AMERICAN JOURNAL OF PHYSIOLOGY, BIOCHEMISTRY AND PHARMACOLOGY, 2018
VOL 7, NO. 1, PAGE 16–23
10.5455/ajpbp.20180507124130

eJManager

ORIGINAL RESEARCH

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Hypoglycemic effect of the methylene chloride-methanolic extract of the fresh and dried fruits of *Gongronema latifolium* in normoglycemic and alloxan-induced diabetic rats

Ifeoma P. Okoli[1,2], Uduma E. Osonwa[3,4], Daniel L. Ajaghaku[5], Amarachukwu U. Anwuchaepe[6]

[1]1Department of Pharmacology and Therapeutics, Imo State University, Owerri, Nigeria

[2]Department of Pharmacology, China Pharmaceutical University, Nanjing, China

[3]Department of Pharmaceutics and Pharmaceutical Technology, Nnamdi Azikiwe University, Awka, Nigeria

[4]Department of Pharmacological and Pharmaceutical Sciences, University of Houston College of Pharmacy, Houston, TX

[5]Department of Pharmacology, Enugu State University of Science and Technology, Enugu, Nigeria

[6]Department of Pharmacognosy, Enugu State University of Science and Technology, Enugu, Nigeria

ABSTRACT

Objective: The study investigated the hypoglycemic effect of the methylene chloride/methanol (1:1) extracts (methylene methanolic extracts) of fresh and dried fruits of *Gongronema latifolium* in both normoglycemic and alloxan-induced diabetic animals. Activities of these extracts were also tested in combination with reference anti-diabetic drug—Glibenclamide.

Methods: Qualitative screening for secondary metabolites and acute toxicities of the extracts was carried out using standard methods and modified Lorke's method, respectively. Diabetes was induced intraperitoneally using alloxan monohydrate (120 mg/kg) and blood samples drawn by tail milking at 0 hour, 30 minutes, 1 hour, 2 hours, and 4 hours, respectively, for the determination of blood glucose. The extracts were tested at 50 and 100 mg/kg, while the combination study was tested at 50 mg/kg of the extracts +5 mg/kg gibenclamide in both alloxan-induced diabetic and normoglycemic rats.

Results: Phytochemical analysis revealed the presence of varying amount of secondary metabolites such as alkaloids, glycosides, tannins, steroids, terpenoids, saponins, and resins. The acute toxicity tests indicated no obvious sign of toxicity or mortality. The fresh and dried fruit extracts produced significant ($p < 0.05$) dose-dependent hypoglycemic activities in both the normoglycemic and alloxan-induced hyperglycemic rats. Co-administration of the fresh fruit extract at 50 mg/kg and glibenclamide at 5 mg/kg showed a significantly ($p < 0.05$) increased activity over glibenclamide alone throughout the duration of the experiment.

Conclusion: This study establishes the anti-diabetic activities of the fresh and dried fruits of *G. latifolium* and proves the treatment benefit in combining the methylene methanolic extract of this fruit with a standard oral hypoglycemic agent.

ARTICLE HISTORY

Received May 07, 2018
Accepted May 08, 2018
Published May 25, 2018

KEYWORDS

Combination; fresh and dried fruits; glibenclamide; *Gongronema latifolium*; hypoglycemic activity

Introduction

Diabetes mellitus is one of the most important of all endocrine diseases and is a disorder in which blood sugar (glucose) levels are abnormally high [1–3]. In diabetes, the body either does not respond properly to its own insulin, produces less insulin, or both, which causes glucose to accumulate in the blood, often leading to various complications [4]. Diabetes mellitus has remained a scourge and a health menace of both the orthodox and traditional personnel in both advanced and developing economies. The study has continued into the possible means of arresting this health hazard. Recently, a lot of attention has been turned to herbs as the panacea [5]. It

Contact Amarachukwu U. Anwuchaepe ✉ amaraanwuchaepe@gmail.com Department of Pharmacognosy, Enugu State University of Science and Technology, Enugu, Nigeria.

has been shown in other literature that *G. latifolium* possesses anti-diabetic activity [6,7].

Gongronema latifolium is a tropical rainforest plant belonging to Asclepiadaceae family [8]. It is a shrub, with milky or less often, clear latex. The leaves are simple, opposite or occasionally whorled, very rarely alternate, usually without obvious stipules. *Gongronema latifolium* is a perennial edible plant with soft and pliable stem. In the South-Eastern States of Nigeria, the plant is known as "Utazi" and used primarily as a staple vegetable/spice [9,10]. Previous investigation on the extract of different parts of the plant showed that it has a good hypoglycemic activity in both normoglycemic and alloxan-induced diabetic rats [11,12], however, nothing is known about the anti-diabetic potentials of the fruit extract. This study investigated the hypoglycemic effect of the methylene methanolic extracts of the fresh and dried fruits of *G. latifolium* in normoglycemic and alloxan-induced diabetic rats.

