www.coast.oaustech.edu.ng
COAST
JOURNAL OF THE SCHOOL OF SCIENCE
Volume 7, Issue 1. May, 2025 ISSN: 2714-3716

Coast, J. Sch. Sci. 7 (1): 1207 - 1221

INHIBITION OF DIGESTIVE ENZYMES AND RAT SENSITIVITY TO *Triumfetta rhomboidea* LEAF EXTRACTS: GLUCOSE LEVEL, HORMONAL CHANGES, OXIDATIVE STRESS INDICES AND THERAPEUTIC INDEX STUDY

***[1]Akintimehin, E. S. and [2]Onoagbe, I. O.**

[1]Biochemistry Unit, Department of Chemical Sciences, School of Sciences, Olusegun Agagu University of Science and Technology, PMB 353, Okitipupa, Ondo State, Nigeria.
[2]Biochemistry Department, Faculty of Life Sciences, University of Benin, Benin City, Nigeria.
Corresponding Author's Email: es.akintimehin@oaustech.edu.ng
https://dx.doi.org/10.4314/coast.v7i1.9

Abstract
Chronic hyperglycemia is one of the major contributory factors that result to acute and chronic complications in diabetes mellitus. These complications are commonly associated with changes in hormonal function and inability of the body to counteract oxidative responses arising from glucotoxicity. The aim of this study is to investigate the effects of aqueous (ATRE) and ethanol (ETRE) extracts of *T. rhomboidea* leaf on carbohydrate digestive enzymes, level of glucose concentration, hormones and antioxidants in healthy experimental rats. Enzymes inhibition study was carried out using alpha amylase and alpha glucosidase inhibition assays. Experimental animals were orally administered varying doses of ATRE and ETRE for 28 days and thereafter sacrificed for sample collections. Obtained blood samples were processed and used for blood glucose concentration and hormonal assays while the tissues supernatant was used to determine oxidative stress indices. Finding from this study revealed significant ($p<0.05$) reduction in blood glucose concentration after 28 days of treatment with effective dose of 800 mg/kg after a dose response study. There were no significant changes in insulin and TSH of animals that were administered varying doses of ATRE and ETRE when compared to the control. Antioxidant systems in liver, kidney and pancreas of treated animals were not adversely affected by the extracts of *T. rhomboidea* leaf. This study has demonstrated that both aqueous and ethanol extracts of *T. rhomboidea* leaf possessed the capacity to lower blood glucose concentration without causing severe changes in normal hormonal function and the antioxidant systems

Keywords: Antioxidant, amylase, diabetes mellitus, insulin, therapeutic index.

Introduction

Diabetes mellitus is one major metabolic disease that is associated with abnormal carbohydrates, proteins and fat metabolisms due to absolute or relative defect in insulin secretion, signals or both. It is a non-communicable disease with multiple etiologies and is usually accompanied with uncontrolled hyperglycemia that over time debilitate body organs and their functions (Karigidi et al., 2020). Other consequences of uncontrolled hyperglycemia such as free

radicals (reactive oxygen species) generation or impaired antioxidant defense system and hormonal imbalances are reported to contribute to severity of diabetes complications (Manjunath et al., 2016, Karigidi and Olaiya, 2020). The dreadful disease is becoming alarming and a menace to mankind as over 500 million people were reported to be diabetic in 2021 and approximately 800 million people were estimated to be diabetic by 2045 with an increasing rate of 45 % (IDF, 2021). Another major concern is the economy economic burden as the direct expenditures due to diabetes are reported to be nearly one million trillion economic USD (IDF, 2021).

In other to combat the dreadful impact of diabetes on humanity, several glucose lowering agents such as biguanides, sulphonylureas, alpha-glucosidase inhibitors, DPP-4 inhibitors and insulin therapy have been developed. These chemical agents exert their therapeutic roles using different mechanisms. One important therapeutic strategy by which antidiabetic agents normalize postprandial hyperglycemia include interruption of glucose absorption through inhibition of carbohydrate linked digestive enzymes (Laoufi et al., 2017). Regardless of their therapeutic roles, the use of these chemically synthesized agents remains challenging in terms of cost, and presents several adverse reactions. Other scientifically proven treatment approach includes diet modification, regular exercise, weight regulation and herbal therapies involving the use of medicinal plants (Bharti et al., 2018).

Several antidiabetic medicinal plants such as *Acacia Arabica* (Shanak et al., 2019), *Annona squamosal* (Kaleem et al., 2006), *Azadirachta indica* (Pingali et al., 2021), *Citrullus colocynthis* (Ahangarpour A, Oroojan, 2013), *Hibiscus rosa sinensis* (Ghosh and Dutta, 2017), *Lantana aculeate* (Kumar et al., 2010), *Phyllanthus amarus*, Populus balsamifera (Harbilas et al., 2012), *Syzygium cumini* (Prabakaran and Shanmugavel, 2017), *Trigonella foenum graecum* (Gupta et al., 2001), *Zingiber officinale* (Islam and Choi, 2008) have been reported to effectively lowers blood glucose concentration by increasing insulin release and plasma insulin levels hence reversing diabetic complications. Other possible mechanisms by which medicinal plants perform their antidiabetic function include enhancement of insulin microcirculation, sensitivities and inhibition of insulin degradative processes, inhibition of pancreatic amylase and intestinal glucosidase (Jarald et al., 2008; Eid and Haddad, 2014).

Triumfetta rhomboidea L. (Family: *Tiliaceae*) is an herbal perennial, pantropical plant that is commonly known as bur weed or chinese burr. It is widely found in tropics and subtropical region of the globe such as India, South America and Africa (Bosch, 2011). Orthodox application of *T. rhomboidea* parts (leaf, flower, root) include, treatments of diseases (diabetes mellitus, tumors, gonorrhea among other ailments), infusion for children, and feed preparation for infant (Bosch, 2011). The local and diverse applications of *T. rhomboidea* leaf for the maintenance of health could be linked to the presence of important bioactive compounds, *in-vitro* radical hunting impact and low toxicity (Akintimehin et al., 2022; Akintimehin and Onoagbe, 2023). In spite the interesting reports on the therapeutic value of medicinal plants, some medicinal plants have been reported to cause changes in cellular functions and hormonal imbalances in normal rat (Tiwari and Kumar, 2017). Similarly, scientifically controlled study on certain parameters such as hormonal

changes and antioxidant level in healthy animals can also be used to predict the possible mechanism by which extract exert it therapeutic function. In this study, we investigated the possible effects of *T. rhomboidea* extracts on carbohydrate digestive enzymes and possible impact on blood glucose concentration, oxidative stress and hormones.

