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Antilipidemic Effect of Ethanolic Extract of *Aloe barbadensis* Gel, *Cymbopogon citratus* Leaves and Its Combination on Alloxan Induced Diabetic Rats

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

This research sought to investigate the antilipidemic effect of ethanolic extract of *Aloe barbadensis*, *Cymbopogon citratus* leaves and its equal combination on induced-alloxan diabetic rat. Plants like *Aloe barbadensis* and *Cymbopogon citratus* are commonly used as home remedies for some

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diseases such as diabetes thereby making them alternative medicines. Ethanolic extract of both plants was extracted using 70% ethanol. The effect of glibenclamide, ethanolic extract of *A. barbadensis* gel, *C. citratus* leaves and its combination after 21 days experimental study showed a significantly increased (P<0.05) high density lipoprotein (HDL) in the induced rats when in comparison with the normal control and diabetic untreated rats. The low density lipoprotein (LDL) significantly showed a (P<0.05) decrease in the rats treated with *A. barbadensis* gel, *C. citratus* leaves and its combined extract compared with those treated with standard drug. The serum total cholesterol (TCHOL), triglyceride (TG) and very low density lipoprotein (VLDL) significantly showed a (P<0.05) decrease in the rats treated with *A. barbadensis* gel, *C. citratus* leaves and its combined extract compared with diabetic untreated rats. This research revealed that ethanolic extract of *A. barbadensis*, *C. citratus* and its combination has antilipidemic effects and holds potential as an alternative therapy for its management.

Keywords: Aloe barbadensis; Cymbopogon citratus; hyperlipidemia; Albino rats; Alloxan.

1. INTRODUCTION

Diabetes is an epidemic endocrine metabolic disorder. It is distinguished by incessant hyperglycemia and hyperlipidemia as a result of interference in carbohyrate and lipid metabolism, a pathological process that may include deficiency in insulin action or/and insulin secretion [1,2]. Type 2 diabetes is the most prevalent and makes up approximately 90% of *Diabetes mellitus* cases. It's primary cause is the body's inability to respond to insulin absorption or sufficient production [3,4]. Diabetes has been recognized as a growing problem in the African region and worldwide at large. According to the World Health Organisation (WHO), 1.6 million deaths are associated with diabetes and the bulk of those affected are the middle-class populations in Asia, Africa and South America [5]. One in twenty-two adults (24 million) in Africa have diabetes and it is estimated to increase by 129% to 55million by 2045 [6]. According to Shi and Hu [7], 1.7% of the Nigerian population is affected with Diabetes mellitus and World Health Organisation estimates that diabetes would rank 7th in the among all causes of death by 2030 [8].

Aloe barbadensis sometimes known as aloe vera is a succulent plant species of genus Aloe that is native to Arabian Peninsula. However, it thrives wild in tropical, semi-tropical and arid climates around the world which explains its widespread distribution. It is a herbaceous or woody plant with huge, thick, fleshy leaves with a sharp tip and a spiky edge that are stemless [9]. Owning to its unique phytoconstituents and special functions, Aloe has garnered numerous accolades like sobriquets namely “The champion among health care medicines” “The best health food in the 21st century” as well as “The new star in plant”. It is also referred to as “All day service” and “Doctor away” in Japan [10]. Aloe vera contains 75 nutrients and more than 200 active compounds like active enzymes, mineral, lignin, sugars, saponins, salicylic, vitamins, amino acids and anthraquinones such as Aloe bitter, Aloe lectin, Aloin and Aloe emodin [11].

Cymbopogon citratus also known as lemon grass, Malabar grass or barbed wire grass is a tall, aromatic perennial plant with slender shape-edge, green, long, glaucoces, linear tapering upwards green leaves which can grow up to 90cm in height and 5m in width, pointed apex, native to Asia [12]. Lemon grass extracts contains several chemical components in its essential oil and aqueous extract [13]. It is also known for its antibacterial, antifungal, anti-inflammatory, antimycobacterial, antidiarrheal, antioxidant, hypolipidemic, hypocholesterolimic and hypoglycemic medical properties [14].

Aloe vera as shown in numerous studies to have antilipidemic impact in animal models [15,16] but limited research has been carried out on its effect with lemon grass and the combination of both plants.

