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Full Length Research Paper

Anti-diabetic activities of *Fleurya aestuans* (L.) Gaudich in alloxan induced rats

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Diabetes mellitus is becoming an increasing concern all over the world and such people especially in some communities have used medicinal plants to treat diabetes and its complications. This investigation aimed to examine the hypoglycemic potential of the methanol extract of *Fleurya aestuans* leaves in normal and alloxan induced diabetic rats. Thirty five Wistar albino rats were grouped into seven different groups of five per group where diabetes was induced in the rats by intra peritoneal administration of alloxan monohydrate (150 mg/kg) except a control group. *F. aestuans* methanol extract at a dose of 100, 200, 300 and 400 mg/kg of body weight were administered at a single dose per day for a period of 10 days to the diabetic rats, respectively. Five mg/kg of standard drug, glibenclamide (a positive control) was given to one of the groups. The effects of *F. aestuans* methanol extract of whole plant, on blood glucose was measured in the diabetic rats. This activity is not dose dependent.

Key words: *Fleurya aestuans* leaves, antidiabetic activity, alloxan, glibenclimide, Wistar rats.

INTRODUCTION

The human population has always been plagued by diseases that have adversely affected health and well-being (Pramodh, 2003). For hundreds of years these ailments were caused by infectious agents and non-communicable diseases which have become the main public health concern in the 21st century (Zimmet et al., 2001). Of these, one particular disease that is increasingly causing greater morbidity and mortality, in both young and old is diabetes mellitus (World Health Organization, 2006).

Diabetes mellitus is a metabolic disease characterized by hyperglycaemia resulting from defects in insulin resistance, secretion and/or action. Several forms of diabetes mellitus are known to occur but type I and II are predominant. Type I diabetes is the auto-immune mediated form of the disease and is characterized by the destruction of the pancreatic beta-cell islets resulting in absolute insulin deficiency resistance while type II diabetes is characterized by insulin resistance of the secreted insulin.

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In comparison, people inflicted with type I diabetes are wholly dependent on exogenous insulin for survival, while people with type II produce insufficient amount of endogenous insulin and sometimes require insulin supplementation for the control of blood glucose concentration either directly or indirectly through the use of hypoglycaemic medications (Shafrir, 1997).

Recently, there has also been a surge in the use of botanicals to manage and control diabetes, due to the common perception that the pharmaceutical products on the market induce severe complications following long term uses (Hanefeld, 1998). Presently, several studies have been dedicated to surveys of these botanicals from across the globe. The African continent has an enormous wealth of plant resources and plants used in traditional medicines which plays a vital role in the life of millions of people throughout Africa (Botha et al., 2001).

In developing nations, many people are still heavily reliant on traditional healers and medicinal plants to meet their daily primary health care needs (Ojewole, 2002), because they presume that these plants are safe based on their long term usage in the treatment of diseases according to the knowledge accumulated over centuries (Fennell et al., 2004).

With much of this documentation being obtained through formal and informal discussions with local communities and traditional medical practitioners, many of the identified remedies need to be ascertained using validated scientific methods to confirm their efficacy.

Fleurya aestuans (L.) Gaudich is an erect annual monoecious herb commonly called the West indian woodnettle, tropical nettleweed and stinging nettle. *F. aestuans* has great medicinal potential and has been reported to treat rheumatism (Alford, 2007) and arthritis (Randall et al., 1999). It has also been reported to have antioxidant, antimicrobial, antiulcer and analgesic properties. Its extract showed *in vitro* inhibition of several key inflammatory events that cause the symptoms of seasonal allergies (Roschek et al., 2009).

Also, *F. aestuans* increases the flow of urine, shrinks inflamed tissues, helps blood circulation and purifies the blood. It is popularly cooked green in many areas due to its high protein content although these have not been scientifically proven.

F. aestuans has been used in various ways in traditional medical practice as a palliative, though they have been no scientific report on the anti-diabetic properties of the plant. Thus, this motivated the present study on the anti-diabetic activity of the leaf extract of *F. aestuans*.

MATERIALS AND METHODS

Plant materials

Fresh plants of *F. aestuans* were collected and authenticated at the Forestry Research Institute of Nigeria (FRIN), Ibadan, Oyo State Nigeria where voucher specimens with voucher number 109710 were prepared and deposited.

Plant extraction

The leaves of *F. aestuans* were carefully separated, air dried to reduce the moisture content for a period of 21 days and powdered and then 150 g of the powdered sample of the plant was extracted with methanol for three days by maceration procedure.

This process was repeated thrice and the whole extract was filtered, concentrated under reduced pressure using rotatory evaporator and dried to a constant weight and stored in a desiccator prior to analysis.

Phytochemical screening

Phytochemical screening was carried out on the powdered sample of the *F. aestuans* to identify the various phyto-constituents. The methods for the screening were carried out following standard procedures (Trease and Evans, 1998).

Experimental animals

Thirty five albino Wistar rats weighing between 120 and 170 g of both sexes were obtained from the Department of Clinical Pharmacy, Faculty of Pharmacy, Olabisi Onabanjo University, Sagamu, Ogun State/ Nigeria. The animals were maintained in cages and fed with water and standard pellets obtained from premier feed, Sagamu, Ogun State/ Nigeria.

The baseline weights and blood glucose levels of the animals were carried out before inducing diabetes in the rats. The rats were divided into seven groups of five rats in a group.

Induction of diabetes

Diabetes was induced in thirty rats that have been fasted for 12 h by a single intra peritoneal administration of freshly dissolved 150 mg/kg alloxan monohydrate in normal saline solution (Szkudelski, 2001). The blood glucose levels (BGL) were monitored daily using the glucometer and touch strips.

Experimental design

The 30 diabetic rats were divided into six groups of five rats each. Group 1 to 4 received 100, 200, 300 and 400 mg/kg methanol extract of *F. aestuans,* respectively. Group five which served as positive control received 5 mg/kg glibenclamide while group six (negative control) received neither extract nor the standard drug. Group seven contained five rats that were dose with 150 mg/kg normal saline only

Statistical analysis

All the values of fasting blood sugar were expressed as mean ± standard error of mean (S.E.M) and analyzed using ANOVA and post hoc Dunnet's test. Differences between groups were considered to be significant at $p<0.05$ levels.

