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In vivo assessment of aqueous extract of African locust beans pulp-mango peel blend as a potential alternative natural sweetener for Alloxan induced diabetic rats

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The search for safer and more nutritious natural sweeteners as alternatives to commercial sweeteners, which are implicated in obesity and diabetes mellitus, has led to the utilization of African locust bean (*Parkia biglobosa*) pulp, which is rich in dietary fibers, soluble sugars, and vitamins. Mango peels, containing antioxidants, fiber, and nutraceuticals, were also used. The pulp and mango peels were blended and used to determine the Incremental Area Under the Curve (IAUC) of the natural sweetener compared to synthetic sweeteners. An aqueous extraction of the viable blend, which had a low glycemic index, was administered to alloxan-induced Wistar rats, along with characterization of soluble sugars. The natural sweetener enhanced the reduction of Low-Density Lipoprotein (LDL), while the commercial sweetener revealed a higher concentration of LDL in the serum of the rats (P > 0.05). Exposure to saccharin increased the levels of Superoxide Dismutase (SOD) in the rats, which was significantly different (P > 0.05) compared to the natural sweetener. The cumulative reduction of LDL, SOD levels, and serum glucose level (6.72 mMol/L) supports the optimal valorization of the African locust bean pulp-mango peel blend as an alternative sweetener for managing diabetes mellitus.

Key words: African locust beans pulp, mango peel, diabetes mellitus, glycemic index and soluble sugars.

INTRODUCTION

Sugars are the building blocks of carbohydrates and are naturally present in food sources such as fruits, vegetables, grains, honey, and dairy products (Jomaa et al., 2021; Tamaeh et al., 2021). Natural sugars are a category of carbohydrate compounds known as sucrose ($C_{12}H_{22}O_{11}$). Related compounds include corn sugar (glucose or dextrose), fruit sugar (fructose or levulose), milk sugar (lactose), and malt sugar (maltose). The main sources of sugar are beet sugar and cane sugar (Zaitoun and Maissam, 2018). Synthetic sweeteners, also known as artificial sweeteners, are sugars added during food preparation. They are non-nutritive and primarily used as food additives to provide a sweet taste with few or no calories. Therefore, synthetic sweeteners such as saccharin, acesulfame potassium, aspartame, neotame, and sucralose are widely recognized as additives that can help reduce energy intake and glycemic properties (Yanna et al., 2013).

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There has been ongoing debate about whether synthetic sweeteners affect glycemic properties and if they are used with the intention of mitigating weight gain and diabetes mellitus (Periyasamy, 2019). Although synthetic sweeteners are intended to assist with weight reduction and diabetes management, they have become a source of health concerns, including obesity and other related metabolic disturbances (Pang et al., 2021). The safety and health benefits of synthetic sweeteners remain controversial, with epidemiological evidence consistently linking them to higher incidences of weight gain and diabetes mellitus (Arshad et al., 2022). The use of synthetic sweeteners can trigger neuroadaptations in the brain's reward system, decoupling eating behavior from caloric needs and leading to compulsive overeating, which often results in an insatiable desire for food and can lead to a condition known as Binge Eating Disorder (BED).

This affects the regulation of blood sugar in the body and can lead to obesity and diabetes mellitus (Freeman et al., 2018). Recent research has increasingly focused on the utilization of natural products as effective therapeutic agents for managing diabetes mellitus, due to their comparatively lower toxicities compared to synthetic compounds (Gan et al., 2021). Natural sweeteners encompass a wide range of compounds, including sugars, sugar alcohols, amino acids, and proteins, which contain beneficial compounds such as terpenoids, glycosides, and polyphenols. These compounds can help offset the negative effects of metabolic disturbances such as obesity (Ariana et al., 2020; Arshad et al., 2022). The demand for naturally derived sweeteners has dramatically increased over the last decade, as consumers become more health-conscious (Laffitte et al., 2017).

The African locust bean (*Parkia biglobosa*) is an important leguminous tree species for food security in Africa, producing about 25 to 52 kg of pods per annum (Nyadanu et al., 2017). A mature fruit contains a yellow pulp with dark brown seeds embedded inside. The pulp is consumed for its sweet taste, while the seeds are processed into a condiment called dawadawa in Hausa, iru in Yoruba (Olalude et al., 2021), and nune among the Tiv people of Benue State. Local products processed from locust bean pulp are made by infusing the fruit pulp in hot water, with the drink often consumed locally as a health tonic (Akubor, 2017).

The locust bean fruit pulp is a good source of nutrients and phytochemicals, including flavonoids, phenols, and carotenoids (Alabi et al., 2005).

Mango peels, on the other hand, are major by-products obtained during the processing of mango fruits and constitute about 15 to 20% of the total weight of the mango fruit (Odunfa, 1983). The peels are a good source of polyphenols, carotenoids, dietary fibers, and other bioactive compounds with various beneficial effects on human health (Ajila et al., 2007). Studies have shown that mango peels contain phytochemical compounds such as anthraquinones, which have antidiabetic properties (Monteiro et al., 2019).

This study aimed to determine the effect of an aqueous extract of African locust bean pulp and mango peel blend as a potential natural sweetener for diabetic rats. The study assessed the glycemic properties, sugar concentration, and investigated the efficacy of the extract in controlling serum blood sugar levels, liver function parameters, lipid profile, and oxidative stress biomarkers. The comparison of this blend to non-nutritive synthetic sweeteners was intended to provide data supporting the utilization of the blend as a functional food for diabetics.

MATERIALS AND METHODS

Chemical and reagents

The reagents and chemicals used included aloxan monohydrate, sodium saccharin, sucralose, chloroform, and glibenclamide (50 mg/kg). HPLC separation was carried out using a carbohydrate column (Grace-Davison Prevail Carbohydrate ES5µ, 150 × 4.6 mm i.d) at NSPRI Lab, Kwara, Nigeria. All other chemicals and reagents were obtained from the National Veterinary Research Institute (NVRI), Vom, Plateau State, Nigeria, and the Postgraduate Lab, Benue State University.

Preparation and formulation of the ALBP-MP blends

The Parkia pods and ripened mangoes were obtained from Ortese Market in Gboko, Benue State, Nigeria. The samples were identified as *P. biglobosa* (African locust beans) and *Mangifera indica* (known locally as "Broken") by agronomists Mr. John Okoh and Mr. Atanu from the Department of Agronomy, Joseph Saawuan Tarkaa University, Makurdi. The pods were sorted for wholesomeness, and then manually split open to remove the pulp along with the attached seeds. The pulp with seeds was oven-dried at 40°C for 6 h to facilitate the removal of the pulp from the seeds. The pulp was then manually separated from the seeds, pounded with a pestle and mortar, and sieved using a 0.5 mm sieve. It was packaged in a well-labeled polythene bag and stored at ambient temperature (Gernah et al., 2007). The mango peels were prepared by manual peeling, oven-dried at 50°C for 6 h, then pounded and sieved using a 0.5 mm sieve. They were packaged in a polythene bag and stored at ambient temperature prior to use (Gernah et al., 2007). The blend was formulated by mixing the African locust bean pulp and mango peel in ratios of 60:40, 50:50, and 40:60, respectively. The formulation was based on similar trials (Ahure and Ariahu, 2013).

