

Hypoglycaemic Activity of Extra Virgin Avocado (*Persea americana*) oil and Extra Virgin Coconut (*Cocos nucifera*) oil on Alloxan-induced Diabetic Rats

ABSTRACT

Diabetes mellitus is a chronic, metabolic disorder characterized by abnormal elevation of blood glucose levels (hyperglycaemia). Despite significant milestones achieved using conventional drugs in managing the disease, there are some drawbacks associated with their use such as drug resistance, adverse drug effects, high cost, and sometimes outright therapeutic failure. The urgent need to bridge this therapeutic lacuna calls for further exploration and investigation of other treatment options. This study therefore aims at evaluating the antidiabetic potentials of virgin avocado oil and virgin coconut oil in alloxan-induced diabetic rats. Animals were divided into 5 groups of five rats each. Group 1 (negative control) received 10 ml/kg/day of normal saline, group 2 (diabetic control) -10 ml/kg/day of normal saline; group 3 received extra virgin avocado oil (1 ml/250 g body weight), group 4 received extra virgin coconut oil (2 ml/kg) bodyweight, and group 5 received 10 mg/kg/day of metformin. Treatments were administered orally for 15 days. Extra virgin avocado oil and extra virgin coconut oil respectively showed significant antidiabetic activities, and may be beneficial for management of diabetes mellitus.

Keywords: Avocado oil, coconut oil, extra virgin, diabetes, alloxan

1. INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder that is characterized by unusually high blood glucose levels known as hyperglycaemia resulting from disruption in carbohydrate, fat and protein metabolism due to defective insulin action, secretion or both [1-3]. The incidence of this disease has been increasing at an alarming rate [4-5]. The two main types of diabetes are type 1 and type 2. Type 1 diabetes which occurs at early stages of life is caused by an autoimmune disorder where the cells of the immune

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system attack the insulin-producing β -cells of the pancreas. Type 2 diabetes on the other hand, develops later ~~later~~ in life, ~~as a result of~~because of malfunctions in metabolic homeostasis. Genetic background, unhealthy eating habits, and sedentary ~~life style~~lifestyle are predisposing factors in type 2 diabetes [6-7]. Globally, 463 million adults were living with diabetes in 2019, and this resulted in about 4.2 million deaths in that year alone. By the year 2045, the number of patients with diabetes is estimated to reach 700 million [1]. Diabetes treatment includes blood sugar monitoring, physical activity, nutritional intervention, self-management education, use of synthetic oral hypoglycaemic drugs, and insulin therapy [8-10]. Despite the significant progress made in the management of Diabetes mellitus, therapeutic outcomes are still unpredictable due to drug resistance, adverse drug effects, and toxicity [11-12]. Toxicity of oral hypoglycaemic agents differs immensely in clinical manifestations and severity [13].

Many plants have been credited to be useful for the management of diabetes, and a reasonably high population of rural dwellers have been depending on medicinal plants for treatment of diabetes due to their affordability and availability [14]. Virgin coconut oil (VCO) is obtained from the fresh mature kernel of the tropical plant, coconut fruit (*Cocos nucifera* L.), by natural or mechanical method without harsh processing such as chemical bleaching, hydrogenation, ~~deodorization~~deodorization, or refining [15-17]. In addition to the above, extra-virgin coconut oil is ~~cold-expressed~~cold expressed (extracted under room temperature), hence the natural content of the oil is not altered. It has been reported that coconut oil has antioxidant and anti-inflammatory properties [18-19]. The oil also been reported to have antiviral, antibacterial, antifungal, antiparasitic, antithrombotic, cardioprotective, hepatoprotective, antidote, antidermatophytic, insect repellent, ~~hypolipidaemic~~hypolipidemic, ~~anticholecystitic~~anticholestatic, anticancer, anticaries, disinfectant, and anti-asthmatic effects [24-25]. Coconut oil contains fatty acids, mostly saturated fatty acid (medium chain fatty acid) and less unsaturated fatty acid. The various fatty acid constituents of coconut oil include lauric acid (50%), myristic acid, caprylic acid, palmitic acid, capric acid, stearic acid, oleic acid, and linoleic acid [17]. The peroxide value of coconut oil was reported to be even lower than that of fresh palm oil, and this was suggested to be the reason for its increased oxidative stability

[20]. The peroxide value of an oil is an indication of its oxidation propensity, and perhaps to serve as a guide on the safety of that ~~particular edible~~ oil [21]. In addition to the peroxide value, high phenolic acid content also contributes to a reduction in the oxidation process [22]. Coconut oil has been reported to contain polyphenols that may significantly reduce lipid peroxidation [23].

