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Hypoglycemic, Hematologic and Hypolipidemic Potential of *Hippophae rhamnoides* (Berry Oil) in Alloxan-induced Diabetic Rats

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ABSTRACT: This study was carried out to evaluate the hypoglycemic, hematologic and hypolipidemic potential of berry oil in alloxan-induced adult Wistar rat. Five groups of Wistar rats (4 rats per group) Group 1 served as "Normal control" rats and received normal rat pellets and water. Diabetes mellitus was induced in Groups 2, 3, 4, and 5 by intraperitoneal injection of alloxan (160 mg/kg). Group 3 rats were treated with glibenclamide (5 mg/kg body weight), groups 4 and 5 received 500 mg/kg and 1000 mg/kg body weight of Berry oil daily respectively for 21 days via oral gavage. Group 2 rats served as the "Negative control". The blood glucose levels was determined using the *Accu-Chek*^{R} test strip method. The rats were sacrificed on the 22nd day and plasma lipids were determined using appropriate assay kits as per manufacturer's protocol. Red blood cell (RBC) counts, packed cell volume (PCV), and haemoglobin values were all significantly raised ($P<0.05$) in treated rats, while the increased White Blood Cell (WBC) value in diabetic rats was lowered. The elevated total cholesterol, triglycerides, low-density lipoprotein cholesterol LDL-C and very low-density lipoprotein cholesterol VLDL-C in diabetic rats were significantly ($P<0.05$) lowered by all doses of Berry oil while the lowered HDL-C was raised.The findings from this study suggest that Berry Oil demonstrated potent hypoglycemic, hematologic and hypolipidemic properties and could be valuable in the treatment and management of diabetes mellitus.

Keywords: *Hippophae rhamnoides* (Berry oil), Glibenclamide; Hematologic, Hypoglycemic, Hypolipidemic, Rat

Introduction

Hippophae rhamnoides L. (Elaeagnaceae) also known as seabuckthorn or Berry oil is a thorny, deciduous, temperate bush plant native to European and Asian countries (Kumar *et al.*, 2011). Hippophae rhamnoides are abundant in nutrients and therapeutic compounds which includes vitamins A and C, alpha-tocopherol and tocotrienols (Kallio *et al.*, 2004) large amounts of carotenoids and vitamin E, minerals (K, Na, Mg, Ca, Fe, Zn, Se), monosaccharides, amino and mineral acids (Sabir *et al.*, 2005), flavonoids such as rutin, quercetin, myricetin, kaempferol, isorhamnetin (Hibasami *et al.*, 2005), fatty acids, glycerolphospholipids, phytosterols, zeaxanthin esters, polyphenolic compounds (Nagi *et al.*, 2005). Its composition varies according to origin, climate, and the extraction procedure used. Vitamin C is one of the major vitamins contained in Hippophae rhamnoides. Because of its components Hippophae rhamnoides has been used for the treatment of several diseases in traditional medicine in various countries throughout the world. seabuckthorn oil (berry oil) is reported to exhibits numerous beneficial actions: antioxidant, anti-inflammatory, antibacterial, anti-ulcer, antineoplastic, immunomodulatory and hepatoprotective (Zakynthinos *et al.*, 2015).

Diabetes mellitus (DM) is a common global public health disease associated with increased incidence and prevalence, it is defined as a group of metabolic diseases characterized by chronic hyperglycemia, due to defective insulin secretion, insulin action or both, resulting in impaired carbohydrate, protein and lipid metabolism (Akah *et al.*, 2009). Particularly in developing and newly industrialized countries (Danaei *et al.*, 2011) concern regarding this chronic disease is focused on serious DM- related complications which can affect multiple vital organ systems, thereby leading to more severe and

irreversible pathological conditions such as nephropathy, retinopathy, vasculopathy, neuropathy and cardiovascular diseases, as well as hepatopathy. Reid 2006**.** Among the pathophysiological anomalies associated with the condition are hyperglycemia and lipid profile abnormalities (Akah *et al.,* 2009; Anemia Akindekle *et al.,* 2012). Treatment is based on oral hypoglycemic agents and insulin which have several side effects. Sea buckthorn oil comes from the seeds and fruit of the sea buckthorn plant. People have used this ancient plant and its oil for centuries as a natural remedy for a variety of conditions However, scientific research proving its benefits is limited and this informed my interest. The Aim of this study is therefore designed to investigate the hypoglycemic, hematologic and hypolipidemic potential of berry oil on alloxan induced diabetic rats.

Materials and methods

Longrich shenxing (Seabuckthorn) berry oil is an all-round health Supplement. Distributed by Longrich Bioscience Company ltd, Address; Longrich Biological Industrial park, Changshu, Jiangsu Province, 215555, Made in China. Purchased from GFGW+93W, Macauly Street, Umuobasi 440233, Umuahia Abia State.