2. MATERIALS AND METHODS

2.1 Experimental Animals

Adult albino rats weighing (150g ± 5g) were sourced from Chris's Experimental Animal Farm and Research Laboratory Awka, Anambra State. The rats were kept in steel cages at room temperature. This was preceded by one (1) week of acclimatization before the start of the experiment and the rats were fed ad libitum with vital growers’ chick mash pellets and clean water for the duration of the experimental period.

2.2 Glibenclamide Preparation

The drug Glibenclamide was procured from Ned King Pharmacy, Awka. A five (5) gram glibenclamide tablet was mashed and stirred in 20 mls of distilled water. For forty minutes, the mixture was kept in an ultrasonic water bath to achieve homogeneity [17]. The suspension was administered to the Group 3 of the induced-alloxan diabetic rats at a dose of 200mg/kg. Fresh preparation of this uncoated suspension was carried out on a daily basis and remnant was discarded when not completely used.

2.3 Preparation of *Aloe barbadensis* Gel Extract

Fresh *Aloe barbadensis* leaves were washed thoroughly with clean running water. Using a sterile knife, the washed leaves were cut lengthwise to extract the translucent gel from the thick epidermis layers. The extracted gel was homogenized using a Sonik Japan SB-735 electric blender. The homogenized mixture was weighed, combined with 70% ethanol and mixed with spatula in a plastic container. The mixture was continually stirred at three (3) hours interval for complete extraction of the phytoconstituents and let to stand for 48 hours. To facilitate the reduction of the ethanolic content, the ethanolic mixture was sieved, filtered using with Whatman's filter paper, put on a flat steel pan and allowed to evaporate in a water bath at 60°C. The collected crude extract was preserved in a refrigerator before the commencement of administration [18] with little modifications.

2.4 Preparation of *Cymbopogon citratus* Leaves Extract

Green *Cymbopogon citratus* leaves were washed, chopped into smaller pieces, and air-dried. Subsequently, the leaves were blended using grinding machine into gritty powder. The coarse powder was measured and mixed with 300ml of 70% ethanol. This mixture was allowed to stand for 48hrs and vigorously stirred every three (3) hours at intervals. The mixture was sieved using muslin cloth and the filtrate was then put in a water bath set at 60°C after being filtered through Whatman's filter paper. The crude extract obtained was collected in an air-tight container and refrigerated before the experiment [19].

2.5 Induction of Diabetes to the Experimental Animals

Alloxan is a urea derivative, cytotoxic glucose analog organic compound chemically synonym to 5, 5-dihydroxyl pyrimidine-2, 4, 6-trione with molecular formulae $C_4H_2N_2O_4$ [20]. Among other known diabetogenic agents such as monosodium glutamate, dithizone, anti-insulin serum agent, high glucose load. One of the most commonly used diabetogenic agents for diabetic studies due to its affordability and availability is alloxan [21]. Alloxan monohydrate used for this experiment was purchased from Sigma Aldrich Missouri, USA. Alloxan suspension was prepared with distilled water and injected intraperitonally using insulin syringe to induce diabetes mellitus at 130mg/kgBW to the experimental albino rats with normal fasted blood glucose levels. In order to lower the risk of hypoglycemia due to destruction of beta cells of the pancreas causing unavailability of blood glucose arising from alloxan induction, 50g of dextrose was dissolved in 1000 millilitres of clean water and given to the induced animals. The animals were tested for diabetes after 3days of induction. An animal with blood glucose level of above 200 mg/dl was termed diabetic [22].

2.6 Experimental Design

After the acclimatization period, the experimental animal's initial weight were recorded and randomly divided into 12 groups. Each of the group had three (3) replicates with 15 albino rats in each. Groups 1 and 2 were the positive and negative controls respectively. Groups 3, 4, 5, 6, 7, 8, 9, 10, 11, and 12 were the treatment groups. The group details are shown below;

Group	Dosage
1	No alloxan induced, no treatment (Positive group)
2	Alloxan induced, no treatment (Negative group)
3	Alloxan induced, treated with glibenclamide
4	Alloxan + 100 of L G extract
5	Alloxan + 200 of L G extract
6	Alloxan + 300 of L G extract
7	Alloxan + 100 of A V extract
8	Alloxan + 200 of A V extract
9	Alloxan + 300 of A V extract
10	Alloxan + 50 of (A V * L G extract)
11	Alloxan + 100 of (A V* L G extract)
12	Alloxan + 150 of (A V* L G extract) In mg/kg

2.7 Lethal Dose Test

According to Nghojuyi et al. [23], the lethal dose of *Aloe barbadensis* extract is reported to be >5000mg/kg. The lethal dose of *Cymbopogon citratus* is been reported to be greater than 5000mg/kg [24]. The lethal dose of equivalent combination of *A*. *barbadensis* and *C*. *citratus* extract is been reported to be >5000mg/kg [25].

