

Corchorus olitorius leaves affect nutrient utilization and improve biochemical indices of alloxan induced diabetic Wistar rats.

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Article

Keywords: Corchorus olitorius leaves, alpha amylase inhibitor, glycosylated hemoglobin, biological value, hyperlipidemia, hyperglycaemia

Posted Date: May 23rd, 2023

DOI: <https://doi.org/10.21203/rs.3.rs-2877214/v1>

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Abstract

BACKGROUND/ OBJECTIVES. The effective management of type 2 diabetes depends on the ability to maintain or improve insulin utilization, regulation of blood glucose, and the control of hyperlipidaemia. The effectiveness of *Corchorus olitorius* leaves in these three domains were evaluated in the present research. The objective of this research was to evaluate the ameliorative effects of *Corchorus olitorius* leaves on alloxan induced diabetes in Wistar rats

METHODS. Nutrient composition of shade dried *C. olitorius* leaves was determined using standard AOAC methods.; alpha-amylase inhibition potentials of *C. olitorius* leaves were determined using four solvent systems. Bioactive compounds in *C. olitorius* were determined using GC-MS. Diets were formulated to contain 0–20% *C. olitorius* and fed to alloxan-induced diabetic rats alongside positive and negative standards for 70 days. Indices of nutritional quality and biochemical indices were compared in diabetic, diabetic/nutritionally managed and normoglycaemic Wistar rats. Histopathology of the pancreas of the three groups of animals were also compared.

RESULTS. Water and 99% ethanol were the best solvents for extracting alpha amylase inhibitor from *C. olitorius* leaves. 9-octadecanoic acid (Z), benzoic acid, 2 phenyl ester, 9-octadeceneZ-methylester and hexadecenoic acids were bioactive compounds identified in *C. olitorius* leaves. Formulated diets containing *C. olitorius* decreased feed intake, body mass gain, HbA1c, fasting blood glucose (FBG), total cholesterol (TC), triglycerides (TGs) in a concentration dependent manner. HDL-C (good cholesterol) increased. *C. olitorius* caused the regeneration of the islets of Langerhans of the alloxan -induced diabetic rats.

CONCLUSION. *Corchorus olitorius* leaves is effective for the control of hyperglycaemia and hyperlipidaemia. Its water and ethanol extracts contain significant quantities of alpha amylase inhibitor that slows down carbohydrate breakdown into sugars thereby controlling the blood glucose level.

INTRODUCTION

Diabetes is a class of metabolic disorders which is caused by decreased pancreatic function where secretion and function of insulin is either limited, impaired or stopped altogether [1]. It is a non-communicable disease that ranks high as a global health problem among others and is a risk factor that can lead to malfunction of other organs and systems in the body if not well managed. It was estimated that 425 million people aged 20–79 years were sick of diabetes mellitus in 2017 and it is projected that it will rise to 629 million by 2045 [2]. Diabetes has been rising more rapidly in low- and middle-income countries than in high income countries [3]. Type 2 diabetes is the most common type of diabetes as it is occurring increasingly and frequently even in children [3]. It is well known that diabetes mellitus is a metabolic, endocrine disorder and is directly connected to carbohydrate, lipid and protein metabolism and as such, nutrition therapy forms an integral part of diabetes management [4]. Ojimekwe and Amaechi [5] reported the importance of *Vernonia amygdalina* and other plant products in the management of type

2 diabetes. Nutrition and diet are the mainstay in type 2 diabetes prevention and management of the complications associated with it.

Treatment of diabetes requires diet and physical activity as well as lowering blood glucose and other risk factors that damage blood vessels [3]. Clinical studies have most times recommended the use of natural or herbal cure for diabetes rather than depending primarily on drugs because of side [6]. It is in view of this that *Corchorus olitorius* leaves (wild jute, bush okra) was investigated for its potential effects on nutrient utilization and biochemical parameters in alloxan-induced diabetic rats. *C. olitorius* is a tropical herb that is upright branching and slightly woody [7]. It is an annual/perennial crop that grows to a height of 3.5m and the edible parts include leaves, seeds and shoots. The leaves are reported demulcent, diuretic, febrifuge, tonic and can be used in the cure of chronic cystitis/gonorrhea and dysuria [8]. *C. olitorius* belongs to the family Malvaceae (formerly Tiliaceae) and is called by many vernacular names such as 'Jew's mallow', 'Krinkrin', 'Tossa jute', 'Bush Okra' and 'West African Sorrel' [9]. It is consumed fresh raw or cooked [10] in soups or as potherb [11]. It is a leafy mucilaginous vegetable and in Nigeria, the leaves are cooked to form a slimy sticky sauce comparable to okra [12]. The leaves can be sundried, milled into flour and stored for a significant time and the dried product can be used for tea and as a soup thickener [13]. It is rich in fiber and helps to control blood pressure, cholesterol build up, and prevents heart disease [12].

METHODS

Plant Collection and Preparation

Fresh *Corchorus olitorus* leaves were procured from Abakpa Market, Abakaliki Ebonyi State, Nigeria. They were picked, washed and drained off water before air drying under shade for 6 days at an environmental temperature ($25 \pm 2^\circ\text{C}$). The air-dried leaves were pulverized and stored in clean, dry airtight container and labeled prior to use.

Determination of Alpha Amylase Inhibitory Activity

Alpha amylase inhibitor (AAI) in the dried *C. olitorius* leaves was extracted using different solvents namely water, chloroform, ethylacetate, absolute ethanol and 70% ethanol using cold maceration method described by Jeremy and Whiteman [14]. The extract was concentrated using a rotary evaporator at a maximum temperature of 40°C and then dried. AAI in each extract was determined by the dinitrosalicylic acid method of Bernfeld (1955) to estimate maltose.

Gas Chromatography-Mass Spectrometry Analysis

Bioactive compounds from ethanol extract of *Corchorus olitorius* leaves was analyzed using gas chromatography-mass spectrometry (QP 2010 plus, Shimadzu, Japan) equipped with flame ionization detector (FID). Helium was used as carrier gas at a flow rate of 3.0ml/min. The column temperature was programmed from 70 to 280°C at the rate of $5^\circ\text{C}/\text{min}$. Injector and detector temperatures were set at

250°C and 260°C, respectively. All quantifications were carried out using a built-in-data handling programme provided by the manufacturer of the gas chromatogram. Interpretation of mass spectrum was conducted using the database of the National Institute Standard and Technology (NIST) version 2.0, 2009 library.