Methods

Plant sample collection and extraction

Fresh leaves of *Triumfetta rhomboidea* leaves were collected along Ugbonla, Ilaje, Ondo State (Latitude: 6 21' 00'' Longitude: 4 48' 00''). Leaf sample was identified and authenticated to obtain voucher number (*UBHT–403*) at Plant Biology and Biotechnology Department, University of Benin. The leaves were washed thoroughly under running water, air-dried at room temperature and pulverized using electrical blender.

The aqueous and ethanol extracts of *T. rhomboidea* leaf (ATRE and ETRE) were prepared as previously described (Akintimehin et al., 2022). Briefly, 1.5 kg of powdered sample were separately weighed in different containers, soaked with distilled water and absolute ethanol respectively for 72 h with constant shaking. Mixtures were double filtered using cheese cloth and further filtered using cotton wool packed glass funnel. Aqueous filtrate was thereafter freeze-dried using a freeze dryer to obtain concentrated aqueous extract of *T. rhomboidea* (AETR), while the ethanol filtrate was concentrated into a slurry paste using rotary evaporator under reduced pressure and further freeze-dried to obtain the ethanol extract of *T. rhomboidea* (EETR). The slurry pastes of each concentrated samples were lyophilized to powder and stored in cool refrigerator (4 °C) till further analysis.

Alpha amylase inhibition assay

Briefly, volume (0.1 mL) of the extract (20 – 100 µg/mL) was premixed with 0.1 mL phosphate buffer (2 mM, pH 6.9) and 0.2 mL of α- amylase (0.02 U/mL) in test-tube and incubated at room temperature (26 °C) for 20 minutes. Next, 0.1 mL starch (1%) solution was introduced to the reaction mixture and incubated in water bath (25 °C) for 5 min. Thereafter, 0.5 mL of dinitrosalicylic acid reagent was added and placed in boiling water bath. After five (5) minutes, test tubes containing reaction mixture were removed, cooled to room temperature and quantified by taken absorbance at 540 nm. Similar assay was also performed for control where the 0.2 mL of the enzyme solution was replaced with a buffer solution. Acarbose was used as reference drug (Bernfeld, 1955). The result was expressed as the concentration of inhibition required to inhibit 50% of alpha amylase activity [IC_{50} (µg/mL)].

Intestinal glucosidase inhibition assay

Portion (0.1 mL) of varying concentrations of extracts (20-100 µg/mL) were pre-incubated with 0.1 mL of α-glucosidase solution and 1.0 mL of phosphate buffer (2 mM, pH 6.9) for 15 minutes in water bath (25 °C). Next, 0.1 mL (3.0 mM) para-nitrophenylglucopyranoside (prepared in 20 mM phosphate buffer (pH 6.9)) was introduced to the solution and left untouched for 20 minutes at room temperature. Thereafter, 2 mL of 0.1 M sodium carbonate was added to mixture to end the reaction and absorbance was taken at 450 nm. The same assay was also performed for control where the 0.1 mL of enzyme was replaced with a buffer solution. Following the same condition, inhibition assay was also performed for standard inhibitor (Acarbose) and final inhibition result was expressed in IC50 (µg/mL). Analyses were performed in triplicate (Kim et al., 2005).

Experimental Animals and protocol

Wistar strain albino rats (male) weighing between 150–170 g was obtained from the

Animal House, Department of Biochemistry, University of Benin. Animals were acclimatized for 14 days in a well-ventilated room, gave free access to water and rat feed. Animal handling is accordance to manual guidelines of laboratory animal care of National research council (NRC, 1997) as approved by the Institution ethical committee (**OAUSTECH/ETHC-BCH/2022/006**).

After acclimatization, a total of fifty-five animals were randomly grouped into 6 groups of 5 animals per group as described in Table 1. heavy metal contamination occurred as a result of anthropogenic activities (Elias and Gbadegesin 2011)Extracts were administered orally using gavage for 28 days. The dose range (100 – 5000 mg/kg) were selected to contain the lower, median and upper limit dose levels. Further, the upper dose limit of 3000 and 5000 mg extract/kg body weight (BW) were considered due to local consumption of *T. rhomboidea* leaf and strong likelihood that findings from this study might have direct relevance for protecting human or animal health.

Table 1. Animal grouping

Grouping	Extract dose	ATRE	ETRE
0	Control (Distilled water)	CTRL0	
1	100 mg extract/kg BW	ATRE1	ETRE1
2	500 mg extract/kg BW	ATRE2	ETRE2
3	1000 mg extract/kg BW	ATRE3	ETRE3
4	3000 mg extract/kg BW	ATRE4	ETRE4
5	5000 mg extract/kg BW	ATRE5	ETRE5

CRTL: Control, ATRE: Aqueous extract of *T. rhomboidea*, ETRE: Ethanol extract of *T. rhomboidea,* BW: Body weight

Termination of extract dosing

After 28th days of extract's administration, animals were fasted overnight and sacrificed for blood sample (via cardiac puncture) and tissue collections. Withdrawn whole blood samples were dispensed in sample bottles for glucose concentration assay (fluoride bottle) and hormonal assays (plain bottle). Collected blood samples were further centrifuged for 10 minutes at 3000 rpm using table top electric centrifuge to obtain the clear yellow supernatant sera. The liver, kidney and pancreas were extracted, cleaned between layers of Whatman filter paper to remove blood stain, excised and homogenized with 0.1 M phosphate buffer (7.4). Homogenate was further centrifuged at 4000 rpm for 10 min to obtain the supernatant for the evaluation of oxidative stress markers.

Dose response study

Dose response study to determine the effective dose (ED_{50}) that would produce best hypoglycemic effects on normal wistar rats was carried out according to the method of Abu et al. (2022). Total of forty-four male rats (wistar strain) used for this study were randomized into 6 cages of 4 animals per cage. Animals in cage 1 represents the control group and were given 1 mL of distilled water, animals in cage 2 to 6 received 200, 400, 600, 800, 1000 mg extract/kg BW of rats. The extracts (aqueous and ethanol) were orally given to animals in a single dose, daily for 14 days. Fasting blood glucose concentration was used as a measure of therapeutic target.