Determination of glycemic index of ALBP-MP blends

To determine the glycemic index of the flour blends, eighteen Wistar albino rats with body weights ranging from 108.5 to 301.3 g were divided into six groups (three rats per group). Each group was housed in a wooden cage in a climate-controlled environment with free access to food and water. The rats were allowed to acclimate to their new environment for 7 days, after which they were reweighed and then fasted for 12 h overnight. Blood glucose concentrations were measured at zero time from the tail vein, followed by the administration of 2.0 g of each blended experimental sample, artificial sweeteners, and standard glucose

(control), which were consumed within 25 min. After consumption, blood glucose levels were measured using an automatic glucose analyzer ('Accu-Chek Active' Diabetes monitoring kit; Normed Pharmacy, Gboko, Nigeria) at 0, 30, 60, 90, and 120-min intervals. The glycemic response was determined as the Incremental Area Under the Blood Glucose Curve (IAUC), measured geometrically from the blood glucose concentration-time graph, excluding the area of the fasting level (baseline) (Akinjayeju et al., 2020).

The glycemic index is:

$$= \frac{(\text{Incemental area under 2h blood glucose curve or food test sample (2.0g)}}{(\text{Incremental area under 2h blood glucose curve for glucose (2.0g)}} \text{x100}$$

Preparation of aqueous extraction of the viable blend

A 50:50 blended sample of African locust bean pulp and mango peels was extracted with 4.5 L of deionized water by maceration over a period of 72 h. The liquid extract obtained was filtered using Whatman filter paper No. 1. The filtrate was then concentrated to dryness (a yellowish-brown solid residue) using a digital aeration oven preset at 50°C. The residue was stored in the refrigerator at 4°C. A fresh stock was prepared as needed. The net weight of the aqueous extract was 39.892 g (Adeneye et al., 2006).

Characterization of sugars in the ALBP-MP

The determination of sugars such as glucose, fructose, maltose, and sucrose was carried out using HPLC (Agilent 1200 series). The mobile phase was prepared by diluting a mixture of 75% acetonitrile and 25% ultrapure water in a volume of 1 L at the time of analysis. The freshly prepared mobile phase was filtered and degassed by vacuum filtration through a 0.45 µm PTFE membrane filter. The sugars were separated using a Grace-Davison Prevail Carbohydrate (ES5 µ, 150 × 4.6 mm i.d.) column equipped with a RID detector. The mobile phase consisted of deionized water with a flow rate of 1.0 ml/min at 25°C, and the injection volume was 5.0 µl (Hadjikinova et al., 2017).

Feeding trials

Anti-diabetic study of aqueous extract of African ALBP-MPwith non nutrient sweetener: Artificial sweetener (Sodium saccharine)

The experimental protocol was approved by the Research and Ethics Committee, College of Health Sciences, Benue State University, with protocol number CHS REC: CREC/DIS/008. Twenty-eight male rats, weighing between 111.2 and 265.5 g, were housed in cages and maintained under standard conditions (12-h light and 12-h dark cycle, 35 to 60°C humidity). The animals were fed a standard diet and provided with water ad libitum. Diabetes was induced in overnight fasted rats by intraperitoneal injection of 150 mg/kg alloxan monohydrate (Oghenesuvwe et al., 2014). After three days, the glycemic levels were measured, and rats with glucose levels higher than 200 mg/dl were selected for the experiment. The aqueous extract of ALBP-MP and normal saline (0.9% w/v NaCl, for the control groups) were administered daily by intragastric injection (gavage) for 14 days (Akinjayeju et al., 2020). The rats were divided into seven groups, with four rats in each group, and treated as follows:

1) GBU: Normal control administered water daily for 14 days;
2) GBV: Diabetic rats+ water daily for 14 days;
3) GBW: Diabetic rats + Glibenclamide (50 mg/kg) daily for 14 days.
4) GBX: Diabetic rats + Extract (400 mg/kg) daily for 14 days.
5) GBY: Diabetic rats + Extract (600 mg/kg) daily for 14 days.
6) GBZ: Diabetic rats + Extract (800 mg/kg) daily for 14 days.
7) SNW: Diabetic rats + Synthetic sweetener (Sodium saccharine) (2 g) daily for 14 days

The blood glucose levels were measured six times. After 14 days, the animals were deprived of food for 12 h and were sacrificed by extracting blood from the jugular vein using a syringe following a razor blade incision.

Serum was obtained by centrifugation at 1500 g for 10 min and then refrigerated at -80°C (Akinjayeju et al., 2020).

The biochemical determination of ALBP-MP and sodium saccharine

The biochemical parameters were analyzed using blood samples, which were first centrifuged at 1,500 × g for 10 min at ambient temperature. The serum was then separated and used for liver function assessment by measuring the enzymes aspartate aminotransferase (AST) (Reitman and Frankel, 1957; Schmidt and Schmidt, 1963), alanine aminotransferase (ALT) (Reitman and Frankel, 1957), and alkaline phosphatase (ALP) (Schebusch et al., 1974). Lipid profile assessment was conducted by measuring total cholesterol (Chol-T) (Allain et al., 1974), triglycerides (TG) (Gordon et al., 1977), high-density lipoprotein (HDL) (Assmann and Antonio, 2004; Gordon, 1977; Friedewald, 1972), and low-density lipoprotein (LDL) (McNamara et al., 1990). Oxidative stress biomarkers were assessed by measuring glutathione (GSH) (Mannervik and Carlberg, 1985), superoxide dismutase (SOD) (Mannervik and Carlberg, 1985), and catalase (CAT) (Mannervik and Carlberg, 1985). Serum glucose was determined using glucose oxidase, an enzyme with a strong affinity for glucose (Washko and Eugene, 1961). These tests were performed using disposable kits obtained from Agappe Diagnostics Switzerland GmbH and Randox Laboratories Ltd, 55 Diamond Road, Crumlin, County Antrim, BT29 4QY, United Kingdom.

Statistical analysis

Measurement were carried out in replicates (Where applicable), and results expressed as mean ± standard error of mean (SEM). Data were analysed by One-way Anova using SPSS (2019) statistical packages for the social science, version 26.0 IBM corp., Armonk, NY, USA. Microsoft excel office (2007) Retrieved from https://office microsoft.com/excel was also used for the analysis where applicable.