Diet formulation

A standard diet containing 83% corn starch, 10% casein, 2% soybean oil, 3% rice bran for fiber and 2% vitamin and mineral mix was prepared. The dried pulverized *Corchorus olitorius* leaves was incorporated at different concentrations of 2.5%, 5.0%, 10.0 and 20% per 100g of standard diet.

Experimental Animals

Weanling albino Wistar Rats (22–26 days old) of both sexes with average initial body weight of 40 ± 2 grams were used for the experiments. The rats were obtained from the Department of Veterinary Medicine, University of Nigeria, Nsukka. They were housed in stainless steel metabolism cages maintained at an ambient temperature of $28 \pm 2^\circ\text{C}$ and a natural light-dark cycle. All the experimental animals were acclimatized for 14 days on the standard diet in accordance with the recommendation in the guide for the care and use of laboratory animals [16]. The experimental protocol was approved by the Institutional Ethics Committee.

Proximate analysis of the formulated diets

Proximate composition of the various diet samples was analyzed by the methods described by AOAC [17].

Induction of diabetes

Diabetes was induced by intra-peritoneal injection of alloxan monohydrate (160mg/kg). After 72 h, fasting blood glucose levels of the rats were determined with a glucometer (Accu-check active Germany).

Experimental design

The method described by Pellet and Young [18] was adopted. Six (6) normoglycemic and 36 diabetic rats were used for the experiment. The normoglycemic rats were assigned group A and diabetic rats were randomly assigned to 5 groups (B-F) of 6 rats each and housed in metabolic cages. Groups A and B received standard feed excluding *C. olitorius* leaves while groups C-F received feed containing 2.5%, 5.0%, 10.0% and 20% *C. olitorius* leaves respectively. The rats were fed *ad libitum* for 70 consecutive days. At first, the animals were used for growth study for a total of 35days to monitor the response of the various animal groups to the different treatments. After 35days of growth study (weaning period), the same group of animals were continuously fed the various treatment diets for another 35days giving a total of 70days. Daily feed and water intake were recorded while body weight was recorded at weekly intervals. Fecal droppings and urine voided for the last 7days of weaning period were collected continuously. The feces were oven dried at 60°C while urine was preserved in 1ml of 0.1N HCl in brown bottles to eliminate microbial growth and prevent nitrogen losses. The urine samples were stored in a deep freezer prior to

nitrogen determination. After the 70th day of feeding, the rats were fasted for 16h and blood samples were collected via ocular puncture into EDTA and plain bottles for whole blood and serum preparations respectively. Thereafter, the rats were sacrificed by cervical dislocation, immediately laparotomised and pancreas were excised and preserved in 10% formalin solution for histopathological study.

Evaluation of nutritional indices:

Nitrogen content of both urine and faecal samples were analyzed using the Kjeldahl method described by James [19]. Protein efficiency ratio (PER), feed efficiency ratio (FER), apparent digestibility (AD), True digestibility (TD), Biological Value (BV) and Net protein utilization (NPU) were calculated.

Biochemical analysis:

Fasting blood glucose (FBG) was determined using a glucometer (Accu-Check, Germany). Glycosylated haemoglobin (HbA1c) was analysed by the method of Trivelli *et al* [20], using a glycohaemoglobin test kit (Teco Diagnostics, USA). Serum total cholesterol, triglycerides, high density lipoproteins were analyzed using their various diagnostic test kits (Randox Laboratory, UK). Serum low density-lipoproteins was calculated using the equation promulgated by Friedwald *et al*. [21] as:

$$\text{LDL Cholesterol (mg/dl)} = \text{Total cholesterol} - \frac{(\text{Triglycerides})}{5} - \text{HDL-Cholesterol}$$

Histopathology:

Excised pancreas of the rats was fixed in 10% formal saline for 24h. They were washed in ascending grades of ethanol, cleared with xylene, embedded in paraffin wax, sectioned with a microtome and stained with hematoxylin and eosin (H and E) and mounted on Canada Balsam (Sigma-Aldrich, St Louis, Mo) [22]. All the sections were examined under a light microscope using 100 and 400 magnifications. Photomicrographs of sections were taken with an Olympus photo microscope (Olympus Scientific Equipment, Ashburn, VA).

Statistical analysis:

One-way analysis of variance (ANOVA) followed by Duncan's Post hoc test was used to separate differences of data collected for the various parameter analyzed. Data obtained were expressed as mean $\pm$ standard deviation and differences in means were significantly different ($p < 0.05$). Statistical analysis was determined using SPSS Statistical Software version 20.

RESULTS AND DISCUSSION

Alpha amylase inhibitory activity

The percentage alpha amylase inhibitory activities of whole *C. olitorius* leaf extracts from different solvents are presented in Fig. 1. Results showed α -amylase inhibitor activities in both polar and non-polar

solvents with highest activity in 99% ethanol and lowest activity in 70% ethanol. α -amylase inhibitory activities exhibited by *C. olitorius* leaves in different solvents depicts the presence of compounds which inhibit α -amylase and they were affected by solvent media. Methanol extract of the leaves was reported to inhibit both α -amylase and α -glucosidase enzymes (Oboh *et al.* [23] and has antioxidant effects (Azuma *et al.*, 1999) [24]. One therapeutic target for delaying oligosaccharide digestion to absorbable monosaccharides in the intestinal brush border is through the inhibition of pancreatic α -amylase and results in reduced postprandial hyperglycemia [25]. The in-vitro inhibition of α -amylase activity of *C. olitorius* leaf extracts indicated they possess bioactive molecules which have potential effects in delaying carbohydrate digestion. Plants that have α -amylase or α -glucosidase inhibitory potentials closely follow the action of acarbose [26]. Hence, with this identified mechanism, it can be deduced that *Corchorus olitorius* leaves has potential therapeutic benefit in managing diabetes mellitus.