The lethal dose (LD_{50}) value for therapeutic index calculation was obtained from previous toxicity report (Akintimehin and Onoagbe, 2023).

Therapeutic index = LD_{50}/ED_{50} where ED_{50} is the effective dose.

Biochemical assays

Blood glucose concentration

Enzymatic oxidation of glucose with glucose oxidase using Randox kit was used to perform blood glucose concentration (Barham and Trinder, 1972).

Hormonal assay procedures

Testosterone was carried out using testosterone-ELISA Kit (Catalog No: E-EL-0155) based on competitive–enzyme linked immunosorbent principle. Thyroid stimulating hormone (TSH) was carried out using rat TSH sandwich ELISA Kit (Catalog No: E-EL-R0976). Insulin assay was carried out using rat Insulin (INS) sandwich ELISA Kit (Catalog Number: CS–E05070r). Assay protocol was according to the manufacturer's manual guide and optical density of the intensity of final colored complex was measured at 450 nm using microplate reader (Maruyama, 1987).

Oxidative stress markers in tissue

Superoxide dismutase (SOD) was determined according to the method of Misra and Fridovich (Misra and Fridovich, 1972) based on the inhibition of adrenalin autoxidation by SOD and determining increasing absorbance (420 nm) for every 60 secs for 3 minutes. Catalase (CAT) was determined according to the method of Cohen et al. (1970) based on the measurement of H_2O_2 decomposition rate after the addition of catalase by reacting with excess KMnO4 and then measuring the residual $KMnO_4$ spectrophotometrically at 480 nm. Glutathione peroxidase (GPx) activity was measured based on the formation of purpurogallin after oxidation of pyrogallol by GPx and quantification of resulting deep brown coloration at 430 nm (Nyman, 1959). Reduced glutathione was estimated by Ellman, (1959). Lipid peroxidation (LPO) was estimated by determining the amount of thiobarbituric acid reactive substances (TBARS) that is produced from reaction mixture containing tissue sample and acids (Gutterridge and Wilkins, 1982).

Statistical Analysis

Data were analyzed using one-way analysis of variance (ANOVA) using Statistical Product and Service Solutions (SPSS) version 17.0 and results were expressed as mean ± SEM. Differences between means were considered to be significant at ($p < 0.05$) using LSD (Least Square Difference) post hoc test.

Results

IC_{50} values of alpha amylase and glucosidase

The inhibitory potentials of *T. rhomboidea* leaf extracts on alpha amylase and glucosidase activities, expressed as inhibition capacity of 50 % (IC_{50}) of enzyme activity is presented in Table 2. Percentage inhibition of alpha amylase and glucosidase respectively revealed the IC_{50} value of 255.53 ± 13.23 µg/mL and 254.24 ± 13.36 µg/mL for ATRE while ETRE demonstrated IC_{50} value of 91.55 ± 4.35 µg/mL and 132.65 ± 10.79 µg/mL for alpha amylase and glucosidase respectively.

Table 2: IC50 values of extracts of *T. rhomboidea* against α-amylase and α-glucosidase

Extract/Standard	Alpha amylase (µg/ml)	Alpha glucosidase (µg/ml)
ATRE	255.53 ± 13.23*	254.24 ± 13.36*
ETRE	91.55 ± 4.35*	132.65 ± 10.79*
Acarbose	21.69 ± 0.87	42.38 ± 2.63

Values are mean ± SD ($n=3$) of IC_{50} values of alpha amylase and glucosidase inhibition by extracts of *T. rhomboidea* leaf and standard drug (acarbose). Value with * indicate significant difference ($p<0.05$).

Results of blood glucose concentration and hormonal study

Data obtained from blood glucose concentration and hormonal analysis of rats that were administered ATRE and ETRE are presented in Figure 1. From this result, only ATRE at 500, 3000 and 5000 mg extract/kg caused a significant (*P<0.05*) decrease in blood glucose concentration relative to control. The level of insulin and TSH in rats that received both extracts of *T. rhomboidea* statistically remain unchanged when compared to the untreated animals. With exception of the groups that received 1000 and 3000 mg ETRE/kg bw that showed significant increase in testosterone, other dose groups demonstrated non-significant difference when compared to the control. TSH (ng/mL) obtained in rats administered with aqueous and ethanol extracts of *T. rhomboidea* was statistically indistinguishable when compared to the control.

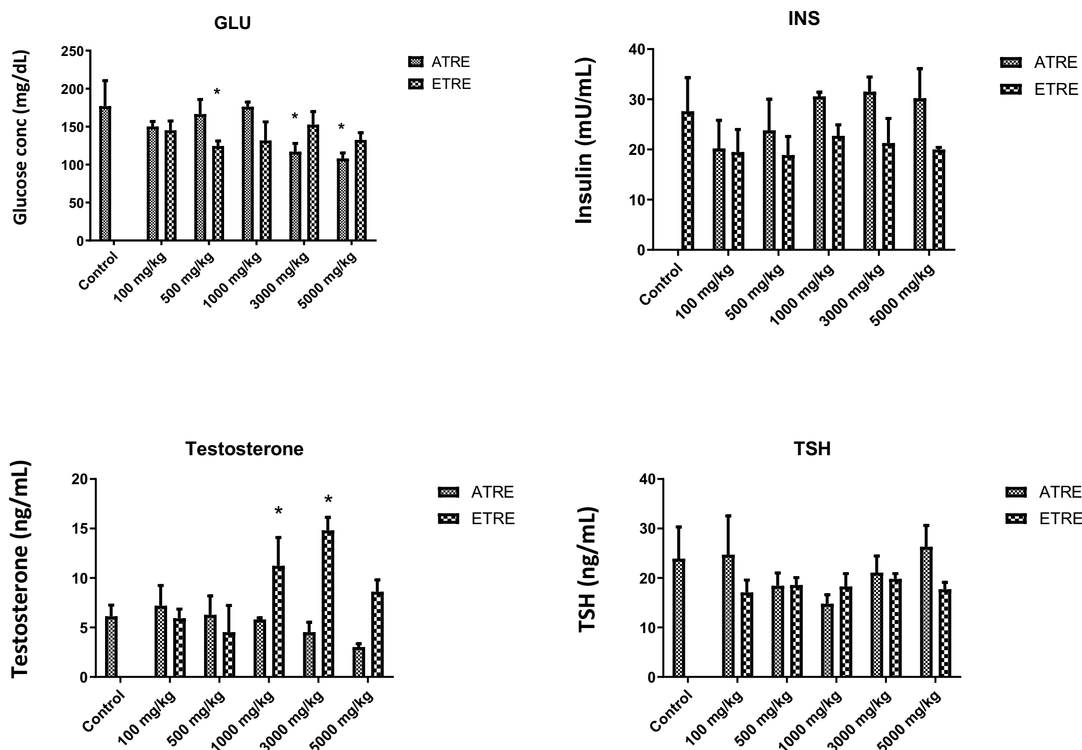

Figure 1: Fasting blood glucose and hormonal results of animals that were administered ATRE and ETRE.

