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ANTIHYPERGLYCAEMIC ACTIVITIES OF PLANT-EXTRACTED SUCCINIC ACID AND HEXAMETHYLCYCLOTRISILOXANE IN DIABETIC ALBINO RATS

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ABSTRACT

This study investigated the *in vivo* activities of these plant-extracted Succinic acid and hexamethylcyclotrisiloxane as antihyperglycaemics in alloxan-induced diabetic Wistar rats. The experiment was conducted in a standard animal house in Enugu State University of Science and Technology (ESUTH), Faculty of Pharmaceutical Sciences, Department of Pharmacology, Agbani, Enugu State. The animal models were randomly divided into seven groups, and Fasting blood glucose level test was conducted to determine the antihyperglycaemic effects of these phytochemicals on alloxan-induced diabetic rats. After four weeks of treatment, the results showed that the diabetic group treated with 100mg/kg b.w of Succinic acid only and 200mg/kg b.w. of combined Succinic acid and Hexamethylcyclotrisiloxane (1:1) for the single effect and combined effect groups, respectively, showed more significant recovery effects on the alloxan-induced diabetic rats than the 100mg/kg b.w. hexamethylcyclotrisiloxane only and 100 mg/kg b.w. of combined Succinic acid and hexamethylcyclotrisiloxane (1:1). Data from this experimental research showed a significant recovery at $P< 0.05$ from hyperglycemia. These findings suggest that Succinic acid and Hexamethylcyclotrisiloxane when administered either as single or combined therapy for four weeks could elicit a dose-dependent effect on blood glucose level normalization and body weight maintenance.

KEYWORDS: Antihyperglycaemics, Succinic acid, Hexamethylcyclotrisiloxane, Diabetes.

INTRODUCTION

In the world, 1 in 8 adults (206 million) are living with diabetes, the number of adults with diabetes is expected to reach 238 million by the year 2030, and 2690 million by the year 2045 (WHO 2016). Experts in the Nigerian health sector have declared that 11.2 million are currently living with diabetes in Nigeria (Uloko, Musa, and Raaham, 2018).

Diabetes mellitus is a type of chronic metabolic disorder categorized by insufficiency in insulin activity and/or insulin secretion. Anomalies in proteins, carbohydrates and lipids metabolism can arise due to the lack of insulin, an anabolic hormone (Colberg, Sigal, & Yardley, 2016; Li *et al*., 2013). Diabetes can be classified as Type 1 diabetes mellitus (T1DM), Type 2 diabetes mellitus (T2DM), gestational diabetes mellitus (GDM), or specific types because of other causes (Colberg, Sigal, & Yardley, 2016). The management of diabetes revolves around controlling blood glucose levels and preventing associated complications. Phytocompounds have gained significant attention for their potential antihyperglycemic effects (Iwuji, Okafor, & Okey-Mbata, 2020; Iwuji *et al*., 2014).

Disturbance of glucose metabolism in conditions of diabetes is accompanied by changes in various body organs, including the blood (Zhang *et al*., 2016; Iwuji et al., 2014). Under conditions of diabetes, a large amount of glucose accumulates and is intensively utilized by insulin-free tissue cells via glycolysis and in the Krebs cycle (Galicia-Garcia *et al*., 2020).

The management of diabetes mellitus has been possible using pharmaceutical drugs. However, the key to strict glycemic control with use of exogenous insulin lies in the creation of delivery methods that emulate physiologic insulin secretion (Chatterjee, Khunti, & Davies, 2017; Sarwan *et al*., 2010). Alternative therapies such as herbal treatment, dietary supplements, hydrotherapy, and yoga therapies are less likely to have the side effects of conventional approaches for diabetes (Awanish *et al*., 2011). Long term diabetes is associated with several morbities such as blindness, poor wound healing, erectile dysfunction, and failure of other organs as time passes (Iwuji, Okafor, & Okey_mbata, 2020; Asrat *et al*., 2019).

Currently available drugs for diabetes have several limitations, including adverse effects and high rates of secondary failure (Wong *et al*., 2016). This situation has led to the search for alternative therapies, such as herbal medicines with improved therapeutic effects (Iwuji *et al*., 2021; Yanardag *et a*l., 2005).