RESULTS

The plant materials (150 g) were extracted and the obtained yield was found to be 12.73%.

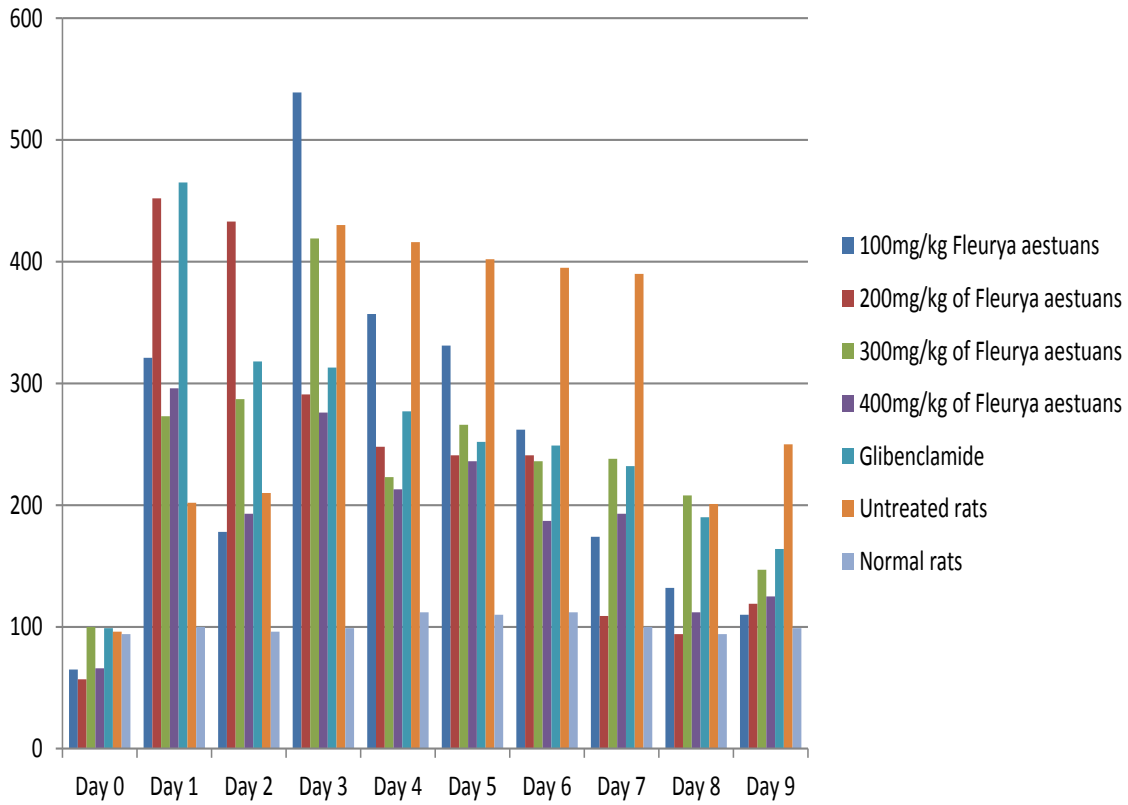

Figure 1. Effect of the 100, 200, 300 and 400 mg/kg methanolic extract of *F. aestuans* on blood glucose level in hyperglycaemic rats compared with the standard drug (5 mg/kg glibenclamide), diabetic untreated rats and normal rats.

DISCUSSION

Alloxan induces diabetes by destroying the beta-cells of islets of langerhans in the pancreas leading to reduction in synthesis and release insulin (Szkudelski, 2001). This model has been used in several studies of anti-diabetic effect of several products (Babu et al., 2002). Phytochemical test carried out on the powdered leaves samples of *F. aestuans* showed that the plant contained tannins, flavonoids, saponin, cyanogenetic glycosides and terpennoids as shown in Table 1. Table 2 depicts the anti-diabetic activities of *F. aestuans*.Oral administrations of methanolic extract of *F. aestuans* caused a significant reduction in the blood glucose levels of the diabetic rats from the 5th day as seen in 100 mg/kg with the value (mean ± SEM) of 539±0.88 to 357±0.33; the group with 200 mg/kg showed a significant reduction in blood glucose levels from the 4th day with the value (mean ± SEM) of 433±85.13 to 291±41.33, the group with 300 mg/kg showed a significant reduction in blood glucose levels from the 5th day with the value (mean ± sem) of 419±77.34 to 223±49.41, the group with 400mg/kg showed a significant reduction in blood glucose levels from the 6th day with the value (mean ± sem) of 236±1.11 to 187±47.41.41 as shown in Figure 1.

The group five which represent the positive control (5 mg/ml of glibenclamide) showed a geometrical reduction in blood glucose from the 2nd day as shown in Table 2. Group six which represent diabetic untreated rats showed a reduction in the blood glucose levels from the 9th day with the value of 390±22.84 to 20±9.82, although no treatment was given. This showed that dietary modification can help in the reduction of blood glucose levels.

Obtained result showed that *F. aestuans* demonstrated a reasonable anti-diabetic activity at concentration between 100 and 200 mg/kg. At these concentrations, the blood sugar levels were drastically reduced especially from day 5 upward. This implied that the activity exerted by the plant was best noticed after the 4th day. These activities (100 and 200 mg/kg) were statistically significant ($P<0.05$). However, at concentrations more than 200 mg/kg, the anti-diabetic attributes of the plant were not significant as the blood sugar reduction was marked by an intermittent low and high levels. Control drug (5 mg/kg glibenclimide) was found to be efficacious in the treatment of induced diabetes but the efficacy of its anti-diabetic activity was not statistically significant among the days ($P<0.05$). Evidence from Table 2 for group six disclosed that controlled diet can lead to

Table 1. Phytochemical screening of *Fleurya aestuans* leaves.

Test	Result
Alkaloids	-
Tannins	+
Flavonoids	+
Saponin glycoside	+
Cardiac glycosides	+
Anthraquinone	-
Cyanogenetic glycoside	+
Terpenoids	+

-, Absent; +, Present.

Table 2. Antidiabetic activity of *F. aestuans* leaves in alloxan induced diabetic rats.