Values are mean ± SEM (*n=5*) and *$p < 0.05$ denote significant difference compared to the control group. Where GLU: Glucose, INS: Insulin, TSH: Testosterone, ATRE: Aqueous extract of *T. rhomboidea*, ETRE: Ethanol extract of *T. rhomboidea*

Oxidative stress indices in organs

The results of oxidative stress marker in liver, kidney and pancreas of animals that were administered graded doses of ATRE and ETRE are presented in Table 3, 4 and 5 respectively. Selected doses of ATRE and ETRE caused significant (*$p<0.05$*) increase in liver and kidney SOD while all the ATRE and ETRE doses caused no significant difference in pancreas SOD compared to the control. Administration of the varying doses of ATRE and ETRE caused improvement in liver and

kidney GSH concentration. Administration of 100 and 500 mg ATRE/kg bw caused significant decrease in pancreas GSH while significant decrease was observed in animals that received high doses of ETRE. Selected doses of ATRE and ETRE caused significant decrease in liver and kidney catalase while catalase activities in the pancreas improved significantly across ATRE and ETRE doses. Activities of GPx in liver of animals that received both ATRE and ETRE was not significant compared to control except animals that were administered 1000 mg ATRE/kg that demonstrated a significant ($p<0.05$) increase. In the kidney, only animals that received varying doses of ATRE demonstrated significant increase in GPx activities while ETRE administration caused no significant difference in GPx activities compared to the control. All doses of ATRE and ETRE caused no significant difference in pancreas GPx activity. ATRE and ETRE at high dose (5000 mg/kg) significantly increased liver MDA. Varying doses of ATRE and ETRE caused no significant difference in kidney MDA except for animals that received 500 mg ETRE/kg which demonstrated significant increase. Only 100 mg ETRE/kg and 3000 mg ATRE/kg caused significant rise in pancreas MDA.

Table 3: Oxidative stress indices in liver of rats administered with extracts of *T. rhomboidea* leaf

Dose (mg/kg)	Extract /d.H_2O	SOD (U/mg Protein) x 10^{-2}	GSH (µmol/mg Protein) x 10^{-2}	CAT (U/mg Protein)	GPx (mmol/mg Protein)	MDA (µmol/mg Protein)
Control	Distilled H_2O	4.84 ± 0.21	6.59 ± 0.86	1.67 ± 0.86	1.67 ± 0.26	41.42 ± 32.15
100	ATRE	3.64 ± 0.28	8.23 ± 2.78	0.50 ± 0.12*	1.34 ± 0.24	23.00 ± 6.69
	ETRE	7.75 ± 1.07*	17.4 ± 5.23*	0.48 ± 0.15*	1.92 ± 0.29	73.73 ± 6.13
500	ATRE	6.51 ± 0.42	13.1 ± 10.0	0.50 ± 0.14*	1.93 ± 0.29	35.87 ± 8.68
	ETRE	4.44 ± 1.12	6.73 ± 1.90	0.35 ± 0.22*	1.68 ± 0.61	63.77 ± 16.11
1000	ATRE	7.04 ± 0.84*	7.81 ± 2.83	0.12 ± 0.08*	4.59 ± 1.73*	61.97 ± 14.71
	ETRE	7.11 ± 0.30*	22.8 ± 4.25*	0.36 ± 0.14*	2.03 ± 0.52	102.84 ± 9.59
3000	ATRE	7.73 ± 0.75*	7.99 ± 1.40	0.09 ± 0.04*	2.73 ± 1.00	93.19 ± 15.45
	ETRE	5.96 ± 0.25	13.0 ± 4.38	0.83 ± 0.31	1.63 ± 0.16	126.91 ± 17.86*
5000	ATRE	6.00 ± 0.68	7.61 ± 2.33	0.14 ± 0.06*	2.14 ± 0.40	173.01 ± 53.86*
	ETRE	5.07 ± 0.29	10.5 ± 1.79	0.20 ± 0.09*	1.13 ± 0.14	219.16 ± 47.91*

Data are mean ± standard error of mean (n = 3 - 5) and data having star sign (*) in each column are significant at $p < 0.05$ when compared to control. Where: ETRE: Ethanol extract of *T. rhomboidea* leaves; ATRE: Aqueous extract of *T. rhomboidea* leaves; CAT: Catalase; SOD: superoxide dismutase, glutathione (GSH) and glutathione peroxidise (GPx)

Table 4: Antioxidant profile in kidney of rats treated with extracts of *T. rhomboidea* leaf

Dose (mg/kg)	Extract / d. H_2O	SOD (U/mg Protein) x 10^{-2}	GSH (µmol/ mg Protein) x 10^{-2}	CAT (U/ mg Protein)	GPx (mmol/ mg Protein)	MDA (µmol/mg Protein)
Control	d. H_2O	5.00 ± 0.52	10.1 ± 1.90	2.06 ± 0.48	1.29 ± 0.03	62.93 ± 20.65
100	ATRE	4.64 ± 0.36	9.86 ± 3.92	0.48 ± 0.05*	2.54 ± 0.23	79.80 ± 3.22
	ETRE	6.63 ± 0.95	4.44 ± 1.69	1.78 ± 1.08	2.01 ± 0.53	119.92 ± 36.08
500	ATRE	5.24 ± 0.73	9.92 ± 4.58	0.51 ± 0.12*	3.23 ± 0.37*	49.50 ± 11.62
	ETRE	7.69 ± 0.56*	4.03 ± 1.69*	0.54 ± 0.14	2.06 ± 0.46	170.38 ± 46.89*
1000	ATRE	4.94 ± 0.57	8.57 ± 3.92	0.68 ± 0.43	3.14 ± 1.77*	78.94 ± 48.32
	ETRE	6.64 ± 0.49	22.6 ± 3.54*	0.65 ± 0.32	1.53 ± 0.29	57.94 ± 15.44
3000	ATRE	7.73 ± 1.33*	8.02 ± 2.33	0.99 ± 0.49	3.07 ± 0.70*	110.10 ± 19.85
	ETRE	4.02 ± 0.51	15.4 ± 3.05*	0.24 ± 0.06*	1.37 ± 0.29	139.74 ± 9.81
5000	ATRE	5.92 ± 0.31	11.9 ± 1.03	0.24 ± 0.10*	2.38 ± 0.15	44.78 ± 18.44
	ETRE	4.05 ± 0.44	18.2 ± 5.26*	1.52 ± 1.33	1.27 ± 0.21	62.93 ± 20.65