Materials and Methods

Materials

Alloxan monohydrate (Sigma Aldrich), Glibenclamide (Aventis Pharm Pty Ltd), Xylene (BDH), Dichloromethane/Methanol 1:1 (Sigma Aldrich), Ethyl-acetate, N-hexane (Sigma Aldrich), Silica gel, Thin layer chromatography (TLC) plate, Soxhlet extractor, Rotary vacuum evaporator, Glucometer with strips (One Touch® by Lifescan).

Plant collection

The fruits of *G. latifolium* were collected from Igbo-ukwu in Aguata Local Government Area of Anambra State, Nigeria. It was identified by Mr. J. M. C. Ekekwe of Botany Department, University of Nigeria, Nsukka Enugu State, Nigeria.

Preparation of the extract

The fruits were grouped into two: the first group was the whole fresh fruits of *G. latifolium* which were cleaned and chopped into smaller bits. They were extracted with methylene chloride/methanol (1:1) using soxhlet extractor and the extract concentrated using a rotary vacuum evaporator at 40°C. The second group of fruits was air dried under room temperature before extraction with the same procedure as the fresh fruit. The extracts obtained were stored in a refrigerator until further use.

Animals

Wistar Albino rats and Swiss Albino mice of both sexes weighing between 98–121 g and 20–30 g, respectively, were obtained from the animal house of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Uyo. They were kept under standard conditions of 12/12 hour light and dark cycle and were fed growers mash (Guinea Feeds Nigeria Ltd) and water *ad libitum*. All animal experiments were conducted in line with the National Institutes of Health Guide for Care and use of Laboratory Animals (Pub. No. 85. Revised 2011) [13]. The animals were handled according to the guidelines approved by the Ethical Committee on animal research, Nnamdi Azikiwe University, Awka, Nigeria.

Phytochemical screening of extracts

Phytochemical analysis of the fresh and dried fruit extracts was performed using standard methods [14,15] for alkaloids, saponins, tannins, flavonoids, glycosides, steroids, and resins.

Acute toxicity studies (LD_{50})

The Lethal dose (LD_{50}) was determined using modified Lorke's method [16] as described in [17,18]. Thirty mice of both sexes were used. A day prior to the test, the animals were fasted overnight for 15 hours with free access to water both in fresh and dried fruit groups. Oral administration of 10, 100, and 1,000 mg/kg body weight, respectively, was given to the animals in the first phase (three per group both for fresh and dried fruits extract). The animals were observed for signs of toxiciy and lethal effect for 24 hours. In the second phase, the remaining 12 animals (two per group both for fresh and dried fruits extract) were given dose of 1,600, 2,900, and 5,000 mg/kg, respectively. They were observed for another 24 hours for signs of toxicity and lethal effect. Then, the LD_{50} was calculated. Using the following equation:

$$LD_{50} = \sqrt{(D_0 \times D_{100})}$$

D_0 = Highest dose that gave no mortality,

D_{100} = Lowest dose that produced mortality.

Determination of hypoglycemic activity of fresh and dried fruits extract in normoglycemic rat

Fifty healthy rats of both sexes were used for the study. The animals were divided into two sets to

Table 1. Phytochemical analysis of fresh and dried fruits of *G. Latifolium*.

Phytochemical	Abundance	
	Dried	Fresh
Flavonoids	-	-
Alkaloids	++	+
Glycosides	+++	+++
Tannins	+++	+++
Terpenoids	++	++
Steroids	+++	++
Saponins	+++	+++
Resins	++	++

Key: - = absent; + = present in trace quantity; ++ = moderate in present; +++ = present in large quantity.

serve for the fresh and dry fruits. Each set was grouped into five groups of five rats each and were fasted for 15 hours with free access to water only. Group 1 received 10 ml/kg of distilled water (vehicle control); groups 2 and 3 received 50 and 100 mg/kg of the extracts, respectively, while groups 4 and 5 received 5 mg/kg of glibenclamide and combination of 50 mg/kg of extracts + 5 mg/kg of glibenclamide, respectively. Blood samples were drawn by tail milking at 0 hour, 30 minutes, 1 hour, 2 hours, and 4 hours, respectively, and blood glucose concentrations were determined using a glucometer.

