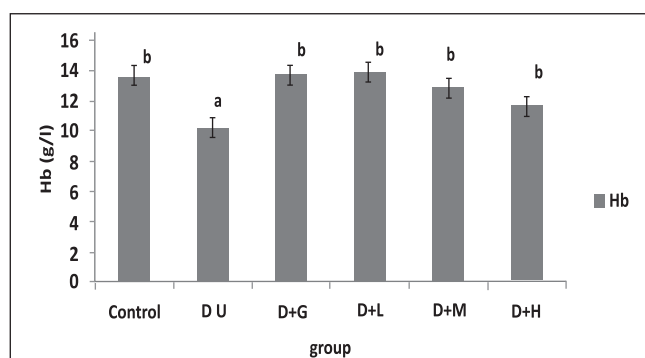


Figure 2: Effect of *S. fistula* leaf extract on haemoglobin concentration of STZ-induced diabetic rats.

Control, DU = Diabetic untreated, D+G = Diabetic treated with glibenclamide (2.5mg/kg), D+L = Diabetic treated with low dose of the extract (28.57mg/kg b.w), D+M = Diabetic treated with moderate dose of the extract (57.14mg/kg b.w), D+H = Diabetic treated with high dose of the extract (114.28 mg/kg b.w). Bars with different alphabets are significantly ($p < 0.05$) different.

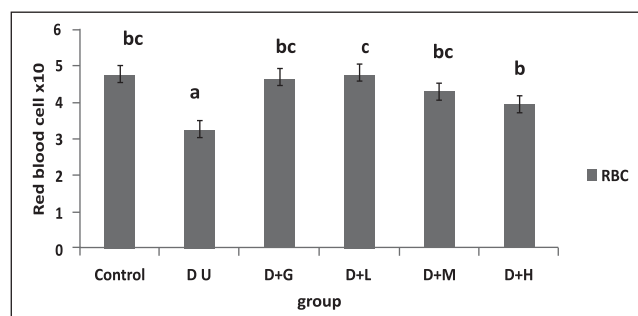


Figure 3: Effect of *S. fistula* leaf extract on RBC count of STZ-induced diabetic rats.

Control, DU = Diabetic untreated, D+G = Diabetic treated with glibenclamide (2.5mg/kg), D+L = Diabetic treated with low dose of the extract (28.57mg/kg b.w), D+M = Diabetic treated with moderate dose of the extract (57.14mg/kg b.w), D+H = Diabetic treated with high dose of the extract (114.28 mg/kg b.w). Bars with different alphabets are significantly ($p < 0.05$) different.

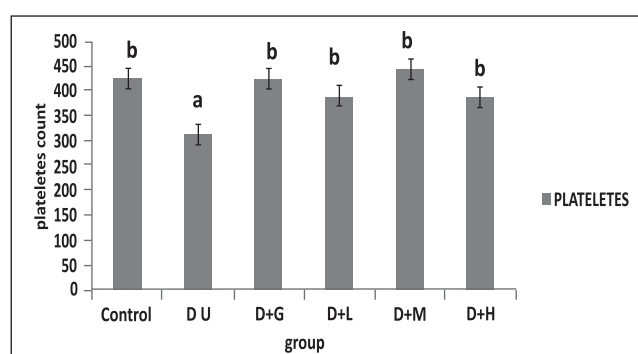


Figure 4: Effect of *S. fistula* leaf extract on platelets count of STZ-induced diabetic rats.

Control, DU = Diabetic untreated, D+G = Diabetic treated with glibenclamide (2.5mg/kg), D+L = Diabetic treated with low dose of the extract (28.57mg/kg b.w), D+M = Diabetic treated with moderate dose of the extract (57.14mg/kg b.w), D+H = Diabetic treated with high dose of the extract (114.28 mg/kg b.w). Bars with different alphabets are significantly ($p < 0.05$) different.

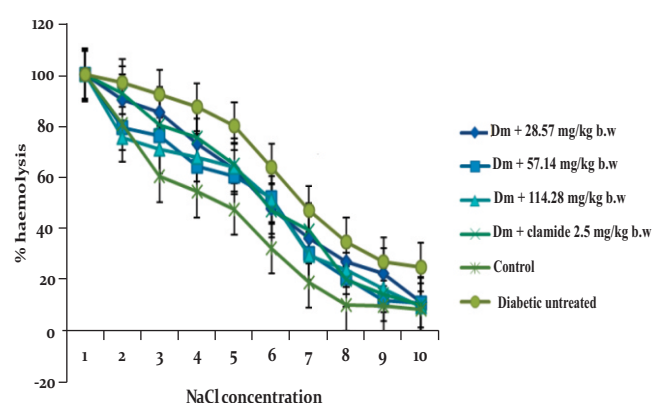


Figure 5: Effect of *S. fistula* leaf extract on osmotic fragility of STZ-induced diabetic rats