Animals: Adult Wistar rats of both sexes (160-180 g) were obtained from the Animal House of Abia State University, Uturu, Abia state. They were fed with standard rat feed, with water *ad libitum* throughout the experimental period, but starved for 12 h before the commencement of the experiment. All animal experiments were conducted in compliance with NIH guidelines for the care and use of laboratory animals. (Pub. No 85-23, Revised, 1985, as expressed by Akah *et al*., 2009). The study was conducted at the Department of Zoology and Environmental Biology, College of Natural Sciences, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.Rats were acclimatized to the condition of their new environment for 14 days prior to the commencement of the experiment.

Induction of diabetes: The Rats were weighed and randomly grouped according to their body weights they were fasted overnight, and diabetes was induced in rats by a single intraperitoneal (I.P) injection of freshly prepared solution of Alloxan monohydrate (160 mg/kg). Eight days later rats with blood glucose concerntration above 190 mg/dl were considered diabetic and 20 of such rats were used for the study. Diabetes was induced in rats by a single intraperitoneal (I.P) injection of freshly prepared solution of Alloxan monohydrate (160 mg/kg). Eight days later rats with blood glucose concentration above 160 mg/dl were considered diabetic and 20 of such rats were used for the study.

Experimental design: Twenty Wistar rats were used for the study. The rats were divided into five (5) groups with each partition consisting of four (4) animals. The berry oil extract was dissolved with 4 mls of *Goya* olive oil.

Group 1: Served as normal control given only water and feed pellets.

Group 2: (Untreated alloxan-induced group) Hyperglycaemic rats were induced with alloxan by intraperitoneal injections given only feed pellets and water.

Group 3: Consist of alloxan-induced hyperglycaemic rats treated with 5 mg/kg body weight) of glibenclamide.

Group 4: This consist of alloxan-induced Hyperglycaemic rats treated with 500 mg/kg BW of berry oil

Group 5: Consist of alloxan-induced hyperglycaemic rats treated with 1000 mg/kg BW of berry oil

The extract were administered with the aid of a gavage acting as an oro-gastric tube. Utmost care was taken not to inflict oral or oesophageal injuries on rats.

Acute and sub-acute effect of berry oil blood glucose levels: On the first day of treatment blood was obtained from the tail of each rat in all groups (1-5) by tail snip method prior to and at 2 and 5 hours following treatment and glucose levels were determined for each rat using a glucose meter following standard procedures prescribed by the producer, Roche diagnostic Company, Germany. For the sub-acute studies, the tests were repeated on day 7, 14 and 21.

Blood collection: At the end of the experimental period of 21 days, the rats were sacrificed after euthanizing them, then whole blood (5 ml) collected into a well labelled specimen bottles (EDTA) containing anticoagulant and lithium heparin bottles and centrifuge for 15 min at 1000 × g within 30 min of collection. The plasma samples were transferred into plain sample bottles and immediately taken to the Michael Okpara University Clinic, Hematology Department.

Hematological Studies: All rats were sacrificed on the 22nd day and blood was collected by cardiac puncture into EDTA bottles to be used for the determination of hematological parameters including: Red blood cell (RBC) counts, Packed cell volumes (PCV), Hemoglobin (Hb) concentrations, White blood cell (WBC) counts, White blood cell differential counts, Platelets counts, Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH) and Mean corpuscular hemoglobin concentration (MCHC). These parameters were obtained at once for each blood sample using an Automated Hematology Analyzer produced by Mindray Company, China. (MC, 2800).

Lipid profile studies: Four milliliters of each blood sample was centrifuged to obtain a clear plasma which was used to estimate total cholesterol, high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C) and triglycerides (TG) using commercial kits and following standard procedures outlined by the producer, Randox Laboratories, UK.

Statistical analysis: Results were expressed as mean ± SEM. Data obtained from the experiment was subjected to analysis of variance (ANOVA) using Statistical Analysis System (SAS) version 9.3 and the treatment means were separated using Least Significance Difference (LSD) at ($P \leq 0.05$). Values of $p<0.05$ were considered significant.

Results

All doses of berry oil significantly ($p<0.05$) decreased blood glucose levels in the diabetic treated rats by the end of the study. By the end of the period 500 and 1000 mg/kg of Berry oil had reduced glucose levels in diabetic rats by 15.68 and 44.480% respectively. The effect of berry oil compared favorably with that of Glibenclamide (5 mg/kg) (Table 1). By the end of the 21 days of sub-acute study berry oil successfully returned blood glucose levels in diabetic rats to normal values. The result of the sub-acute effect was also significantly ($p<.05$) different from that of the negative control group but compared favorably with the effect of Glibenclamide (Table 1).