Determination of hypoglycemic activity of fresh and dried fruits extract in alloxan-induced diabetic rat

Diabetes was induced in 50 rats by a single intraperitoneal injection of alloxan monohydrate (120 mg/kg) according to the method of Tomita [19]. The fasting glucose level was determined 72 hours post-induction and rats of blood glucose > 160 mg/dl were used for further assay. The grouping, dosing, and blood sample collection were as in the normoglycemic rats.

Data analysis

Results were presented as mean ± Standard error due to mean (SEM) and analysed using SPSS Version 18.0. Significant differences in means were tested using analysis of varaince (ANOVA). Graphical plots were done with the microsoft excel® 2010. Differences between means were considered significant at $P < 0.05$.

Table 3. ANOVA results for hypoglycemic effect of fresh and dried fruit extract in normoglycemic rats.

Time	*F* value		*P* value		Remarks	
	Dried	Fresh	Dried	Fresh	Dried	Fresh
30 minutes	2.550	3.055	0.071	0.041	-	*
1 hour	4.487	4.275	0.009	0.012	*	*
2 hours	9.333	9.065	0.000	0.000	*	*
4 hours	13.486	9.917	0.000	0.000	*	*

*Significant at $p < 0.05$.

Table 2. Acute toxicities of the extracts of fresh and dried fruits of *G. Latifolium*.

Phases	Dose (mg/kg)	Mortality	
		Dried	Fresh
Phase 1	10	0/3	0/3
	100	0/3	0/3
	1,000	0/3	0/3
Phase 2	1,600	0/2	0/2
	2,900	0/2	0/2
	5,000	0/2	0/2

Results

The result of phytochemical analysis of the fresh and dried fruit extracts is as shown in Table 1. Flavonoids were absent in both the extracts, while alkaloids and steroids were present in varying quantity; terpenoids and resins were present in moderate amounts; glycosides, tannins, and saponins were present in large amounts.

Acute toxicity tests of the fresh and dried fruits extract (Table 2) showed the absence of mortality in all the groups throughout the testing peroid. The LD_{50s} for the fresh and dried fruit extracts were estimated to be above 5,000 mg/kg.

The extract of the fresh fruit showed a dose-dependent hypoglycemic effect in non-diabetic rats (Fig. 1). The level of significance is as shown in Table 3. Both the 50 mg/kg and 100 mg/kg doses had hyperglycemic activities less than the 5 mg/kg glibenclamide. Co-administration of the extract at 50 and 5 mg/kg glibenclamide showed significantly ($p < 0.05$) an increased activity than glibenclamide alone within 1 hour post-administration. The extract of the dried fruit showed a dose-dependent hypoglycemic effect in non-diabetic rats (Fig. 2). The level of significance is as shown in Table 3. However, at the two dose levels (50 and 100 mg/kg), the hypoglycemic activities were less than that of 5 mg/kg of glibenclamide. Co-administration of the extract at 50 mg/kg and glibenclamide 5 mg/kg showed a significantly ($p < 0.05$) increased activity over glibenclamide alone within 1 hour post-administration.

The fresh fruit extract showed a dose-dependent hypoglycemic effect in the alloxan-induced diabetic