Group	Mean value (%)											
	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	F value	P value
100 mg/kg *Fleurya aeustrans*	65±15.4	321±0.00 (0)	178±1.20 (44.5)	539±0.88 (67.9)*	357±0.33 (11.2)*	331±0.58 (3.1)*	262±28.5 (18.4)	174±1.45 (45.8)	132±2.30 (58.9)	110±10.3 (65.7)	109.4	<0.05
200 mg/kg *Fleurya aestuans*	57±2.88	452±73.1 (0)	433±85.1 (4.2)	291±41.3 (35.6)	248±81.8 (45.1)	241±72.2 (46.7)	241±72.2 (46.7)	109±34.0 (75.9)	94±27.2 (79.2)	119±11.0 (73.7)	7.22	<0.05
300 mg/kg Fleurya aestuans	100±7.44	273±61.6 (0)	287±63.0 (5.1)*	419±77.3 (53.5)*	223±49.4 (18.3)	266±25.2 (2.6)	236±53.2 (13.6)	238±99.7 (12.8)	208±78.4 (23.8)	147±80.7 (46.2)	1.95	>0.05
400 mg/kg *Fleurya aestuans*	66±4.48	296±87.4 (0)	193±61.8 (34.8)	276±1.08 (6.8)	213±76.8 (28.0)	236±1.11 (20.3)	187±47.4 (36.8)	193±51.3 (34.8)	112±4.33 (62.2)	125±25.3 (57.8)	1.13	>0.05
Glibenclamide (5 mg/kg)	99±7.46	465±20.8 (0)	318±81.3 (31.6)	313±77.2 (32.7)	277±85.7 (40.4)	252±64.4 (45.8)	249±63.4 (46.5)	232±42.4 (50.1)	190±76.7 (59.1)	164±80.2 (64.7)	2.08	>0.05
Untreated	96±1.15	202±1.08 (0)	210±79.8 (4.0)*	430±22.4 (11.3)*	416±22.2 (105.9)*	402±24.7 (99.0)*	395±16.8 (95.5)*	390±22.89 (3.1)*	201±79.8 (0.5)	250±1.02 (23.8)*	4.96	<0.05
Normal rats	94±9.44	100±7.44 (0)	96±1.15 (4)	99±7.46 (1)	112±4.33 (12)*	110±10.3 (10)*	112±4.33 (12)*	100±7.44 (0)	94±22.7 (6)	99±7.46 (1)	19.4	<0.05

* Percentage increase in blood glucose level.

reduction of blood glucose levels, while group seven further stressed that in non-diabetic rats with inappropriate balanced diet may also lead to diabetes. Partial restoration of the pancreatic islet cells after treatment with the extract indicate that the possible mechanism by which the methanolic extract of *F. aestuans* reduced blood glucose levels of the diabetic rats may be either by increasing the pancreatic secretion of insulin from the islet of Langerhan's or it is released from bound insulin. Similar result has been reported (Pari et al., 2004).

Recently, Mgbeje et al. (2016) found that there was a significant reduction in the blood glucose levels of rats treated with n-hexane fraction of *Heinsia crinite* whencompared with the diabetic control (Mgbeje et al., 2016). Also, this significant reduction was found in the administration of n-hexane and methanol leaf fractions of *Nauclea latifolia* to the diabetic rats which were dose dependent in both the n-hexane and the methanol fractions (Effiong et al., 2014). However, Momoh et al. (2014) and Momoh et al. (2014) showed that there was statistically significant reduction ($P<0.05$) of *Vernonia amygdaline* not only in the glucose levels but also in the association of polytriads symptoms (Momoh et al., 2014). Methanolic leaves extract of *Jatropha curcas* showed that different concentrations exhibited a profound reduction ($P<0.005$) in the blood sugar levels of the diabetic albino rats (Momoh et al., 2014). All these results agree with the obtained results regarding the methanol extract of *F. aestuans* on the alloxan-induced diabetic rats.

Conclusion

In conclusion, *F. aestuans* is more effective at lower concentration in the treatment of diabetic rats. Further studies can be carried out to investigate the lethal dose and isolate the active compounds with the structural elucidation of the isolated compounds.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Haematology and serum biochemistry of alloxan-induced diabetic rats administered with extracts of *Phragmanthera incana* (Schum.) Balle

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This study evaluated the toxic changes that may accompany treatment of diabetes with *Phragmanthera incana*, a mistletoe species growing on two plant hosts [*Cola nitida* (Kolanut; PICN) and *Theobroma cacao* (Cocoa; PITC)]. The toxic potential of this treatment regimen was evaluated using the effect of the extracts PICN and PITC on the haematology and serum chemistry of the diabetic rats. Alloxan-induced diabetic rats were treated with the extracts at doses of 200, 400 or 800 mg/kg or glibenclamide for 14 days. Blood samples were collected on day 15 for haematology and serum biochemistry. Haematological parameters analyzed were packed cell volume, haemoglobin, red blood cells, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), white blood cell count, platelet count, lymphocytes, neutrophil, monocytes and eosinophil. Serum biochemical parameters analyzed were total protein, albumin, globulin, albumin-globulin ratio, aspartate transaminase, alanine transaminase, alanine phosphatase, blood urea nitrogen, creatinine, cholesterol, bilirubin and glucose. The results showed that *P. incana* extracts, regardless of the host plant decreased blood glucose and cholesterol levels. Although it depressed packed cell volume (PCV), it also alleviated other complications of diabetes such as liver and kidney injury, and may possess hepatoprotective effect.

Key words: *Phragmanthera incana*, *Cola nitida*, *Theobroma cacao*, diabetes, haematology, serum biochemistry.

INTRODUCTION

Mistletoe, commonly known as bird lime, all heal, devil's fuge, Iscador is a general term for woody shoot parasites in several plant families, especially in Loranthaceae and Viscaceae families (Polhill and Wiens, 1998; Watson,

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2001; Judd et al., 2002). Mistletoe is especially interesting botanically because it is a hemiparasite (Adesina et al., 2013). Mistletoe is also capable of growing on its own like other plants as it can produce its own food by photosynthesis (Williams, 1990; Hoagy, 2008). However, it is more commonly found growing as a parasitic plant (Runyon et al., 2009).