RESULTS AND DISCUSSION

Measurement of blood glucose response

Blood glucose level (glycaemia) measures the concentration of glucose in the bloodstream. The *in-vivo* blood glucose response of albino rats fed with samples 1, 2, and 3 (Sample 1 = 50:50, Sample 2 = 60:40, Sample 3 = 60:40), commercial synthetic sweeteners for diabetic patients (sodium saccharin and sucralose), and 100% glucose (standard glucose) was determined at 0, 30, 60, 90, and 120 min using the oral glucose tolerance test (OGTT), as shown in Figures 1 and 2. The OGTT is used to evaluate insulin release and insulin resistance

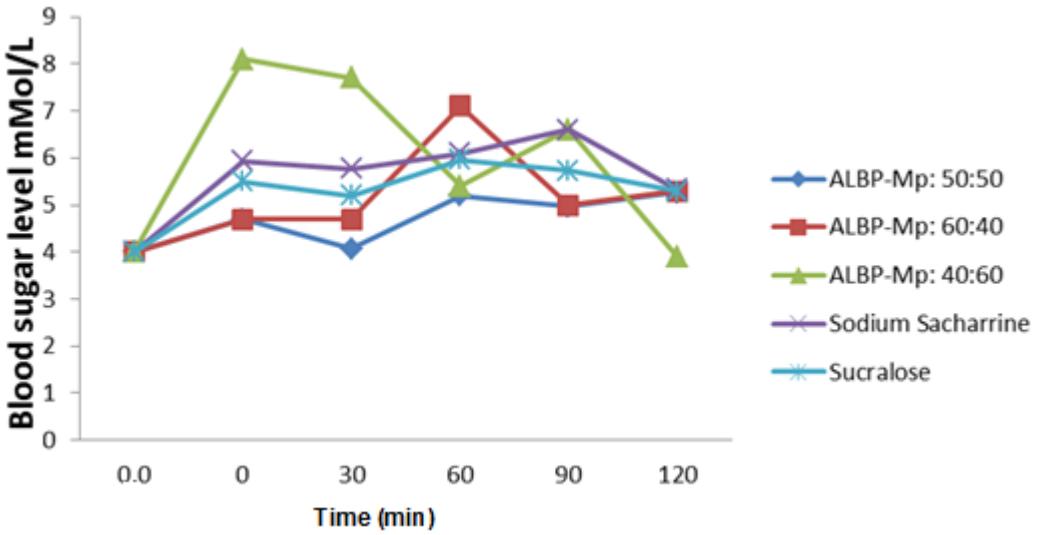

Figure 1. IAUC of ALBP-MP samples and synthetic sweeteners.

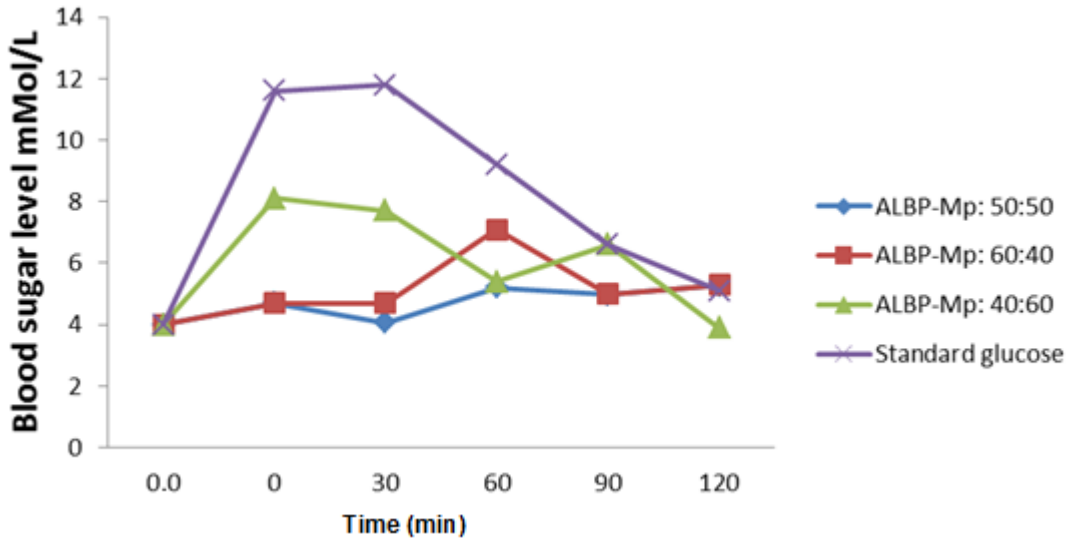

Figure 2. IAUC of ALBP-MP samples and standard glucose.

(Akinjayeju et al., 2020).

As shown in Figure 2, the blood glucose concentration of the control group rose from about 4 to 11.8 mMol/L within the first 30 min of administration and then decreased to 5.1 mMol/L over the subsequent 120 min. This indicates a high absorption and metabolizing rate of blood glucose in the rats, which may suggest a higher risk of diabetes (Anitha et al., 2012). The glucose concentration of rats fed sodium saccharin rose from 4 to 6.6 mMol/L within 90 min and dropped to 5.3 mMol/L within 120 min, while sucralose rose from 4 to 5.9 mMol/L within 60 min and declined to 5.3 mMol/L within 120 min. The low glucose concentrations clearly indicate that synthetic sweeteners are non-nutritive. These sweeteners are known to induce changes in the hypothalamus, which may affect satiety signals differently from natural sweeteners. This can lead to a disconnect between eating behavior and caloric needs, potentially resulting in compulsive overeating and subsequent exhaustion of cells and insulin, often leading to type 2 diabetes (Freeman et al., 2018; Mengqiu, 2023).

Conversely, the blood glucose concentration for the blends was as follows: Sample 1 (50:50) rose from 4 to 5.2 mMol/L in 60 min and then dropped to 4.96 mMol/L within 90 min; Sample 2 (60:40) rose to 7.1 mMol/L within 60 min and dropped to 5.3 mMol/L within 120 min;

Table 1. The quantity of glucose response and and IAUC in blends of ALBP-MP, Synthetic sweeteners and standard glucose.

Min/Groups/IAUC	0	30	60	90	120	IAUC mmol.min/L
1	4.70±0.55^{b}	4.06±0.56^{b}	5.20±1.35^{a}	4.96±0.63^{a}	5.26±0.11^{a}	576
2	4.73±1.83^{b}	4.76±1.55^{b}	7.16±2.88^{a}	5.00±0.80^{a}	5.33±1.95^{a}	654
3	8.16±3.19^{ab}	7.76±5.65^{ba}	5.43±1.46^{a}	6.60±3.30^{a}	3.90±0.78^{a}	771
S.S	5.93±0.68^{b}	5.76±0.50^{b}	6.10±0.85^{a}	6.60±1.31^{a}	5.33±0.05^{a}	723
S.L	5.50±0.70^{b}	5.20±1.17^{b}	5.96±1.24^{a}	5.73±0.65^{a}	5.30±1.00^{a}	669
S.G	11.63±2.56^{a}	11.86±5.15^{a}	9.26±4.13^{a}	6.60±3.30^{a}	5.13±0.75^{a}	1078

Means of triplicate determination reported within rows with the same supercript are not significantly (P>0.05) different. A=(ALBP-MP =50:50), C = (ALBP-MP =40:60), B = (ALBP-MP =60:40), S.S = sodium saccharine, S.L= sucralose, S.G = Standard glucose (Glucose D).