2.8 Biochemical Assays

Triglycerides, High Density Lipoprotein (HDL) and Total Cholesterol were measured using Randox test kit and spectrophotometer after collecting blood samples using syringe into anticoagulant bottles which was centrifuged and the serum used for analysis in accordance the manufacturer's instructions [26]. The formula below was used to compute the Very Low Density Lipoprotein (VLDL) and Low Density Lipoprotein (LDL) respectively [27].

VLDL= TG/5

LDL= Total Cholesterol - (HDL + VLDL)

2.9 Statistical Analysis

Using the Statistical Package for Social Science software for windows version 25, data on the lipid profile levels were reported as Mean ± SEM and compared among groups with One Way ANOVA Tests at (P<0.05) significant level.

3. RESULTS

The result revealed an increase in High-Density Lipoprotein level in groups administered both individual and combined ethanolic extract as shown in Table 1 and Figs. 1, 2, 3, 4, 5. This marked increase in the HDL concentration shows an increase significantly (*P*<0.05) in the albino rats administered with standard drug (glibenclamide), ethanolic extracts of *A*. *vera*, *C*. *citratus* and its combination when compared with the positive and negative controls. There was a decrease in the groups of rat treated with 300mg/kgBW of both the individual and combined ethanolic extracts when compared with other dosages of extract and this noted decrease was significant (*P*<0.05) when compared with groups treated with glibenclamide and negative control.

The LDL of the negative control group significantly (*P*<0.05) increased when compared with normal non-diabetic rats. It also showed a significantly (*P*<0.05) decreased LDL in the group of animals treated with individual and combined extract respectively when compared with those treated with standard drug.

Table 1. Effect of *A*. *barbadensis* gel, *C*. *citratus* leaves and its combined extract on lipid profile levels of induced-alloxan diabetic rats expressed as Mean ± SEM

Groups	Lipid profile parameters ± SEM				
	HDL (mg/dl)	LDL (mg/dl)	TCHOL (mg/dl)	TRIG (mg/dl)	VLDL (mg/dl)
1	74.46 ± 31.48*f*	58.74 ± 5.12	67.12 ± 2.45	84.75 ± 3.17	31.57 ± 13.97
2	90.26 ± 5.46*f*	75.52 ± 38.80	103.90 ± 39	234.86 ± 57.95	46.96 ± 11.59
3	126.54 ± 12.26*ac*	105.85 ± 19.54	93.30 ± 47.87	150.75 ± 16.35	30.15 ± 3.28
4	130.04 ± 2.88*ac*	58.71 ± 24.18	85.33 ± 34.32	108.97 ± 10.57	20.94 ± 2.21
5	114.47 ± 6.67*a*	80.31 ± 5.24	61.54 ± 9.84	130.12 ± 19.97	26.79 ± 3.34
6	92.93 ± 8.14	51.62 ± 13.64	111.17 ± 27.11	93.10 ± 5.05	18.07 ± 1.26
7	129.56 ± 12.94*ac*	84.59 ± 8.29	72.14 ± 4.73	135.86 ± 5.63	27.18 ± 1.13
8	111.82 ± 4.86*a*	75.71 ± 12.50	93.08 ± 21.52	127.77 ± 11.59	25.54 ± 2.31
9	102.81 ± 0.13	48.57 ± 17.74	80.87 ± 18.19	131.91 ± 5.97	26.38 ± 1.19
10	130.64 ± 1.33*ac*	69.28 ± 10.65	124.79 ± 32.99	146.96 ± 28.10	29.38 ± 5.62
11	103.64 ± 15.68	63.47 ± 29.32	79.41 ± 11.52	150.70 ± 48.09	30.14 ± 9.63
12	94.33 ± 5.50	46.33 ± 11.97	65.95 ± 3.11	89.88 ± 18.15	17.97 ± 3.64

[a] Significant increase with respect to normal control; [c] Significant increase with respect to diabetic untreated; [f] Significant decrease with respect to standard drug.