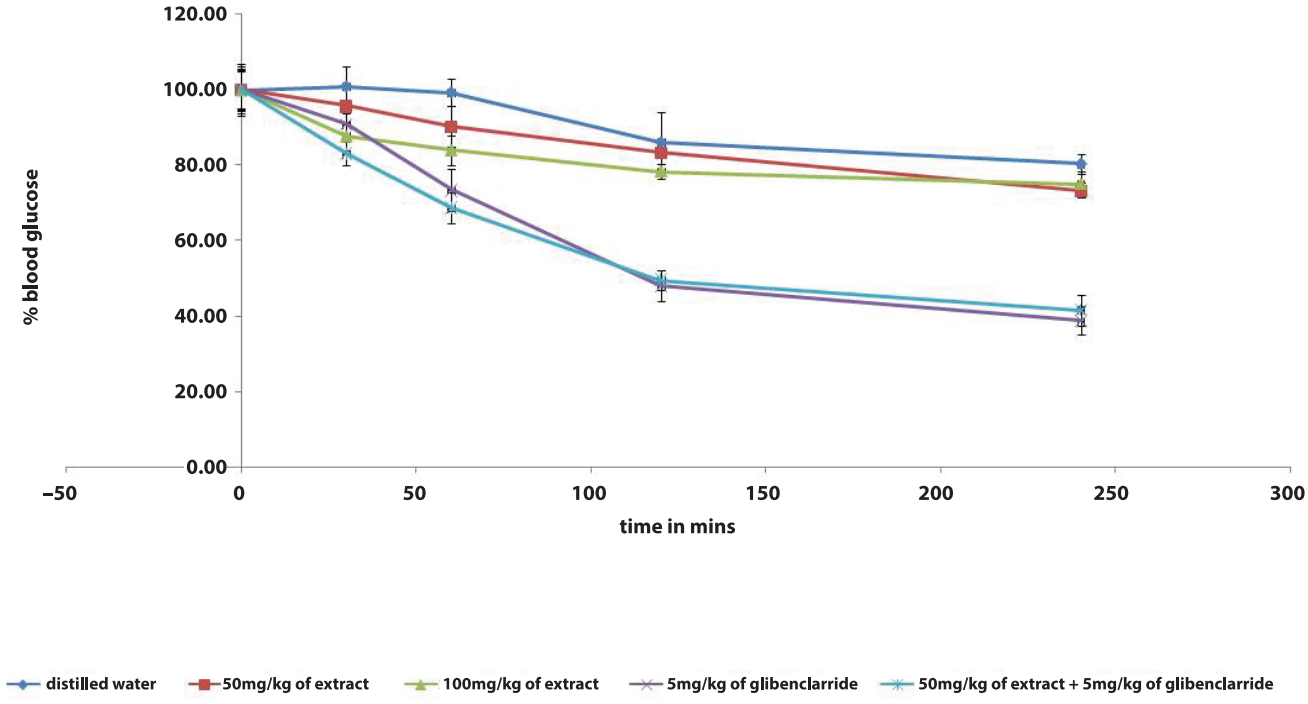

Figure 1. Hypoglycemic effect of fresh fruits extract of *G. latifolium* in non-diabetic rats.

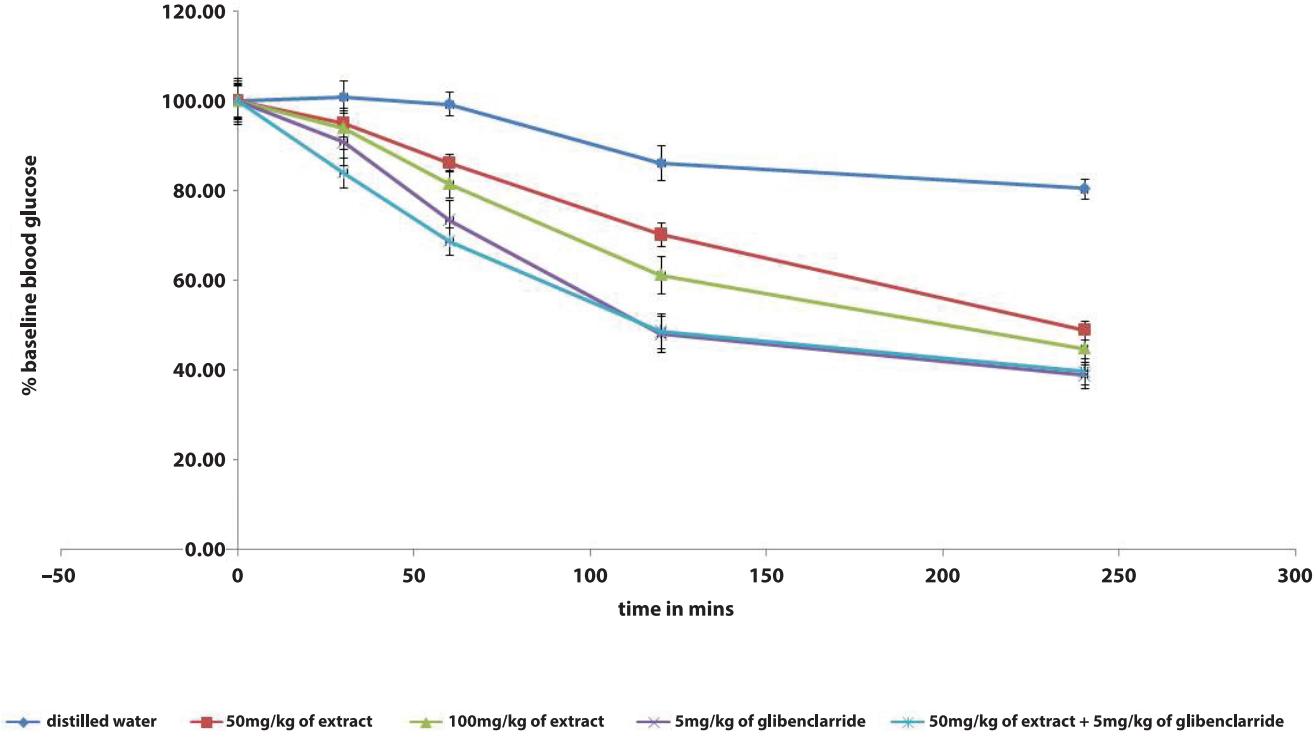

Figure 2. Hypoglycemic effect of dried fruits extract of *G. latifolium* in non-diabetic rats.

rats (Fig. 3). The level of significance is as shown in Table 4. At the 50 and 100mg/kg dose levels, the hypoglycemic activity was close to that of 5 mg/kg of glibenclamide. Co-administration of the extract at 50 mg/kg and 5 mg/kg glibenclamide showed a significantly ($p < 0.05$) increased activity over glibenclamide alone throughout the duration of the experiment.