Table 1: Effect of Berry oil and alkaline water on blood glucose concentration of alloxan-induced diabetic mice

Group	Treatment	Initial FBG (mg/dl)	Final FBG (mg/dl)	Fall in Glucose Level
1	Control	83.75±2.3^{c}	84.75±2.85^{c}	-1.15±1.61^{d}
2	Diabetic untreated	540.0±24.85^{a}	533.0±24.31^{a}	-2.45±0.75^{d}
3	Standard drug (Glibenclamide)	458.50±26.22^{b}	200.75±6.51^{d}	55.78±2.91^{a}
4	500mg Berry oil	527.75±31.30^{ba}	444.0±20.68^{2}	15.68±1.48^{a}
5	1000mg Berry oil	523.75±25.89^{ba}	291.50±20.04^{c}	44.48±1.18^{b}

**Group 1: Normal control, Group 2: Untreated diabetic control, Groups 4 and 5 were treated with 500 mg/kg and 1000mg/kg body weight of berry oil extract respectively while Group 2 (glibenclamide). Values are presented as mean ± SEM (n = 5). P value less than 0.05 (p<0.05) indicates significant change, by one-way Duncan's multiple range ANOVA.*

The effect of berry oil on haematological parameters of alloxan-induced diabetic mice is shown in Table 2. All doses of berry oil significantly ($P<0.05$) raised RBC, PCV and HB values and lowered WBC counts in the diabetic treated rats. MCV, MCH, MCHC, and Platelets values were significantly affected.

Table 2: Effect of Berry oil on hematological parameters of alloxan-induceddiabetic mice

**Group 1: Normal control, Group 2: Untreated diabetic control, Groups 4 and 5 were treated with 500 mg/kg and 1000mg/kg body weight of berry oil extract respectively while Group 2 (glibenclamide). Values are presented as mean*

Group	HB (g/dl)	PCV (%)	RBC (10^{6}/µL)	WBC (10^{3}/µL)	MCV (fL)	MCH (Pg)	MCHC (g/dL)	PLT
Control	18.70±0.73^{a}	49.25±1.1^{a}	7.81±0.19^{a}	9.10±0.24^{c}	63.10±0.36^{c}	23.95±0.46^{a}	3.77±0.06^{a}	108.75±3.94^{c}
Diabetic untreated	14.10±0.19^{c}	40.00±0.41^{c}	6.42±0.07^{c}	15.40±0.70^{c}	62.32±0.52^{c}	21.95±0.13^{b}	3.55±0.03^{b}	144.25±2.02^{a}
Standard drug (glibenclamide)	16.77±0.19^{b}	45.50±0.96^{b}	6.93±0.09^{b}	9.81±0.39^{c}	65.75±2.15^{ba}	24.25±0.37^{a}	3.70±0.11^{b}	126.25±2.84^{b}
500 mg berry oil	16.30±0.15^{b}	43.50±0.65^{b}	6.84±0.05^{b}	12.65±0.19^{b}	63.55±0.47^{ba}	23.80±0.20^{a}	3.75±0.06^{a}	128.25±1.55^{b}
1000 mg berry oil	16.67±0.09^{b}	45.50±0.29^{b}	6.84±0.05^{b}	12.62±0.55^{b}	62.52±0.45^{a}	24.37±0.06^{a}	36.67±0.03^{b}	123.50±1.76^{b}

± SEM (n = 5). P value less than 0.05 (p<0.05) indicates significant change, by one-way Duncan's multiple range ANOVA.

The elevated total cholesterol, triglycerides, LDL-C and VLDL-C in diabetic rats were significantly ($P<0$ .05) lowered by all doses of berry oil while the lowered HDL-C was raised (Table 3).

Table 3: Effect of Berry oil on lipid profile of alloxan-induced diabetic mice.

Group	Treatment	TAG (mg/dl)	Cholesterol (mg/dl)	HDLC (mg/dl)	VLDLC (mg/dl)	LDLC (mg/dl)
1	Control	79.44±1.56^{c}	67.68±0.99^{c}	40.03±0.13^{c}	15.89±0.31^{c}	11.76±0.87^{c}
2	Diabetic untreated	100.59±1.47^{a}	111.55±4.88^{a}	40.99±0.63^{c}	20.12±0.29^{a}	50.44±4.71^{a}
3	Standard drug (Glibbenclamide)	89.80±0.64^{b}	92.28±1.09^{b}	43.99±0.61^{b}	17.94±0.13^{b}	30.38±1.59^{b}
4	5000 mg berry oil	91.23±0.66^{b}	94.70±1.71^{b}	45.49±0.73^{ba}	18.22±0.13^{b}	30.99±2.04^{b}
5	1000 mg/kg berry oil	91.80±0.87^{b}	91.57±0.67^{b}	46.42±0.64^{a}	18.36±0.17^{b}	26.79±0.72^{b}