Mistletoe is used mainly in Europe as an adjuvant therapy with other drugs and or radiation for treatment of cancer (Maier and Fiebig, 2002; Elluru et al., 2009). While American mistletoe is toxic, European mistletoe is considered to have medicinal properties till today. Mistletoe extracts represent the most unorthodox oncology therapy in Germany (Bock et al., 2004; Mengs et al., 2005). In Nigeria, the Hausa and Fulani tribes of Northern Nigeria use mistletoe in the treatment of cancers and inflammations (Abubakar et al., 2007). Mistletoe has been used in medicine to prove much of its older frame as “all healer”. In addition to its use for treatment of cancers and as an immune booster, the white-berried mistletoe (*Viscum album*) has also been documented as a traditional treatment for diabetes and high blood pressure (Orhan et al., 2005). The African mistletoe, *Loranthus bengwensis* L. (Loranthaceae), has been widely used in Nigeria folk medicine to treat diabetes mellitus (Ibatomi et al., 1994). A recent study on another Nigerian mistletoe *Phragmanthera incana* (Schum.) Balle from the family Loranthaceae showed it has potent antidiabetic effect (Ogunmefun et al., 2016).

Prior to the introduction of insulin in 1922, the treatment of diabetes mellitus relied heavily on dietary measures which included the use of traditional plant therapies. People in many countries still depend on medicinal plants for the management of diabetes mellitus especially in developing countries where western medical resources are meager (Bnouham et al., 2006). A number of medicinal or culinary herbs have been reported to yield hypoglycaemic effects on diabetic conditions. These include bitter melon, *Momordica charantia* (Srivastava et al., 1993; Raman and Lau, 1996); onions and garlic, *Allium cepa*, *A. sativum* (Koch and Lawson, 1996) and holy basil, *Ocimum sanctum* (Rai et al., 1997). Some other common botanicals demonstrating *in vivo* hypoglycaemic activities in animals include juniper berries (Sanchez de Medina et al., 1994) and alfalfa (Gray and Flatt, 1997).

Marles and Farnsworth (1994) however cautioned that one- to two-thirds of the 1123 plants that affect blood glucose may be dangerous and many of their constituents are hypoglycaemic due to metabolic or hepatic toxicity. Initial toxicological evaluation of the extract of *P. incana* on Wistar rats showed it was safe (Ogunmefun et al., 2013). The caution documented by Marles and Farnsworth (1994) and more recently other researchers such as Eddouks et al. (2002) and Hilmi et al. (2013) informed the study on evaluation of the toxic potentials of *P. indica* using the effect of the methanol extract on haematology and biochemistry of alloxan- induced diabetic Wistar rats administered with the extract. *P. incana* growing on kolanut (*Cola nitida*) and cocoa (*Theobroma cacao*) were investigated in the study.

MATERIALS AND METHODS

Plant sample collection

P. incana (Schum.) Balle, mistletoe growing on Cocoa (*T. cacao*) and Kolanut (*C. nitida*) was collected at Alesan Obolode, Owo, Ondo State, Nigeria. Identification and authentication was done at the Forestry Research Institute of Nigeria (FRIN) herbarium. A voucher specimen of *P. incana* with Forestry Herbarium Index (FHI) 108925 was submitted at the Botany Department herbarium of the University of Ibadan, Nigeria with University of Ibadan herbarium (UIH) number 22332.

Methanol extract preparation

The samples were washed under running water, air dried after which the dried samples were ground to powder and kept dry in an air-tight container. Cold extraction method with methanol for 72 h at room temperature was used (Ogbole et al., 2013). 500 g of powdered mistletoe samples harvested from Cocoa and Kolanut were extracted separately with one litre of methanol each after which concentration of the filtrates were done using rotary evaporator and the extracts were further concentrated on water bath at a low temperature of 40°C to remove all solvents.

Experimental animals

Wistar rats were obtained from and housed at the Experimental Animal House of the Department of Veterinary Physiology, Biochemistry and Pharmacology, University of Ibadan, Ibadan, Nigeria. The animals were fed with commercial pelletized rat ration and portable water *ad libitum.* The animals were handled humanely in compliance with the Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria guidelines for the use of laboratory animals.

Induction of diabetes

Blood glucose of rats of average weights 150 g was determined using an AccuChek® active glucometer and only normoglycemic rats were included in the study. Diabetes was induced by intraperitoneal administration of alloxan monohydrate (100 mg/kg). Blood glucose levels were monitored and rats with blood glucose levels of ≥150 mg/dl 48 h after administration of alloxan were included in the groups. Diabetic rats were randomly and equally divided into 8 groups of five rats each. A ninth group of normoglycemic rats were included in the study as non-diabetic untreated control group.

Management of diabetes

Rats in groups 1 to 3 were administered with the extract of *P. incana* harvested from *C. nitida* (PICN) at doses of 200, 400 or 800 mg/kg, rats in groups 4 to 6 were administered with the extract of *P. incana* harvested from *T. cacao* (PITC) at the same dose rate as

above. Group 7 rats were the positive control group and were administered with glibenclamide, a sulfonylurea antidiabetic drug at the dose of 0.07 mg/kg. The rats in group 8 were diabetic but untreated serving as negative controls for the study, while group 9 rats were non-diabetic (normoglycemic) and untreated throughout the course of the study. All treatment groups were administered with the extract or drug for 14 days.

Sample collection and analysis

Blood samples were obtained from the retro-orbital sinus on day 15 to determine haematological and biochemical parameters. Haematological parameters were determined using the method of Jain (1986). The haematological parameters determined were packed cell volume (PCV), red blood cell count (RBC) and other red cell indices such as haemoglobin concentration, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC), white blood cell count (WBC) and its differentials; neutrophils and lymphocytes and platelet count. Biochemical parameters determined include total protein (TP) using the method of Weichselbaum (1946), albumin (Alb) using the method of Doumas et al. (1971), globulin (Glob), albumin-globulin ratio (Alb/Glob), aspartate transaminase (AST) and alanine transaminase (ALT) followed the method described by Reitman and Frankel (1957) following the principle according to Schmidt and Schmidt (1963), alanine phosphatase (ALP) using the assay method first described by King and Armstrong (1934), modified by Ohmori (1937) and later improved by Hausamen et al. (1967), blood urea nitrogen (BUN) using the method of Weatherburn, 1967, creatinine (Crt) assay was based on the principle according to Henry (1974), cholesterol (Chol) was determined according to the procedure of Fiedewald et al. (1972), bilirubin (Bil) using the procedure described by Jendrassik and Grof (1938) and glucose using method of analysis by Trinder (1969).