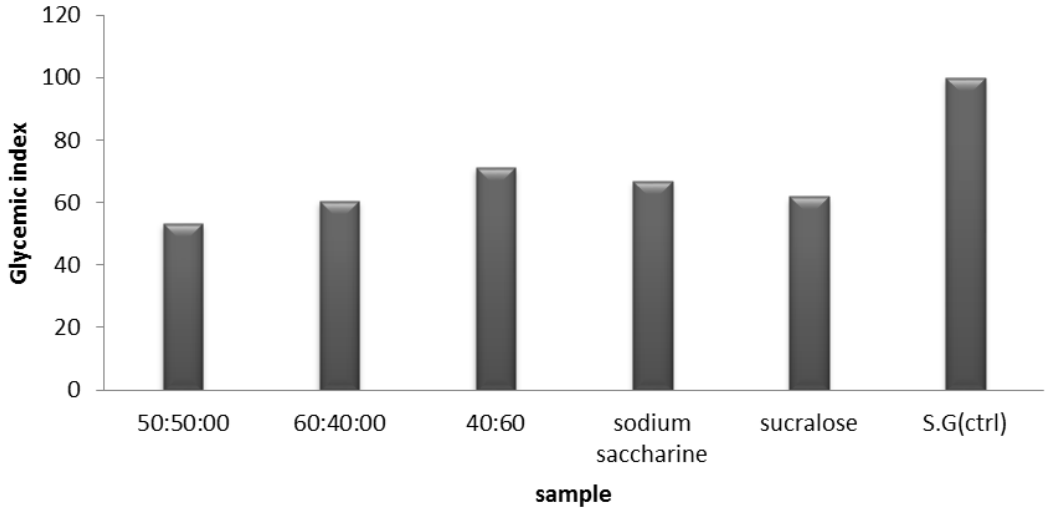

Figure 3. The Glycemic index of samples of ALBP-MP, synthetic sweeteners and control (standard glucose).

Sample 3 (40:60) rose to 7.7 mMol/L in 30 min and dropped to 3.9 mMol/L within 120 min, as shown in Figure 1 and 2. When comparing the blood glucose response curves of these samples to the control (standard glucose) and synthetic sweeteners, it was found that Sample 1 (50:50) exhibited a better glucose response curve, with an IAUC of 576 mMol·min/L. The IAUC for saccharin and sucralose were 723 and 669 mMol·min/L, respectively, which were lower than those of Samples 2 and 3 but still less than that of Sample 1, shown in Table 1. This suggests that Sample 1 has a lower digestion and absorption rate, better regulates blood glucose levels, and has a high potential for managing and controlling type 2 diabetes.

The glycemic index concept extends the fiber hypothesis by suggesting that fiber consumption reduces the rate at which nutrients enter the bloodstream from the gut (Jenkins et al., 2014). It also measures the rate at which carbohydrates are digested and subsequently increase blood glucose levels (Akinjayeju et al., 2020; Akinjayeju, 2015). The glycemic index values for the three samples were 53.4% for Sample 1 (50:50), 60.6% for Sample 2 (60:40), and 71.4% for Sample 3 (40:60), as shown in Figure 3. The high glycemic index in Sample 3 could be attributed to the high percentage of mango peel in the formulation. Sample 1 (53.4%) had a lower glycemic index than the recommended value for low glycemic index foods (<55) and was significantly different from the glycemic index of sodium saccharin (67%) and sucralose (62%) used in the study. Studies have shown that low glycemic index foods are ideal for diabetic patients, as they are associated with a reduced risk of diabetes, body weight, and serum cholesterol (Akinjayeju et al., 2020). In comparison to the synthetic sweeteners used in the study (sodium saccharin and sucralose), the glycemic index values for Sample 2 (60:40) and Sample 3 (40:60) were similar, but Sample 3 showed a significantly higher (P<0.05) glycemic index at 71.4%, as