Medicinal plants (such as *Mangiferin indica* and *Annona muricata*) play a major role in the introduction of new therapeutic agents and have received much attention as sources of biologically active substances (Dabelea *et al*., 2009; Iwuji *et al*., 2013). Also, numerous bioactive compounds and phytochemicals have been reported to be present in *Annona muricata* (Soursop) (Ogbonna et al, 2023; McKeigue, Shah, & Marmot, 1991). Edeoga *et al*. (2005) reported the presence of alkaloids, flavonoids, and phenols in high quantities, especially in fruit pulp and leaf.

Mangifera indica (Mango) belongs to the family *Anacardiaceae* and has been said to be a vital traditionally significant and one of the most economically important tropical fruit crops globally (Agu *et al*., 2017). Nigeria is known as one of the largest mangos producing countries. Mango leaves (MLs) have been used in traditional medicine to treat hyperglycemia, diarrhea, bronchitis, etc. (Barreto *et a*l., 2008). Leaves of the mango plant have been studied for their health benefits, which are attributed to a plethora of phytochemicals. Fruit acidity of mango is attributed mainly to the content citric and malic acids. Several epidemiological studies have proved the activities of these phytochemicals against chronic diseases like diabetes, cancers, cardiovascular and neurodegenerative diseases (Kulkarni & Rathod, 2014). The phytochemical element of interest from MLs in this study is succinic acid gotten from the saponin fraction of the ethanolic extract of *Mangifera indica* leaves (Rasouli, Furzaei & Khodarahmi, 2017).

Ana *et al. (*2018) the efficacy of Succinic acid from Mango leaves in treating diabetic neuropathies in geriatric patients with type 2 diabetes and found that it plays a positive role in management of elderly patients with type 2 diabetes. Saleem *et al*. (2019) evaluated the anti-diabetic potential of MLs extract. The authors found that the administration of MLs extract in chemically induced diabetic mice reduced the postprandial glucose level, prevented the surge of glucose in the blood, and improved the lipid profile along with body weight.

Bhuvaneshwari *et al*. in 2014 investigated the anti-diabetic activity of tender and mature leaves of totapuri cultivar of mango, and the authors found that tender leaves extract (500 mg/kg) efficiently inhibited the α-amylase with IC50 22.01 µg/mL, while mature leaf extract (500 mg/kg) exhibited the α-glucosidase inhibition with IC50 21.03 µg/mL. Their findings suggest that bioactive compounds from the ML can be effective in reducing the risk of hyperglycemia.

Annona mucarita L. (*Magnoliales: Annonaceae*) commonly called soursop due to the soured and acidic nature of the matured and ripe fruit pulp is a tropical plant known for its edible fruit which has many medicinal usefulness. It is a small, upright evergreen tree growing 5–10 meters in height. *Annona muricata* L. is a species of the *Annonaceae* family that has been widely studied in the last decades due to its therapeutic potential (Saleem *et al*., 2019). In vivo studies of the crude extracts and isolated compounds of A. muricata were shown to possess hypoglycemic activity (Bhuvaneshwari, khan, & Devi, 2014). Numerous bioactive compounds and phytochemicals have been reported to be present.

Ogbonna *et al*. (2023) reported the ethanol extraction of hexamethylcyclotrisiloxane from the flavonoid fraction of *Annona muricata* (soursop leaf) using cold maceration.

A study on the antihyperglycemic activities of *Annona muricata* (Linn) by David *et al* (2008) showed that daily intraperitoneal administration of 100mg/kg of extracts of A. muricata to diabetic rats for 15 consecutive days caused a statistically significant increase in the body weight of diabetic animals despite the decrease in food and fluid intake observed in these animals.

Arroyo *et al*. (2009) conducted a randomized, parallel grouped, double blind phase II clinical trial, in patients with type 2 diabetes mellitus. Groups of patients were given 1, 2 or 3 capsules of ethanol extract from A. muricata leaves (180 mg) plus 5 mg of glibenclamide for 30 days, and another group only received glibenclamide. The results of this study showed a decrease in the blood glucose or glycemia level in patients receiving extract of *A. muricata* compared to patients who did not receive it.