HDL: High Density Lipoprotein; LDL: Low Density Lipoprotein; TCHOL: Total Cholesterol; TRIG: Triglyceride VLDL: Very Low Density Lipoprotein

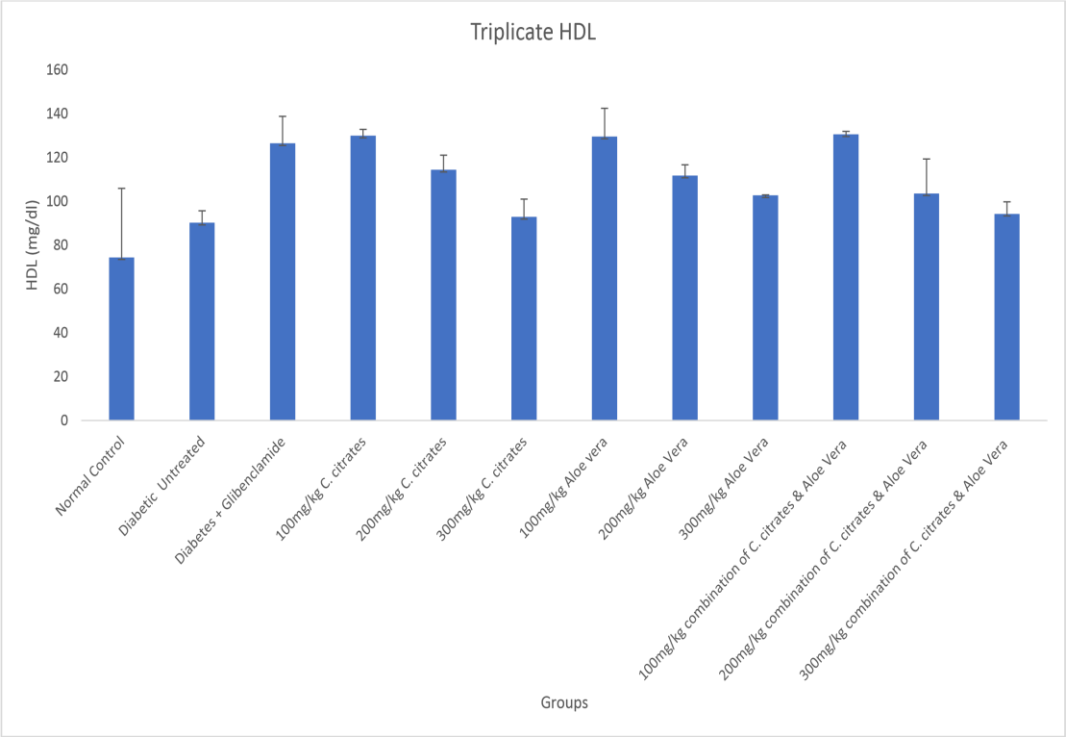

Fig. 1. Effect of *A. barbadensis*, *C. citratus* and its combined extract on HDL level of alloxan-induced diabetic rats