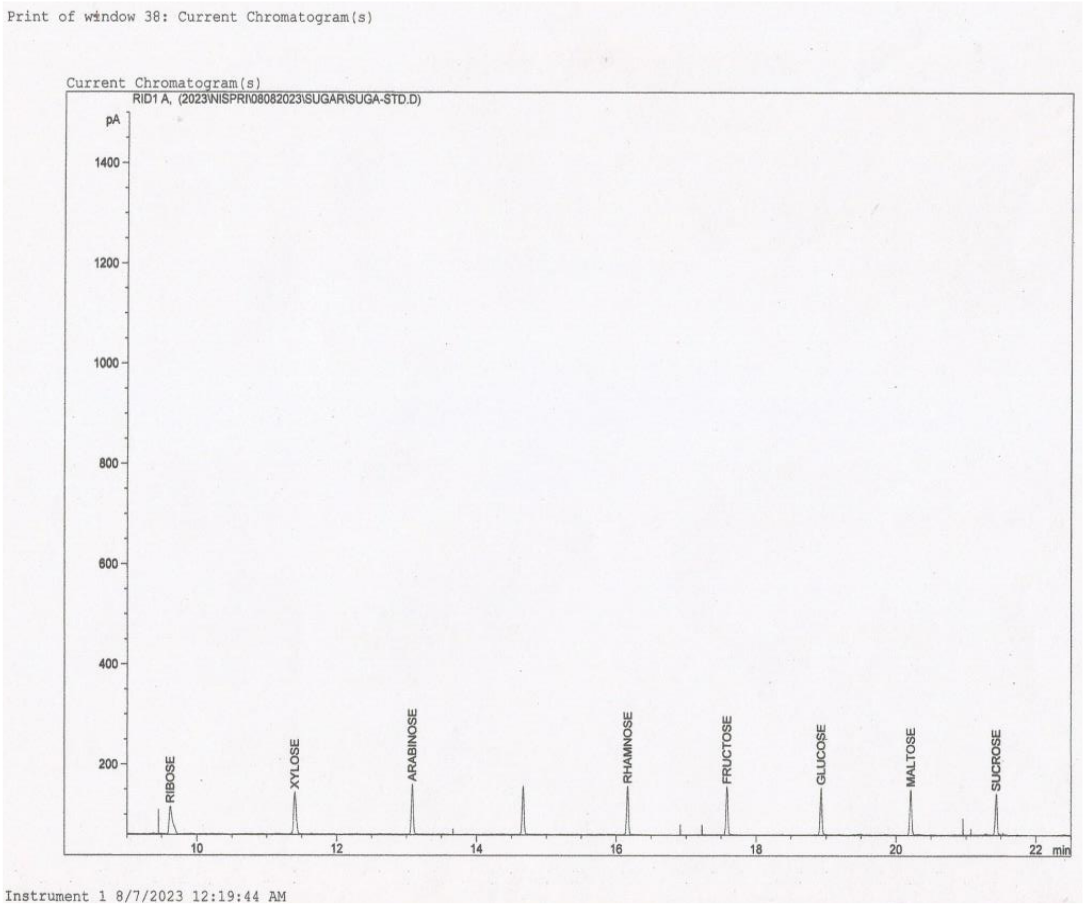

Figure 4. HPLC-RID overlay of chromatogram of standard sugars during calibration of eight (8) carbohydrates standard.

shown in Figure 3. The low glycemic property of Sample 1 (50:50) indicates that this blend has strong potential as a natural sweetener for diabetic patients. These findings align with reports that natural fruits, with their bioactive components, can substantially reduce the risk of diabetes by targeting the pathophysiological mechanisms of obesity and diabetes (Anne-Helen et al., 2008; Samir et al., 2011). Therefore, consuming natural sweeteners from blends of African locust bean pulp fruit and mango peel may lead to a gradual and slower increase in blood glucose concentration, making it suitable for managing or controlling diabetes.

Glycemic Index of ALBP-MP blends and synthetic sweeteners

Characterization of soluble sugars

The peaks in the HPLC chromatograms, as shown in Figures 4 and 5, were identified by comparing the retention times of soluble sugars in the samples with those of the standards. Under standard chromatographic conditions, the sugars were eluted within 22 min, achieving good separation among the individual sugars. The retention times of soluble sugars in the standard and the aqueous extract of ALBP-MP are listed in Table 2.

The results of the sugar concentration, shown in Table 3, indicate that fructose is the predominant soluble sugar in the aqueous extract blend of Parkia pulp fruit and mango peel, accounting for 14.6%. This concentration corresponds to the range of fructose reported in peach fruit, which is 7 to 14% (Nowicka et al., 2019). Fructose from fruit is less glucogenic and has a lower glycemic index. Natural sweeteners derived from fruits may serve as an alternative for diabetic patients who prefer sweet foods but are susceptible to high postprandial glucose concentrations (Uusitupa, 1994). Other sugars present in high concentrations in the extract include glucose (13.20%), maltose (0.6%), and rhamnose (0.5%), shown in Table 3. The concentrations of glucose and fructose in the extract are lower than those reported for grape juice (Rizzon and Miele, 2012).

Fruits exhibit a wide range of soluble sugar concentrations; the total sugar content in the aqueous extract (ALBP-MP) ranged from 0.5 g/100 to 14.6/100 g.

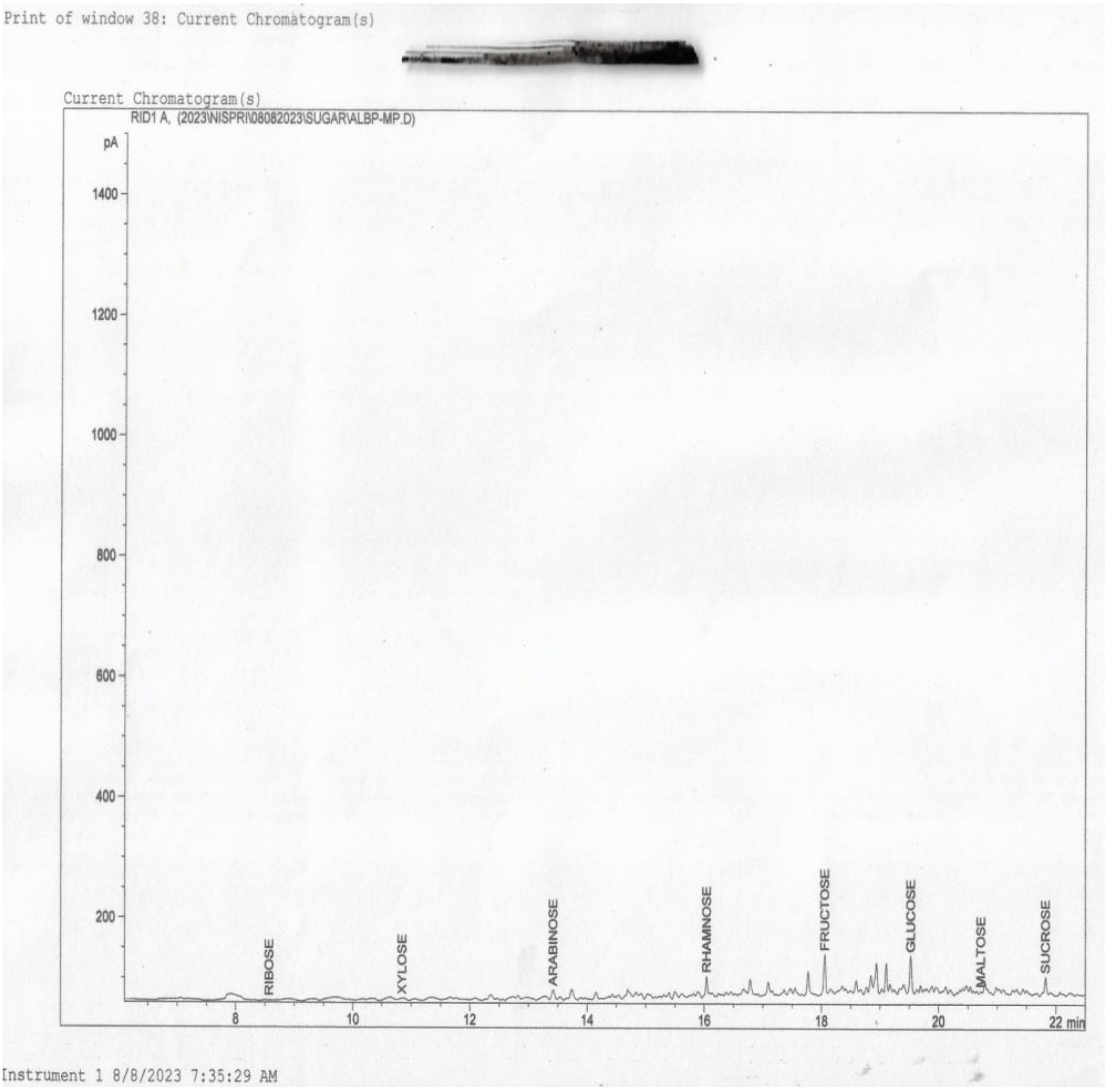

Figure 5. HPLC-RID overlay of chromatogram of ALBP-MP extract.