Data are mean ± standard error of mean (SEM) of at least three experimental rats and values bearing asterisk signs (*) in each column are significant at $p < 0.05$ relative to control. Where: ATRE: Aqueous extract of *T. rhomboidea* leaves; ETRE: Ethanol extract of *T. rhomboidea* leaves; SOD: superoxide dismutase; CAT: Catalase; glutathione (GSH) and glutathione peroxidase (GPx).

Table 5: Antioxidant profile in pancreas of rats treated with extracts of *T. rhomboidea* leaf

Dose (mg/kg)	Extract/ d. H_2O	SOD (U/ mg Protein) x 10^{-2}	GSH (µmol/mg Protein) x 10^{-2}	CAT (U/ mg Protein)	GPx (mmol/ mg Protein)	MDA (µmol/mg Protein)
Control	Distilled H_2O	4.70 ± 1.05	46.10 ± 12.6	0.40 ± 0.06	1.22 ± 0.13	30.05 ± 12.26
100	ATRE	3.56 ± 0.96	10.70 ± 4.45*	2.63 ± 1.72	1.28 ± 0.04	34.95 ± 6.00
	ETRE	4.74 ± 1.10	11.0 ± 1.52*	1.82 ± 1.28	0.90 ± 0.18	92.15 ± 19.83*
500	ATRE	4.75 ± 0.73	12.70 ± 4.69*	3.91 ± 1.41	2.73 ± 1.51	45.97 ± 23.59
	ETRE	6.78 ± 1.57	22.60 ± 7.20	5.09 ± 2.44*	2.65 ± 0.52	66.54 ± 15.68
1000	ATRE	9.09 ± 4.80	18.30 ± 6.03	4.97 ± 4.60*	2.88 ± 1.40	23.11 ± 8.40
	ETRE	7.49 ± 1.12	38.70 ± 12.00	5.20 ± 2.12*	1.34 ± 0.35	68.08 ± 11.86
3000	ATRE	6.07 ± 2.27	33.00 ± 21.4	5.09 ± 2.60*	2.17 ± 0.87	112.06 ± 14.15*
	ETRE	8.71 ± 1.83	11.90 ± 4.41*	2.00 ± 0.99	1.29 ± 0.51	58.44 ± 7.53
5000	ATRE	3.85 ± 1.86	32.10 ± 3.90	5.43 ± 1.77*	2.19 ± 0.40	51.98 ± 30.06
	ETRE	6.46 ± 0.70	16.30 ± 2.16*	4.43 ± 2.24*	0.99 ± 0.27	30.05 ± 12.26

Values are mean ± standard error of mean (n = 3 - 5) and values having asterisk (*) along similar column showed significant difference ($p < 0.05$) relative to group that did not receive extract. Where: ATRE: Aqueous extract of *T. rhomboidea* leaves; ETRE: Ethanol extract of *T. rhomboidea* leaves; CAT: Catalase; glutathione (GSH); SOD: superoxide dismutase and glutathione peroxidise (GPx).

Dose response result

The result of dose response of animals to the oral administration of ATRE and ETRE is presented in Figure 2. ATRE doses at 200 and 400 mg/kg resulted in significant increase in blood glucose concentration while only ATRE and ETRE dose at 800 mg extract/kg caused significant decrease in fasting blood glucose concentration with therapeutic index (LD_{50}/ED_{50}) of 6.25.