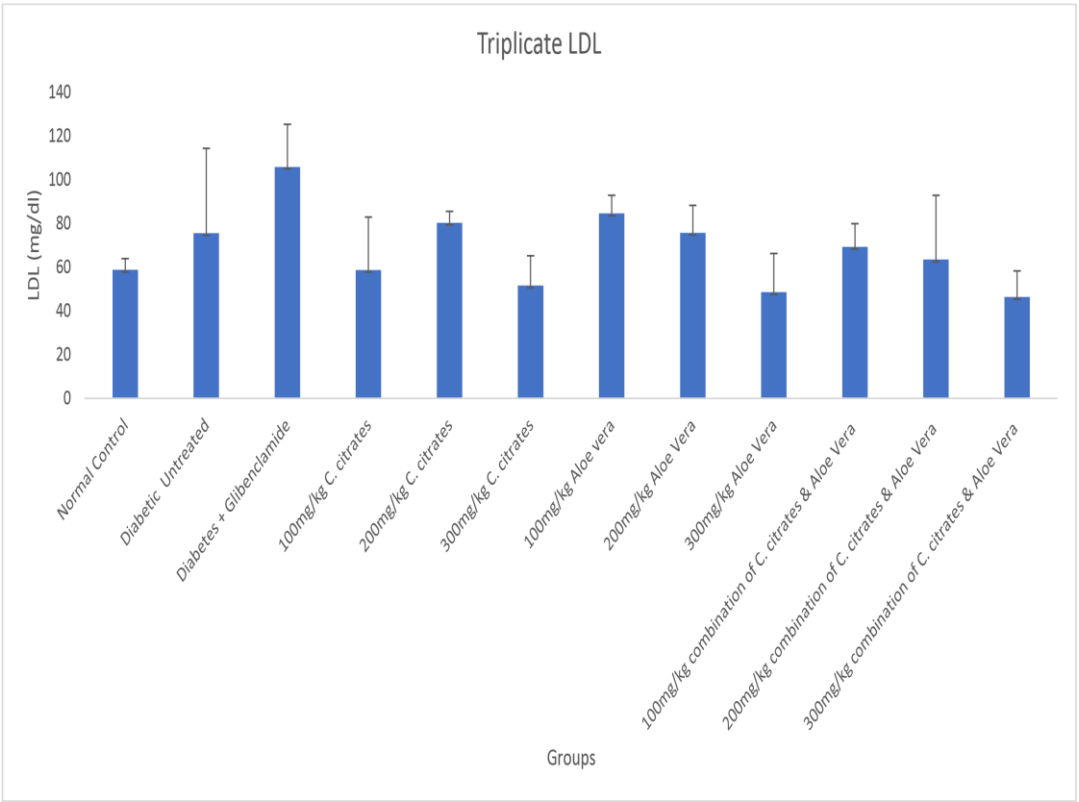

Fig. 2. Effect of *A. barbadensis*, *C. citratus* and its combined extract on LDL level of alloxan-induced diabetic rats

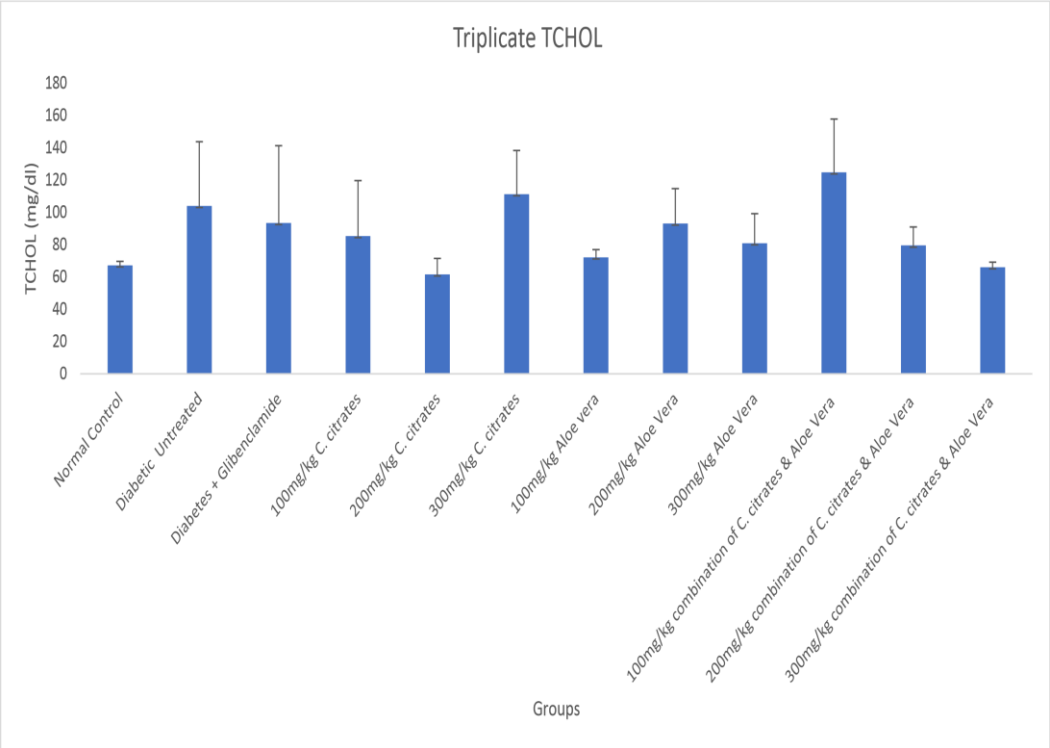

Fig. 3. Effect of *A. barbadensis*, *C. citratus* and its combined extract on TCHOL level of alloxan-induced diabetic rats

