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Antidiabetic and Cytotoxic Effect of Aqueous and Methanolic Leaf Extract of *Portulaca oleracae*

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Abstract

Portulaca oleracea is one of the most used medicinal plants listed by the World Health Organization for diabetes. In the present study, the Antidiabetic and cytotoxic effect of the aqueous and methanolic leaf extract of *Portulaca oleracae*was evaluated.Forty-two animals were randomly assigned into seven groups consisting of six animals (n=6). Group I was normal control. Group II-III were normal animals administered with 500 and 1000 mg/kg extract. Group IV-VII were alloxan-induced type 2 diabetes animals. Group V-VI were treated with 500 and 1000 mg/kg extract while Group VII was treated with Glibenclamide. All treatments were administered once a day via oral gavage for 21 days. Blood sample was collected for biochemical essay and organs processed histologically using molten paraffin wax method.The acute toxicity values for methanol and aqueous extracts of *Portulaca oleracae* weregreater than 5000 mg/kg. Aqueous extract showed the highest reduction in blood glucose level compared to the glibenclamide and methanolic extract. Isolated organs of the treated diabetic animals incontestable showed that *Portulaca oleracea*extract improved the kidney shrunken glomeruli and liver centrilobular necrosis. Besides, the acute toxicities of both methanol and aqueous extracts showed no treatment-related mortality at the tested doses.According to the result, *Portulaca oleracea* is a good source of antidiabetic drug. Both the aqueous and methanolic extracts improved the histology of the anatomic liver, kidney, and heart.

Keywords: Portulaca oleracea, biochemicals, centrilobular necrosis, histopathology.

Introduction

Diabetes mellitus (DM) is a widespread metabolic disorder characterized by the lack of the body's ability to regulate blood glucose due to impaired insulin secretion, insulin action or even both[1].In 2021, the global diabetes-related health expenditures were estimated to be 966 billion USD and is projected to reach 1,054 billion USD by 2045[2]. Besides, this projection only considered demographic changes in populations relating to ageing and urbanization, and not changes in risk factor prevalence or survival, it is possible that the projection is underestimated. The rising

prevalence of diabetes is attributed principally to drug resistance and by this phenomenon, the number of people living with diabetes will continue to increase in the aging population[3].

To obtain a distinct solution for diabetes-related health complications, an ethnopharmacological survey represents a major reference point in unveiling the treasure of natural resources[4,5]. *Portulaca oleracae* (family Portulacaceae) is an herbaceous shrub found in India and other tropical countries Nigeria inclusive. The indigenous names for *Portulaca oleracae*in Hausa and Igbo languages are pasa kasa andnti oke respectively[2]. *Portulaca oleracae* provides a rich source of nutritional benefits when eaten raw as a green and cooked as a sauce in soup[6]. Besides, the nutritional composition includes carbohydrates, proteins, saponins, tannins, and essential amino acids (linoleic acid and oleic acid), and vitamins[7,8].

Previous studies also reported the wound healing[9], antifungal[10], antifertility[11], antiinflammatory[12], and antidiabetic effects[13] of *Portulaca oleracae.* However, diabetes affects several organs of the body with serious systemic involvements which are common problems[14, 15]. Therefore, the aim of the current study is to verify the antidiabeticand cytotoxic activities of the aqueous and methanolic leaf extracts of *Portulaca oleracae* as a raw drug for diabetes especially after synthetic drugs resistance awareness.

Materials and Methods

Chemicals used

Alloxan monohydrate (Chemical Co. St. Louis, USA) and Randox reagent test kits (Randox Laboratories Limited, United Kingdom) for ALT, AST, AP, and E/U/Cr were purchased and used.

Preparation of *Portulaca oleracea* Aqueous and Methanol Extracts

Fresh *Portulaca oleracea* was collected and identified in the Department of Botany, University of Nigeria, Enugu Campus, Nigeria, where voucher specimen was deposited. The Aqueous and methanol extracts of the plant were prepared according to the method as described by Gamde *et al.*[16]. Five hundred grams (500 g) of dried *Portulaca oleracea* was macerated in 1000 mL of distilled water for 24 hours and then filtered. The plant remains from the aqueous extract were resuspended in methanol. The filtrates were evaporated to dryness in an oven at 40^{o}C to yield dark residues.