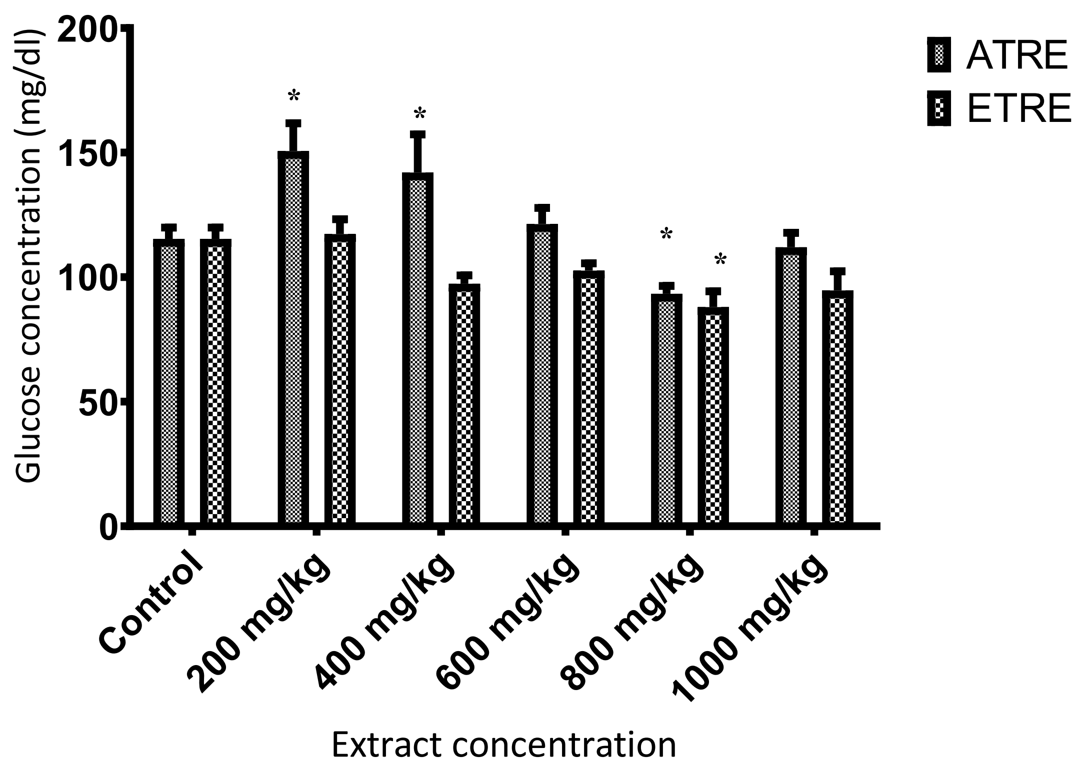

Figure 2: The result of dose response study

Discussions

Uncontrolled postprandial glucose concentration is one major risk factor in the development of the metabolic disease such as diabetes mellitus. One distinctive approach for maintaining postprandial hyperglycemia is by delaying the uptake of dietary carbohydrate in the gut. Inhibition the enzymes such as alpha amylase and intestinal glucosidase are reported to be strong therapeutic strategy for controlling postprandial hyperglycemia in diabetes (Kamtekar et al., 2014). In this study, the extracts of *T. rhomboidea* leaf and standard drug inhibited alpha amylase and glucosidase in the order of acarbose<ETRE<ATRE as revealed by the IC_{50} values. The high inhibitory capacity of an agent can be illustrated with lower IC_{50} values of the tested samples. The lower IC_{50} value of ethanol as demonstrated in this study could suggest high antihyperglycemic potential when compared to it aqueous counterpart (Kazeem et al., 2013). The ability of the ETRE to demonstrate high inhibitory potential as compared to ATRE could be linked to high flavonoid and total antioxidant capacity that was obtain from previous study (Akintimehin

et al., 2022). Vital polyphenolic compounds such as flavonoid and other phytocompounds have been reported to have positive correlation and potentially inhibit intestinal glucosidase and pancreatic amylase activities (Williams, 2013).

Despite the lesser ability of ATRE to potentially inhibit amylase and glucosidase *in-vitro*, administration of the ATRE for 28 days in experimental rats caused significant decrease in blood glucose concentration at 500, 3000 and 5000 mg extract/kg. This observation suggest that ATRE might be using other mechanism to lower blood glucose concentration order than digestive enzyme inhibition. Several mechanistic approach by which phytocompounds from plant sources reduce blood glucose concentration include: stimulation of insulin production, regulation of glucose transporters, regulation of metabolic pathways (glycolysis, glycogen synthesis), pancreatic beta cell regeneration among other documented mechanisms (Bharti et al., 2018).

The non-significant changes that were observed in level of insulin and TSH of animals that were administered ATRE and ETRE could indicate that extract did not cause impairment in the synthesis and overall physiology of the hormones. Further, the slight increase in the level of insulin of animals that received ATRE could be responsible for the value that was obtained for the blood glucose concentration in this study. Insulin and TSH are important hormones that play in crucial role in normal body metabolism. While insulin specifically triggers different metabolic reaction that consequently lowers blood glucose concentration, TSH is a thyrotropin that regulate the activities of other pituitary hormones. TSH is synthesize and secreted in the anterior lobe of the pituitary gland and regulate the activities of other hormones (Sacher and Richard, 2000). Testosterone is a sex hormone that is crucial for the physical development and function of the male reproductive system. From this study, ATRE did not alter the level of testosterone relative while ETRE at 1000 and 3000 mg/ extract/kg significantly elevated testosterone. Studies have shown that chemicals that are capable of altering androgens (such as testosterone) production might consequently have deleterious effects on male genital tract development (Fisher, 2004).

Oxidative stress is characterized by perturbation in the parallel level of oxygen-centered reactive species (ROS) generation and the convertible ability by internal antioxidant system. Several reports have implicated the pivotal role of cellular oxidations in pathogenesis of diseases like diabetic mellitus. However, scavengers (antioxidant) of reactive species have also been reported to have alleviative effects on oxidative stress associated diseases (Oyenihi et al., 2016).

Combination of *in-vivo* antioxidants such as reduced glutathione, superoxide dismutase, catalase can collaboratively form effective defenses against ROS upsurge. From this study, administration of ATRE and ETRE in experimental rats did not adversely alters the oxidative defensive mechanisms in liver, kidney and pancreas. Selected doses of ATRE and ETRE significantly improved the activities of SOD, GSH and GPx in both liver and kidney while the activity of catalase reduced significantly ($p<0.05$) these organs. The reduction in catalase activity could be due to production of peroxides in the extract's metabolites or interference of catalase activity with some bioactive compounds in both ATRE and ETRE. In respective of the factor that resulted to the decline catalase activity in both liver and kidney, the compensatory hydrogen peroxide scavenging antioxidant (GPx) was

elevated in both organs. GPx is an important supportive antioxidant that also help in the decomposition of excess hydrogen peroxide and other form peroxides to water thereby halting the propagation of oxidative reactions (Omodanisi et al., 2017)

In the pancreas, all enzymic antioxidants were not affected following the administration of ATRE and ETRE while GSH concentration reduced significantly in selected doses. Glutathione is one of the abundant non enzymic intracellular antioxidant that serves as cofactor of some enzymes GPx and glutathione–S–transferases. Through enzymatic action of glutathione-S-transferases and peroxidases, highly reactive species could also be detoxified by GSH [30]. From this study, the significant reduction in pancreas GSH level could be due continuous oxidation due to its conjugation with bioactive ingredients in extract. Studies have shown that skeletal radicals that are produced from dietary ascorbate and tocopherol constantly regenerate to their active states through conjugation with GSH, resulting in GSSG/GSH ratio (Valko et al., 2007; Akintimehin et al., 2021). Malondialdehyde (MDA) is an important parameter for measuring the extent of lipid oxidation in the body cells. From this study, the significant increase that was observed in liver, kidney and pancreas MDA of animals that received selected doses of ATRE and ETRE could be due to the presence of some bioactive compounds in the extract. Previous studies have shown that dietary bioactive compounds in plant extracts can occasionally cause slight surge in cellular oxidation due to their pro-oxidative activities when in their carbon skeletal form (Halliwell, 2007; Oso et al., 2019). In a dose response study, safe doses of ATRE and ETRE from previous study (Akintimehin and Onoagbe, 2023) was used to establish the effective dose(s) using fasting blood glucose as measure of the therapeutic index. From this findings, the significant reduction of fasting blood glucose concentration at 800 mg/kg for both ATRE and ETRE could suggest that both extracts are most effective at the 800 mg/kg body weight.

Conclusions

In conclusion, this study has demonstrated that both aqueous and ethanol extracts of *T. rhomboidea* possessed the capacity to lower blood glucose concentration without causing severe changes in hormonal function and the antioxidant system. Possible mechanisms by which *T. rhomboidea* leaf extracts performed the blood glucose reduction could be linked to inhibition of carbohydrate digestive enzymes, maintenance and stability of insulin synthesis and function.