Statistical analysis

The mean and the standard error of mean (Mean ±SEM) were used in the analysis of the data from this study. The mean ±SEM of five replicates were subjected to DUNCAN multiple range test where the effects of the extracts were compared to those of the three types of control, that is, the control (normoglycemic non-diabetic rats), glibenclamide and the diabetic untreated groups. $P<0.05$ is considered significant for the parameters examined.

RESULTS

Haematology

Packed cell volume (PCV)

There was a significant ($P<0.05$) decrease in the PCV of all diabetic rats compared to the normoglycemic control (37.6±1.07%), with the most marked decrease observed in rats administered with extract of *P. incana* harvested from *C. nitida* (PICN) at the dose of 400 mg/kg (24.0±0.63%). The diabetic untreated rats (30.4±1.29%) had the least decrease observed. No significant ($P>0.05$) difference was observed between rats administered with glibenclamide (25.3±1.45 %) and the other treatment groups (Table 1).

Red blood cells (RBC)

Red blood cell counts (RBC) of all diabetic rats were significantly ($P<0.05$) decreased compared to the control rats ($6.76±0.19×10^6$ µL), with the most significant decrease observed in rats administered with glibenclamide ($4.06±0.25×10^6$ µL).

For rats administered with the extracts, rats administered with the extract *P. incana* harvested from *T. cacao* (PITC) at the dose of 800 mg/kg ($4.34±0.28 ×10^6$ µL) had the least RBC. There was no significant ($P>0.05$) difference in the mean red blood cells of all diabetic treated rats compared to the diabetic untreated rats ($4.68±0.21×10^6$ µL) (Table 1).

Haemoglobin (HB)

A significant decrease in haemoglobin concentration (Hb) of diabetic rats was observed when compared to the normoglycemic rats (11.5±0.33 g/dl). Hb of all treatment groups were decreased compared to diabetic but untreated rats (9.62±0.46 g/dl), with significant decreases observed in rats administered with PITC at 400 and 800 mg/kg (7.30±0.31 and 7.72±0.55 g/dl) (Table 1).

Mean corpuscular volume (MCV)

MCV of all rats administered with the extracts were non-significantly ($p>0.05$) decreased compared to the normoglycemic control rats (55.62±1.35ƒl), except in rats administered with PITC at a dose of 800 mg/kg (59.89±2.78 ƒl). Rats administered with glibenclamide (62.62±2.74 ƒl) and the diabetic but untreated rats (65.01±1.88 ƒl) had increased MCV values (Table 1).

Mean corpuscular haemoglobin (MCH)

The same pattern as with MCV was observed for MCH. MCH of the normoglycemic rats (17.01±0.34 ρg) was higher than that of all rats administered with the extract except for rats administered with PITC at 800 mg/kg (17.96±1.27 ρg). MCH of normoglycemic rats were lower than that of rats administered with glibenclamide (20.11±0.53 ρg) and diabetic untreated rats (20.55±0.62 ρg) (Table 1).

Mean corpuscular haemoglobin concentration (MCHC)

The mean MCHC of rats administered with PICN and 200 mg/kg of PITC were higher than that of normoglycemic rats (30.59±0.53 g/dl) and diabetic untreated rats (31.62±0.32 g/dl). Rats treated with glibenclamide

Table 1. Packed cell volume and red blood cell indices of rats administered with extracts of *Phragmanthera incana* harvested from *Cola nitida* (kolanut; PICN) and *Theobroma cacao* (cocoa; PITC).

Extract mg/kg	PCV (%)	RBC (X10^{6}µL)	HB (g/dl)	MCV (*f*l)	MCH (ρg)	MCHC (g/dl)
PICN 200	24.2±0.74^{c}	4.44±0.31 ^{b}	8.58±0.50^{bcd}	55.77±4.53^{abc}	19.49±0.81^{ab}	35.57±2.35^{ab}
PICN 400	24.0±0.63^{c}	4.97±0.22^{b}	9.24±0.13^{bc}	48.56±2.11^{c}	18.74±0.90^{abc}	38.62±1.27^{a}
PICN 800	25.8±0.97^{c}	4.94±0.28^{b}	8.00±0.50^{bcd}	52.85±3.37^{bc}	16.43±1.45^{bc}	30.91±0.90^{bcd}
PITC 200	25.7±1.45^{c}	5.01±0.40^{b}	8.40±0.50^{bcd}	52.02±5.73^{bc}	16.94±1.44^{abc}	32.75±1.00b^{c}
PITC 400	27.0±3.00^{bc}	5.00±0.56^{b}	7.30±0.31^{d}	54.815.79^{abc}	15.14±2.39^{c}	27.58±2.71^{d}
PITC 800	25.8±1.36^{c}	4.34±0.28^{b}	7.72±0.55^{cd}	59.89±2.78^{ab}	17.96±1.27^{abc}	29.98±1.62^{cd}
Diabetic	30.4±1.29^{b}	4.68±0.21^{b}	9.62±0.46^{b}	65.01±1.88^{a}	20.55±0.62^{a}	31.62±0.32^{bcd}
Glibenclamide	25.3±1.45^{c}	4.06±0.25^{b}	8.13±0.32^{bcd}	62.62±2.74^{ab}	20.11±0.53^{ab}	32.19±0.96^{bcd}
Control	37.6±1.07^{a}	6.76±0.19^{a}	11.5±0.33^{a}	55.62±1.35^{abc}	17.01±0.34^{abc}	30.59±0.53^{bcd}

Mean ± SEM; n=5; Values with different alphabets in the same column are significantly different at p>0.05. PCV = Packed Cell Volume; RBC = Red Blood cell; HB = Haemoglobin; MCV = Mean Corpuscular Volume; MCH = Mean Corpuscular Haemoglobin; MCHC = Mean Corpuscular Haemoglobin Concentration

Table 2. White blood cell indices and platelet count of rats administered with extracts of *P. incana* harvested from *C. nitida* (kolanut; PICN) and *T. cacao* (cocoa; PITC).