There is need therefore to assess and compare the antihyperglycaemic activities of the single and combined phytocompounds (Succinic acid and hexamethylcyclotrisiloxane) isolated from *Mangifera indica* (Mango) and *Annona muricata* (soursop) leaves by Ogbonna *et al*. (2023).

MATERIALS AND METHODS

Plant Specimen and Preparation

The plant materials used were: *Mangifera indica* (Mango leaf) and *Annona muricata* (Soursop leaf). 200g each of leaf samples were gathered from the Botanical Garden at the Crop Science Department of the Federal University of Technology Owerri. A qualified Botanist identified the leaves, which were subsequently washed, air-dried at room temperature for 4 weeks, and then pulverized into powder using an electric blender. To prevent moisture and contamination, the powdered samples were stored in airtight ziplock bags (Ogbonna *et al*., 2023).

Extraction of Phytochemicals, Analysis, Isolation and Storage

Phytochemicals were extracted from the samples using 95% ethanol at a ratio of 1:6 (w/v) of sample to solvent. The crude ethanolic extraction process was employed, and the resulting extract was stored in a properly labeled, airtight specimen bottle. The powdered material was soaked at room temperature for 3 days, periodically agitated to ensure complete extraction, and then filtered using a folded muslin cloth. The obtained extract was concentrated under vacuum using a rotary evaporator, with the heating bath set at 45°C, analysis and isolation of crude extracts was carried out. Then the sourced phytochemicals were stored in the refrigerator (Ogbonna *et al*., 2023).

Animal Handling and Experimental design

The animals used were forty two(42) albino Wistar rats (Rattus norvegicus), weighing between 100 -150g. Laboratory cages at ambient temperatures (25 $\pm 2^0$C) and humidity (50-60%) were used to house them. They were maintained on a 12: 12-hour light/dark cycle in the animal house facility for a period of two (2) weeks acclimatization to the experimental condition. The animals were fed with rat feed (top feed Nig. Limited) and allowed access to water. They were randomly divided into seven (7) Groups consisting of six (6) animals per cage. The first three (3) groups were the control groups, while the other groups served as the test(treated) Groups.

Relevant approval for the animal experimental study was obtained from the institution.

Induction of Diabetes

The animals were fasted for 24 hours, and diabetes was induced by a single intraperitoneal injection of freshly prepared solution of alloxane. The dose of alloxane was 140mg/kg bodyweight.

The animals were given glucose solution in drinking water immediately after injection of alloxane to overcome drug induced hypoglycemia. The induction of diabetes was confirmed by collecting blood sample of animals through tail tipping method. After 24-48 hours, animals with fasting blood glucose levels above 160mg/dl were considered diabetic.

Table 1: Treatment regimen.

Group	No of animals per group	Treatment and dosage given
1	6	Normal Control: fed on rat feed and water only.
2	6	Untreated Control: induced with diabetes but not treated.
3	6	Standard Treatment Control: induced with diabetes and treated with Metformin (500mg/kg b.w).
4	6	Treated with Succinic acid and Hexamethylcyclotrisiloxane (1:1, 100 mg/kg b.w).
5	6	Treated with Succinic acid only (100mg/kg bodyweight).
6	6	Treated with hexamethylcyclotrisiloxane only (100 mg/kg b.w)
7	6	Treated with Succinic acid and hexamethylcyclotrisiloxane (1:1, 200 mg/kg b.w).

The treatment regimen was observed for a period of four (4) weeks.

Estimation of Weight Change

The changes in the weight of the animals were calculated and presented as percentage weight change per week using the formula:

$$Wy\text{-}Wx \times 100/ Wx \quad \text{-------- eqt 1}$$

Where Wx = weight at the beginning of the week.
Wy = weight at the end of the week.

Fasting Blood Glucose level (FBGL) Test

Principle

The principle of a fasting blood glucose level test is to measure the concentration of glucose (sugar) in the blood after a period of fasting.

Procedure

- The FBGL of the rats was assessed during this study starting from the pre-Induction to post- Induction of diabetes mellitus and its treatment.