Table 2. Validation parameters for determination of sugars in ALBP-MP by HPLC-RID.

Sugar RID	RT(Min)(extract	RT(Min)(Standards)	Range (µL) (N=3)	Regression eqn.	R^2
Glucose	19.521	18.50	0.025-5.0	Y=266.8x-253.6	1
Fructose	18.056	17.30	0.025-5.0	Y=279.5x-264.9	1
Sucrose	21.823	21.00	0.025-5.0	Y=215.9x-215.8	1
Maltose	20.719	20.30	0.025-5.0	Y=67.75x-67.20	1
Rhamnose	16.039	16.30	0.025-5.0	Y=147.4x-146.9	1
Arabinose	13.420	13.30	0.025-5.0	Y=94.27x-94.27	1
Xylose	10.842	11.50	0.025-5.0	Y=72.48x-72.48	1
Ribose	8.573	9.50	0.025-5.0	Y=26.94x-26.94	1

RID = Refractive Index Detector; RT = Retention Time; R^2= Linear Correlation Coefficient.

This is less than the highest soluble sugar concentration reported for grapefruit (16.5 g/100 to 26.3/100 g) and peach (2.2/100 to 20/100 g) but more than that of tomatoes (1.4/100 to 5/100 g) (Zhanwu et al., 2016).

Table 3. Concentration of soluble sugars in aqueous extract of ALBP-MP.

Parameter	Concentration (g/100 g)
Glucose	13.20022±0.00038[b]
Fructose	14.60828±0.01434[a]
Sucrose	0.12615±0.04531[e]
Maltose	0.54995±0.00009[c]
Rhamnose	0.48014±0.03439[d]
Arabinose	0.00003±0.00006[f]
Xylose	0.00003±0.00005[f]
Ribose	0.00000±0.00000[f]

All determination was done in triplicate and results expressed as g/100g of ALBP-MP.

Mango fruits are known to contain glucose, fructose, and sucrose, with sucrose being the most abundant (Medicott and Thompson, 1983). However, the extract showed a low concentration of sucrose (0.13%). The rhamnose concentration in the aqueous extract of ALBP-MP was also low, consistent with the low rhamnose content reported in mango peel (Ajila and Prasada, 2013). Although arabinose is reported as a major sugar in mango peel (Ajila and Prasada, 2013), its concentration, along with xylose and ribose, in the aqueous extract was low. Xylose, a sucrose inhibitor, has been shown to significantly lower blood glucose levels in normal rats when present in lower concentrations (Eunju, 2023). The reported concentrations of glucose (0.25 mg/100 g), sucrose (0.48 mg/100 g), and fructose (0.20 mg/100 g) in African locust bean pulp fruit (Alabi et al., 2005) were lower compared to those present in the aqueous extract of ALBP-MP.

The reference values for serum concentrations are reported as follows: AST - 70 µ/L (Olaf et al., 2007), ALT - 35 µ/L (Olaf et al., 2007), and ALP - 41 µ/L (Tanko et al., 2018). The liver function test parameters reported in this study were generally below these reference values, except for GBX (400 mg/kg). Poor control of blood glucose levels is a key contributor to associated metabolic consequences such as microvascular complications (e.g., retinopathy, neuropathy, and nephropathy) or macrovascular complications (e.g., myocardial infarction, heart failure, and stroke) (Noriega-Cisneros, 2012; Rathore et al., 2018). The AST values for groups administered ALBP-MP extract, with the exception of group GBZ, were not significantly different ($P<0.05$) compared to group SNW, shown in Table 4. Although the concentration of AST in SNW (39.5 µ/L) was higher than in GBW (36 µ/L), GBX (38 µ/L), and GBY (31.75 µ/L), it was significantly ($P<0.05$) decreased compared to GBZ. High and low doses of saccharin fed to rats have been reported to have AST concentrations of 63.7 and 86.4 µ/L, respectively (Kamal and Hessah, 2015). The concentration of AST in rats fed aspartame is reported to be 76.82 µ/L (Magda et al., 2018), which is significantly higher than the AST values obtained from rats fed the aqueous extract and synthetic sweeteners.

Furthermore, the ALT concentration showed that the ALBP-MP group GBX (12.00 µ/L) and SNW (11.25 µ/L) were not significantly different from each other but were significantly decreased ($P<0.05$) compared to the negative control (GBV) and the standard drug. However, these values were significantly higher compared to the first positive control group (GBU), shown in Table 4. This result contrasts with the high ALT concentration (194.36 µ/L) reported in rats fed aspartame (Magda et al., 2018), but it was not significantly different ($P<0.05$) from the 13.5 µ/L ALT concentration in rats fed saccharin (Kamal and Hessah, 2015). Other studies have reported increased ALT levels ranging from 40 to 80 µ/L in rats fed saccharin and aspartame (Muthear et al., 2022). Overall, the present study did not record elevated levels of AST and ALT in the SNW group compared to the negative control and positive control groups (GBU and GBV).

The concentration of ALP in groups GBY and GBZ was significantly decreased ($P<0.05$) compared to the non-nutritive synthetic sweetener (SNW), the negative control (GBV), and the positive control (standard drug: GBW), shown in Table 4. There was no significant difference between GBY and GBZ compared to the positive control group (GBU) without aloxan monohydrate induction. Although the synthetic sweetener (sodium saccharin) performed better than the standard drug and GBX, it was significantly ($P<0.05$) increased compared to the results of the aqueous extract of ALBP-MP in groups GBY and GBZ and the control group (GBU), respectively. Higher concentrations of ALP have been reported in Wistar rats fed saccharin at high and low doses and aspartame (Kamal and Hessah, 2015; Magda, 2018). However, the result for group GBX was significantly ($P<0.05$) increased compared to the synthetic sweetener. This indicates that the aqueous extract (ALBP-MP) is nutritive and has a negligible tendency to induce liver dysfunction. It is less likely to trigger the release of abnormal levels of glucose

Table 4. The liver function test of rats fed aqueous extract of ALBP-MP and synthetic sweetener.