The dried fruit extract showed a dose-dependent hypoglycemic effect in the alloxan-induced diabetic

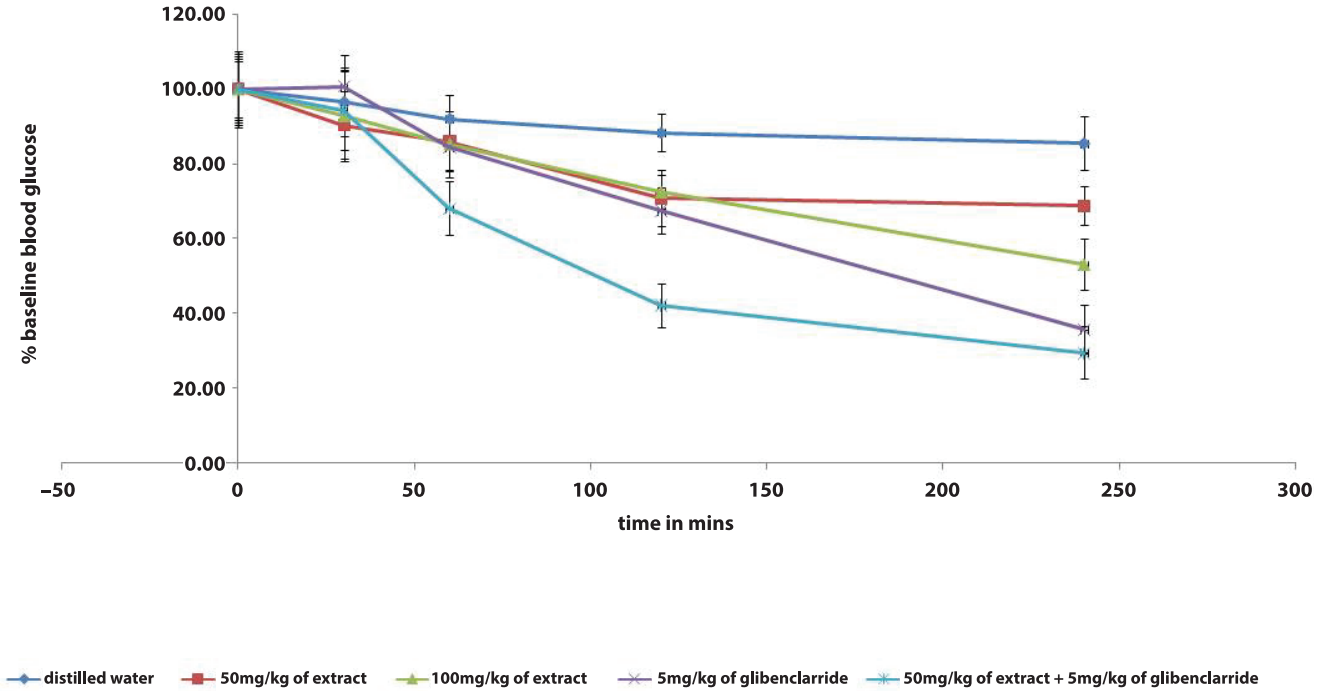

Figure 3. Hypoglycemic activity of *G. latifolium* fresh fruits extract in diabetic rats.

rats (Fig. 4). The level of significance is as shown in Table 4. At the two dose levels 50 and 100 mg/kg, the hypoglycemic effects were significantly ($p < 0.05$) greater than that of 5 mg/kg of glibenclamide within 30 minutes of drug administration. Co-administration of the extract at 50 mg/kg and 5 mg/kg glibenclamide showed a much increased activity over glibenclamide alone for the first 2 hours of the experiment.

The effect of state of drying of *Gongronema* fruits on the hypoglycemic activity of the extracts in both the diabetic and non-diabetic rats (Figs. 5 and 6) revealed the dried fruit extract to show significantly ($p < 0.05$) a higher hypoglycemic effect than that of the fresh fruit.