Avocado oil is obtained from the pulp of the avocado fruit (*Persea americana* Mill.). It is a fruit that is native to Central America. Today, it is cultivated and harvested worldwide, growing mainly in warm temperate and subtropical climates [26-27]. Extra-virgin avocado oil is obtained from the avocado fruit, mostly by cold pressing, and without undergoing alterations in its nature by the addition of chemicals or subsequent processing [32]. It is viscous and dark green in colour (due to chlorophylls and carotenoids contents), ~~and~~ ~~and~~ has a mild taste [32]. Avocado oil is rich in monounsaturated fatty acids with oleic acid content being the highest (50.95 %). Other fatty acid constituent includes palmitic acid (28.21 %), linoleic acid (13.87 %), palmitoleic acid (5.69 %), stearic acid (0.69 %), and linolenic acid (0.58 %) [28]. The oil contains bioactive compounds such as phytosterols (β -sitosterol), carotenoids (e.g., tocopherols, tocotrienols) as well as polyphenols, lecithin, minerals, and vitamins, making it of particular interest for research aimed at the prevention and mitigation of several disease conditions [18, 26]. Avocado oil has been reported to have anti-inflammatory [29], antihypertensive [30], and antioxidant [31] potentials. This study aims at evaluating the hypoglycaemic activity of virgin avocado oil and virgin coconut oil in alloxan-induced diabetic rodent ~~model~~.

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2. METHODS

2.1 Preparation of Extra Virgin Coconut oil

Five (5) mature coconut fruits were ~~cracked~~cracked, and the fleshly edible endosperm removed, chopped into pieces and blended to obtain a milky slurry. The slurry was

strained using sieve cloth to obtain coconut milk which was allowed to stand for about 16 hours. The top paste was skimmed ~~off, and~~ ~~off and~~ kept frozen in a freezer for 24 hours. Thereafter, it was removed from the refrigerator, allowed to defrost, the oil settling out at the top carefully separated, and filtered into an air-tight container.

2.2. Preparation of Extra Virgin Avocado oil

Ten (10) mature, high-quality avocado fruits were bought from Uyo market, in Uyo Local Government Area of Akwa Ibom State, Nigeria. They were washed and kept until soft.

The pulp was removed, ~~mashed~~ ~~mashed~~, and spread on a large surface open container kept under room temperature. During this period, the pulpy mash was turned occasionally for faster drying. After about 4 days, droplets of the oil were seen on the surface (this shows its ready for extraction). The oil was squeezed from the paste using sieve cloth and then filtered to remove unwanted particles.

2.3. Experimental Animals

A total of 25 rats of both sexes, weighing (100-140g) were obtained from the animal house of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Uyo. They were maintained on standard animal pellets and water *ad libitum*, housed in cages to acclimatize to the animal house, and maintained under standard conditions (25- 28 °C) with 12h dark/12h light cycles.

2.4. Induction of Diabetes mellitus

The animals were fasted ~~overnight~~ ~~overnight~~, and experimental diabetes was induced by single intra-peritoneal injection of freshly prepared solution of alloxan (150mg/kg bodyweight) in distilled water. Negative control rats were injected with distilled water alone. After 72 hours, rats with ~~hyperglycaemia~~ ~~hyperglycemia~~ (blood glucose level

>200mg/dl were considered as diabetic and used for the treatment [33]. Blood glucose levels were checked using a glucometer (Accu-Chek®, Roche Diagnostics, Germany).

2.5. Experimental Design

Animals were divided into 5 groups consisting of five rats per group. Rats of each group were orally pre-treated as follows:

Group 1 (Negative control): Nondiabetic rats given 10ml /kg/day of normal saline orally for 15 days.

Group 2 (Diabetic control): Diabetic rats given 10ml/kg/day of normal saline orally for 15 days.

Group 3 (Avocado oil): Diabetic rats given extra virgin avocado oil 1ml/250g body weight orally for 15 days [34].

Group 4 (Coconut oil): Diabetic rats given extra virgin coconut oil 2ml/kg bodyweight orally for 15 days [35].

Group 5 (Positive control): Diabetic rats given 10mg/kg/day of metformin orally in aqueous solution for 15 days

Oral administration was carried out with the aid of a rubber cannula attached to a calibrated syringe. Blood glucose level was checked before administration of extracts (0 hour), and at 1_hr, 2_hrs, 4_hrs, 6_hrs, 5_days, 10_days, and 15 days after daily treatments. Blood was obtained from the tail veins of the animals. Animals were weighed using electronic balance on days 0, 5, 10, and 15 of treatment.