List of Abbreviations

ETRE: Ethanol extract of *T. rhomboidea* leaves; ATRE: Aqueous extract of *T. rhomboidea* leaves; CAT: Catalase; SOD: superoxide dismutase, glutathione (GSH) and glutathione peroxidase (GPx); IC_{50}: 50% Inhibition capacity; TSH: Thyroid stimulating hormone.

Declarations

Competing interests

As declared by all authors, no competing interests exist.

Funding

Not applicable

Authors' contributions

The research work was carried out in collaboration between all authors. IOO and ESA conceived and designed the study. Authors ESA managed experimental protocols, performed the experimental analyses and data analysis under the supervision of IOO. Author ESA managed the literature searches and wrote the first draft of the manuscript with the assistant of IOO. All authors read and approved the final draft of

the manuscript.

REFERENCES

Abu O. D., Onoagbe I. O., Ojo I. (2022) Dose response study of aqueous extract of *Dialium Guineense stem bark. Am J Biomed Sci Res.* 15(2). *AJBSR.MS.ID.002099.* https://doi.org/10.34297/AJBSR.2022.15.002099

Ahangarpour, A., and Oroojan, A. (2013) Effect of crust and seed hydro-alcoholic and aqueous extracts and pulp hydroalcoholic extract of Citrullus colocynthis on glucose, insulin and FIRI level in insulin resistant male rat's. *Horizon Med Sci.* 19(3): 149-54.

Akintimehin, E. S. and Onoagbe, I. O. (2024). Acute and sub-chronic toxicity studies of aqueous and ethanol extracts of *Triumfetta rhomboidea* (Tiliaceae) Leaf in healthy albino rats. *Vegetos* 37: 2532 – 2541. https://doi.org/10.1007/s42535-023-00762-7

Akintimehin, E. S., Karigidi, K. O., Omogunwa, T. S. Adetuyi F.O (2021). Safety assessment of oral administration of ethanol extract of *Justicia carnea* leaf in healthy wistar rats: hematology, antioxidative and histology studies. *Clin Phytosci.* 7: 2. https:// doi. org/ 10. 1186/s40816-020- 00234-4

Akintimehin, E., Onoagbe, I. and Abu O. (2022). Proximate composition, qualitative phytochemicals screening and In-vitro antioxidant potential of *Triumfetta rhomboidea* leaves extracts. *Trends in Sci* 19(24): 3033. https://doi.org/10.48048/tis.2022.3033

Barham, D. and Trinder, P. (1972) Improved Color Reagent for the Determination of Blood Glucose by the Oxidase System. *Analyst.* 97: 142-145. http://dx.doi.org/10.1039/an9729700142

Bernfeld, P. (1955). "Amylase, α and β," in Methods in Enzymology, S. P. Colowick and N. O. Kaplan, Eds., pp. 149–158, Academic Press, New York, NY, USA.

Bharti, S. K., Krishnan, S., Kumar, A. and Kumar, A. (2018). Antidiabetic phytoconstituents and their mode of action on metabolic pathways. *Therap Adv Endocrinol Metab.* 9(3): 81– 100. http://doi.org/10.1177/2042018818755019

Bosch, C. H. (2011) *Triumfetta rhomboidea* Jacq. [Internet] Record from PROTA4U. Brink, M. & Achigan-Dako, E.G. (Editors). PROTA (Plant Resources of Tropical Africa /Ressources végétales de l'Afrique tropicale), Wageningen, Netherlands. http://www.prota4u.org/search.asp

Cohen, G., Dembiec, D. and Marcus, J. (1970) Measurement of catalase activity in tissue extracts, *Anal. Biochem.* 34: 30-38.

Eid, H. M. and Haddad, P. S. (2014). Mechanisms of Action of Indigenous Antidiabetic Plants from the Boreal Forest of Northeastern Canada. *Adv. Endocrin.* Article ID 272968, 11 pages http://dx.doi.org/10.1155/2014/272968

Ellman, G. L. 1959. Tissue sulfhydryl groups. *Arch. Biochem. Biophys.* 82: 70-77

Fisher, J. S. (2004). Environmental anti-androgens and male reproductive health: focus on phthalates and testicular dysgenesis syndrome. *Reproduction*, *127*: 305–315.

Ghosh, A., and Dutta, A. (2017) Antidiabetic

effects of ethanolic flower extract of Hibiscus rosa sinensis (L) on alloxan induced diabetes in hyperlipidaemic experimental Wister rats (WNIN). *Int J Eng Develop Res* 5(4):674–679

Gupta, A., Gupta, R., and Lal, B. (2001). Effect of Trigonella foenum-graecum (fenugreek) seeds on glycaemic control and insulin resistance in type 2 diabetes mellitus: a double blind placebo controlled study. *J Assoc Physicians India* 49:1057–1061

Gutterridge, J. M. C. and Wilkins, C. (1982). Copper dependent hydroxyl radical damage to ascorbic acid. Formation of thiobarbituric acid reactive product. Febs Letters, 137: 327–340.

Halliwell B. (2007). Dietary polyphenols: good, bad or indifferent for your health? *Cardiovasc Res.* 73(2):341–7.

Harbilas, D., Brault, A., Vallerand, D. et al. (2012) "Populus balsamifera L. (Salicaceae) mitigates the development of obesity and improves insulin sensitivity in a diet-induced obese mouse model," *J. Ethnopharma*, 141(3): 1012– 1020.

International Diabetes Federation (IDF), 2021. IDF Diabetes Atlas 2021 – 10th edition. Online version of IDF Diabetes Atlas: www.diabetesatlas.org

Islam, M. S., Choi, H. (2008) Comparative effects of dietary ginger (Zingiber officinale) and garlic (*Allium sativum*) investigated in a type 2 diabetes model of rats. *J Med Food* 11(1):152–159

Jarald, E. E. Joshi, S. B. and Jain, D. C. (2008). Diabetes and Herbal Medicines. *Iranian J Pharmacol Therap*. 7: 97-106. http://dx.doi.org/1735-2657/08/71-97-106

Kaleem, M., Asif, M., Ahmed, Q., Bano, B. (2006). Antidiabetic and antioxidant activity of Annona squamosa extract in streptozotocin-induced diabetic rats. *Singapore Med J.* 47(8): 670-5.