Extract (mg/kg)	WBC (X10^{3}µL)	Lymph (%)	Neut (%)	Mono (%)	Eosin (%)	Platelets (10^5/µl)
PICN 200	1.48±0.19^{a}	65.60±3.49^{abc}	30.2±2.73^{abc}	1.60±0.40^{a}	1.4±0.68^{a}	1.92±0.26^{ab}
PICN 400	1.48±0.58^{a}	66.60±3.61^{abc}	29.2±3.18^{abc}	1.80±0.49^{a}	2.4±0.51^{a}	2.06±0.22^{ab}
PICN 800	1.37±0.07^{a}	71.40±1.36^{ab}	24.4±1.83^{bc}	2.20±0.20^{a}	2.2±0.58^{a}	2.24±0.23^{b}
PITC 200	1.49±0.13^{a}	65.67±4.84^{abc}	29.33±5.33^{abc}	2.67±0.33^{a}	2.33±0.33^{a}	2.49±0.91^{a}
PITC 400	1.31±0.09^{a}	71.67±3.76^{ab}	24.0±5.20^{bc}	1.33±0.88^{a}	3.0±1.00^{a}	1.55±0.30^{ab}
PITC 800	1.33±0.09^{a}	70.20±3.14^{ab}	24.2±2.69^{bc}	2.60±0.25^{a}	2.8±0.58^{a}	1.87±0.21^{ab}
Diabetic	0.81±0.02^{b}	75.00±3.66^{a}	21.2±3.38^{c}	1.60±0.40^{a}	2.2±0.75^{a}	1.97±0.25^{ab}
Glibenclamide	1.20±0.26^{a}	57.33±1.76^{c}	38.0±2.00^{a}	2.33±0.67^{a}	2.33±0.67 ^{a}	2.12±0.65^{ab}
Control	1.25±0.03^{a}	63.00±3.44^{bc}	32.4±3.44^{ab}	2.00±0.55^{a}	2.00±0.32^{a}	1.56±0.23^{ab}

Mean ± SEM; n=5; Values with different alphabets in the same column are significantly different at p>0.05; WBC = White Blood Cells; Lymph = Lymphocytes; Neut = Neutophils; Mono = Monocytes; Eosin = Eosinophils.

(32.19±0.96 g/dl) also showed a non-signifcantly increased MCHC levels compared to the normoglycemic rats (Table 1).

White blood cells (WBC)

There was a non-significant increase in the mean WBC of rats administered with PICN and PITC compared to the normoglycemic control rats (1.25±0.03×10^3 µL). Rats administered with glibenclamide (1.20±0.26×10^3 µL) showed a mild decline in the WBC, but diabetic untreated rats (0.81±0.02×10^3 µL) showed a significant decrease in WBC compared to all other groups (Table 2).

Lymphocytes

There was a non-significant (P>0.05) increase in the lymphocytes of rats administered the extracts of PICN and PITC compared to the normoglycemic rats (63.00±3.44%). Diabetic untreated rats (75.00±3.66%) however, showed a significant (P<0.05) increase compared to the normoglycemic rats, while rats administered with glibenclamide had a lower percentage of lymphocytes (57.33±1.76%) (Table 2).

Neutrophils

The reverse of our observation for lymphocytes was seen for the neutrophils with rats administered with glibenclamide (38.0±2.00%) having higher neutrophil count compared to all other groups. Diabetic untreated rats (21.2±3.38%) significantly (P<0.05) had the least neutrophil count. Normoglycemic rats had 32.4±3.44% of WBC as neutrophils and this was non-significantly (P>0.05) higher than that observed in PITC at 400 mg/kg

Table 3. Serum protein and enzymes level of rats administered with extracts of *P. incana* harvested from *C. nitida* (kolanut; PICN) and *T. cacao* (cocoa; PITC).

Extract mg/kg)	TP (g/dl)	Alb (g/dl)	Glob (g/dl)	Alb/Glob ratio	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
PICN 200	8.22±0.22^{ab}	4.72±0.18^{a}	3.50±0.13^{ab}	0.74±0.05^{ab}	45.60±0.51^{ab}	29.20±0.73^{a}	116.40±9.68^{ab}
PICN 400	8.02±0.19^{ab}	4.84±0.19^{a}	3.18±0.16^{b}	0.62±0.05^{b}	42.40±1.50^{b}	28.40±1.63^{a}	105.60±11.21^{ab}
PICN 800	8.40±0.13^{a}	4.88±0.10^{a}	3.52±0.15^{ab}	0.66±0.04^{ab}	44.80±1.66^{ab}	29.20±1.16^{a}	92.60±12.96^{b}
PITC 200	8.43±0.12^{a}	4.53±0.33^{a}	3.90±0.26^{a}	0.83±0.12^{a}	44.00±2.08^{ab}	29.33±2.19^{a}	139.33±4.06^{a}
PITC 400	8.10±0.38^{ab}	4.87±0.38^{a}	3.47±0.03^{ab}	0.67±0.07^{ab}	46.67±1.33^{a}	31.67±0.88^{a}	119.00±10.41^{ab}
PITC 800	8.26±0.12^{ab}	4.68±0.11^{a}	3.58±0.15^{ab}	0.74±0.04^{ab}	45.20±1.16^{ab}	30.80±0.49^{a}	134.00±16.75^{a}
Diabetic	7.00±0.17^{c}	4.10±0.20^{b}	2.90±0.07^{c}	1.41±0.05^{c}	50.20±0.97^{c}	35.20±1.24^{b}	144.40±9.92^{c}
Glibenclamide	7.57±0.62^{b}	4.37±0.27^{a}	3.20±0.40^{b}	0.70±0.06^{ab}	44.33±0.88^{ab}	31.00±0.58^{a}	137.33±2.40^{a}
Control	8.50±0.05^{a}	4.94±0.12^{a}	3.56±0.09^{ab}	0.68±0.04^{ab}	47.00±0.45^{a}	28.40±0.81^{a}	104.60±5.91^{ab}

Mean ± SEM; n=5; Values with different alphabets in the same column are significantly different at p>0.05; TP = Total protein; Alb = Albumin; Glob = Globulin; Alb/Glob = Albumin/Globulin ratio; AST = Aspartate transaminase; ALT = Alanine transaminase; ALP = Alanine phosphate.

which had the least neutrophil count of 24.0±5.20% (Table 2).

Monocytes and eosinophil

There was no significant (P>0.05) difference in the mean monocytes and eosinophil of all extract treated groups compared to that of diabetic untreated, glibenclamide and normoglycemic control groups (Table 2).