2.6 Statistical Analysis

Results were expressed as mean \pm standard error of mean (SEM). Statistical analysis was carried out using SPSS version 23 statistical software. Statistical significance was

determined using one-way Analysis of Variance (ANOVA) followed by Tukey's post hoc test. Values of $p < 0.05$ were considered to be significant.

3. RESULTS

3.1 Effect of Avocado oil and Coconut oil on Body weight of Alloxan-induced Diabetic Rats

The effect of Avocado oil and Coconut oil on body weight of alloxan-induced diabetic rats is shown in table1. After 5 days of experiment, there was no significant difference ($p > 0.05$) in body weight of rats in all the groups except for rats in the groups treated with coconut oil which showed significant ($p < 0.05$) increase in body weight compared to the diabetic control group. On days 10 and 15, rats in the diabetic control group showed significant ($p < 0.05$) decrease in body weight compared to those in the negative control group. The effects of the two oils and Metformin were comparable to each other as they showed significant ($p < 0.05$) increase in body weight relative to those in the diabetic control group, with avocado oil causing the most improvement in the body weight after 15 days.

Table 1. Effect of avocado oil and coconut oil on body weight of alloxan-induced diabetic rats

Group	Dose	Body weight (g)			
		Day 0	Day 5	Day 10	Day 15
Group 1 (Negative control)	10 ml/kg normal saline	121.80±4.20	124.00±4.12	145.60±3.36	142.00±2.21
Group 2 (Diabetic control)	10 ml/kg normal saline	113.60±4.18	114.00±4.15	115.200±3.84 ^a	115.20±4.34 ^a
Group 3 (Avocado oil)	1ml/250g body weight	112.00±3.87	113.00±4.64	134.20±2.64 ^b	144.20±2.65 ^b
Group 4 (Coconut oil)	2ml/kg body weight	108.00±5.33	134.00±1.55 ^b	132.00±3.29 ^b	138.00±3.11 ^b
Group 5 (Positive control)	Omeprazole 10mg/kg	106.20±3.93	116.60±6.91	134.20±5.00 ^b	135.40±4.54 ^b

Data are expressed as mean ± SEM. Significant at ^ap<0.05 when compared to negative control; ^bp<0.05 when compared to diabetic control (n=5).

3.2 Antidiabetic Effect of Avocado oil and Coconut oil on Alloxan-induced Diabetic Rats in Acute Study

The antidiabetic effect of avocado oil and coconut oil on alloxan-induced diabetic rats in acute study is as shown in table 2, and figure 1. Avocado oil significantly ($p < 0.05$) reduced the mean blood glucose level when compared to the diabetic control starting from the 2nd hour and continuing to the 6th. Coconut oil also reduced blood glucose level when compared to the diabetic ~~control~~ [control](#), but the effect started after 4 hours and continued to the 6th hour under evaluation. The antidiabetic effects of the two oils (particularly avocado oil) were comparable to that of the standard drug, Metformin.

Table 2. Antidiabetic effect of avocado oil and coconut oil on alloxan-induced diabetic rats in acute study

Group	Dose	Mean blood glucose level (mg/dl)				
		0hr	1hr	2hr	4hr	6hr
Group 1 (Negative control)	10 ml/kg normal saline	99.40±0.75	97.60±0.	96.40±0.93	95.20±1.07	94.20±1.53
Group 2 (Diabetic control)	10 ml/kg normal saline	326.40±12.97 ^a	374.20±10.59 ^a	374.20±21.78 ^a	395.40±28.90 ^a	417.20±18.39 ^a
Group 3 (Avocado oil)	1ml/250g body weight	344.60±7.97 ^a	312.40±19.28 ^a	284.00±15.24 ^{a, b}	256.20±11.05 ^{a, b}	245.00±7.92 ^{a, b}
Group 4 (Coconut oil)	2ml/kg body weight	355.00±16.79 ^a	359.00±3.79 ^a	340.00±12.72 ^{a, c}	275.00±8.34 ^{a, b}	261.20±8.97 ^{a, b}
Group 5 (Positive control)	omeprazole 10mg/kg	296.28±13.12 ^a	362.40±27.43 ^a	270.60±11.07 ^{a, b}	247.40±2.36 ^{a, b}	233.20±4.32 ^{a, b}

Data are expressed as mean ± SEM. Significant at ^ap<0.05 when compared to negative control; ^bp<0.05 when compared to diabetic control; ^cp<0.05 when compared to metformin (n=5)