Discussion

The hypoglycemic effect of methylene methanolic extract of fresh and dried fruits of *G. latifolium* in both the normoglycemic and alloxan-induced glycemic rats was evaluated to justify the ethno-medicinal use of this plant part as an anti-diabetic agent. The concomitant use of medicinal plants with synthetic drugs is a growing inclination particularly in Africa due to the difficulty in successfully treating some chronic diseases like diabetes with synthetic drug only [20]. More so, Gabriel et al. [12] reported better anti-diabetic benefit associated with combining the leaf extract of this plant with glibenclamide than using them singly, thus this study further investigated the hypoglycemic effect of the fresh and dried fruit extracts of *G. latifolium* singly and in combination with glibenclamide in both the diabetic and non-diabetic rats.

Table 4. ANOVA results for hypoglycemic effect of fresh and dried fruit extract in glycemic rats.

Time	*F* value		*P* value		Remarks	
	Dried	Fresh	Dried	Fresh	Dried	Fresh
30 minutes	4.633	1.038	0.008	0.412	*	-
1 hour	2.459	3.257	0.079	0.033	*	*
2 hours	7.364	8.221	0.001	0.000	*	*
4 hours	17.993	15.901	0.000	0.000	*	*

*Significant at $p < 0.05$.

The phytochemical analysis of the fresh and dried fruit extracts of *G. latifolium* revealed varying concentration of alkaloids steroids, terpenoids, resins, glycosides, tannins, and saponins. These secondary metabolites have been shown to produce potent anti-diabetic activities via various mechanisms. Tannins have been reported to maintain blood glucose levels, glucose uptake, and insulin secretion as well as the modulate immune function to prevent specific Diabetis mellitus [21]. Different types of alkaloids have been reported to show good anti-diabetic acivities via numerous mechanisms like inhibition of glucosidase as well as the inhibition of the activity of disaccharides in

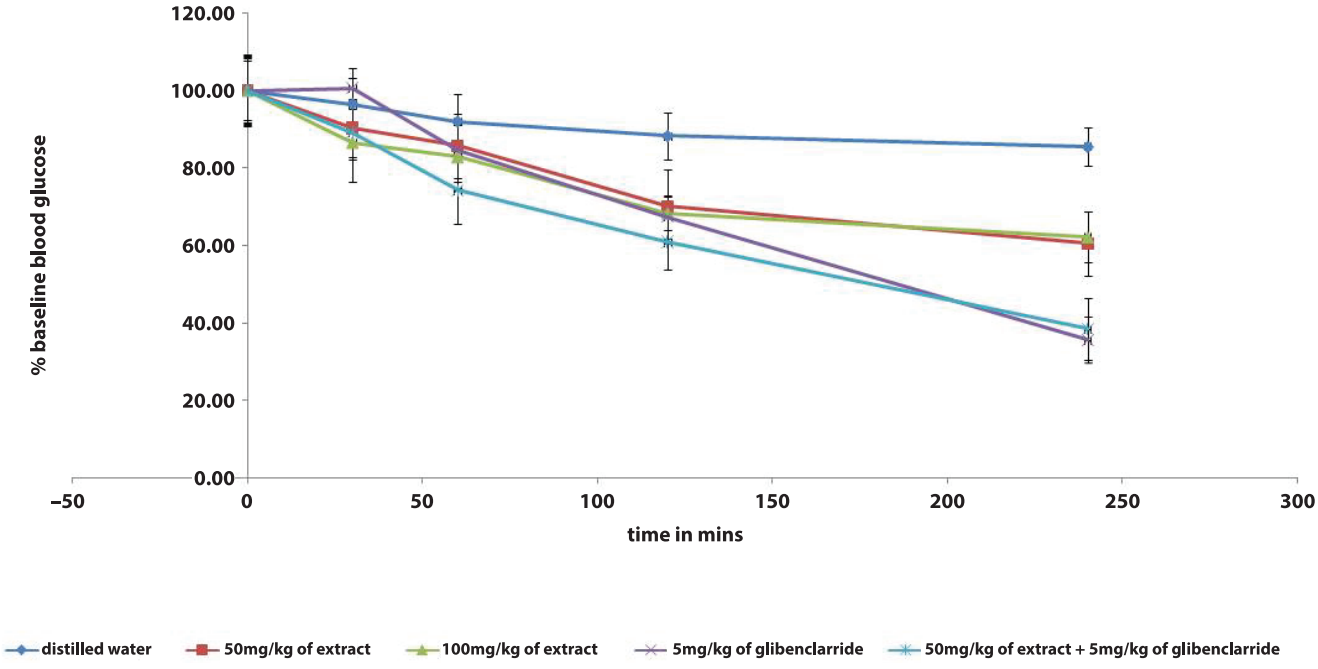

Figure 4. Hypoglycemic effect of *G. latifolium* dried fruits extract in diabetic rats.