Kamtekar, S., Keer, V. and Patil, V. (2014). Estimation of Phenolic content, Flavonoid content, Antioxidant and Alpha amylase Inhibitory Activity of Marketed Polyherbal Formulation. *J Appl Pharmaceut Sci* 4(09): 061-065.

Karigidi, K. O. and Olaiya, C. O. (2020). Antidiabetic activity of corn steep liquor extract of Curculigo pilosa and its solvent fractions in streptozotocin-induced diabetic rats. *J Tradit Complement Med* 10: 555–564.

Karigidi, K. O., Akintimehin, E. S., Omoboyowa, D. A., Adetuyi, F. O. and Olaiya, C. O. (2020). Effect of Curculigo pilosa supplemented diet on blood sugar, lipid metabolism, hepatic oxidative stress and carbohydrate metabolism enzymes in streptozotocin-induced diabetic rats. *J Diabetes Metab Disord* 19: 1173–84.

Kazeem, M.I., Raimi, O.G., Balogun, R.M., Ogundajo, A.L. (2013). Comparative study on the α-amylase and α-glucosidase inhibitory potential of different extracts of *Blighia sapida* Koenig. *American J Res Communication.* 1(7): 178-192

Kim, Y. M., Jeong, Y. K., Wang, M. H., Lee, Y. H. and Rhee, H. I. (2005). Inhibitory effect of pine extract on alpha-glucosidase activity and postprandial hyperglycaemia. *Nutri* 21:756–761

Kumar, K. V., Sharief, S. D., Rajkumar, R., Ilango, B., Sukumar, E. (2010).

Antidiabetic potential of *Lantana aculeata* root extract in alloxan-induced diabetic rats. *Int J Phytomed* 2: 299-303.

Laoufi H, Benariba N, Adjdir S, Djaziri R (2017). *In vitro* α-amylase and α-glucosidase inhibitory activity of *Ononis angustissima* extracts. J. Appl. Pharm Sci 7 (02):191-198 http://doi.org.10.7324/JAPS.2017.70227

Manjunath, A., Haseena, S., Kusal, K. D., Shaik, H. S. (2016). Effect of Nigella Sativa seed and thymoquinone on reproductive parameters in sterptozotocin induced diabetic and normal male albino rats. *Inter J Integrat Med Sci* 3: 248-252.

Maruyama, Y. (1987): Sex-steroid-binding plasma protein (SBP), Testerterone, Oestradiol and DHEA in Prepuberty and Puberty. *Acta Endocrinological.* 114: 60-67

Misra, H. P. and Fridovich, I. (1972). The role of superoxide anion in the auto-oxidation of epinephrine and a simple assay for superoxide dismutase. *J. Biol. Chem.* 247:3170-3175.

National Research Council. Occupational Health and Safety in the Care and Use of Research Animals. Washington, DC: The National Academies Press 1997. https://doi.org/10.17226/4988.

Nyman, M. (1959). Serum hatoglobin; methodological and clinical studies. *Scandinavian J. Clin Lab. Invest.* 11(Supp 39): 1 – 169.

Omodanisi, E. I., Aboua, Y. G. and Oguntibeju, O. O. (2017) Assessment of the Anti-Hyperglycaemic, Anti-Inflammatory and Antioxidant Activities of the Methanol Extract of Moringa Oleifera in Diabetes-Induced Nephrotoxic Male Wistar Rats. *Molec* 22: 439. doi:10.3390/molecules22040439

Omonkhua, A. A. and Onoagbe, I. O. (2012). Long-term effects of three hypoglycaemic plants (*Irvingia gabonensis, Urena lobata and Carica papaya*) on the oxidative status of normal rabbits. Biokemistri. *Inter J Nigerian Society Experi Bio.* 24(2): 82-89

Oso, B. J, Oyewo, E. B. and Oladiji, A. T. (2019). Influence of ethanolic extracts of dried fruit of Xylopia aethiopica (Dunal) A. Rich on haematological and biochemical parameters in healthy Wistar rats. *Clin Phytosci.* 2019; 5:9. https://doi.org/10.1186/s40816-019-0104-4.

Oyenihi, O. R., Afolabi, B. A, Oyenihi, A. B., Ogunmokun, O. J. and Oguntibeju, O. O. (2016). Hepato- and neuro-protective effects of watermelon juice on acute ethanol-induced oxidative stress in rats. *Toxicol Report* 3: 288 – 294. http://dx.doi.org/10.1016/j.toxrep.2016.01.003

Patel, D. K., Kumar, R., Laloo, D. and Hemalatha, S. (2012). Diabetes mellitus: An overview on its pharmacological aspects and reported medicinal plants having antidiabetic activity. *Asian Pac J Trop Biomed* 2(5): 411-420. https://doi.org/10.1016/S2221-1691(12)60067-7

Pingali, U., Vuppalanchi, B., Nutalapati, C., and Gundagani, S. (2021) Aqueous

Azadirachta indica (Neem) extract attenuate insulin resistance to improve glycemic control and endothelial function in subjects with metabolic syndrome. *J Med Food.* https://doi.org/10.1089/jmf.2020.4838

Prabakaran, K., and Shanmugavel, G. (2017). Antidiabetic activity and phytochemical constitients of Syzygium cumini seds in Puducherry region. *South India Intern J Parmachem Res* 9(7):985–989

Sacher, R., Richard, A. M. (2000). Widmann's Clinical Interpretation of Laboratory Tests, 11th ed. F.A. Davis Company

Shanak, S., Saad, B., and Zaid, H. (2019) Metabolic and Epigenetic Action Mechanisms of Antidiabetic Medicinal Plants Evidence-Based. *Complement Alter Med.* Article ID 3583067, 18 pages https://doi.org/10.1155/2019/3583067

Tiwari, P. and Kumar S. P. (2017). Plants altering hormonal milieu: A review. *Asian Pac J Reprod* 6(2): 49-53. http://doi.org/10.12980/apjr.6.20170201

Valko, M., Leibfritz, D., Moncol, J., Cronin, M. T. D., Mazur, M. and Telser, J. (2007). Free radicals and antioxidants in normal physiological functions and human disease. *Inter J Biochem Cell Bio.* 39(1):44–84. https://doi.org/10.1016/j.biocel.2006.07.001

Williams, G. (2013). Possible effects of datary polyphenols on sugar absorption and digestion. *Mol. Nutri Food Res.* 57:48-57.