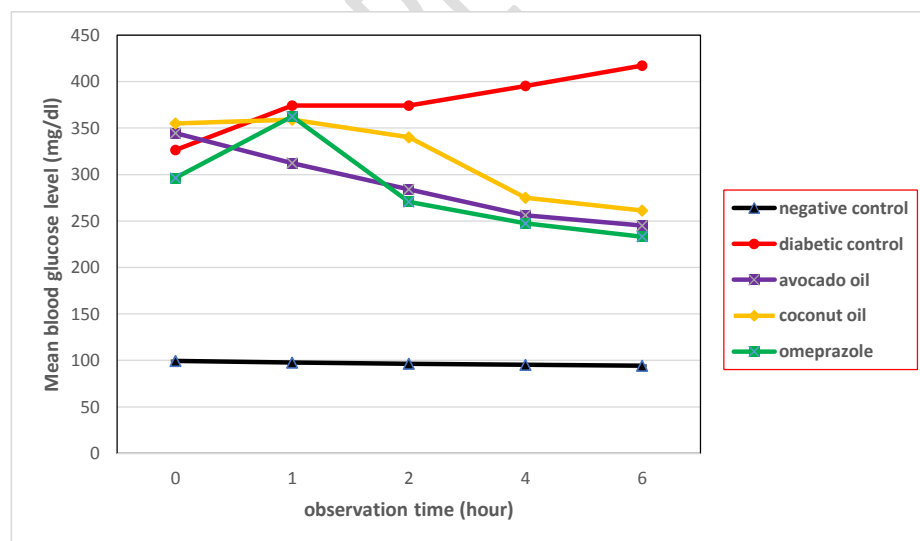


Fig. 1: Antidiabetic effect of avocado oil and coconut oil on alloxan-induced diabetic rats in acute study

3.3 Antidiabetic Effect of Avocado oil and Coconut oil on Alloxan-induced Diabetic Rats in Prolonged Study

The antidiabetic effect of avocado oil and coconut oil on alloxan-induced diabetic rats in prolonged study is as shown in table 3 and figure 2. Avocado oil significantly ($p < 0.05$) sustained the reduction in blood glucose level throughout the 15-day period of the experiment (155.60 ± 9.16 , 110.20 ± 1.83 and 98.60 ± 0.93 for days 5, 10 and 15 respectively) when compared to the diabetic control values of 418.80 ± 2.20 , 404.20 ± 6.15 and 341.20 ± 10.08 for the same period. On its part, coconut oil also significantly ($p < 0.05$) reduced blood glucose level in the prolonged study (174.40 ± 4.18 , 108.60 ± 3.56 and 97.60 ± 0.93 for days 5, 10 and 15 respectively) when compared to the values of diabetic control for the same interval. There were no significant differences between the blood glucose levels of rats treated with avocado oil, coconut oil and Metformin, they were comparable at the respective intervals of 5, 10 and 15 days. It is worthy of note that after 15 days of treatment, blood glucose levels of avocado oil, coconut oil and Metformin-treated rats were respectively reduced to approximately the same level as that of negative control.

Table 3. Antidiabetic effect of avocado oil and coconut oil on alloxan-induced diabetic rats in prolonged study

Group	Dose	Mean blood glucose level (mg/dl)			
		DAY0	DAY5	DAY10	DAY15
Group 1 (Negative control)	10 ml/kg normal saline	99.40±0.75	94.20±1.53	95.20±1.12	95.20±0.86
Group 2 (Diabetic control)	10 ml/kg normal saline	326.40±12.97 ^a	418.80±2.20 ^a	404.20±6.15 ^a	341.20±10.08 ^a

Group 3 (Avocado oil)	1ml/250g body weight	344.60±7.97 ^a	155.60±9.16 ^b	110.20±1.83 ^b	98.60±0.93 ^b
Group 4 (Coconut oil)	2ml/kg body weight	355.00±16.79 ^a	174.40±4.18 ^b	108.60±3.56 ^b	97.60±0.93 ^b
Group 5 (Positive control)	omeprazole 10mg/kg	296.28±13.12 ^a	160.00±8.1 ^b	106.60±4.08 ^b	96.60±4.40 ^b

Data are expressed as mean ± SEM. Significant at ^ap<0.05 when compared to negative control; ^bp<0.05 when compared to diabetic control (n=5).