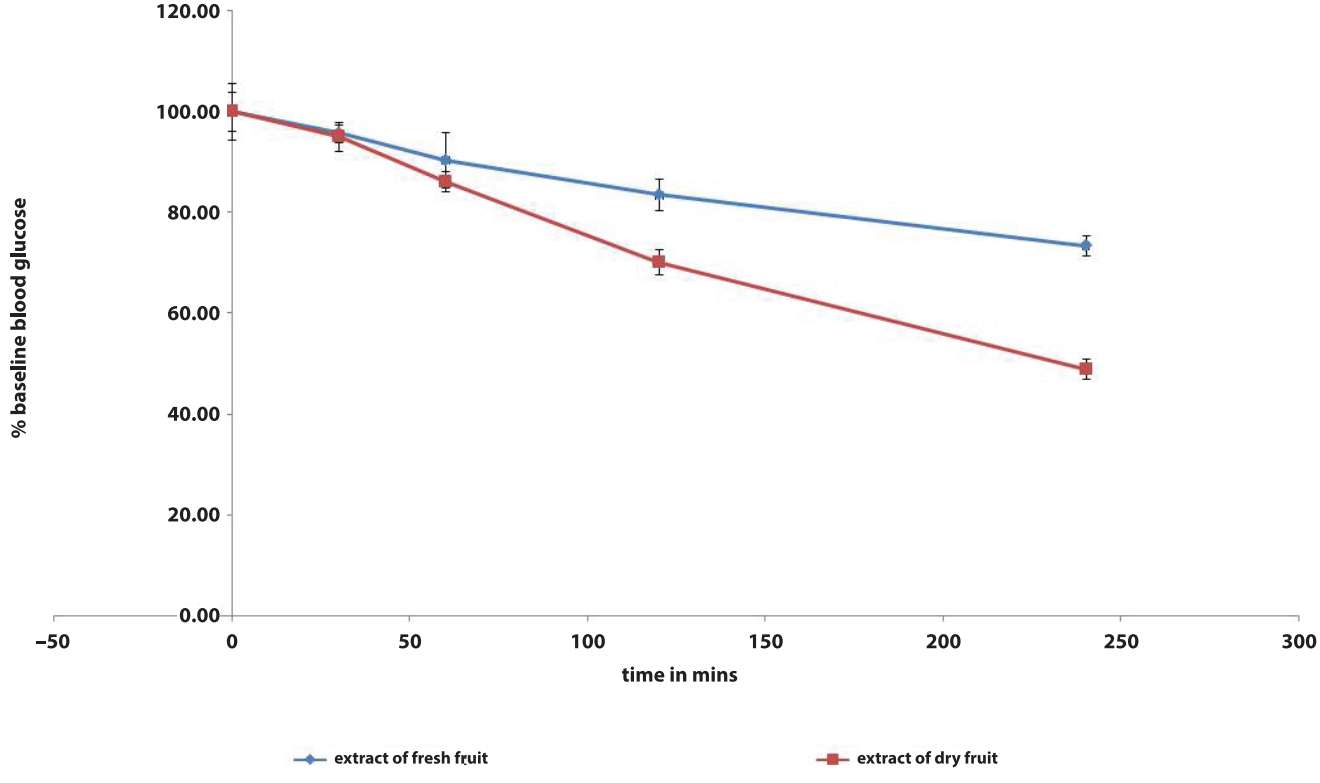

Figure 5. Effect of state of drying of *Gongronema* fruits on the hypoglycemic activity of the extract in non-diabetic rats.

Caco-2 cells [22]. Triterpenoid and steroidal glycosides have been reported to inhibit glucosidase, increase glucose uptake into the muscles, lower blood glucose, and increase insulin secretion in pancreatic β-cells [23]. Resins have been reported to affect hepatic glucogenesis, induce glucose uptake, etc [24,25].