- The rats were weighed using a digital scale and their weights recorded.
- They were then placed on an overnight fast for 12-14 hours with access to water.
- Their Baseline glucose level was checked using a glucometer and tail tipping blood sample collection method and recorded.
- The rats were then induced with alloxane and allowed to stay for about 48 hours after Induction and were given feed and water. After the manifestation of diabetes, treatment commenced according to the animal grouping and treatment plan.
- Subsequently blood samples were collected at intervals(weekly) after every overnight fast to check the glucose level of the rats and ascertain the effect of the treatment given. This was always measured with a glucometer and recorded.

Statistical Analysis

All data were expressed as Mean ± Standard deviation error and analyzed using the Analysis of Variance (ANOVA) at P< 0.05 levels of significance. The analysis was done with the use of SPSS (version 23).

RESULTS

The results of the study on the effects of Succinic acid and Hexamethylcyclotrisiloxane both singly and combined, on the fasting blood glucose levels (using weight as an index) of the experimental rats is presented in Tables 1 and 2. While the percentage glucose change and percentage weight change are shown on tables 3 and 4.

Table 1: The results of the determination and comparison of the individual and combined effects of Succinic acid and hexamethyl cyclotrisiloxane on fasting glucose level in diabetic Wistar rats.

BLOOD GLUCOSE LEVEL (mg/dl)						
	Initial	**Confirm**	**WK1**	**WK2**	**WK3**	**WK4**
GRP 1	94.83±5.04	96.50±2.43	96.00±4.98	95.67±3.39	94.20±2.68	93.16±4.40
GRP 2	91.33±7.99	321.17±108.97*	363.67±74.58*	396.50±71.07*	404.67±68.64*	411.67±64.10*
GRP 3	91.67±6.68	415.83±61.16*	312.17±77.51*	102.50±14.69^{a}	94.17±16.17^{a}	87.67±8.80^{a}
GRP 4	92.83±10.72	295.67±66.51*	249.00±46.15^{*,a}	153.83±38.02^{a}	105.67±13.49^{a}	95.67±12.03^{a}
GRP 5	93.50±8.96	346.17±66.51*	151.50±32.70^{a,b}	113.50±15.82^{a}	108.67±12.55^{a}	97.00±9.01^{a}
GRP6	94.67±5.61	325.00±82.50*	284.33±70.94^{a,d}	203.33±69.77^{*,a}	167.00±57.31^{*,a,b}	100.67±2.94^{a}
GRP 7	97.00±10.95	322.17±67.44*	192.00±68.55^{a,b}	151.17±30.08^{a}	119.17±9.98^{a}	92.33±13.25^{a}

Values expressed as mean ± SD, n = 6.

*=sig. diff. from GRP1 at p<0.05; ^{a}=sig. diff. from GRP2 at p<0.05; ^{b}=sig. diff. from GRP3 at p<0.05; ^{c}=sig. diff. from GRP4 at p<0.05; ^{d}=sig. diff. from GRP5 at p<0.05.

Table 2: The results of the determination and comparison of the individual and combined effects of Succinic acid and Hexamethylcyclotrisiloxane on Weight of Experimental Diabetic Rat.

WEIGHT OF EXPERIMENTAL RATS (g)						
	Initial	**Confirm**	**WK1**	**WK2**	**WK3**	**WK4**
GRP 1	119.5±6.66	121.83±4.58	125.83±3.31	128.00±4.86	132±4.47	131.67±6.92
GRP 2	120.17±7.60	104.83±6.97*	92.16±8.86*	82.00±6.07*	76.17±5.23*	70.33±2.58*
GRP 3	113.33±9.75	97.50±3.51*	93.67±2.58*	93.67±4.55^{*,a}	94.00±3.90^{*,a}	101.67±4.0^{*,a}
GRP 4	119.33±10.01	103.67±7.17*	108.18±6.79^{*,a}	107.17±5.46^{*,a,b}	109.33±5.92^{*,a,b}	113.33±7.94^{*,a,b}
GRP 5	112.00±4.10	101.83±3.12*	101.00±2.97*	106.17±3.97^{*,a,b}	107.16±2.56^{*,a,b}	109.17±7.78^{*,a}
GRP6	111.17±2.93	95.33±7.29*	97.00±4.78*	98.17±2.32^{*,a,b,c}	100.33±3.27^{*,a,c}	103.83±2.93^{*,a}
GRP 7	120.83±5.31	105.5±6.74*	101.67±4.55*	96.50±4.37^{*,a,c,d}	97.00±4.65^{*,a,c}	103.00±5.44^{*,a}

Values expressed as mean ± SD, n = 6.