Dm + 28.14 mg/kg bw is Diabetic group treated with 28.57 mg/kg bw of aqueous leaf extract of *Senna fistula*; Dm + 57.14 mg/kg bw is Diabetic group treated with 57.28 mg/kg bw of aqueous leaf extract of *Senna fistula*; Dm + 114.28 mg/kg bw is Diabetic group treated with 114.57 mg/kg bw of aqueous leaf extract of *Senna fistula*; Dm + clamide 2.5 mg/kg bw is Diabetic group treated with 2.5 mg/kg bw of glibenclamide.

Figure 6 shows the effect of *S. fistula* leaf extract on Glucose-6-phosphate dehydrogenase (G6PDH) activity of STZ-induced diabetic rats. Glucose-6-phosphate dehydrogenase (G6PDH) activity in STZ-induced diabetic rats decreased significantly ($p < 0.05$). But treatment of diabetic animals with the extract caused a significant ($p < 0.05$) increase in the activity of G6PDH, however, the values obtained in extract-treated diabetic rats did not differ significantly ($p > 0.05$) from the glibenclamide-treated animals.

Figure 7 depicts the effect of administration of aqueous extract of *Senna fistula* leaves on malondialdehyde (MDA) level of streptozotocin-induced diabetic rats. MDA value was significantly elevated in STZ-induced diabetic rats. Treatment with different doses of the extract caused a significant decrease ($p < 0.05$) in MDA level of diabetic rats, which compared favourably with the control and glibenclamide-treated rats.

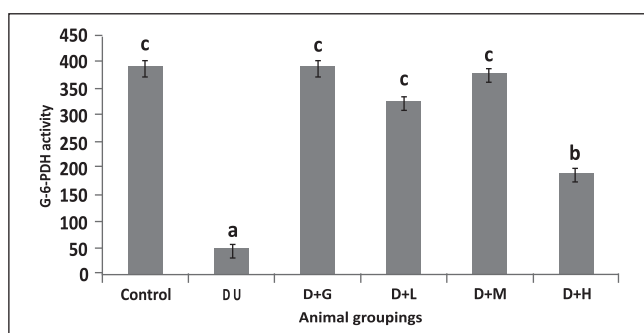


Figure 6: Effect of *S. fistula* leaf extract on G-6-PDH activity of STZ-induced diabetic rats

Control, DU = Diabetic untreated, D+G = Diabetic treated with glibenclamide (2.5mg/kg), D+L= Diabetic treated with low dose of the extract (28.57mg/kg b.w), D+M = Diabetic treated with moderate dose of the extract (57.14mg/kg b.w), D+H = Diabetic treated with high dose of the extract (114.28 mg/kg b.w). Bars with different alphabets are significantly ($p < 0.05$) different.

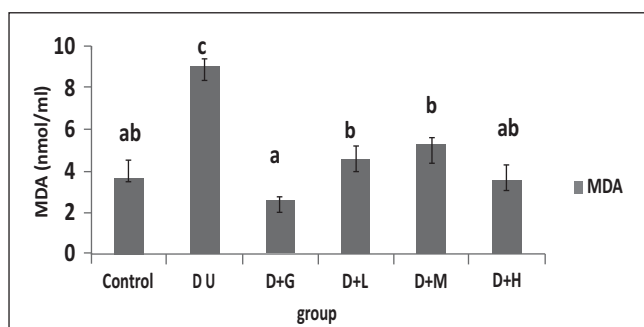


Figure 7: Effect of *S. fistula* leaf extract on MDA level of STZ-induced diabetic rats

Control, DU = Diabetic untreated, D+G = Diabetic treated with glibenclamide (2.5mg/kg), D+L= Diabetic treated with low dose of the extract (28.57mg/kg b.w), D+M = Diabetic treated with moderate dose of the extract (57.14mg/kg b.w), D+H = Diabetic treated with high dose of the extract (114.28 mg/kg b.w). Bars with different alphabets are significantly ($p < 0.05$) different.