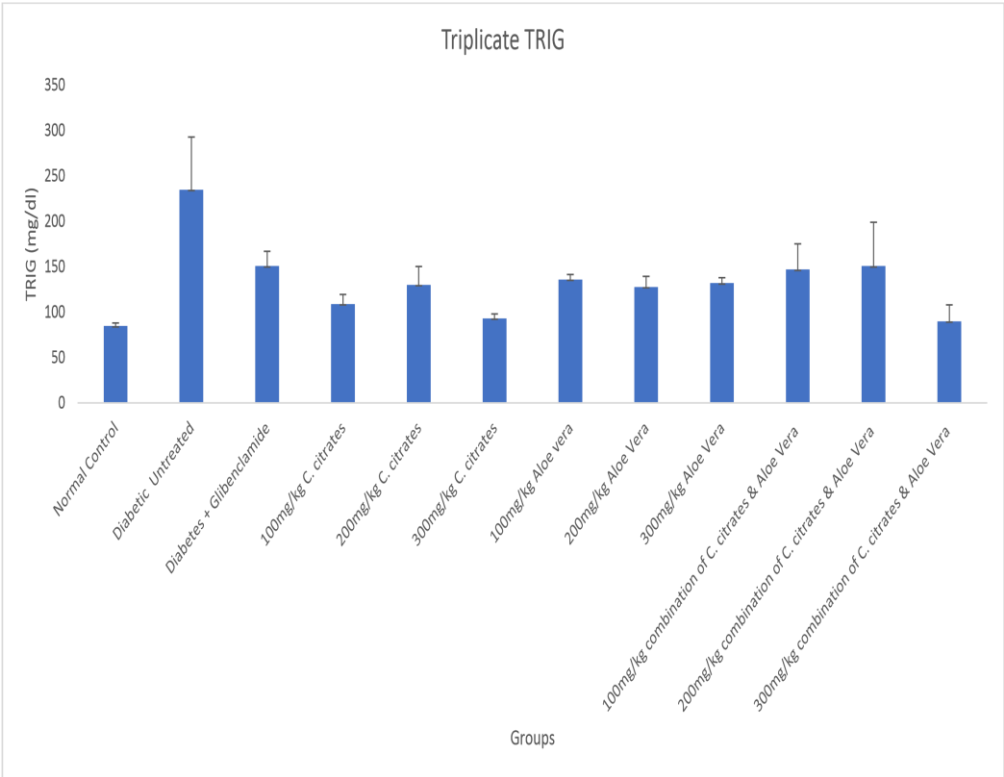

Fig. 4. Effect of *A. barbaensis*, *C. citratus* and its combined extract on TRIG level of alloxan-induced diabetic rats

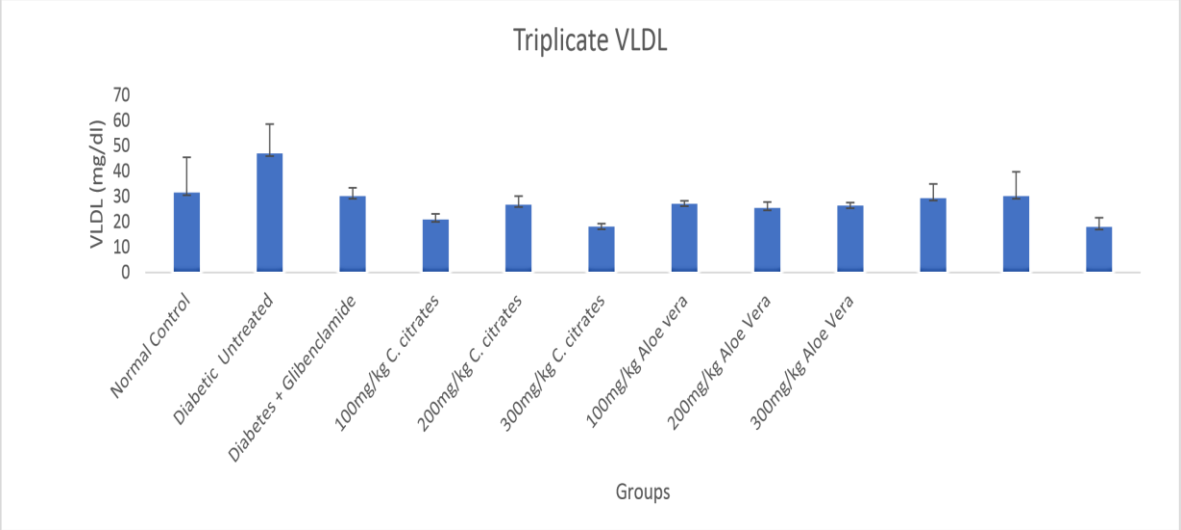

Fig. 5. Effect of *A. barbadensis*, *C. citratus* and its combined extract on VLDL level of alloxan-induced diabetic rats