PO Acute toxicity study

Acute toxicity study was carried out according to the guidelines of Organization of Economic Company and Development (OECD) 425 (OECD, 2001). The PO extracts were administered in a single dose using oral gastric tube. Animals were deprived of food 3 h prior to the extract dosing. After each extracts were administered, physical observations was done at critical interval of 30 min for 4 hours and after 24 hours for behavioral change or death. The PO extract dosage 250, 500, 1000, 2000, 4000 mg/kg body weight were determined. The Up and Down method for oral acute toxicity study was carried out to determine the acute toxicity in Rabbits as described by Gamde *et al.* [18]. Briefly, animals were dosed one at a time and observed for survival or death. If a rabbit survives, the subsequent dose for the next rabbit was increased but if the rabbit die, the dose was reduced. Each rabbit was observed at least for 24 hours before dosing the next rabbit to arrive at the least dose lethal to the rabbits.

Experimental Animals

The study was approved by the Animal Welfare and Ethics Committee of University of Nigeria, Enugu Campus, Nigeria. The male and female rabbits used in the study were obtained from the Animal House of the Department of Zoology, University of Nigeria, Enugu Campus, Nigeria. Animals were kept in metal cages and maintained under standard laboratory conditions. The animals were fed with chow and allowed free access to clean water *ad libitum*. All experimental procedures were in accordance to the United States National Institute of Health (NIH) Guide for Care and Use of Laboratory Animals.

Induction of hyperglycemia

Hyperglycemia was induced by 150 mg/kg alloxan monohydrate after a16 hours fast.The fasting blood sugar (FBS) was determined 72 hours after induction, a fasting blood glucose test confirmed hyperglycemia (14.0 mmol/L), after which *Portulaca oleracea*was orally administered and animals whose fasting blood sugar was and above were considered diabetic as described with little modification[19].

Induction of Diabetes Type II

Fresh 5% percent alloxan monohydrate was prepared in saline and it was used to induce diabetes using intraperitoneal injection of 150 mg/kg body weight of animals.

Experimental Design

Forty-two animals were randomly assigned into seven groups consisting of six animals each.
Group I was normal animals control
Group II was animals administered with 500 mg/kg PO
Group III was animals administered with 1000 mg/kg PO
Group IV Diabetes induced animals without treatment
Group V Diabetic animals treated with 500 mg/kg PO
Group VI Diabetic animals treated with 100o mg/kg PO
Group VII Diabetic animals treated with 2.5 mg/kg Glibenclamide

Glibenclamide and PO extract were administered via oral gavage to the animals once a day for 21 consecutive days. Animals were euthanized and blood samples were collected by cardiac puncture for biochemical essays. The liver, heart, and kidney were excised via abdominal incisions and and processed using molten paraffin wax for histological examination.

Biochemical assays

Serum triglyceride (TG) was determined by the method described by Fossati *et al*.[17]. Serum total cholesterol (TC) was determined according to the method of Roeschlau *et al*.[18] while LDL-cholesterol was determined by the formula described by Friedewald *et al*.[19]

Statistical Analysis

Results were analyzed using IBM SPSS (version 25) by one-way analysis of variance (ANOVA)and differences were considered statistically significant at $p< 0.05$.

Results

Toxicity of *PO* Extract

The acute toxicities of both methanol and aqueous PO extracts showed no death nor treatment-related mortality at the tested doses. The extracts seems to be safe up to a dose of 5000 mg/kg body weight. Therefore, the acute toxicity (LD_{50}) value is considered to be greater than 5000 mg/kg.

Effect of extract on blood glucose

The baseline blood glucose levels were determined at the beginning of the study ($p >0.05$). Following the induction of hyperglycemia, animals with blood glucose values ±200 mg/dL ($p>0.05$) were considered diabetic. Active constituents of the aqueous *PO* extract showed the highest reduction in blood glucose levels compared with the glibenclamide and methanolic extract. Oral administration of the methanolic and aqueous extracts of *Portulaca oleracea*at 500, and 1000 mg/kg body weight showed significant reduction of the blood glucose levels compared to the control groups (Table 1 and 2).