Gas Chromatography- Mass Spectrometry Results of ethanol extract of *Corchorus olitorius* leaves

Table 1 shows results on gas chromatography-mass spectrometry analysis of ethanol extract of *Corchorus oiltorius* leaves. Results indicated the presence of 9 compounds. The predominant compounds include benzoic acid phenyl ethyl ester (15.54%), hexadecanoic acid (12.29%), octadecenoic (Z)-methyl ester (13.06%) and 9-octadecenoic (Z) (48.45%). The respective compounds have been reported to have various beneficial biological activities. Hexadecanoic acid, octadecenoic (Z)-methyl ester and 9-octadecenoic acid are polyunsaturated fatty acids while benzoic acid 2-phenyl ethyl ester is an essential oil and classified as a flavoring agent and occurs as a volatile compound in foods. On the contrary, 2-Dodecenal was found to be the major component alongside other compounds namely 2-methy-1-penten-4yn-3-ol, 2,4-Decadienal and ethanone were found in appreciable quantities [27]. Hexadecanoic acid and 9-Octadecenoic acid methyl ester has been reported to exhibit both antioxidant and hypocholesterolemic activities [28], while oleic acid has anti-inflammatory and hypocholesterolemic activities [29]. Lipid peroxidation and oxidative stress are closely related in inducing elevated blood sugar levels [30]. Polyunsaturated fatty acids have antioxidant potentials and are important in preventing lipid peroxidation [30]. The presence of these compounds in *C. olitorius* leaves are responsible for its nutritional and health benefits.

Table 1
Bioactive compounds identified in the ethanol extract of *Corchorus olitorius* leaves.

Peak No	Retention Time (mins)	Identity of Compound	Concentration (%)	Molecular Weight	Molecular Formula
1	16.33	Benzoic acid, 2-phenyl ethyl ester	15.54	226	C ₁₅ H ₁₄ O ₂
2	21.33	1,6,10-Dodecatrien-3-ol-1,7,11-trimethyl (E)+/- trans Nerolidiol	0.26	222	C ₁₅ H ₂₆ O
3	24.73	3,7,11,15-tetramethyl-2-hexadecen-1-ol	2.38	296	C ₂₀ H ₄₀ O
4	25.71	Hexadecanoic acid methyl ester	3.05	270	C ₁₇ H ₃₄ O ₂
5	26.32	Hexadecanoic acid	12.29	256	C ₁₆ H ₃₂ O ₂
6	27.46	9-Octadecenoic (Z)-methyl ester	0.06	298	C ₁₉ H ₃₆ O ₂
7	27.62	Octadecanoic acid methyl ester	48.45	282	C ₁₉ H ₃₈ O ₂
8	28.06	9-Octadecenoic acid (Z)	4.37	298	C ₁₈ H ₃₄ O ₂
9	30.73	9,12-Octadecadienoyl chloride			C ₁₈ H ₃₁ ClO

Proximate composition of standard diet and diets containing different quantities of *Corchorus olitorius* leaves.

Incorporation of *C. olitorius* leaves resulted to a concentration dependent significant ($p < 0.05$) increase in the moisture, ash, crude fiber, fat and crude protein content of the ration when compared with the standard ration (Table 2). Carbohydrate content of rations containing *C. olitorius* leaves significantly ($p < 0.05$) decreased in a concentration dependent manner when compared with carbohydrate content of the standard ration. This agrees with the findings of Ranawara *et al.* [31] who reported the addition of vegetables (broccoli, carrot, tomato, beetroot) respectively to wheat flour in the production of bread resulted to an increase in moisture, protein, fat contents while total carbohydrate decreased when compared with carbohydrate content of plain bread made from wheat flour alone. Similarly, El-Khatib and Muhieddine [32] also reported an increase in fiber and other nutrients content of bread to which pumpkin flour was added while carbohydrate decreased when compared with plain bread. Idris *et al.* [33] reported *C. olitorius* leaves to be a good source of minerals, lipids, protein and carbohydrates which are important as energy source in human and animal nutrition.

Table 2

Proximate composition of standard ration and rations containing *Corchorus olitorius* leaves at varied concentration.

Parameter	Standard diet	2.5% CO	5% CO	10% CO	20% CO
Moisture	6.52 ^d ± 0.18	6.98 ^d ± 0.16	7.73 ^c ± 0.09	9.64 ^b ± 0.06	11.08 ^a ± 0.14
Ash	2.58 ^c ± 0.13	2.79 ^b ± 0.02	2.83 ^b ± 0.04	3.82 ^a ± 0.05	3.91 ^a ± 0.12
Crude Fiber	2.60 ^d ± 0.07	2.85 ^d ± 0.12	3.82 ^c ± 0.12	4.75 ^b ± 0.05	5.50 ^a ± 0.15
Fat	2.68 ^d ± 0.04	3.39 ^c ± 0.09	3.84 ^c ± 0.04	4.05 ^b ± 0.06	5.85 ^a ± 0.05
Crude Protein	16.95 ^e ± 0.05	19.38 ^d ± 0.13	21.60 ^c ± 0.15	23.56 ^b ± 0.13	25.71 ^a ± 0.37
Carbohydrate	56.97 ^c ± 0.16	65.11 ^a ± 0.31	60.17 ^b ± 0.22	54.18 ^d ± 0.10	47.95 ^e ± 0.39

Values are means of triplicate determinations ± standard deviation. Means in the same row bearing different superscripts are significantly different ($p < 0.05$). CO = *Corchorus olitorius* leaves.

Effect of *Corchorus oiltorius* leaves on nutrient utilization in the experimental animals.

The effect of the incorporation of *C. olitorius* leaves on nutrient utilization of diabetic rats compared with normoglycemic and diabetic rats fed standard rations (i.e rations not containing *C. olitorius* leaves) is shown in Table 3 Results indicated varied responses. *C. olitorius* leaf supplemented diets resulted to a significant ($p < 0.05$) decrease in feed intake (FI), body mass gain (BMG), protein efficiency ratio (PER), endogenous fecal nitrogen (EFN), endogenous urinary nitrogen (EUN), %nitrogen retention (%NR), apparent digestibility (AD) and net protein utilization (NPU) of treated diabetic groups when compared with normoglycemic and diabetic groups fed plain standard diets. Feed intake (FI) was relatively higher in diabetic untreated and diabetic rats fed 2.5% *C. olitorius* leaves. A similar finding was reported by Aluwong *et al.* [34] where FI of alloxan-induced diabetic rats increased when compared with the diabetic treated group. The higher FI observed in the diabetic untreated and 2.5% *C. olitorius* leaves treated group could be attributed to the deficiency and/or absence of insulin, which induces impairment in glucose transport resulting to energy deficiency in cells hence, increased feeding to compensate for the energy deficiency [35].