A significant (P<0.05) decrease in Total Cholesterol at differing degrees in the treatment groups was seen when compared with the negative control. The negative control group showed a significant (P<0.05) increase in TCHOL in comparison to the normal non-diabetic group. The modulatory impact of the combined ethanolic extract on the total cholesterol level is dose-dependent i.e. decrease with increasing extract concentration.

There was a decreased (P<0.05) significance in the serum Triglyceride level of the treated groups as shown in Table 1 when compared with the negative control group. The negative control group showed a significant (P<0.05) increase compared with normal non-diabetic group.

The result also revealed a significant (P<0.05) decrease in the serum VLDL level of the groups treated with both the individual and combined ethanolic extract combined with the negative control and normal non-diabetic group. The negative control group showed a significant (P<0.05) increase compared with the normal non-diabetic group.

4. DISCUSSION

Diabetes showed an elevated lipid profile in the experimental rats as distinct in the lipid profile of the negative control rats as compared to the normal non-diabetic rats. This finding is in contrast with Mitra et al. [28] who reported that alloxan elevated Total Cholesterol (TC), Triglyceride (TG), Low density lipoprotein (LDL) and Very low density lipoprotein (VLDL) but reduced High density lipoprotein (HDL) in diabetic rats. The result also showed that administration of the *Cymbopogon citratus*, *Aloe barbadensis* and combined extract modulated the lipid profile much more than the standard antidiabetic drug glibenclamide as seen in LDL, VLDL, and Triglycerides.

The lipid profile study revealed that group of rats ad extracts of *A. barbadensis*, *C. citratus* caused a decrease in TCHOL, TRIG, VLDL and LDL and significant increase (P<0.05) in HDL when compared with the negative control groups of rat. This result is consistent with Mohammad et al. [29] and Ewenighi et al. [30] who noted that both extracts reduced triglycerides, very low density lipoprotein, cholesterol and low density lipoprotein respectively and Fatimah [31] who added that the potential of the aloe vera gel on lipid profile was attributed to glycoprotein. This potential is also attributed to the fact that *A. barbadensis* has a radical scavenging activity, rich in polysaccharide and hinders thromboxane formation [32]. The effect of combined ethanolic extract also recorded the same result as individual ethanolic aloe vera and lemon grass respectively. This effect could be due to phytochemical reaction of phytoconstituents of both plant extract. Edom et al. [33] and Can et al. [17] also reported that administration of *A. barbadensis* extract significantly reduced the lipid profile level and increased the high density cholesterol level. The observations in this study are at odds with those of Nur et al. [34] who

examined the impact of *A. barbadensis* on serum lipid profiles in the rat after being exposed to cigarette smoke and reported that treatment with aloe vera extract reduced all elevated lipid profile level but did not increase high density lipoprotein. It is also in contrast with Hasan and Abdullah [16] who reported that a significant reduction was observed in the triglyceride level (P<0.05) but slightly reduced in the cholesterol level after three weeks of treatment. They also added that no change was observed in the high density lipoprotein when compared with diabetic and treated group but a slight decrease was observed in induced animals treated with glibenclamide drug. This observation varied with those of Adegbegi et al*.* [35] who stated that the lipid profiles of rats treated with both aqueous and ethanolic extracts of *C. citratus* were not significant in comparison with normal control while rats administered with ethanolic extract was decreased when in comparison with those administered aqueous extract and the normal non-diabetic rats [23,24,7].

5. CONCLUSION

The study revealed the antilipidemic properties of *Cympobogon citratus, Aloe barbadensis* and it's proportionate combination at varied dosage on diabetic-induced rats and therefore can be used as adjunctive therapy to subjugate diabetes.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

ETHICAL APPROVAL

The Nnamdi Azikiwe University-Animal Research Ethics Committee acknowledged this research with an ethical approval that was assigned the reference number; NAU/AREC/2022/00011.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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