Group/Parameter (µ/l)	GBU	GBV	GBW	GBX	GBY	GBZ	SNW	Ref. Value
AST	27.00±2.16^{a}	50.50±20.14^{ab}	36.00±4.08^{a}	38.33±8.37^{a}	31.75±8.22^{a}	64.50±30.11^{b}	39.50±2.38^{a}	70.00
ALT	10.50±0.57^{a}	20.75±6.94^{c}	17.00±.00^{abc}	12.00±.00^{ab}	18.75±5.56^{bc}	24.00±3.82^{c}	11.25±0.95^{ab}	35.00
ALP	14.43±2.29^{a}	21.30±3.69^{ab}	41.55±9.05^{c}	55.63±5.55^{d}	14.20±6.71^{a}	18.78±5.91^{ab}	26.87±2.63^{b}	41.00

Means within a row with the same supercripts are not significantly (P>0.05) different. GBU=Positive ctrl, GBV=negative control group (150mg/kg) alloxan, GBW= positive control (standard drug 50mg/kg glibenclamide), GBX=Aqueous extract (400 mg/kg), GBY=Aqueous extract (600 mg/kg), GBZ=Aqueous extract (800 mg/kg) and SNW=Synthetic sweetener (sodium saccharine), Ref. Value= reference values.

to the liver for processing and may instead provide diabetic tissues with metabolizable energy, vitamins, and antidiabetic phytochemicals such as anthraquinones and alkaloids for proper metabolism. In contrast, synthetic sweeteners like sodium saccharin have been implicated in altering the gut microbiome and inducing glucose intolerance due to the loss of natural satiety from the absence of calories, potentially increasing the risk of type 2 diabetes, especially in men (De Koning et al., 2011).

Overall, the trend observed in this study showed that liver function parameters increased in the group administered SNW (saccharin) compared to those fed the extract of ALBP-MP. This increase may indicate changes in hepatic function, confirmed by the rise in cholesterol, low-density lipoprotein, and superoxide dismutase reported in this study. This may lead to stress on the liver, which is associated with the use of synthetic sweeteners like saccharin (Kamal and Hessah, 2015).

Results for biochemical test of aqueous extract of ALBP-MP and Non nutrient sweetener (sodium saccharine)

Liver function test

Aberrations in lipid metabolism are implicated in the development of diabetes mellitus (DM) and diabetes dyslipidemia (DD) (Vasilios et al., 2018). Glucose and lipid metabolism are interconnected and play a role in diabetic dyslipidemia, which is characterized by elevated triglycerides, low levels of high-density lipoprotein cholesterol (HDL-C), and high levels of low-density lipoprotein cholesterol (LDL-C) particles (Klaus, 2015).

The reference values for lipid profile parameters (cholesterol) were higher than those reported in this study, except for the triglyceride (T.G) parameter (Ihedioha et al., 2013). HDL values were also higher than the reference values reported by Ihedioha et al. (2013), except for groups GBV, GBY, and GBZ. LDL values were significantly lower than the reference values, except for the negative control group (GBV) and the commercial non-nutritive sweetener (SNW).

In this study, the lipid profiles of Wistar rats fed the aqueous extract of ALBP-MP and the non-nutritive sweetener (sodium saccharin) were analyzed and compared, as shown in Table 5. The results showed that the cholesterol concentration in the synthetic sweetener (sodium saccharin) group was significantly higher (P>0.05) compared to the cholesterol concentrations in groups GBX, GBY, and GBZ, which were administered varying concentrations (400, 600, and 800 mg/kg) of the aqueous extract of ALBP-MP. Total cholesterol has been reported to be highest in the lipid profiles of male rats fed aspartame and sucralose (Nermin et al., 2020). The present findings align with those of studies showing very high total cholesterol concentrations of 125 and 127.6 mg/dl in Wistar rats fed high and low doses of saccharin, respectively (Kamal and Hessah, 2015). This indicates that the cholesterol (T.Chol) concentration of wistar rats administered ALBP-MP extract was within the standard range and significantly (P<0.05) lower compared to the non-nutritive synthetic sweetener sodium saccharin (SNW). There was no significant (P<0.05) difference in cholesterol (T.Chol) concentration between the group administered the drug and group GBX (ALBP-MP). However, the standard drug group (GBW) showed a significant difference (P<0.05) when compared to groups GBY and GBZ of the ALBP-MP and the synthetic sweetener.

The total cholesterol concentration for the negative control group (induced without treatment) was significantly different (P<0.05) from that of the positive control (group without alloxan induction), the treated group (ALBP-MP), the standard drug, and the non-nutritive sweetener (sodium saccharin). This discrepancy may be due to alloxan induction, which could have reduced the metabolic activity of the rats in group GBV, leading to decreased physical activity (Murwan et al., 2016). Recent studies have shown that Parkia pulp can serve as a substitute for artificial sweeteners in composite

Table 5. The lipid profile test of wistar rats fed aqueous extract of ALBP-MP and synthetic sweetener.

Group/Parameter (mg/dl)	GBU	GBV	GBW	GBX	GBY	GBZ	SNW	Ref. value
(CHOL-T)	71.19±3.27^{d}	20.87±11.62^{a}	51.27±4.10^{c}	44.28±7.02^{c}	40.03±16.26^{bc}	30.42±6.25^{ab}	83.95±2.63^{e}	95.24
(TG)	83.07±3.56^{d}	76.50±1.66^{c}	61.70±1.20^{a}	67.23±1.85^{b}	67.78±1.08^{b}	70.99±5.13^{b}	67.53±2.94^{b}	50.27
(HDL-c)	46.92±1.15^{bc}	24.33±8.50^{a}	53.83±7.56^{c}	40.49±13.71^{abc}	31.14±5.28^{ab}	31.21±5.41^{ab}	52.06±5.92^{c}	35.00
(LDL-c)	7.14±3.06^{a}	17.07±8.48^{ab}	14.56±4.23^{ab}	9.65±9.89^{ab}	13.39±5.43^{ab}	12.48±7.10^{ab}	18.13±3.37^{b}	20.28

Means within a row with the same supercripts are not significantly (P>0.05) different. GBU=Positive ctrl, GBV=negative control group (150mg/kg) alloxan, GBW= positive control (standard drug 50mg/kg glibenclamide), GBX=aqueous extract (400 mg/kg), GBY=aqueous extract (600 mg/kg), GBZ=aqueous extract (800mg/kg) and SNW=Synthetic sweetener (sodium saccharine); Ref. Value: reference values.

biscuits and snacks (Kazeem et al., 2019) without posing a risk of dyslipidemia that could lead to diabetes mellitus.

Cholesterol is physiologically important, but high blood levels are a major risk factor for atherosclerosis (Ihedioha et al., 2013). The serum triglyceride (T.G) levels in the non-nutritive synthetic sweetener (sodium saccharin: 67.3 mg/dl) showed no significant (P<0.05) difference compared to the groups administered varying concentrations of ALBP-MP, shown in Table 5. However, the serum triglyceride level in the group administered the standard drug was significantly (P<0.05) decreased compared to those in the aqueous extract and synthetic sweetener groups.