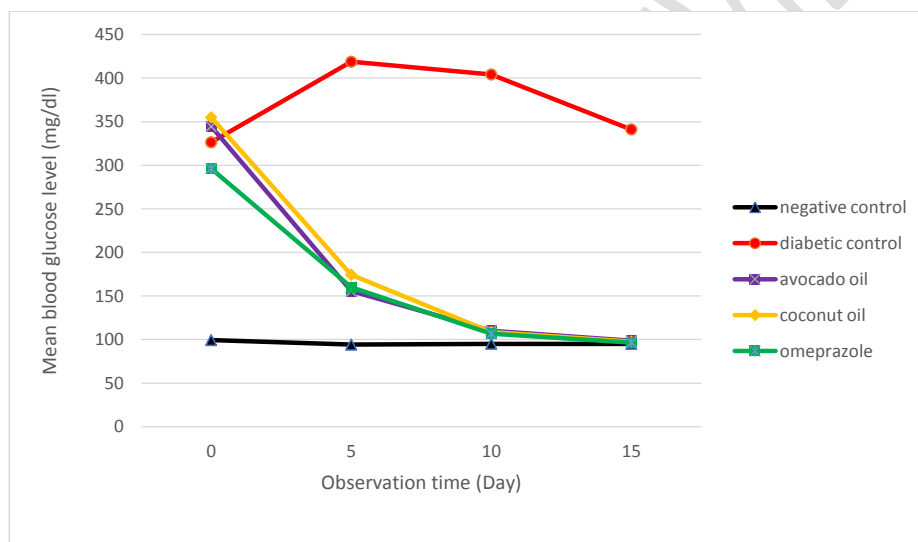


Fig. 2: Antidiabetic effect of avocado oil and coconut oil on alloxan-induced diabetic rats in prolonged study

4. DISCUSSION

Diabetes mellitus is not merely a disease of blood level elevation. In most cases, it involves a plethora of complications in peripheral tissues such as the kidneys, liver, cardiovascular system, retina, and the nervous system. Current therapies are not

always effective in completely combating this malady [1], hence the urgent need to explore alternative treatments such as the use of plant-derived substances. Alloxan (2,4, 5, 6-pyrimidinetetrone) is a popular diabetogenic agent commonly used to induce type 2 Diabetes mellitus in animal models [36]. Alloxan and its reduction product, dialuric acid form superoxide radicals which undergo dismutation to hydrogen peroxide. Simultaneously, there is a massive increase in cytosolic calcium concentration which causes rapid destruction of β cells of the pancreas, resulting in decreased insulin secretion [37]. From the result, administration of alloxan caused hyperglycaemia in the diabetic control group when compared to rats in the negative control group given normal saline instead of alloxan. Generally, body weights are decreased in diabetic animals [38]. In this study, the decrease in body weight was diminished by both avocado and coconut oils, and their effects were comparable to that of the standard drug, metformin. This effect may be beneficial to the diabetic animals. Medium chain fatty acids and triglycerides present in coconut oil metabolizes fast and may help in stimulating weight loss in diabetic patients with obesity [16].

In this research, coconut oil reduced the blood glucose levels of alloxan-induced diabetic rats in both acute and prolonged studies. This may be due to the presence of bioactive constituents present in the oil. Coconut has been reported to contain bioactive, antioxidant constituents which include ferulic, *p*-coumaric, vallinic, and protocatechuic acids suggested to have several beneficial health effects [39-40]. In diabetic patients with hypoglycaemia, antioxidants may play a vital role in enhancing insulin [sensitivity](#), [or sensitivity](#) or may reduce insulin resistance and injury to pancreatic beta cells by scavenging reactive oxygen species (ROS) [16]. Lauric acid, present in coconut oil has also been reported to possess insulinotropic properties [41]. Also in this experiment, avocado oil has reduced the blood glucose levels of alloxan-induced diabetic rats in both acute and prolonged studies. Torres and co-workers [42] have reported the presence of phytochemicals such as alkaloids, saponins, unsaturated steroids and triterpenoids (Leucoanthocyanins) in the ethanolic extract of avocado fruit. Tocopherols have also been identified in the acetone extract of avocado [43]. Avocado oil retains most of the bioactive substances and carotenoids present in the fruit [44]. The

antidiabetic activity of avocado oil seen in this study may be attributable to these bioactive constituents present in the oil.

5. CONCLUSION

In conclusion, extra virgin avocado oil and extra virgin coconut oil respectively showed significant hypoglycaemic ~~activity, and activity and~~ may be beneficial in management of diabetes mellitus. However, further studies should be carried out at the cellular and molecular levels to unravel the exact mechanism(s) of action.

CONSENT

It is not applicable

ETHICAL APPROVAL

The procedures were performed according to the guidelines on the use of ~~animals,~~ ~~and animals and~~ approved by the Institutional Animal Ethical Committee of the Faculty of Pharmacy, University of Uyo, Nigeria (Ethical Approval No: FPharm/EC/011).

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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Comment [A3]: Add same pattern of articles in each reference. Do for all reference.

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