Effect of extract on liver and kidney functions

In this study, high blood glucose significantly ($P< 0.05$) raised serum creatinine and hepatic enzymes (AST and ALT) levels for 21 days. However, active constituents of PO significantly reduced the raised hepatic enzymes and creatinine. PO extracted by methanol showed the highest reduction in a dose-related manner as compared with the aqueous extract and control (Table 3).

Anti-hyperlipidemic effect of pericarp extract

PO leaf extract on lipid profile on alloxan induced diabetes is presented in Table 4. There was a significant difference ($p<0.05$) in the LDL and total cholesterol contents of diabeteic animals treated with 1000 mg/kg PO aqueous extract as compared with the aqueous extract and control.

Histopathology

Figure 3. Histopathology of diabetic animals illustrating shrunken glomeruli with decreased mesangium size while the liver exhibited centrilobular necrosis. However, animals treated with 1000 mg/kg PO revealed improved anatomic histology of the liver and kidney. No pathological changes was seen in the heart

.

Table 1: Effect of Methanolic Extract on Blood Glucose (Mmol/L)

Treatment groups	0 hr	10 days	21 days
Normal control	5.34±0.15$^{\#}$	5.56±0.21$^{\#}$	5.41±0.18$^{\#}$
Normal ＋500 mg/kg *PO*	5.37±0.03$^{\#}$	5.96±0.21$^{\#}$	5.32±0.14$^{\#}$
Normal ＋1000 mg/kg *PO*	5.57±0.16$^{\#}$	5.63±0.25$^{\#}$	5.30±0.38$^{\#}$
DM control	15.24±0.15*	14.98±0.14*	14.99±0.28*
DM ＋500 mg/kg *PO*	14.92±0.50$^{*\#}$	11.16±0.47*$^{\#}$	9.29±0.33*$^{\#}$
DM ＋1000 mg/kg *PO*	15.20±0.16*	10.09±0.07*$^{\#}$	7.61±0.34*$^{\#}$
DM + 2.5 mg/kg Glib.	15.14±0.09*	10.45±0.18*$^{\#}$	6.74±0.19*$^{\#}$

Data are expressed as the mean ± standard deviation per group. *Mean values were significantly different with respect to the Normal control at $P\leq 0.05$. $^{\#}$Mean values were significantly different compared to the diabetes group (Alloxan-induced DM only) at $P\leq 0.05$. N means normal, DM diabetes, Glib Glibenclamide and PO *Portulaca oleracae*

Table 2: Effect of Aqueous Extract on Blood Glucose (Mmol/L) level

Treatment groups	0 hr	10 days	21 days
Normal control	5.35±0.15$^{\#}$	5.55±0.21$^{\#}$	5.31±0.17$^{\#}$
Normal ＋500 mg/kg *PO*	5.36±0.03$^{\#}$	5.94±0.21$^{\#}$	5.33±0.14$^{\#}$
Normal ＋1000mg/kg *PO*	5.51±0.16$^{\#}$	5.63±0.25$^{\#}$	5.31±0.38$^{\#}$
DM control	15.14±0.15*	14.98±0.14*	14.99±0.28
DM ＋500 mg/kg *PO*	15.42±0.22*	11.72±0.46*$^{\#}$	9.35±0.28*$^{\#}$
DM ＋1000 mg/kg *PO*	15.05±0.23*	7.87±0.31*$^{\#}$	6.66±0.19*$^{\#}$
DM + 2.5 mg/kg Glib.	**15.14±0.09***	**10.45±0.18*$^{\#}$**	**6.74±0.19*$^{\#}$**

Data are expressed as the mean ± standard deviation per group. *Mean values were significantly different with respect to the Normal control at $P\leq 0.05$. $^{\#}$Mean values were significantly different compared to the diabetes group (Alloxan-induced DM only) at $P\leq 0.05$. DM means diabetes, Glib Glibenclamide, and PO means *Portulaca oleracae.*