The HDL-C serum concentration for group GBX, which was fed the aqueous extract of ALBP-MP, was above the reference concentration. HDL-C values in the ALBP-MP group were significantly (P<0.05) higher than those in the negative control (GBV) but significantly (P<0.05) lower than those in the group administered the synthetic sweetener (SNW), shown in Table 5. There was no significant difference between the HDL-C levels in the group administered glibenclamide (standard drug) and the non-nutritive sweetener (SNW). Groups GBY and GBZ had HDL-C levels lower than the reference value and were significantly (P<0.05) decreased compared to SNW.

High levels of HDL-C have significant physiological benefits. HDL-C is a type of lipoprotein that transports atheromas from tissues and cells to the liver for processing into bile and excretion, thereby clearing blood vessels for efficient nutrient distribution. The high concentration of HDL-C in the aqueous extract of ALBP-MP (GBX) suggests that ALBP-MP can positively impact blood vessel health. Although the HDL-C concentration in the synthetic sweetener (sodium saccharin) was higher than that in ALBP-MP, this may be attributed to the low crude fat content of Parkia pulp (Afolayan et al., 2014). Reports indicate that HDL-C levels in rats fed synthetic sweeteners increase slightly but insignificantly (Nermin et al., 2020), with a slight increase in HDL-C concentrations ranging from 0.7 to 1.11 mg/dl (Jiddah et al., 2022).

The Low-Density Lipoprotein Cholesterol (LDL-c) concentration in groups GBX, GBY, and GBZ administered the aqueous extract of ALBP-MP was significantly (P<0.05) lower compared to the synthetic sweetener (SNW). However, there was no significant difference when the extract was compared to the control group (GBW), although it was significantly (P<0.05) lower than in the first control group (GBU). These findings are consistent with other studies that reported high LDL-c concentrations of 31.0 and 26.23 mg/dl in rats fed a saccharin diet (Kamal and Hessah, 2015). High LDL-c levels are a major risk factor for the development of atherosclerosis, hepatic dysfunction, and ultimately diabetes mellitus (Ihedioha et al., 2013). The results align with findings that long-term administration of sucralose in mice significantly increased serum levels of LDL-c (Farid et al., 2020). The greatest increase in LDL-c concentration has also been reported in groups supplemented with high doses of aspartame (Nermin et al., 2020). Additionally, increases in LDL-c have been observed in rats fed blends of aspartame and cyclamate (Jiddah et al., 2022). Elevated LDL-c levels are considered a predictive biomarker of poor glycemic control in individuals with diabetes mellitus (Artha et al., 2019).

The reference values reported by Pereira et al. (1994) for Glutathione Reductase (GR), Superoxide Dismutase (SOD), and Catalase (CAT) were lower than those reported in the present study, except for GR in the synthetic sweetener (sodium saccharine) group, which had a lower concentration compared to the reference value. The study indicated a significant (P<0.05) decrease in GR levels in the group administered the synthetic sweetener (sodium saccharine) compared to the negative control (GBV) and the groups administered the extract (GBY and GBZ).

Table 6. Oxidative stress biomarkers of rats fed aqueous extract of ALBP-MP and synthetic sweetener.

Group/Parameter	GBU	GBV	GBW	GBX	GBY	GBZ	SNW	Ref. value
GR (µg/mL)	4.31±1.19[a]	7.00±2.86[b]	4.56±1.19[a]	4.75±1.27[a]	5.62±0.47[ab]	5.68±0.12[ab]	3.76±0.54[a]	4.01
SOD (µmoleSOD/mL)	3.25±0.53[a]	1.81±0.55[a]	2.38±0.08[a]	2.89±0.89[a]	5.62±3.64[a]	2.49±2.69[a]	11.37±2.37[b]	0.65
CAT (µmoleH_20_2/mLn/mg protein)	13.94±0.77[a]	11.48±4.82[a]	17.29±1.98[a]	16.77±3.75[a]	18.83±10.44[a]	16.06±3.97[a]	16.23±1.68[a]	1.23

Means within a row with the same supercripts are not significantly (P>0.05) different. GBU=Positive ctrl, GBV=negative control group (150 mg/kg) alloxan, GBW= positive control (standard drug 50 mg/kg glibenclamide), GBX=aqueous extract (400 mg/kg), GBY=aqueous extract (600 mg/kg), GBZ=aqueous extract (800 mg/kg) and SNW=synthetic sweetener (sodium saccharine); Ref. values: reference values.

Table 7. Serum glucose level of rats fed aqueous extract and synthetic sweetener.

Parameter	Serum glucose oxidase (mMol/L)
GBU	6.58±1.16[a]
GBV	18.58±1.47[c]
GBW	5.24±2.43[a]
GBX	6.72±4.16[a]
GBY	10.77±3.78[b]
GBZ	10.83±1.96[b]
SNW	4.59±0.28[a]

However, there was no significant (P>0.05) difference in GR levels when compared to group GBX and the two positive controls (GBU and GBW). The SOD concentration in the group fed the synthetic sweetener (sodium saccharine) was significantly (P<0.05) higher compared to all groups fed ALBP-MP, shown in Table 6. A very high SOD concentration (73.58 µ/g) has also been reported in rats fed saccharine (Kamal and Hessah, 2015). Non-nutritive synthetic sweeteners are known to increase oxidative stress and induce mitochondrial changes (Griebsch et al., 2023). There were no significant (P>0.05) differences in catalase levels across all groups.

Oxidative stress biomarkers

Serum glucose test

The serum glucose level in the group administered the synthetic sweetener was significantly (P<0.05) lower compared to the extract (ALBP-MP) administered in groups GBY and GBZ, shown in Table 7. A decrease in serum glucose levels in rats fed non-nutritive sweeteners has been reported by other researchers (Eman et al., 2019). This is likely due to the fact that synthetic sweeteners contain few or no calories. Furthermore, the serum glucose level in the synthetic sweetener group did not differ significantly (P>0.05) from the extract in group GBX. There was also no significant (P>0.05) difference between the synthetic sweetener and the two control groups (standard drug; GBW and GBU).

Conclusion

The results from this study indicated that the African locust bean pulp-mango peel blend has a low glycemic index compared to artificial sweeteners. The characterization of soluble sugars revealed high fructose content (15%) relative to glucose and sucrose, with sucrose present at a very low concentration of 0.13%.

Xylose was also detected in small amounts; it is known to inhibit sucrose, which is implicated in raising blood sugar levels. The biochemical assays of the aqueous extract showed positive effects on important parameters such as LDL and SOD, compared to the synthetic sweeteners. This suggests that the use of ALBP-MP could help mitigate some downstream metabolic effects commonly associated with diabetes, such as retinopathy, neuropathy, and nephropathy.

The study provides valuable insights and significant benefits for diabetes management in experimental rats. However, further research is needed to assess the antidiabetic potential of the ALBP-MP aqueous extract, including studies on amylose and amylopectin structures of carbohydrates, as well as hematological and histological parameters, to establish the African locust bean pulp-mango peel blend as a functional food for diabetics.

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CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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