Normoglycemic animals fed standard diets had a significantly ($p < 0.05$) high PER, FER, %NR, AD, TD and NPU than the diabetic untreated and diabetic treated groups. Zhang *et al.* [36] reported a decrease in NPU as dietary fiber concentration increased; this could be attributed to decreased bioavailability of amino acids. There were no significant differences ($p < 0.05$) in BMG of normoglycemic and diabetic untreated groups despite the high feed intake of the diabetic untreated group. This could be attributed to alleviation of catabolic processes in the diabetic untreated group [34]. BMG significantly ($p < 0.05$) decreased in a concentration dependent manner in the diabetic treated groups. Gomaa *et al.* [37] reported that *C. olitorius* leaf extract significantly reduced weight gain and visceral white adipose tissue. Weight increase

is a major risk factor in the development of diet related diseases such as cardiovascular diseases, stroke, type diabetes and certain cancers [38]. Increase in vegetable consumption results in weight loss [39].

Table 3

Effect of *Corchorus olitorius* leaves on nutrient utilization in normoglycemic and diabetic Rats.

Parameter	Normal Control	Diabetic Control	2.5% CO	5% CO	10% CO	20% CO
FI (g)	282.38 ^{bc} ± 38.8	330.68 ^a ± 25.9	332.05 ^a ± 14.2	302.38 ^b ± 10.4	260.23 ^c ± 6.7	190.30 ^d ± 6.2
PI (g)						
BMG (g)	47.84 ^d ± 6.6	56.02 ^c ± 4.4	64.59 ^a ± 2.7	65.40 ^a ± 2.3	61.23 ^b ± 1.6	48.93 ^d ± 1.6
PER	87.57 ^b ± 10.4	80.23 ^b ± 2.8	109.72 ^a ± 6.8	85.47 ^b ± 10.9	60.30 ^c ± 7.5	38.68 ^d ± 3.4
FER		1.44 ^c ± 0.1				
NI (mg)	1.85 ^a ± 0.1	0.24 ^c ± 0.0	1.71 ^b ± 0.1	1.30 ^d ± 0.1	0.98 ^e ± 0.1	0.79 ^f ± 0.1
FN (mg)	0.31 ^a ± 0.0	1519.77 ^b ± 118.8	0.33 ^a ± 0.0	0.28 ^b ± 0.0	0.23 ^c ± 0.0	0.20 ^d ± 0.0
UN (mg)	1297.76 ^c ± 178.2		1752.05 ^a ± 74.6	1773.55 ^a ± 62.0	1661.25 ^a ± 42.6	1327.42 ^c ± 43.2
EFN (mg)		155.64 ^d ± 16.5				
EUN (mg)	108.22 ^e ± 24.9	38.97 ^c ± 0.1	167.31 ^d ± 7.2	191.93 ^c ± 8.6	227.10 ^b ± 9.7	277.92 ^a ± 12.3
NR	41.10 ^b ± 3.5	35.49 ^c ± 1.3	62.07 ^a ± 1.4	62.86 ^a ± 0.8	58.85 ^a ± 0.4	44.37 ^b ± 4.7
NR%						
AD%	39.19 ^c ± 4.4	10.45 ^c ± 0.5	53.36 ^a ± 3.0	44.85 ^b ± 4.9	35.58 ^c ± 3.4	28.20 ^d ± 1.6
TD	11.93 ^c ± 1.75	1325.16 ^b ± 102.6				
BV			17.71 ^a ± 1.1	14.20 ^b ± 1.9	10.48 ^c ± 1.4	7.54 ^d ± 0.6
NPU	1148.78 ^c ± 153.2	87.20 ^b ± 0.2	1522.67 ^a ± 74.6	1519.60 ^a ± 53.4	1375.31 ^b ± 34.0	1005.13 ^d ± 31.2
	88.56 ^a ± 0.4	88.11 ^{cd} ± 4.3				75.73 ^e ± 0.2
	91.77 ^a ± 0.8	87.43 ^c ± 0.3	86.89 ^b ± 0.7	85.64 ^c ± 0.2	82.79 ^d ± 0.3	
	88.74 ^a ± 0.7	96.26 ^a ± 0.3	90.44 ^{ab} ± 0.6	89.19 ^b ± 0.2	86.33 ^d ± 0.3	79.07 ^e ± 0.3
	95.30 ^b ± 0.5	84.17 ^a ± 0.3				76.95 ^f ± 0.3
	84.56 ^a ± 0.4		87.39 ^b ± 0.6	86.67 ^d ± 0.3	83.54 ^e ± 1.3	94.87 ^c ± 0.2
			94.79 ^c ± 0.2	94.99 ^c ± 0.0	95.04 ^{bc} ± 0.1	73.04 ^e ± 0.3

Values are means ± standard deviation. Means in the same row bearing different superscripts are significantly different ($p < 0.05$). CO = *Corchorus olitorius* leaves. FI = Feed Intake, PI = Protein Intake, BMG = Body Mass Gain, PER = Protein Efficiency Ratio, FER = Feed Efficiency Intake, NI = Nitrogen Intake, FN = Fecal Nitrogen, UN = Urinary Nitrogen, EFN = Endogenous Fecal Nitrogen, EUN = Endogenous Urinary Nitrogen, NR = Nitrogen Retention, %NR = Percentage Nitrogen Retention, AD = Apparent Digestibility, TD = True Digestibility, BV = Biological Value, NPU = Net Protein Utilization

Parameter	Normal Control	Diabetic Control	2.5% CO	5% CO	10% CO	20% CO
			82.83 $\pm$ 0.7	82.32 ^c $\pm$ 0.3	81.02 ^d $\pm$ 2.6	
Values are means $\pm$ standard deviation. Means in the same row bearing different superscripts are significantly different ($p < 0.05$). CO = Corchorus olitorius leaves. FI = Feed Intake, PI = Protein Intake, BMG = Body Mass Gain, PER = Protein Efficiency Ratio, FER = Feed Efficiency Intake, NI = Nitrogen Intake, FN = Fecal Nitrogen, UN = Urinary Nitrogen, EFN = Endogenous Fecal Nitrogen, EUN = Endogenous Urinary Nitrogen, NR = Nitrogen Retention, %NR = Percentage Nitrogen Retention, AD = Apparent Digestibility, TD = True Digestibility, BV = Biological Value, NPU = Net Protein Utilization						

Effects of Corchorus olitorius leaves on biochemical parameters of diabetic Rats.