Platelets

Platelet counts of all groups non-significantly (P>0.05) increased compared to the normoglycemic rats ($1.56±0.23×10^5$/µl), except for rats administered with PITC at 400 mg/kg ($1.55±0.30×10^5$/µl) which was slightly decreased (Table 2).

Serum biochemistry

Total protein (TP)

The mean total protein was non-significantly (P>0.05) lowered in all rats administered with the extracts, but there was a significant (P<0.05) decrease in the diabetic untreated rats (7.00±0.17 g/dl) compared to the normoglycemic control rats (8.50±0.05 g/dl). Rats administered with glibenclamide (7.57±0.62 g/dl) also showed a significant (P<0.05) decrease in their total protein compared to the normoglycemic rats (Table 3).

Albumin (ALB)

The mean albumin was non-significantly (P>0.05) lowered in rats administered with PICN or PITC extract, or even glibenclamide (4.37±0.27 g/dl) compared to the normoglycemic control rats (4.94±0.12 g/dl). Diabetic untreated rats (4.10±0.20 g/dl) had the most marked decline in total protein levels compared to the normoglycemic rats (Table 3).

Globulin (GLB)

The same trend observed for albumin levels was also seen in the globulin levels, except for a non-significant (P>0.05) increase in globulin levels of rats administered with PITC at a dose of 200 mg/kg (3.90±0.26 g/dl) which was increased compared to that of normoglycemic rats (3.56±0.09 g/dl) (Table 3).

Albumin-Globulin ratio (Alb/Glob ratio)

The mean Alb/Glob ratio of rats administered with the extracts (0.62±0.05 - 0.83±0.12) or glibenclamide (0.70±0.06) was non-significantly different compared to normoglycemic control (0.68±0.04).

However, the mean Alb/Glob ratio for diabetic but untreated rats (1.41±0.05) was significantly (P<0.05) lower than all treated and normoglycemic control rats (Table 3).

Aspartate transaminase (AST)

The mean AST value for all rats administered with PICN, PITC or glibenclamide were non-significantly decreased compared to the normoglycemic controls (47.00±0.45 IU/L) except for rats administered with PICN at a dose of 400 mg/kg (42.40±1.50 IU/L), which decreased significantly

Table 4. Serum biochemistry of rats administered with extracts of *P. incana* harvested from *C. nitida* (kolanut; PICN) and *T. cacao* (cocoa; PITC)).

Extract (mg/kg)	BUN (mg/dl)	Crt (mg/dl)	Chol (mg/dl)	Bil (mg/dl)	Gluc (mg/dl)
PICN 200	11.00±0.55^{b}	0.58±0.06^{a}	52.80±5.34^{a}	0.24±0.04^{ab}	99.6±11.2
PICN 400	11.60±0.24^{ab}	0.62±0.07^{a}	50.80±4.87^{a}	0.20±0.04^{ab}	78.8±14.56
PICN 800	12.20±0.24^{ab}	0.70±0.03^{a}	54.20±6.53^{a}	0.20±0.04^{ab}	127.2±10.16
PITC 200	12.00±0.58^{ab}	0.57±0.07^{a}	54.33±7.51^{a}	0.17±0.03^{ab}	130.8±62.08
PITC 400	12.00±0.58^{ab}	0.70±0.06^{a}	47.33±5.36^{a}	0.20±0.06^{ab}	98.2±24.64
PITC 800	12.00±0.32^{ab}	0.70±0.04^{a}	51.40±5.80^{a}	0.14±0.02^{ab}	175.2±17.84
Diabetic	12.40±0.24^{a}	0.80±0.05^{b}	72.60±3.93^{b}	0.26±0.02^{a}	417.2±54.56
Glibenclamide	11.67±0.67^{ab}	0.53±0.13^{a}	49.33±9.61^{a}	0.20±0.06^{ab}	159±58.00
Control	12.20±0.37^{ab}	0.54±0.05^{a}	65.80±6.79^{a}	0.12±0.02^{b}	81.4±10.32

Mean ± SEM; n=5; Values with different alphabets in the same column are significantly different at p>0.05; BUN = Blood Urea Nitrogen; Crt = Creatinine; Chol = Cholesterol; Bil = Bilirubin; Gluc = Glucose.

(P<0.05). On the converse, diabetic untreated rats (50.20±0.97 IU/L) had a significant (P<0.05) increase in AST levels compared to all treatment groups and the normoglycemic group of rats (Table 3).

Alanine transaminase (ALT)

There was no significant (P>0.05) difference in the mean ALT values for all groups except diabetic untreated rats (35.20±1.24 IU/L) which was significantly (p<0.05) higher compared to normoglycemic rats (28.40±0.81 IU/L). ALT level of diabetic untreated group was also significantly (p<0.05) higher than those of all treated groups (Table 3).

Alanine phosphate (ALP)

Rats administered with PICN extract showed a non-significant (P>0.05) change in their ALP levels compared to the normoglycemic rats (104.60±5.91 IU/L), while rats administered with PITC extract showed a significant increase in ALP levels (119.00±10.41 - 139.33±4.06 IU/L).

Rats administered with glibenclamide (137.33±2.40 IU/L) and diabetic untreated rats (144.40±9.92 IU/L) also had significantly (P<0.05) increased ALP levels compared to the normoglycemic rats (Table 3).

Blood urea nitrogen (BUN) and creatinine (Crt)

There was no significant (P>0.05) difference between the BUN and creatinine levels of treated rats (extract or glibenclamide) and the normoglycemic rats. However, diabetic untreated rats had significantly increased BUN (12.40±0.24 mg/dl) and creatinine levels (0.80±0.05 mg/dl) compared to normoglycemic controls (12.20±0.37 and 0.54±0.05 mg/dl) (Table 4).

Cholesterol (Chol) and bilirubin (Bil)

Mean cholesterol levels of PICN (50.80±4.87-54.20±6.53 mg/dl), PITC (47.33±5.36 - 54.33±7.51 mg/dl) or glibenclamide (49.33±9.61 mg/dl) treated rats significantly (P>0.05) decreased compared to the diabetic untreated rats (72.60±3.93 mg/dl), but the decline was not significant in comparison to normoglycemic rats (65.80±6.79 mg/dl) (Table 4).

Mean bilirubin levels of all treated rats were non-significantly (P>0.05) increased compared to the normoglycemic rats (0.12±0.02 mg/dl), but the diabetic untreated rats (0.26±0.02 mg/dl) had a significantly increased bilirubin level (Table 4).