Table 3; Effect of Extract on Liver and Kidney Functions

Group	U (Mmol/l)	Cr (Mmol/l)	AST (Mmol/l)	ALT(Mmol/l)	ASP (Mmol/l)	TB (Mmol/l)
Normal control	8.23±0.54#	59.45±4.38#	27.58±1.47#	25.01±0.92#	39.05±4.10	1.50±0.21
N+1000 mg aq *PO*	6.13±0.62*	55.25±10.19	36.00±4.30*	44.25±3.88*	58.25±7.16*	2.60±0.80
N+1000 mg/kg Met. *PO*	4.88±0.55*	71.00±7.99*	40.75±6.94*	48.25±9.67*	44.00±9.00*	1.80±0.00
DM control	5.88±.20*	77.75±4.19*	42,25±6.65*	46.00±8.63*	39.75±7.02	1.80±0.00
DM+2.5 mg/kg Glib.	2.95±0.22*#	58.25±5.68#	31.25±5.54#	48.00±3.11*	55.75±4.78*#	1.8±0.00
DM+500 mg/Kg Met*PO*	1.63±0.11*#	64.00±5.40*#	28.50±3.07#	68.50±5.12*#	72.25±3.35*#	1.80±0.00
DM+500 mg/kg Aq *PO*	5.53±1.60*	53.50±9.13#	41.50±1.04*	95.50±22.22*#	43.00±16.62*	1.80±0.00
DM+1000 mg/kg Met *PO*	3.23±0.77*#	49.25±4.44#	26.0±04.49#	59.50±10.90*#	50.50±8.42*#	1.80±0.00
DM+1000 mg/kg Aq *PO*	3.35±0.27*#	72.75±8.01*	44.00±8.65*	56.75±4.42*#	84.25±21.10*#	1.80±0.00

Data are expressed as the mean ± standard deviation per group. *Mean values were significantly different with respect to the Normal control at $P \leq 0.05$. #Mean values were significantly different compared to the diabetes group (Alloxan-induced DM only) at $P \leq 0.05$. N means normal, Aq aqueous, Met methanol, DM diabetes and PO *Portulaca oleracae*

Table 4: Effect of Extract on Lipid Profile

Group	LDL (Mmol/l)	TC (Mmol/l)	TG (Mmol/l)
Normal control	0.25±0.08#	1.16±0.04#	1.61±0.01
N+1000 mg/kg aq *PO*	0.06±0.01*#	2.47±0.17*	1.41±0.02*
N+1000 mg/kg Met. *PO*	0.70±0.00*#	2.27±0.11*	1.34±0.03*
DM control	0.26±0.01	3.31±0.01*	2.13±0.06*
DM +2.5 mg/kg Glib.	0.06±0.00*#	2.56±0.02#	1.41±01#
DM+500 mg/kg Met*PO*	0.16±0.01#	3.11±0.02*#	1.65±0.02#
DM+500 mg/kg Aq *PO*	0.12±0.01#	3.13±0.01*	1.46±0.01#
DM+1000 mg/kg Met*PO*	0.18±0.01#	3.05±0.01*	1.49±0.01#
DM+1000mg/kg Aq *PO*	0.09±0.00*#	2.76±0.00*#	1.41±0.01#

Data are expressed as the mean ± standard deviation per group. *Mean values were significantly different with respect to the Normal control at $P \leq 0.05$. #Mean values were significantly different compared to the diabetes group (Alloxan-induced DM only) at $P \leq 0.05$. N means normal, LDL low density lipoprotein, TC total cholesterol, TG triglyceride, Aq aqueous, Met methanol, DM diabetes and PO *Portulaca oleracae*