Table 4 shows results on biochemical parameters of normoglycemic, diabetic and diabetic treated rats. Haemoglobin A1c (HbA1c) and fasting blood glucose (FBG) were significantly ($p < 0.05$) higher in the diabetic untreated group while it decreased in the diabetic treated group in a concentration dependent manner. High HbA1c and FBG in diabetic condition due to the administration of alloxan is attributed to the damage to pancreatic beta cells resulting to deregulation of blood glucose, hence resulting to an increase in blood glucose concentration [40] and the generation of reactive oxygen species [41]. Tabesh *et al.* [42] found out that the consumption of vegetables and fruits generate antioxidants which defend and help reduce HbA1c in diabetic patients. Therefore, the gradual reduction in HbA1c observed in the diabetic treated groups could be attributed to the presence of antioxidants and other bioactive compounds in *C. olitorius* leaves and these could ameliorate oxidative reactions.

With respect to the lipid profile, it was observed that total cholesterol (TC), triglyceride (TG) and low-density lipoprotein cholesterol (LDL-C) were higher in the diabetic untreated and group fed 2.5% *C. olitorius* leaves. The increase in TC and TGs could be attributed to reduced lipoprotein lipase activity secondary to decreased serum insulin levels because of the diabetic condition [34]. High density lipoprotein cholesterol (HDL-C) significantly ($p < 0.05$) increased in a concentration dependent manner in the diabetic treated group while it was quite low in the diabetic untreated as well as the group fed 2.5% *C. olitorius* leaves. The reduction in HDL-C may be attributed mainly to low density lipoprotein which favor the hydrolysis of stored triglycerides in the adipocytes thereby discharging them into the circulation resulting to a decrease in serum HDL-C concentrations [43]. Low, high density lipoprotein cholesterol and increased triglyceride concentration predisposes individuals to dyslipidaemia and may put up likelihood of cardiovascular disease [44]. The reduction observed in our findings for serum lipids with an increase in serum HDL-C in the diabetic treated groups fed whole *C. olitorius* leaves agrees with the findings of Gomaa *et al.* [37] who reported that the leaf extract was effective in preventing hyperlipidaemia and decrease serum glucose.

Table 4

Effects of *Corchorus olitorius* leaves on biochemical parameters of normoglycemic and diabetic Rats.

Parameter	Normal Control	Diabetic Control	2.5% CO	5%CO	10%CO	20%CO
HBA1 _c (%)	4.05d ± 0.1	10.65a ± 2.0	7.95b ± 0.98	6.98b ± 0.5	6.53bc ± 0.9	5.30cd ± 0.4
FBG (mg/dl)	93.17d ± 12.8	212.50a ± 35.9	161.17b ± 9.1	128.17c ± 3.3	118.67c ± 2.6	98.33d ± 7.7
TC (mg/dl)	165.39d ± 14.18	251.28a ± 13.5	246.80a ± 1.4	206.80b ± 10.4	188.46c ± 16.3	162.31d ± 9.8
TG (mg/dl)	117.82e ± 6.7	195.77a ± 5.9	177.63b ± 16.7	144.23c ± 5.3	132.92d ± 11.0	113.85e ± 3.7
HDL-C (mg/dl)	54.14ab ± 6.4	27.44d ± 8.3	24.36d ± 1.0	42.12c ± 4.2	50.23bc ± 9.6	61.93a ± 5.2
LDL-C (mg/dl)	86.44d ± 12.0	184.69a ± 14.3	186.91a ± 8.1	135.83b ± 13.0	112.05c ± 17.2	77.57d ± 13.8

Values are means ± standard deviation. Means in the same row bearing different superscripts are significantly different ($p < 0.05$). CO = *Corchorus olitorius* leaves. HBA1_c = Hemoglobin Alc, FBG = Fasting blood glucose, TC = Total cholesterol, TG = Triglycerides, HDL-C = High density lipoprotein cholesterol, LDL-C = Low density lipoprotein cholesterol.

Histopathology Photomicrograph sections of Pancreas of Rats after 70days feeding with *Corchorus olitorius* leaves are shown in Fig. 2. The diabetic untreated group showed loss of islet cells and necrosis of acini. Alloxan monohydrate caused considerable damage to pancreatic beta cells as shown by the histopathological assay. *Corchorus olitorius* leaves gradually regenerated the islet and acini cells in a concentration dependent manner in the diabetic treated groups.

Figure 2: Photomicrograph sections (H and E X400) of Pancreas of normoglycemic (positive control), diabetic untreated (negative control) and diabetic treated (2.5%CO, 5%CO, 10%CO and 20%CO) groups. CO = *Cochorus olitorius*: AC = Acini: IC = Islet cells.

CONCLUSION

Corchorus olitorius leaves exhibited alpha amylase inhibition activities; hypoglycemic and hypolipidemic activities and reversed pancreatic damage in alloxan-induced diabetic rats. It had varied effects on nutritional indices at various concentrations. *Corchorus olitorius* leaves at 10% inclusion significantly improved biochemical parameters but at an inclusion rate of 2.5% it had better effects on nutrient utilization. Both the results of its content of poly unsaturated fatty acids and cholesterol lowering potentials suggest that it could be more effective in the prevention of dyslipidaemia.

Abbreviations

apparent digestibility (AD), body mass gain (BMG), Biological Value (BV); *Corchorus olitorius* (CO); endogenous fecal nitrogen (EFN), endogenous urinary nitrogen (EUN); Fasting blood glucose (FBG) feed intake (FI), feed efficiency ratio (FER), Haemoglobin A1_c (HbA1_c); High Density Lipoprotein Cholesterol (HDL-C); low-density lipoprotein cholesterol (LDL-C); Protein efficiency ratio (PER); True digestibility (TD); Net protein utilization (NPU); % nitrogen retention (% NR); total cholesterol (TC), triglyceride (TG).