Glucose level

Rats administered with PICN and PITC extracts showed significantly (P<0.05) lower blood glucose levels compared to the diabetic control (417.2±54.56 mg/kg) and glibenclamide treated rats (159±58.00 mg/dl), but comparable to that observed in the normoglycemic control rats (81.4±10.32 mg/dl) especially at 400 mg/kg (78.8±14.56 and 98.2±24.64 mg/kg) (Table 4).

DISCUSSION

Findings from this study showed that treatment of diabetes mellitus with extracts of *P. incana* which is traditionally practiced in South West Nigeria is a safe practice. Judging by the effect of this hemi-parasitic plant on the blood picture of diabetic rats, it was observed that packed cell volume of the diabetic rats decreased which is a typical symptom of diabetes mellitus. Oyedemi et al. (2011) reported that the occurrence of anaemia in diabetes is due to the increased non - enzymatic

glycosylation of red blood cell (RBC) membrane proteins. Also, the oxidation of proteins and hyperglycaemia in diabetes mellitus causes an increase in the production of lipid peroxides that lead to haemolysis of RBC (Arun and Ramash, 2002).

Treatment of diabetic rats with extract of *P. incana* (PICN and PITC) improved the RBC count, which glibenclamide a known anti-diabetic agent was unable to reverse, but a further decline in RBC was observed. An earlier report by Ogunmefun et al. (2013) noted that *P. incana* does not cause anaemia, but this study has shown that anaemia which results due to the diabetes was essentially not reversed by *P. incana*. The decline in mean corpuscular volume, haemoglobin and its concentrations are in agreement with the findings of earlier researchers such as Arun and Ramash (2002) that noticed a drastic reduction in the levels of red blood cell (RBC), haemoglobin (Hb), haematocrit (PCV) and mean corpuscular haemoglobin concentration (MCHC) of diabetic animals.

This was also observed by Baskar et al. (2006) who reported antihyperglycemic activity of aqueous root extract of *Rubia cordifolia* in streptozotocin - induced diabetic rats. The alterations in these haematological parameters have also been reported in humans (Balasubraimanian et al., 2009). Clinically, MCV and MCHC levels are lowered in cases of iron deficiency, sideroblastic anaemia, thalassemia and lead poisoning while they are elevated in liver diseases, megaloblastic anaemia, folic acid and vitamin B_{12} deficiency (Janz et al., 2013). Findings from our study suggest that the anaemia observed in extract treated rats may clinically be due to iron deficiency while the anaemia in the diabetic untreated and glibenclamide treated rats may tend towards deterioration of liver function.

The white blood cell (WBC) population markedly declined in the diabetic untreated rats, but was non-significantly increased in the extract treated rats. Lymphocyte counts were particularly increased which may be indicative of increase immune response such as observed in serum sickness, aplastic anaemia, leukaemia and immune diseases (Scheinberg and Young, 2012). On the other hand, neutrophil count reduced indicating increased risk of infection which is usually associated with neutropenia (Hsieh, 2007). On the contrary, platelet count increased, but this further supports our findings with red and white cell indices, which point towards the presence of some form of anaemia. Clinically increased platelet (thrombocytosis) is observed in cases of acute blood loss, infection, Iron (Fe) deficiency, haemolytic anaemia or polycythaemia Vera (Skoda, 2009).

Serum biochemistry data clearly shows that diabetes is accompanied by impaired hepatic function typified by markedly decreased total protein and its constituent fractions especially albumin, as well as increased expression of liver enzymes. All these were successfully reversed by the treatment of diabetic rats with the extract. Our result shows that the extract had a more profound hepatoprotective effect than even glibenclamide. Normally, plasma proteins are produced by hepatocytes and hepatic damage is usually indicated by decreased protein synthesis and increased expression of liver specific-enzymes alanine transaminase (ALT) and aspartate transaminase (AST) (Nyblom et al., 2006). Increased expression of alkaline phosphatase (ALP) is more specific for biliary tract damage, obstruction or infection (Aabakken et al., 2007) which was markedly increased in diabetic untreated rats.

As earlier mentioned, diabetes is accompanied by increased haemolysis, which consequently will result in increased excretion of heme as a by-product of hemolysis (Woollard et al., 2009). The increase in bilirubin levels in all groups of rats in this study also supports our hypothesis that there was a degree of increased excretion of heme, but was significantly ($P<0.05$) increased in the diabetic untreated rats.

The mean BUN and creatinine levels were significantly increased in diabetic untreated rats which may be indicative of renal injury (Mazze et al., 2000; Waikar and Bonventre, 2006). The primary metabolite derived from dietary protein and tissue protein turnover is urea while muscle creatinine catabolism results in production of creatinine (Thurman and Parikh, 2008). The extract treated rats however showed a non-significant decline in their BUN levels, while creatinine levels increased. It can be inferred that the extract did try to reverse the renal damage but glibenclamide did a better job at the reversal.

The high cholesterol level in diabetic untreated group can be attributed to the diabetic condition which normally lowers the more beneficial cholesterol; high density lipoprotein (HDL) and increases the harmful cholesterols; triglycerides (TG) and low density lipoprotein (LDL), eventually increasing the overall cholesterol level and may result in serious cardiovascular complications (Henry, 2001). The extract showed better glycemic control compared to glibenclamide in lowering blood cholesterol and glucose levels, which is the desired effect of an oral hypoglycemic agent. A previous toxicological evaluation of the *P. incana* showed that the hemiparasitic plant had good hypolipidemic properties, particularly by significantly lowering LDL (Ogunmefun et al., 2013). It also has very minimal hypoglycemic property in normoglycemia, but profound antihyperglycemic properties (Ogunmefun et al., 2016).

In conclusion, it can be inferred from this study that the extract of *P. incana* regardless of its host plant, not only decreased blood glucose and cholesterol levels, but also alleviated some complications of diabetes such as liver and kidney injury. The PCV was depressed, but RBC count improved. Traditional therapy with *P. incana* extract may need to be in combination with a hematinic to prevent development of anaemia. Also, further studies

may be warranted to ascertain its effect on cardiovascular complications of diabetes and its potential as a hepatoprotective agent.

Conflict of interests

The authors hereby disclose that there is no conflict of interest pertaining to this research work.

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