**Group 1: Normal control, Group 2: Untreated diabetic control, Groups 4 and 5 were treated with 500 mg/kg and 1000mg/kg body weight of berry oil extract respectively while Group 2 (glibenclamide). Values are presented as mean ± SEM (n = 5). P value less than 0.05 (p<0.05) indicates significant change, by one-way Duncan's multiple range ANOVA.*

Discussion

The field of herbal medicines research has gained significant importance in the last few decades and the demand to use natural products in the treatment of diabetes is increasing worldwide. Available literature reports shows that there are more than 400 plant species showing anti-diabetic activity. The effects of these plants have been shown to delay the development of diabetic complications and correct some metabolic abnormalities. In the past few years some of the new bioactive drugs isolated from hypoglycaemic plants showed anti-diabetic activity with more efficacy than oral hypoglycaemic agents used in clinical therapy (Mahamed *et al*., 2006).

The aim of this study was to demonstrate the effect of berry oil in the reduction of blood glucose in alloxan-treated rats administering 500mg/kg and 1000mg/kg BW Of the extract. The study also determined the effect of the extract on body weight, haematological parameters and lipid profile parameters in alloxan-treated rat. Induction of diabetes was by alloxan monohydrate, glibenclamide served as standard drug, and berry oil extracts of (500 mg/kg and 1000 mg/kg) were used for study. Previous studies have shown *Hippophae rhamnoides* to possess phytochemicals (flavonoids, e.t.c.), secondary metabolites like tannins, steroids, cardiac glycosides (Gajalakshmi *et al*., 2012). The presence of flavonoids, tannins, saponins, phytosterols and phenolic in the extract may contribute the hypoglycemic and hypolipidemic effect (Tripoli *et al*., 2007).

All doses of berry oil significantly (P<.05) lowered blood glucose levels within the 5 hours of acute study and returned the blood glucose levels in diabetic rats to normal values by the end of 21 days of treatment. The effect of Berry oil compared favourably with that of glibenclamide, which is considered hypoglycemic drug used as reference. The results therefore suggest that berry oil contain active principles with hypoglycemic properties. The extract may have achieved this hypoglycemic effect by increasing insulin selection and peripheral utilization of glucose in diabetic rats, inhibition of endogenous glucose production, inhibition of intestinal glucose absorption and/or regenerating existing beta cells. These mechanisms have all been reported to be responsible for lowering blood sugar levels (Colak *et al*., 2012).

Results of the hematological studies indicate that anemia is a pathophysiology associated with diabetes mellitus as the diabetic rats had significantly (P< .05) lowered RBC, PCV, HB, MCH, MCV and MCHC values when compared to the normal control rats. The results agree with Akindele *et al*. (2012) who reported that in diabetes mellitus, there is the development of anemia, particularly, the hypochromic type, due to fall in the iron content of the body resulting from oxidation stress associated with the condition. All doses of berry oil restored the values of these parameters to normal in the diabetic treated rats by the end of the 21 days of treatment, suggesting that Berry oil has anti-anemic activity. This effects of berry oil could be due to its high iron content Saliu (2012) and/or the ability to improve bone marrow functions Akomas *et al*. (2014).

In the lipid profile studies, the elevated total cholesterol, triglycerides, low-density lipoprotein cholesterol (LDL-C), very low-density lipoprotein cholesterol (VLDL-C) with decreased high density lipoprotein cholesterol (HDL-C) observed in the untreated diabetic group agreed with existing literature report that the development of diabetes mellitus is usually accompanied by a marked increase in blood cholesterol, triglycerides, LDL-C, VLDL-C and a reduction in HDLC (Akah *et al*., 2009). The lowering of cholesterol, triglycerides, LDL-C and VLDL-C and increase in HDL-C observed in the groups treated with 5000 and 1000 mg/kg of berry oil. This could be attributed to the high content of essential oil as reported in the literature (Hibasami *et al*., 2005).

Conclusion

This study suggested that *Hippophae rhamnoides (*berry Oil) may also be a safe and potent agent capable of attenuating hematological and lipid profile anomalies associated with diabetes mellitus. However, further studies are required to delineate its mode of actions.

Competing interest

Authors hereby declare that no competing interest exist as far as this work is concerned.

Ethical approval

Authors declare that this work is not against public interest and that all animal experiments were conducted in line with the NIH guidelines for care and use of laboratory animals, Pub. No. 85-23, Revised, 1985 and approved by the University's Animal Ethics Committee.

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