Declarations

ACKNOWLEDGMENTS

We thank the staff of College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria for providing the space and expertise for animal experiments.

AUTHOR CONTRIBUTIONS

PO conceptualized the research. NO carried out the work. The two authors drafted and edited the manuscript. All authors approved the manuscript.

FUNDING

This research was not sponsored by any funding organization

CONFLICT OF INTERESTS

The authors declare that there are no conflicts of interest.

References

1. American Diabetes Association (ADA). Diagnosis classification of Diabetes mellitus. Diabetes Care. 2011; (Suppl 1), S62-S67. <https://doi.org/10.2337/dc10-S062>
2. Aynalem SB, Zeleke AJ. Prevalence diabetes mellitus and its risk factors among individuals aged 15 years and above in Mizan –Aman town, Southwest Ethiopia, A cross sectional study. J Endocrinol. 2018; Article ID 9317987. 17pages <https://doi.org/10.1155/2018/9317987>. eCollection 2018
3. WHO (2022) <https://www.who.int/news-room/fact-sheets/detail/diabetes> document. Accessed 5March, 2023.
4. Folorunso O, Oguntibeju OO. The role of nutrition in the management of diabetes mellitus: Diabetes mellitus Insights and perspective. Intechopen. 2013; 5772/48752 (<https://www.intechopen.com/books/diabetes>).

5. Ojimelukwe PC, Amaechi NC. 2019. Composition of *Vernonia amygdalina* and its Potential Health Benefits. *Int J Environ Agric Biotechnol*. 2019; 4 (6), 1838-1846 <https://doi.org/10.22161/ijeab.46.34>
6. Rashmi S, Shilpy S. Herbs and botanic ingredients with beneficial effects on blood sugar in pre-diabetes. *Herbal Med*. 2016; 2:1 <https://doi.org/10.1021762472-0151.100011>
7. Dayal B, Singh N. *molokhia*- the wealth for a better health *Int J Res*. 2015; 2(2), 658-661. <http://internationaljournalofresearch.org/>
8. Chopra RN, Nayar SL, Chopra IC. Glossary of Indian medicinal plants. Quarterly Review of Biology. Council of Scientific Industrial Research, New Delhi. Council of Scientific & Industrial Research, New Delhi. 1958;33 (2), pp156. <https://doi.org/10.1086/402350>
9. Fondio L, Gubben GJH. *Corchorus olitorius* L. [Internet] Record from PROTA4U. In: Brink M, Achigan-Dako EG. 2011. (Editors). PROTA- Plant Resources of Tropical Africa, Wageningen, Netherlands
10. Usher G. A dictionary of plants used by man. Constable. 1974; ISBN0094579202
11. Facciola S, Cornucopia- A source. Book of Edible plants. Kampong Publications,1990; ISSB 0-96280.870-9. 1990
12. Medical Health Guide. Saluyot. *Corchorus olitorius*. Medical health guide.com. 2011; (Accessed 7-5-20).
13. Jeremy CKL, Whiteman M. Antioxidant activities of some Chinese herbs. *Free Radical Biol. Med*. 2003; 26, 1231-1237. <https://doi.org/10.1093/ecam/nem054>
14. Bernfeld P. Amylases, alpha and beta. In: Colowick SP, Kaplan NO. (Eds.). *Methods in Enzymology*. 1955; New York Academic Press. pp. 149-158
16. AOAC (2005). Association of Official Analytical Chemists Official Methods of Analysis. Washington DC
17. Pellet PL, Young VR. Evaluation of protein in experimental animals. In: *Nutritional evaluation of protein foods* (1st Ed) Tokyo. 1980. The United Nations University, pp 41-57. <https://doi.org/10.4236/fns.2013.44048>
18. James CS. *Analytical Chemistry of Foods*.1995; Black Academic and Professional, London.
19. Trivelli LA, Ranney PH, Lai HT. Glycohaemoglobin in diabetic subjects. *N. Engle J Med*. 1971; 284, 353-357. <https://doi.org/10.1111/jdi.12241>
20. Friedwald WT, Leve IR, Fredrikson SD. Estimation of the concentration of low-density lipoprotein separation by three different methods. *Clinical Chem*. 1972; 18, 499-502. <https://doi.org/10.1002/jcla.23429>
21. Berrougui H, Ettaib A, Gonzalez MDH, Sotomayor MA, Bennani-Kabchi N, Hmamouchi M. Hypolipidemic and hypocholesterolemic effect of argan oil (*Argan spinosa* L.) in meriones shawl rats. *J Ethnopharmacol*. 2003; 89, 15-18. [https://doi.org/10.1016/s0378-8741\(03\)00176-4](https://doi.org/10.1016/s0378-8741(03)00176-4)