Histopathology result

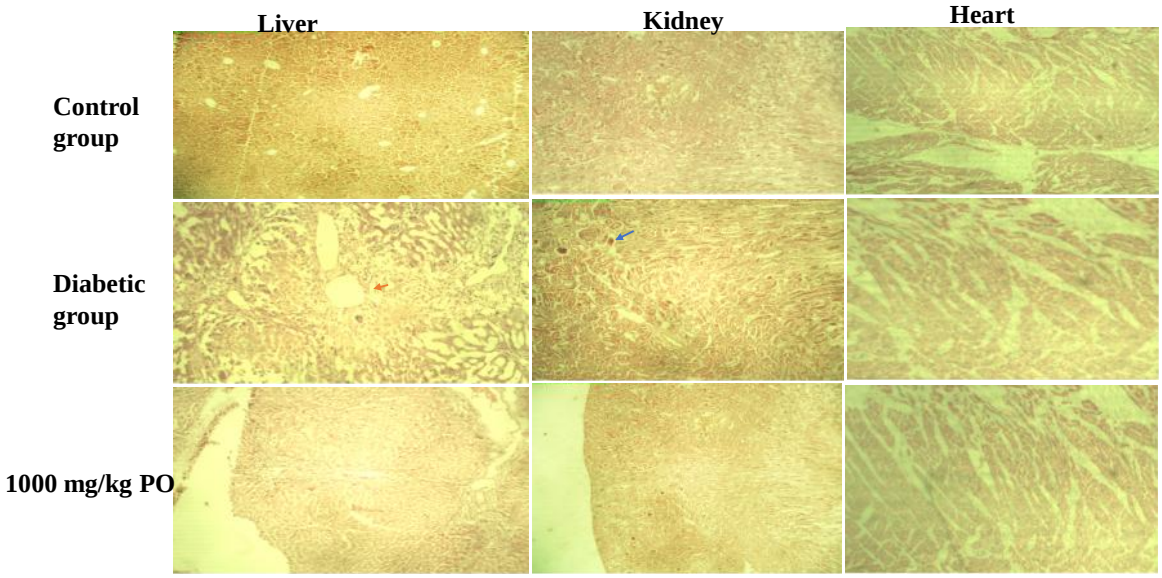

Fig. 1 Histopathological sections of the diabetic animals showed centrilobular necrosis (red arrow) and glomerular shrinkage (blue arrow). Animals treated with 1000 mg/kg PO revealed improved anatomic histologies of the liver and kidney. The regular cardiac muscles indicated no histopathological change when compared to the control (H &E stain. X 100).

Discussion

In this study, the hypoglycemic effect of aqueous and methanolic leaf extract of *Portulaca oleracea*were observed in diabetic rabbits comparing to normal ones. Active constituents of the aqueous leaf extract demonstrated the highest reduction in blood glucose levels followed by glibenclamide and methanolic leaf extract comparing with the normal control. *P. oleracea* increases the stimulation of insulin secretion and glucose uptake, which can aid in the recovery process of diabetic patient[1]. A number of plants have also been reported to have hypoglycemic and insulin releasing stimulatory effects[20,21]. Flavonoids, tannins, and Ca^{+} were identified in *portulaca oleracea* to modulate calcium-mediated mechanism for insulin release[7]. In another study, *P. oleracea* reduces blood glucose and suppresses body weight gain. Although there were differences in diet calories, the present finding is consistent with Won *et al*[22]reports.

Documented evidence abounds that endocrine disorders such as lipids, carbohydrates, and protein metabolism are common in chronic diabetes[23].In the present study, *Portulaca oleracea* shows antihyperlipidemic effect on the experimental model. The low serum LDL-cholesterol and VLDL-cholesterol levels accompanying increase in HDL-cholesterol in the alloxan-induced animals treated with *Portulaca oleracea* extract suggest restraint of hyperlipidemia due to the phytochemicals identified[24]. Similar study has been reported by Wong *et al*[22] and Maqsood *et al.*[23].

In addition, many in-vitro and in vivo studies have demonstrated that diabetes and its treatments is associated with various dysfunctions and organs failure[25,26,27]. Isolated organs of the treated diabetic animals incontestable showed that *Portulaca oleracea*extract improved the kidney shrunken glomeruli and liver centrilobular necrosis. Besides, the acute toxicities of both methanol and aqueous PO extracts showed no treatment-related mortality at the tested doses. Hence, investigating the diabetes-related cellular toxicities and developing new drug is essential.

Conclusion

According to the result of this study, *Portulaca oleracea* is a good source of antidiabetic drug.Both the aqueous and methanolic leaf extracts of *Portulaca oleracea* improved the histology of the anatomic liver, kidney, and heart.

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