DISCUSSION

In this study, the decrease in the values of some haematological parameters (PCV, RBC, Hb) studied in the diabetic rats agrees with previous reports documenting

alterations in haematological parameters in diabetes mellitus.[19,23] The decrease could be due to reduction in erythropoietin (EPO) secretion from possibly a damaged kidney a common complication in diabetes. On the other hand the increased blood indices in the diabetic rats treated with aqueous extract of *S. fistula* leaves could be related to the mineral constituents of the leaf extract of *Senna fistula* which include protein, Zn, Ca, K, Mn, Fe, P and others, [17] most of these mineral constituents are well known haematological factors that have influence on the production of blood from the bone marrow.[24]

Studies have reported changes in platelets morphology in diabetic state.[25-26] Different parameters can be used to reflect the condition of platelets such as platelet count, mean platelets volume (MPV) and others. In this study, platelet count was determined in both diabetic untreated and extract-treated diabetic rats. The platelets count was lowered in diabetic untreated group when compared with the control and extract-treated group. This is in line with the work of Hekimsoy *et al.*,[27] This decrease in platelet counts in diabetic untreated group may be due to decrease in platelet survival time as a result of defect in platelet morphology.[28]

Osmotic fragility test is a standard test used to confirm the role of factors affecting the physical integrity of red blood cells,[29] and diabetes has been reported to cause change in the membrane properties of red blood cells.[30] The increase in membrane osmotic fragility in diabetic rats reported in this study is in agreement with previous work documenting increased fragility of red blood cells exposed to hyperglycaemia.[18] The effect of the extract may be mediated by the presence of high amount of ascorbic acid (one of its chemical constituents) which has been previously reported.[17] Ascorbic acid is an antioxidant which is known to prevent oxidative damage to cell membrane, which could result in increased membrane fluidity and leading to loss of membrane integrity.[31] Therefore, as a result of membrane-stabilizing effect of ascorbic acid in the aqueous extract of *Senna fistula*, there is an increased resistance of RBC to lysis, thereby increasing the lifespan of RBC. This may translate to increased RBC count, packed cell volume and haemoglobin concentration in extract-treated groups observed in this study. Red blood cells (RBCs) are susceptible to oxidative stress and become haemolysed if oxidative stress is high. Diabetes is a condition that is associated with increased oxidative stress so, in diabetes, RBCs are faced with increased oxidative stress. G-6PD is the rate-limiting enzyme in the pentose – phosphate pathway, which produces Ribose 5- phosphate and NADPH especially in red blood cell metabolism. Studies have shown that G6PD is the major source of NADPH for the antioxidant system and other critical enzyme.[32-36] NADPH is used by glutathione and thioredoxin system to regenerate reduced forms that will then be used in antioxidant roles. Hyperglycaemia and diabetes have been shown to cause a decrease in G6PD activity in endothelial cells, kidney, liver and red blood cells which lead to oxidative damage, cellular dysfunction and organ damage.[37-40]

In the present study, glucose -6- phosphate dehydrogenase activity was found to decrease significantly in STZ-induced diabetic rats, administration of the extract increased the activity of G6PDH in diabetic rats which are comparable to that of the control and glibenclamide-treated rats. Thus, the decrease in G6PDH in diabetic untreated rats

may not be unconnected to increase in oxidative load present in diabetic rat, therefore, treatment with the extract increased the activity of G6PDH enzymes and may help to control free radical generated during diabetes. The decrease in G6PDH activity in diabetic rats could also be connected to increase in haemolysis of RBC as seen in erythrocyte fragility test; because studies have reported that decreased G6PDH activity is associated with increased haemolysis.[41]

Lipid membrane of cells are important for the maintenance of cellular integrity and survival.[42] In diabetes mellitus, there is increased production of free radical or impaired antioxidant defences.[31] This increased free radical production in DM reacts with membrane lipids causing elevated level of lipid peroxide which leads to cellular damage. In this study, MDA level (product of lipid peroxidation) increased significantly in diabetic rats which agrees with many reports documenting elevated level of MDA in diabetes mellitus.[43-45] Administration of aqueous extract of *Senna fistula* significantly reduced the elevated MDA level in STZ-induced diabetic rats. The reduced level of MDA in aqueous extract of *Senna fistula*-treated rats may be due to either increasing the synthesis of the antioxidant molecules or the presence of some secondary metabolites and antioxidant compounds in the plant such as ascorbic acid (vitamin C), flavonoids and antioxidant minerals (Zn, Mn). These compounds work in synergy with each other and against free radicals.

CONCLUSION

We conclude that this plant extract has the potential to reduce the diabetes-induced disturbances of packed cell volume, red blood cell count, platelets count and haemoglobin level by reduction in the level of lipid peroxidation and increased G6PDH activity, thereby reducing haemolysis which eventually elongated the life span of blood cells.

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Conflict of Interest

The authors declare no competing interests.

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