22. Oboh G, Ademiluyi AO, Akinyemi AJ, Henle T, Saliu JA, Schwarzenbolz U. Inhibitory effect of polyphenol-rich extracts of jute leaf (*Corchorus olitorius*) on key enzyme linked to type 2 diabetes (α -amylase and α -glucosidase) and hypertension (angiotensin I converting) in vitro. J Funct Foods. 2012; 4, 450-458. [https://doi.org/ 10.1016/j.jff.2012.02.003](https://doi.org/10.1016/j.jff.2012.02.003)
23. Azuma K, Nakayama M, Koshioka M, Ippoushi K, Yamaguchi Y, Kohata K, Yamauchi Y, Ho H, Higashio H. Phenolic antioxidants from the leaves of *Corchorus olitorius* L. J Agric Food Chem. 1999; 47, 3963-3966. [https://doi.org/ 10.1021/jf990347p](https://doi.org/10.1021/jf990347p)
24. Sim L, Jayakanthan K, Mohan S, Nasi R, Johnston BD, Pinto BM, David RR. 2010. New glucosidase inhibitors from an ayurvedic herbal treatment for type2 diabetes: structures and inhibition of human intestinal maltase, glucoamylase with compounds from *Salacia reticulata*. Biochem 2010. 49(3), 443-451. [https://doi.org/ 10.1021/bi9016457](https://doi.org/10.1021/bi9016457). PMID: 20039683.
25. Ezurike UF, Prieto JM. The use of plants in the traditional management of diabetes in Nigeria: Pharmacological and toxicological considerations. J. Ethnopharmacol. 2014;155, 857-924. [https://doi.org/ 10.1016/j.jep.2014.05.055](https://doi.org/10.1016/j.jep.2014.05.055)
26. Orieke DC, Ohaeri OI, Ijeh II, Ijioma NS. Phytocomponents and acute toxicity evaluation of *Corchorus olitorius* leaf extract. Euro J Med Plants. 2018; 23(1), 1-16. <https://doi.org/10.9734/EJMP/2018/38739>
27. Osuntokun OT, Ayegbusi BL, Yusuf-Babatunde AM, Ige OO. Assessment of antimicrobial, phytochemical screening and gas chromatography-mass spectrometric profile of crude *Chrysophyllum albidum* essential oil. Chem Res J 2017; 2(5), 68-85
28. Duke, J.A., Phytochemical and ethnobotanical databases <http://search.conduit.com/results.aspx?q=dr+duke's+phytochemical+ethnobotanical+databases&ctid> 2014; (Accessed 27-11-2014).
29. Guldas M, Ziyank-Demirtas S, Sahan Y, Yildiz E, Gurbuz O. Antioxidant and anti-diabetic properties of *Spiriluna platensis* produced in Turkey. Food Sci Technol. 2020; [https://doi.org/ 10.1590/fst23920](https://doi.org/10.1590/fst23920)
30. Ranawara V, Campbell F, Bestwick C, Nicol P, Milne L, Duthie G, Raikos V. Breads fortified with freeze-dried vegetables: Quality and nutritional attributes. Part II: Breads not containing oil as an ingredient. Foods. 2016; 5(3), 62. [https://doi.org/ 10.3390/foods5030062](https://doi.org/10.3390/foods5030062)
31. El-Khatib S, Muhieddine M. Nutritional profile and medicinal properties of pumpkin fruit pulp. In: Salanta, L.C (Ed). The health benefits of foods-Current knowledge and further development. IntechOpen. 2019; [https://doi.org/ 10.5772/intechopen.89274](https://doi.org/10.5772/intechopen.89274).
32. Idris S, Yisa J, Ndamitso MM. Nutritional composition of *Corchorus olitorius* leaves. Anim Prod Res Adv. 2009; 5(2) <https://doi.org/10.4314/apra.v5i2.49827>
33. Aluwong T, Ayo JO, Kpukple A, Oladipo OO. Amelioration of hyperglycaemia, oxidative stress and dyslipidaemia in alloxan-induced diabetic wistar rats treated with probiotic and vitamin C Nutrients. 2016; 8(51), 1-15. [https://doi.org/ 10.3390/nu8050151](https://doi.org/10.3390/nu8050151)
34. Wong KK, Tzeng ES. Appearance of different diabetic symptoms after streptozotocin administration: A comparison study. Biochem. Mol. Biol. Int. 1993; 30, 1035-1041

35. Zhang W, Li D, Liu L, Zang J, Duan Q, Yang W, Zhang L. The effects of dietary fiber level on nutrient digestibility in growing pigs. *J Animal Sci Biotechnol*. 2013; 4(1), 17 <https://doi.org/10.1186/12049-1891-4-17>
36. Gomaa AA, El-Sers DA, Al-Zokeim NI, Gomaa MA. Amelioration of experimental metabolic syndrome induced in rats by orlistat and *Corchorus olitorius* leaf extract: role of adipocytokines. *J Pharmacy Pharm*. 2018; 71(2):281-291. <https://doi.org/10.1111/jphp.13032>
37. Ng M, Fleming T, Robinson M, Thomson B, Graetz N, Margono C, Mullany EC, Biryukov S, Abbafati C, Abera SF. Global, regional and national prevalence of overweight and obesity in children and adults during 1980-2013: A systematic analysis for the global burden of disease study. *Lancet*. 2014; 384, 766-781. <https://doi.org/10.1093/ecam/nem054>
38. Nour M, Lutze SA, Grech A, Allman-Farinelli M. The relationship between vegetable intake and weight outcomes: A systematic review of cohort studies. *Nutrients*. 2018. 10 (11), 1626. <https://doi.org/10.3390/nu1011626>
39. Verma L, Singour PK, Chaurasiya PK, Pawar RS, Patil UK. Effect of ethanolic extract of *Cassia occidentalis* Linn for the management of alloxan-induced diabetic rats. *Pharmacog*. 2010; 2, 132-137. <https://doi.org/10.4103/0974-8490.65506>. PMID: 21808555; PMCID: PMC3141303
40. Das J, Vasan U, Sil PC, Taurine exerts hypoglycemic effect in alloxan-induced diabetic rats, improves insulin mediated glucose transport signaling pathway in heart and ameliorates cardiac oxidative stress and apoptosis. *Toxicol. Appl. Pharmacol*. 2012; 258, 296-308. <https://doi.org/10.1016/j.taap.2011.11.009>
41. Tabesh M, Hariri M, Askari G, Ghiasvand R, Tabesh M, Heydari A, Darvishi L, Khorvash F. 2013. The relationship between vegetables and fruits intake and glycosylated hemoglobin values, lipid profiles and nitrogen statues in type II inactive diabetic patients. *Int J Preventive Med*. 2013; 4(Suppl 1), S63-S67. PMID: 23717773; PMCID: PMC3665029
42. Rifia N, Warnick GR. Lipids, lipoproteins, apolipoproteins and other cardiovascular risk factors. 2006; In: Burtis, C.A and Ashwood, E.R. (Eds). *Tietz Textbook of Clinical Chemistry and Molecular Diagnostics*. Elsevier Saunders, St. Louis Mo USA. Pp 903-981. [https://doi.org/10.1016/0021-9150\(90\)90047-m](https://doi.org/10.1016/0021-9150(90)90047-m)
43. Salwa WR. Health implications of fructose consumption: A review of recent data. *Nutr. Metab*. 2010; 7:82. <https://doi.org/10.1186/1743-7075-7-82>. World Health Organization. Diabetes Newsletter 2020 (Accessed 19-5-2020).