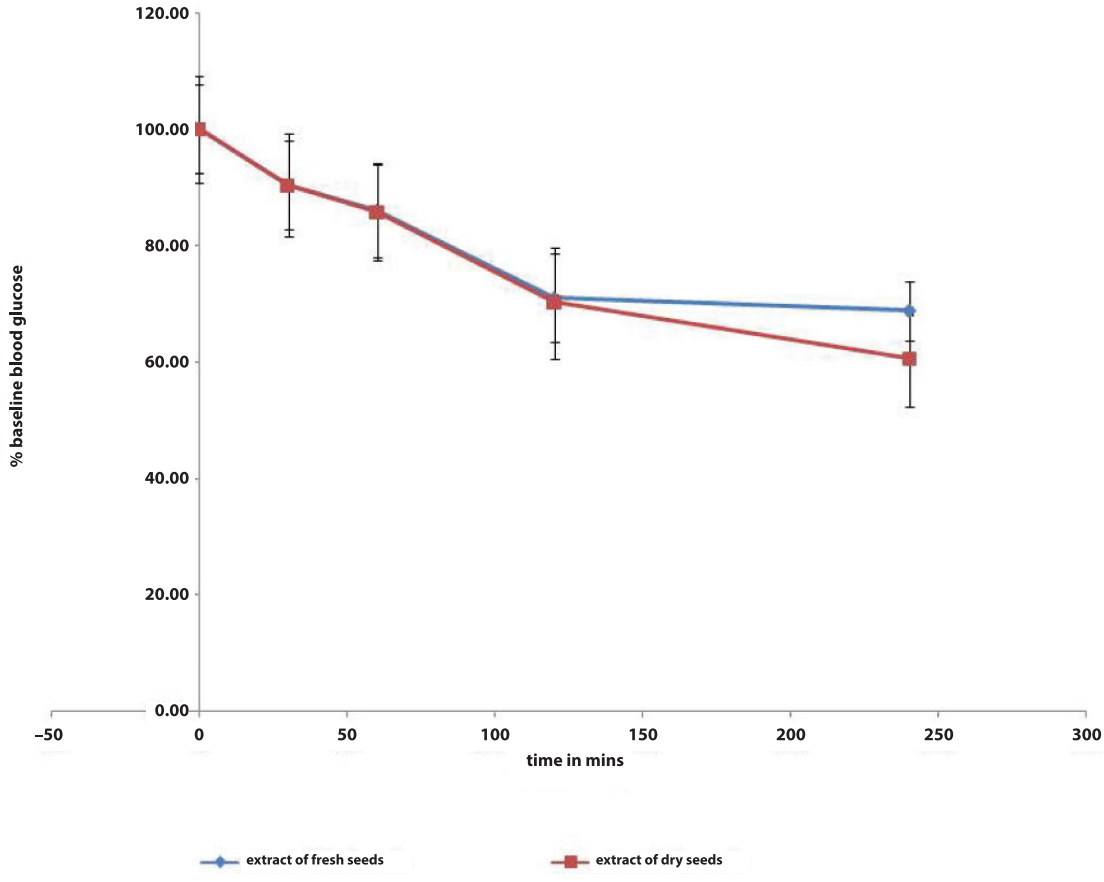

Figure 6. Effect of state of drying of *Gongronema* fruits on the hypoglycemic activity of the extract in diabetic rats.

The oral acute toxicity studies of the methylene methanolic extracts of fresh and dried fruits of *G. latifolium* revealed that the extracts did not cause any death or sign of toxicity in mice, suggesting that the fruit is safe when orally consumed acutely [6,14,26].

The methylene methanolic extracts of fresh and dried fruits of *G. latifolium* at the two doses used lowered blood glucose level in non-diabetic rats although not comparable to that of gibenclamide, thus indicating its hypoglycemic activity that is possibly the mechanism of action through which the fruit ameliorates diabetes, and thus establishes its anti-diabetic potential. However, in alloxan-induced diabetic rats, the anti-diabetic activities recorded at the two doses were comparable to that of glibenclamide 3 hours 30 minutes post-treatment. More so, the combination of the fresh fruit extract and glibenclamide significantly reduced the glucose level in diabetic rats better than glibenclamide alone, thus suggesting that there is a great treatment benefit when the fresh fruit is taken with gibenclamide in this ratio (50 mg/kg and glibenclamide 5 mg/kg). Akah et al. [27] reported that the islet beta cells which produce insulin are destroyed on injection of alloxan in rats. Thus, regeneration of the islet beta cells may be the mechanism of action of this co-administration therapy.

Generally, the dried fruit extract recorded better glucose lowering activity than the fresh fruit extract in both the non-diabetic and diabetic rats suggesting that the anti-diabetic benefit of *G. latifolium* fruit will be better obtained in the dried fruit. The better anti-diabetic activity recorded by the dried fruit extract may be attributed to the phytochemical constituents revealed in this plant material, most especially the higher abaundance of steroids and alkaloids which has been shown to produce potent anti-diabetic activities.

Conclusion

Generally, the anti-diabetic benefit of *G. latifolium* fruit will be better obtained in the dried fruit; however, co-administration of the fresh fruit extract with glibenclamide can be employed for better control of hyperglycemic states. The study shows the extracts to be safe for short-term human consumption.

Thus, further studies on the long-term use of this plant part and toxicity of the co-administration of the fresh fruit extract and gibenclamide should be investigated.

Conflict of Interest

There are no conflicts of interest.

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