*=sig. diff. from GRP1 at p<0.05; ^{a}=sig. diff. from GRP2 at p<0.05; ^{b}=sig. diff. from GRP3 at p<0.05; ^{c}=sig. diff. from GRP4 at p<0.05; ^{d}=sig. diff. from GRP5 at p<0.05.

Table 3: Calculated Weekly Percentage Weight Change in treated diabetic rats.

WEEKLY WEIGHT CHANGE (g) IN EXPERIMENTAL DIABETIC WISTAR RATS					
	Confirm (%)	**Wk1 (%)**	**Wk2 (%)**	**Wk3 (%)**	**Wk4 (%)**
Grp1	1.95	3.28	1.73	3.13	-0.25
Grp2	-12.62	-12.09	-11.02	-7.11	-7.67
Grp3	-13.17	-3.93	0.00	0.35	8.16
Grp4	-13.12	4.35	-0.93	2.02	3.67
Grp5	-9.08	-0.79	5.12	0.93	1.88
Grp6	-14.25	1.75	1.20	2.20	3.49
Grp7	-12.69	-3.63	-5.08	0.52	6.19

Table 4: Calculated Percentage Weight Change(%) in the Last Week of Treatment.

PERCENTAGE WEIGHT CHANGE (%)	
Grp 1	8.08
Grp 2	-32.91
Grp 3	4.28
Grp 4	9.32
Grp 5	7.21
Grp 6	8.92
Grp 7	-2.36

DISCUSSION

The process of lowering blood glucose levels involves multiple mechanisms, and the treatments with Succinic acid and Hexamethylcyclotrisiloxane, the bio-active constituents found in Mango and Soursop leaves appeared to have a positive impact on glucose regulation by possibly improving peripheral tissue glucose utilization according to Jung *et al*. (2006).

Single Effects of plant extracts containing Succinic Acid and Hexamethyl Cyclotrisiloxane

These results align with David *et al*. (2008), as similar effects on fasting blood glucose levels (FBGL) after treatment with A. Muricata was observed. Additionally, Alimohammadi *et al*. (2013) reported comparable findings regarding body weight gain when using Nigella sativa extract, likewise Olga *et al*. (2019) after the administration of the red and yellow fruits of Cornelian cherries, after 14 days.

Combined Effects of Succinic acid and Hexamethylcyclotrisiloxane

This study findings are in line with Rossetti *et al*. (2008) who noted a significant reduction in blood glucose levels with metformin usage. In this study, the synergistic effect managed to reduce blood glucose levels to a significantly normal range, similar to the observations of Lattibeauidiere & Alexander (2022) when using Oleic acid and Succinic acid. Although their study did not return blood glucose levels to basal, it demonstrated a slight difference. As blood glucose levels decreased, we also observed an increase in body weight gain within tissues, consistent with the findings of Wodu, Iwuji & Adienbo (2017).

CONCLUSION

This study hypothesizes that the significant reduction in blood glucose levels, weight gain and maintenance after four weeks of treatment may be attributed to the phytochemicals' ability to enhance the utilization of glucose by peripheral tissues. These phytochemicals are believed to include various bioactive constituents such as phenols, terpenoids, alkaloids, flavonoids, saponins, and tannins, which are present in Mango and Soursop leaves, as mentioned by Jung *et al*. (2006). The phytocompounds are safe (Rasouli, Furzaei & Khodarahmi, 2017) and can be possibly used in the management of diabetes mellitus, and its related weight effect; it also suggests that these substances may have a role in mitigating weight loss associated with diabetes.

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