Figures

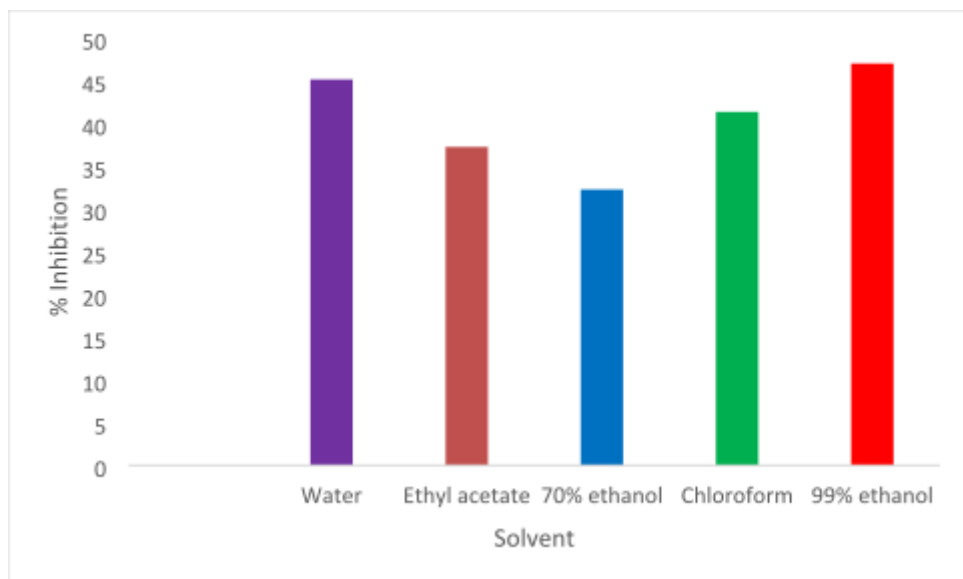


Figure 1

Alpha amylase inhibitory activities of different extracts of *Cochorus olitorius*

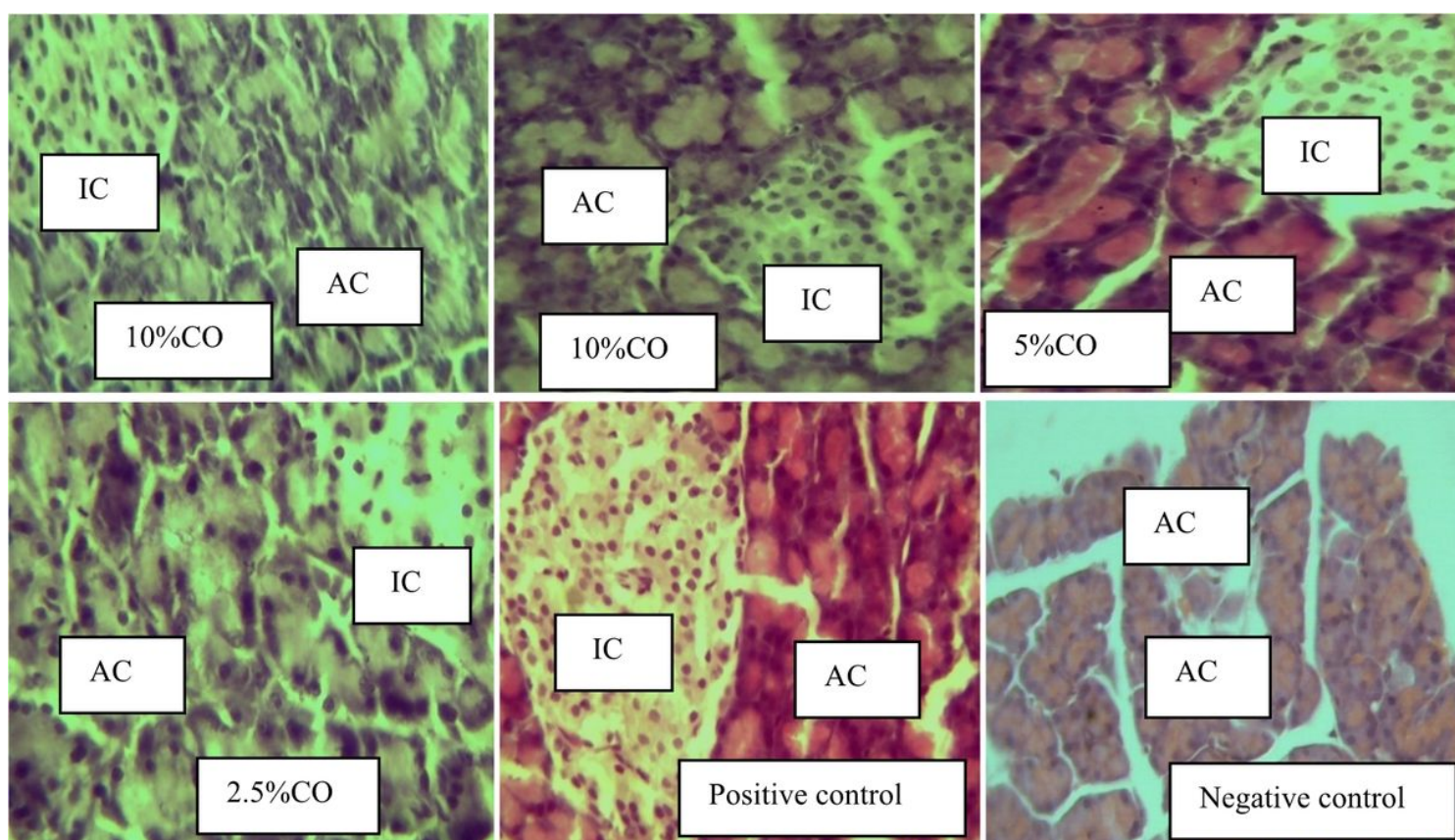


Figure 2

Photomicrograph sections (H and E X400) of Pancreas of normoglycemic (positive control), diabetic untreated (negative control) and diabetic treated (2.5%CO, 5%CO, 10%CO and 20%CO) groups. CO= *Cochorus olitorius*. AC = Acini: IC = Islet cells.